E-435

Applied Reproductive Strategies in Beef Cattle

Conference Proceedings

November 12 - 13, 2005

Memorial Student Center
Texas A&M University
College Station, Texas

Presented by:

North Central Region Bovine
Reproduction Task Force

Texas Agricultural Experiment Station
Texas Cooperative Extension
Texas A&M Department of Animal Science
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PROCEEDINGS

APPLIED REPRODUCTIVE STRATEGIES IN BEEF CATTLE

November 12 and 13, 2005

Memorial Student Center, Texas A&M University
College Station

Edited by G.L. Williams and D.W. Forrest

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Applied Reproductive Strategies in Beef Cattle

November 12 - 13, 2005
Memorial Student Center
Texas A&M University, College Station

Objectives of Applied Reproductive Strategies in Beef Cattle

- **Improve the understanding of the physiological processes of the estrous cycle, the procedures available to synchronize estrus and ovulation, and the proper application of these systems for AI in *Bos taurus* and *Bos indicus*-influenced cattle**
- **Improve the understanding of biological and managerial factors that influence fertility in AI programs, including male fertility and herd health, and the use of adjunct technologies**

Schedule and Proceedings Contents

SATURDAY, NOVEMBER 12

7:15-8:00 Registration

– Morning Sessions –

Synchronization of Estrus and Ovulation: Tools and Systems
Moderator: Dr. David Forrest

10:10 Break

Moderator: Dr. Paul Harms

11:45 Questions for Morning Speakers

12:00-1:30 Lunch and Trade Show

– Afternoon Sessions –

Synchronization of Estrus and Ovulation: The *Bos indicus* Influence
Moderator: Dr. Bruce Carpenter

3:20 Break

Biological and Managerial Factors Influencing Reproductive Efficiency
Moderator: Dr. Ron Randel

5:05 Questions for afternoon speakers

5:20 Producer Panel: Utilizing Advanced Reproductive Technologies (Dr. David Patterson, Moderator)

5:45 Adjourn

SUNDAY, NOVEMBER 13
– Morning Sessions –

Biological and Managerial Factors Influencing Reproductive Efficiency, Cont.
Moderator: Dr. Tom Welsh, Jr.

9:45 Break

Herd Health Programs and Evaluation of Reproductive Potential
Moderator: Dr. Floron (Buddy) Faries

11:40 Questions for Morning Speakers

12:00-1:30 Lunch and Trade Show

– Afternoon Sessions –

Adjunct Reproductive Technologies
Dr. Duane Kraemer

3:00 Break

3:15: Cloning Bovine Embryos: Current Status and Future Application
Dr. Mark Westhusin, Texas A&M University 309

3:45 Questions for Afternoon Speakers

4:00 Veterinary Panel: Utilizing Advanced Reproductive Technologies
(Dr. Dan Posey, Moderator)

4:30 Adjourn

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PHYSIOLOGICAL PRINCIPLES UNDERLYING SYNCHRONIZATION OF ESTRUS

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Introduction

Reproductive efficiency is the most important factor impacting the economics of a cow calf operation. The economic value of reproduction for commercial beef producers was reported to be five times greater than calf growth (Trenkle and Willham, 1977). Maximizing reproductive efficiency depends upon the successful completion of the following events: a heifer must reach puberty before the start of the breeding season, conceive early in the breeding season, calve unassisted, raise the calf to the time it is marketed, and the heifer/cow must conceive in time to calve early during the subsequent calving season. Any interruption in the preceding cycle will constitute reproductive loss, which is estimated to cost the US beef industry around $500 million annually (Bellows et al., 2002). Therefore, minimizing reproductive loss needs to be a high priority.

Recent years have witnessed the rapid development of technologies utilized to increase reproductive efficiency and (or) improve the genetic merit of a herd. Some of these technologies include: estrous synchronization, artificial insemination, gender-selected semen, in vitro embryo production, embryo transfer, ultrasonography, transgenics, and cloning. Of the preceding reproductive technologies, estrous synchronization and artificial insemination are among the most powerful and applicable technologies for genetic improvement of beef herds (Seidel 1995). The development of new and improved methods of synchronizing estrus and ovulation depends on our understanding of the physiological and hormonal mechanisms controlling the estrous cycle and the initiation of estrous cyclicity in prepubertal heifers and postpartum cows. Although estrous synchronization products and protocols have changed over time, the basic physiological principles underlying how these products work have not. An understanding of the bovine estrous cycle and how estrous synchronization products work will facilitate the application of these technologies in groups of cycling and anestrous females. This article reviews the endocrine regulation of the estrous cycle with specific emphasis on the regulation of growth of a dominant follicle and the lifespan of the corpus luteum. In addition, emphasis will be given to estrous synchronization products that are commercially available, and the physiologic mechanisms by which these products synchronize estrus and (or) ovulation in cattle.

Principles of the Bovine Estrous Cycle

Characteristics of the Estrous Cycle

In cattle, the estrous cycle normally varies from 17 to 24 days and the duration of estrus is generally 10 to 18 hrs; however, considerable variation exists among individual animals (range < 8 to > 30 hr; O'Connor and Senger, 1997). There are a number of estrous detection aids available to assist producers; however, the HeatWatch electronic estrous detection system provides information on the intensity of estrus. Rorie et al., (2002) utilized the HeatWatch system with 500 Angus cows to evaluate the effect of estrous intensity on fertility. Estrus was synchronized with the Select Synch protocol (GnRH followed seven days later with an injection of prostaglandin $F_{2\alpha}$). Length of estrus ranged from 0.5 to 24 hr and there was no effect of length of estrus on pregnancy status. However, pregnant cows were mounted more times per estrus than nonpregnant cows. These data are similar to another study with Angus cows in which pregnant cows were mounted more times per estrus than nonpregnant cows (Kuhlman et al., 1998).

A seasonal effect on estrous behavior has been reported in Angus x Hereford cows located in Oklahoma (White et al., 2002). In the preceding study, the length of estrus was greater in summer compared to winter or spring; however, cows were mounted more frequently per estrus in winter compared to summer or spring. Therefore, estrous detection may need to occur more frequently in winter compared to spring or summer; whereas, in summer estrous detection may need to occur for a longer duration at each check. In this study, there was no effect of season on the interval from the onset of estrus to ovulation (Mean = 31 hr).

In contrast to other livestock species, cattle ovulate following the end of estrus (approximately 28 to 32 hours after the onset of estrus or 12 to 20 hr following the end of estrus). Although characteristics of the estrous cycle are similar among most beef breeds, important differences have been reported between Bos Taurus and Bos Indicus breeds (Galina et al., 1987, Inskeep et al., 1982). In general, it is more difficult to detect estrus in Bos Indicus females compared to Bos Taurus females. This is likely because Bos Indicus females are reported to have a shorter duration of behavioral estrus compared to Bos Taurus females (Brewester and Cole, 1941, Plasse et al., 1970). In addition, Bos Indicus females had a decreased interval from onset of estrus to ovulation (Randel, 1976), decreased magnitude of the preovulatory luteinizing hormone surge (Randel, 1976), smaller corpora lutea (Irvin et al., 1978), and lower luteal phase concentrations of progesterone (Adeyemo and Heath, 1980) than Bos Taurus females.

Hormonal Patterns During the Estrous Cycle

The estrous cycle is divided into three stages (follicular phase, estrus, and luteal phase) and is regulated by hormones secreted by the hypothalamus (gonadotropin releasing hormone [GnRH]), anterior pituitary gland (follicle stimulating hormone [FSH] and luteinizing hormone [LH]), ovary (estradiol and progesterone), and uterus (prostaglandin $F_{2\alpha}$; $PGF_{2\alpha}$). The preceding hormones serve as chemical messengers that

travel in the blood to specific target tissues which contain receptors that are hormone specific and regulate the phases of the estrous cycle. The combination of hormone secretion and metabolism (liver, kidneys, and lungs) maintain the correct hormonal balance during the follicular phase, estrus, and luteal phase of the cycle. For a list of hormones, their biological functions, their role in estrous synchronization, and product names see Table 1.

A preovulatory follicle and the subsequently formed corpus luteum are the two primary ovarian structures that regulate the estrous cycle through secretion of estradiol and progesterone, respectively. Changes in a preovulatory follicle and corpus luteum, patterns of secretion of LH, estradiol and progesterone, and changes in ovarian blood flow during the ruminant estrous cycle are depicted in Figure 1.

Table1. Reproductive hormones, their functions during the estrous cycle, roles in estrous synchronization, product name, dosages, and route of administration.

Hormone	Endocrine Gland	Function of Hormone	Biological Action in Estrous Sync.	Product Name	Dosage	Route of Administration
Progesterone	Corpus luteum	Inhibit estrus Inhibit ovulation Prepares animal for pregnancy Maintenance of pregnancy	Inhibit estrus Inhibit ovulation Induce cyclicity Dominant follicle turnover	Melengestrol Acetate (MGA®) EAZI-BREED CIDR®	0.5 mg/hd/day 1 CIDR per animal (1.38 g prog)	Feed Vaginal insert
Prostaglandin $F_{2\alpha}$	Uterus	Induce luteal regression	Induce premature luteal regression	Lutalyse® ProstaMate® In Synch® Estrumate® estroPLAN®	5 ml 5 ml 5 ml 2 ml 2 ml	im inject im inject im inject im inject im inject
GnRH	Hypothalamus	Controls secretion of LH Induces gonadotropin surge	Synchronize follicle wave Induce ovulation	Cystorelin® Factryl® Fertagyl® OvaCyst®	2 ml 2 ml 2 ml 2 ml	im inject im inject im inject im inject
Follicle Stimulating Hormone (FSH)	Anterior Pituitary Gland	Initiation of a follicular wave	Superovulation	Follitropin®	Depends on application	im inject
Luteinizing Hormone (LH)	Anterior Pituitary Gland	Stimulated by GnRH Induction of ovulation Oocyte maturation Luteal tissue formation	Synchronize follicular wave Induction of ovulation	N/A	N/A	N/A
Estradiol	Ovarian follicle	Estrous behavior Induction of gonadotropin surge Sperm transport	Dominant follicle turnover Estrous behavior	N/A	N/A	N/A

GnRH = gonadotropin releasing hormone; prog = progesterone; N/A = not applicable

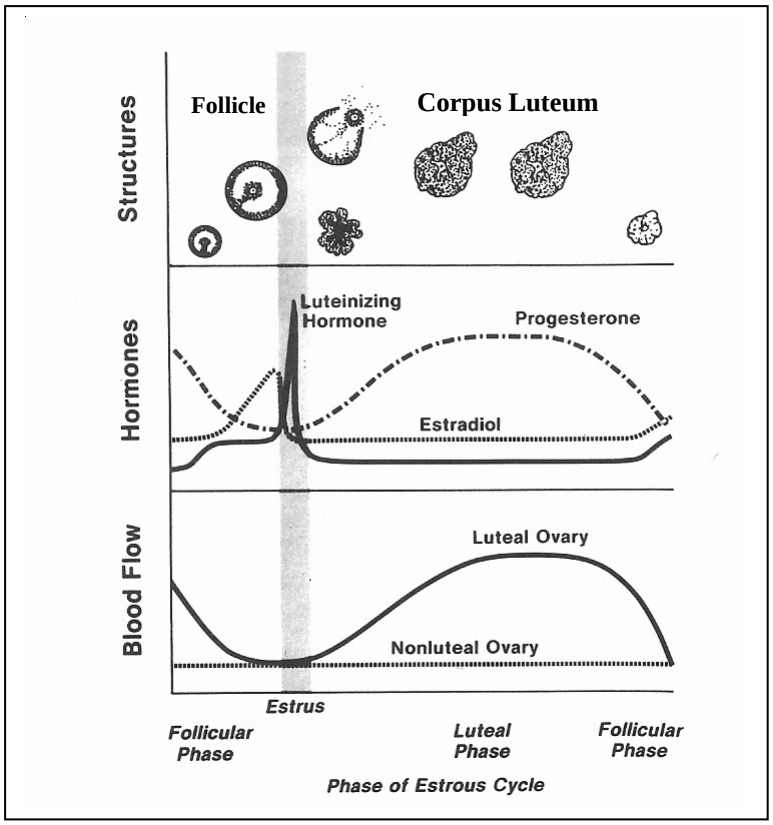

Figure 1. Changes in ovarian structures (preovulatory follicle and corpus luteum), hormones (luteinizing hormone, estradiol, and progesterone) and ovarian blood flow (ovary containing [luteal ovary] or not containing [nonluteal ovary] a corpus luteum) during the three phases of the estrous cycle (follicular, estrus, and luteal phase; Modified from Garverick and Smith, 1993).

Follicular Phase

The follicular phase (proestrus) begins with the initiation of corpus luteum regression (luteolysis) and ends with the onset of estrus. Luteolysis is accompanied by a rapid decrease in progesterone resulting in a decrease in the negative feedback on pituitary LH secretion. As circulating concentrations of progesterone decrease, LH pulse frequency increases followed by a rapid increase in follicular estradiol secretion. The production of follicular estradiol results from the coordinated actions of LH and FSH on theca and granulosa cells, respectively (Fortune, 1986; Fortune 1988). Thecal cells have membrane receptors that bind LH resulting in the synthesis of androgens that subsequently diffuse through the basement membrane into granulosa cells. Following FSH binding to membrane receptors on granulosa cells there is an increase in aromatase activity, that converts androgens to estradiol. Increased circulating concentrations of estradiol initiate estrous behavior and induce the preovulatory gonadotropin surge, which is essential for ovulation. In addition, estradiol can act within granulosa cells to increase LH receptor concentration and thereby prepare the preovulatory follicle to respond to the gonadotropin surge (Richards, 1980).

Regulation of Follicular Waves: Two general patterns of antral follicular development are present in mammals. In cattle, sheep, and horses, dominant ovulatory sized follicles develop in sequential waves during both the follicular and luteal phases of the cycle (Figure 2). In primates, pigs, and rodents, however, dominant ovulatory follicles only develop during the follicular phase of the cycle (Fortune, 1994). The bovine estrous cycle usually consists of two to three follicular waves and each wave begins with the recruitment of a cohort of antral follicles from a pool of growing small follicles. One follicle is subsequently selected from this cohort for continued growth and becomes dominant. The remaining follicles in the cohort become atretic. During a nonovulatory follicular wave, the dominant follicle eventually becomes atretic and a new follicular wave is initiated. A viable dominant follicle present at luteolysis will generally become the ovulatory follicle (Adams, 1999). The estrous cycle length of cows that have three follicular waves is generally longer (20-24 days) compared to cows with two follicular waves (18-20 days).

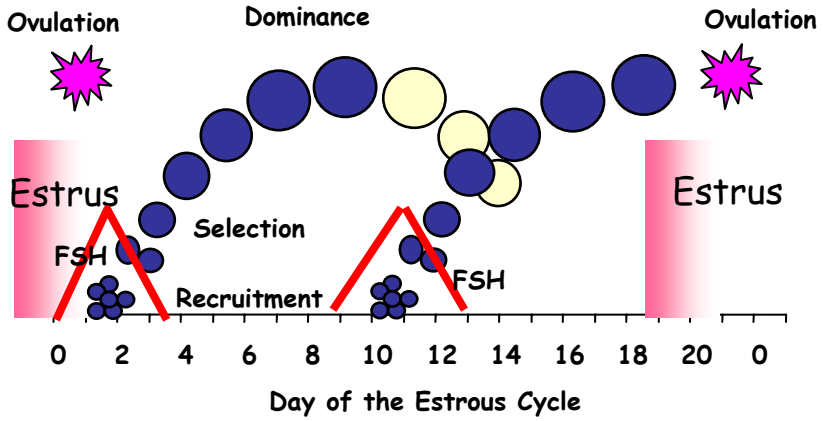

Figure 2. Relationship between circulating concentrations of follicle stimulating hormone (FSH) and stages of a bovine follicular wave (recruitment, selection, and dominance). A transient increase in FSH initiates recruitment of a cohort of follicles, from which a single follicle is normally selected to become the dominant follicle. If the corpus luteum regresses in the presence of a viable dominant follicle ovulation will occur (second follicular wave). However, in the absence of luteal regression, the dominant follicle becomes atretic (regresses; light circles; Modified from Kojima and Patterson, 2003).

In cattle, follicular waves can be detected during most reproductive states including the prepubertal period, estrous cycle, gestation, and postpartum anestrous period (Adams, 1999). The only exception to the continuous growth and development of follicular waves in cattle is during the last 21 days of gestation. During this time follicles greater than 6 mm in diameter have not been detected (Ginther et al., 1996a). Following parturition, follicular waves resumed following a rise in circulating concentrations of FSH (Schallenberger and Prokopp, 1985), and the first dominant follicle appeared between days 7 and 15 postpartum in both beef and dairy cows (Murphy et al., 1990; Crowe et al., 1993).

Recruitment. Follicular waves have been studied most extensively in cattle and consist of the following three stages: recruitment, selection, and dominance. Recruitment of a cohort of follicles, around 3 mm in diameter, is stimulated on each ovary by a transient rise in FSH (Figure 2). Inhibition of both FSH and LH arrested follicular growth at two to four mm, however, when physiological levels of FSH were infused for 48 hours follicular growth from five to eight mm was stimulated (Gong et al., 1996). The peak concentration of FSH occurred when the future dominant follicle attained a mean diameter of approximately four mm, after which concentrations of FSH declined (Figure 2; Ginther et al., 1996b), and were at basal concentrations by the time follicular selection occurred (Ginther et al., 2000a). The mechanism responsible for the initial decline in FSH concentration is unknown, however, estradiol and inhibin are follicular products that probably play a major role in the decline of FSH (Adams, 1999).

Selection. Follicular *s*election is the process by which a single follicle from the recruited cohort is selected to continue to grow and become dominant, while the remaining follicles of the cohort undergo atresia. With the decline in circulating FSH concentrations, small follicles are presumably unable to continue growth and the selected follicle (dominant follicle) may shift its dependency from FSH to LH (Ginther et al., 1996b). The decreased circulating concentrations of FSH at the time of selection are likely important for the selection of a single dominant follicle (Figure 2). The decline in circulating concentrations of FSH is presumably driven by increasing concentrations of estradiol (and perhaps inhibin) produced by the cohort of recruited follicles (Ginther et al., 2000b). Increased concentrations of estradiol and inhibin may feed back on the hypothalamic-pituitary axis to selectively suppress FSH secretion (Martin et al., 1988). At follicular deviation, the selected follicle continues to grow while the subordinate follicles enter atresia (Ginther et al., 1996b). In cattle, deviation usually occurs when the largest follicle reaches a diameter of approximately 8 mm, approximately 2.7 days after the initiation of a follicular wave (Ginther et al., 1997; Ginther et al., 1999) or 61 hours after the LH surge (Kulick et al., 1999).

Dominance. The dominance phase of the follicular wave occurs when a follicle has been selected and continues to grow at a faster rate than the largest subordinate follicle, and inhibits the emergence of a new follicular wave (Ginther et al., 1996b). Following selection and establishment of a dominant follicle, follicular recruitment is inhibited until dominance is lost or ovulation occurs. Inhibition of follicular recruitment may be mediated by inhibiting the transient rise in circulating concentrations of FSH (Adams, 1999). An alternative hypothesis is that the dominant follicle directly inhibits growth of small follicles through the secretion of a factor(s) that acts directly on other follicles in the ovary. Regardless of the mechanism, destruction of a dominant follicle results in a transient rise in circulating concentrations of FSH and subsequent initiation of a new follicular wave (Adams et al., 1992).

Estrus Phase

Increasing circulating concentrations of estradiol following luteolysis initiate estrous behavior, increase uterine contractions (facilitate sperm transport), and induce the preovulatory gonadotropin surge. The preovulatory gonadotropin surge coordinates the following events that are critical to the establishment of pregnancy: resumption of meiosis within the oocyte, follicular rupture, and luteinization of follicular cells. LH is generally considered to be the primary gonadotropin that controls the preceding events; however, FSH also has been shown to cause ovulation and luteal tissue formation (Galway et al., 1990). The end of the estrus phase of the cycle is marked by follicular rupture, which is the culmination of a complex cascade of events leading to the activation of proteolytic enzymes that digest the follicular wall and allows the egg (oocyte) to be released for fertilization. This process is similar to mechanisms associated with inflammation. Injection of GnRH will induce a surge of LH within 2 to 4 hours and ovulation of a dominant follicle will occur 24 to 36 hr after injection (Figure 3).

Estrus and ovulation are not always linked and frequently occur as independent events. The incidence of anovulatory estrus in peripuberal heifers was 22% and 13% for years 1 and 2, respectively and this phenomenon has been called nonpuberal estrus (Nelsen et al., 1985; Rutter and Randel, 1986). The incidence of nonpuberal estrus may be affected by age, breed, and photoperiod or season of the year (Nelsen et al., 1985). Formation of a cystic follicle can also result in estrous behavior without ovulation; however, the incidence of cystic follicles is low in beef cattle. Cystic follicles are normally treated by injecting GnRH, to luteinize the follicular tissue followed by an injection of $PGF_{2\alpha}$ seven days later to regress the luteal tissue.

Alternatively, ovulation without estrus is not uncommon in beef cattle. The first ovulatory estrus in heifers and postpartum cows is preceded by a transient increase in progesterone (short luteal phase; Gonzalez-Padilla et al., 1975). This is presumably due to ovulation without estrus. Increased concentrations of progesterone may be involved in preparation of the uterus for the possibility of pregnancy or in the establishment of patterns of gonadotropin secretion characteristic of cycling females. Short-term exposure of prepuberal heifers or anestrous postpartum beef cows to a progestin (Melengestrol Acetate [MGA] or Controlled Internal Drug Release [CIDR]) has been used extensively in estrous synchronization protocols to mimic this short period of progesterone exposure and will be discussed in more detail later.

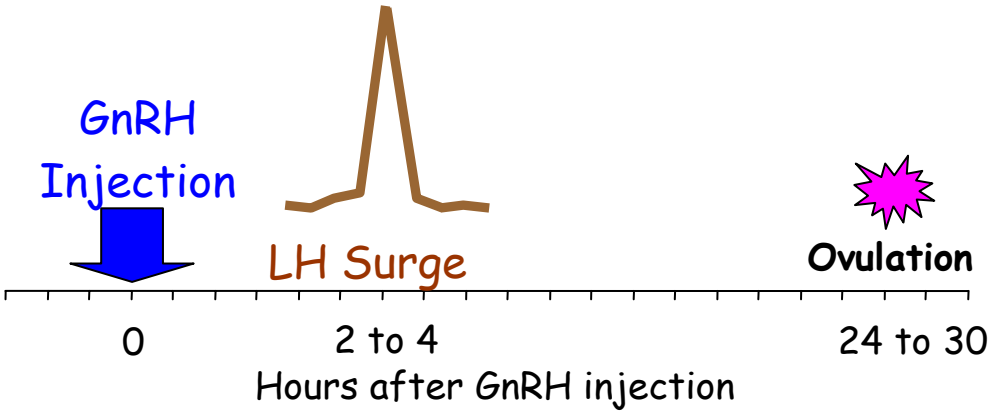

Figure 3. Injection (im) of GnRH will induce a surge of LH within 2 to 4 hr and ovulation of a viable dominant follicle (≥ 10 mm) will occur within 24 to 36 hr (Modified from Kojima and Patterson, 2003).

Luteal Phase

The luteal phase spans the time of corpus luteum formation and maintenance which begins with ovulation and ends with luteolysis (Figure 4). Progesterone is the primary secretory product of the corpus luteum and is regulated by secretions of the anterior pituitary, uterus, ovary, and embryo (Niswender et al., 1976). The regulation of progesterone secretion is likely controlled by a balance of luteotropic (stimulate progesterone) and luteolytic (inhibit progesterone) stimuli, given that both types of stimuli are secreted concurrently during the estrous cycle. In ruminants, LH is considered to be the primary luteotropic hormone and concentration of luteal LH receptors is positively correlated with changes in progesterone and luteal growth (Niswender et al., 2000). Corpora lutea receive the majority of the ovarian blood flow (Figure 2) and blood flow to the luteal ovary and progesterone secretion are highly correlated (Niswender et al., 1976). Progesterone has a central role in the regulation of the estrous cycle as it determines estrous cycle length and is required for the maintenance of pregnancy.

In cattle, $PGF_{2\alpha}$ is the uterine luteolysin and is commonly used to synchronize estrus in cattle. In the absence of an embryo, the uterine concentrations of $PGF_{2\alpha}$ increase during the late luteal phase and $PGF_{2\alpha}$ is secreted as pulses into the uterine veins on days 17 to 20 following estrus (Figure 4; day 0 = estrus; Inskeep and Murdoch, 1980). $PGF_{2\alpha}$ is transported from the utero-ovarian vein into the ovarian artery via a counter-current transfer mechanism (Hixon and Hansel, 1974; McCracken et al., 1972) and is transported to the corpus luteum. $PGF_{2\alpha}$ may have both a direct and an indirect effect on a ruminant corpus luteum to cause luteolysis. In the presence of an embryo, pulsatile secretion of $PGF_{2\alpha}$ is reduced and the corpus luteum does not regress. Maintenance of high circulating concentrations of progesterone in pregnant animals prevents the expression of estrus and ovulation.

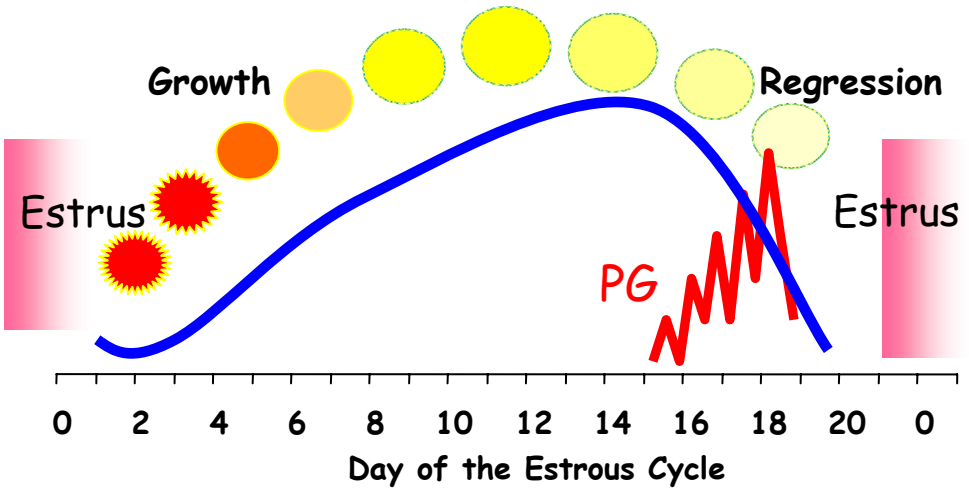

Figure 4. Changes in corpus luteum development, circulating concentrations of progesterone, and circulating concentrations of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) during the luteal phase of the bovine estrous cycle are depicted above. Luteal secretion of progesterone inhibits the expression of estrus, inhibits ovulation, and is essential for the maintenance of pregnancy. In the absence of an embryo, $PGF_{2\alpha}$ is secreted as pulses that cause a precipitous decrease in progesterone and regression of the corpus luteum. Products that mimic the action of progesterone (progestins) are commonly used in estrous synchronization. Progestin administration in cows that have experienced corpus luteum regression will delay the expression of estrus and ovulation until after progestin withdrawal (Modified from Kojima and Patterson, 2003).

Follicular Determinants of Corpus Luteum Function

Corpora lutea are a continuation of follicular maturation; consequently, changes in the hormonal stimulation of a preovulatory follicle may have a subsequent effect on luteal progesterone secretion. The endocrine microenvironment of a preovulatory follicle is unique relative to surrounding nonovulatory follicles and is important for preparation of follicular cells for luteinization and secretion of progesterone (McNatty et al., 1975). McNatty et al.(1979) suggested that development of a normal corpus luteum may depend upon a preovulatory follicle meeting the following criteria: 1) an adequate number of granulosa cells, 2) an adequate number of LH receptors on granulosa and thecal cells, and 3) granulosa cells capable of synthesizing adequate amounts of progesterone following luteinization. Furthermore, the ability of luteinized human granulosa cells to secrete progesterone increased when the cells were collected from follicles having increased follicular fluid concentrations of estradiol compared to granulosa cells collected from follicles that had lower concentrations of estradiol (McNatty et al., 1979). Premature induction of ovulation in ewes was associated with luteal insufficiency (Murdoch et al., 1983). These data are relevant to fixed-time insemination protocols in which physiologically immature dominant follicles are induced to ovulate at AI and the subsequent circulating concentrations of progesterone are lower than in cows in which a larger dominant follicle is induced to ovulate with GnRH (Perry et al., 2005). Inadequate luteal function following induced ovulation may be due to a reduced number of follicular

cells and/or inadequate preparation of follicular cells for luteinization and secretion of progesterone.

Estrous Synchronization Products and Mechanism of Action.

Effective estrous synchronization protocols are designed to synchronize follicular maturation with the onset of corpus luteum regression. In general, development of estrous synchronization protocols in cycling animals has involved the following three approaches: 1) Inhibit ovulation following spontaneous corpus luteum regression (long-term progestin treatment), 2) Induction of corpus luteum regression ($PGF_{2\alpha}$ treatment), and 3) a combination of 1 and 2. Most of the protocols utilized today can be categorized under the third approach. The first approach requires long-term progestin treatment (14 days) and is effective at synchronizing estrus; however, fertility at the synchronized estrus is frequently reduced due to the presence of persistent follicles (see section below). The second approach results in good fertility; however, animals that are in the first 5 to 6 days of their cycle will not respond to the $PGF_{2\alpha}$ injection, resulting in a reduced synchronization response. The third approach allows effective synchronization of estrus, regardless of stage of the cycle, without compromising fertility. This is particularly true when an injection of GnRH is administered at the beginning of progestin treatment to ovulate a dominant follicle and synchronize a new follicular wave. The following section will focus on specific estrous synchronization products and how they work. Subsequent papers in the proceedings will provide detailed information on specific estrous synchronization protocols.

Hormonal Management of the Luteal Phase for Synchronization of Estrus

Successful estrous synchronization protocols require control of the timing of both dominant follicle development and luteal regression. During the estrous cycle when a corpus luteum is present and circulating concentrations of progesterone are high, standing estrus and ovulation are inhibited; however, when the corpus luteum regresses and progesterone concentrations decrease, circulating concentrations of estradiol increase and the animal returns to standing estrus. Progestins mimic the actions of progesterone produced by the corpus luteum and inhibit estrus/ovulation. Progestins can delay the interval to estrus when luteal tissue is not present by inhibiting estrus and ovulation. Following the removal of the progestin, progesterone concentrations will be low and standing estrus and ovulation will occur.

Progestins

Two progestin products that are commercially available for estrous synchronization include Melengestrol Acetate (MGA) and the CIDR (Controlled Internal Drug Release). In cycling cows and heifers, administration of MGA or CIDRs does not affect the time of corpus luteum regression. However, once corpus luteum regression has occurred, progestin administration can prevent a cow or heifer from showing estrus and ovulating. Consequently, progestin administration in cows that have experienced

corpus luteum regression will delay the expression of estrus and ovulation until after progestin withdrawal.

Role of Progestins in Anestrus. At the start of a breeding season, most herds consist of a mixture of cycling and anestrous females. An effective estrous synchronization protocol must be able to induce a fertile estrus or ovulation in both anestrous and cycling heifers and cows. A short luteal phase usually occurs in prepuberal heifers and postpartum beef cows following the first ovulation (Perry et al., 1991; Werth et al., 1996). This short exposure to progesterone is believed to be necessary for reprogramming the reproductive axis to resume normal estrous cycling. Therefore, in herds that have a large proportion of prepuberal heifers or anestrous cows, progestin pretreatment before induction of ovulation can initiate estrous cycling status and eliminate or at least reduce the occurrence of short estrous cycles.

Administration of low levels of a progestin (i.e. MGA) in the absence of a corpus luteum, can result in the formation of a persistent follicle (see below). However, the effect of progestin treatment on persistent follicle formation differs between cycling and anestrous animals. Administration of low concentrations of progestins did not induce persistent follicle formation in early postpartum anestrous dairy heifers (Rhodes et al., 1997) or anestrous postpartum beef cows (Perry et al., 2002). It is not clear why persistent follicles did not form in anestrous cows.

Progestin Administration and Formation of Persistent Follicles. Persistent follicles are characterized by an extended dominant follicle life span and increased estradiol production (Zimbelman and Smith, 1966b; Siriois and Fortune, 1990; see review by Fortune and Rivera, 1999). Treatment of cycling heifers or cows with low levels of a progestin, following luteolysis, resulted in the formation of persistent follicles that had a large diameter, extended lifespan, and increased production of estradiol (Zimbelman and Smith, 1966a; Sirois and Fortune, 1990; Fortune et al., 2001). Administration of low (subluteal) concentrations of progestins to cattle, in the absence of luteal tissue, increased LH pulse frequency (Savio et al., 1993; Kojima et al., 1995; Kinder et al., 1996); however, midluteal phase concentrations of progesterone decreased LH pulse frequency and persistent follicles did not form (Sirois and Fortune, 1990; Savio et al., 1993). Thus, the formation of persistent follicles has been associated with increased LH pulse frequency, and infusion of exogenous LH induced persistent follicle formation (Duffy et al., 2000).

Insemination immediately following long-term progestin treatment and ovulation of a persistent follicle has been associated with decreased fertility (Mihm et al., 1994). No difference was reported in fertilization rate following ovulation of persistent follicles, but fewer zygotes developed into embryos containing 16 or more cells compared to ovulation of oocytes from control follicles (Ahmad et al., 1995). Decreased fertility following formation and ovulation of persistent follicles may result from alterations in the uterine environment due to increased estradiol secretion (Butcher and Pope, 1979) and (or) premature resumption of meiosis due to prolonged exposure to increased LH pulse frequency (Mattheij et al., 1994).

Progestin Administration-Management Tips. Melengestrol acetate is an orally-active progestin and each animal must receive the appropriate daily dose of MGA throughout the treatment period. The effect of MGA treatment (14 days) on cows in different stages of the estrous cycle is illustrated in Figure 5. If you detect an animal in standing estrus while feeding MGA then it is likely the animal did not receive the appropriate dose of MGA. Melengestrol acetate should be fed at a dose of 0.5 mg/hd/day in 2 to 5 lb of a highly palatable carrier. The MGA should not be top-dressed on a large amount of feed such as silage. If cattle are on a lush pasture it can be helpful to remove salt from the pasture and include the salt (0.5 oz/cow/day) in the MGA carrier. In addition, it is a good idea to feed carrier alone for several days before administering the MGA so that the cattle become accustomed to coming to the bunk. There should be a minimum of 18 in. of bunk space for heifers and 24 in. for cows. Remember to not inseminate cattle at the estrus immediately following long-term (14 days) MGA treatment since fertility will be reduced due to the ovulation of persistent follicles (see previous section).

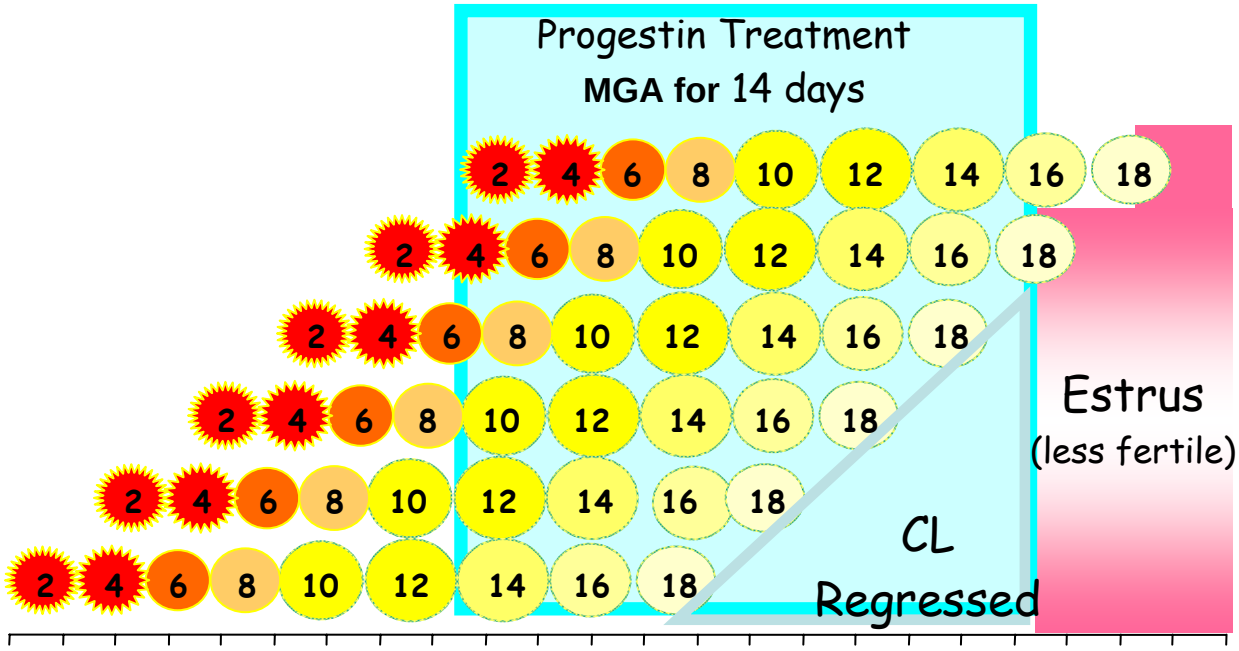

Figure 5. Effect of 14 days of MGA feeding on estrous synchronization of cows in different stages of the estrous cycle. Circles represent development and regression of corpora lutea. Numbers inside each circle represent days of the cycle. In this diagram, spontaneous luteal regression occurs around day 17 to 18 of the cycle. Note that at the end of progestin treatment all corpora lutea have regressed or are in the process of regressing (Modified from Kojima and Patterson, 2003).

In the absence of a corpus luteum, a CIDR functions as an artificial corpus luteum by releasing progesterone and thereby suppressing estrus and ovulation for seven or more days. CIDR's consist of a "T" shaped nylon backbone that is coated with a silicone layer

containing 10% progesterone by weight. The CIDR's are inserted into the vagina with a lubricated applicator following disinfection of the applicator and vulva. CIDR's are easily removed by pulling the flexible nylon tail. Although a small amount of vaginitis is a common observation at CIDR removal, fertility is not compromised. The retention rate of CIDR's is approximately 95%. If the retention rate is considerably less then 95% the device may have been inserted incorrectly or other animals may be pulling the CIDR's out by biting on the nylon tails. In the latter case, the problem can be remedied by trimming the nylon tails.

Prostaglandin $F_{2\alpha}$

Prostaglandins are naturally occurring compounds that are produced by most cells in the body and have a variety of biological actions. Prostaglandin $F_{2\alpha}$ is a naturally occurring luteolytic hormone that has also been utilized to synchronize estrus and induce abortion in cattle through induction of corpus luteum regression. In the absence of an embryo, uterine concentrations of $PGF_{2\alpha}$ increase during the late luteal phase. $PGF_{2\alpha}$ is secreted in pulses and transported to the corpus luteum via a counter-current mechanism. The mechanisms associated with $PGF_{2\alpha}$ –induced luteolysis are not completely understood; however, $PGF_{2\alpha}$ probably has both a direct and indirect (decreased blood flow) action. Luteal cells are known to have $PGF_{2\alpha}$ receptors on the plasma membrane and direct inhibitory effects of $PGF_{2\alpha}$ on luteal progesterone secretion have been demonstrated (Niswender et al., 2000). In addition, $PGF_{2\alpha}$ is known to reduce luteal blood flow due to vasoconstrictor activity (Niswender and Nett, 1988).

Administration of $PGF_{2\alpha}$ to domestic ruminants does not induce luteolysis during the early luteal phase (Figure 6). For purposes of estrous synchronization, injection of $PGF_{2\alpha}$ is only effective in cycling heifers and cows (approximately day 6 to 16 following estrus; day 0 = estrus). Although functional $PGF_{2\alpha}$ receptors and signal transduction mechanisms are present in developing ovine corpora lutea (Tsai et al., 1997; Tsai and Wiltbank, 1998), the acquisition of luteolytic capacity is not established until after day 4 postestrus (Tsai and Wiltbank, 1998).

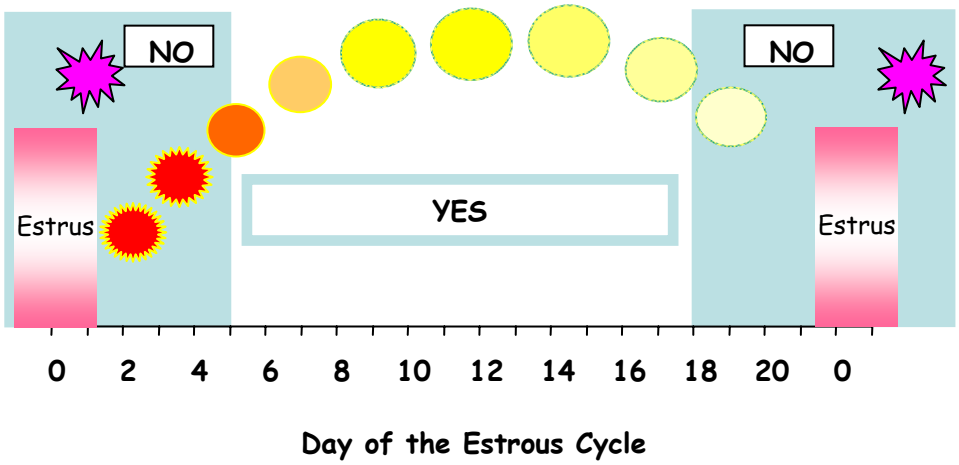

Figure 6. Effect of stage of the bovine estrous cycle on luteal responsiveness to $PGF_{2\alpha}$ Bovine corpora lutea will not respond to an injection of $PGF_{2\alpha}$ during the first five days

of the cycle. Therefore, $PGF_{2\alpha}$ should not be injected at the beginning of progestin treatment (Modified from Kojima and Patterson, 2003).

Injection of $PGF_{2\alpha}$ into prepuberal heifers or anestrous cows is not effective due to the absence of luteal tissue. Furthermore, $PGF_{2\alpha}$ treatment will not induce cycling activity in noncycling cattle. Therefore, when using $PGF_{2\alpha}$ alone to synchronize estrus it is important to assess the proportion of cycling animals before initiating the treatment. In herds containing both cycling and noncycling females , the most effective estrous synchronization protocols combine treatment with a progestin and an injection of $PGF_{2\alpha}$. In pregnant feedlot heifers, $PGF_{2\alpha}$ is highly effective at inducing abortion before 100 days of gestation.

Hormonal Management of Follicular Waves for Synchronization of Estrus

The development of effective protocols for fixed-time insemination is dependent upon the precise synchronization of follicular waves culminating in a fertile ovulation at a predetermined time. Two approaches that have been used to synchronize bovine follicular waves include: 1) ovulating/destroying the dominant follicle and thereby initiating a new follicular wave, and 2) prolonging the lifespan of a dominant follicle (persistent follicle).

Initiation of a new follicular wave occurs following ovulation or turnover (atresia) of the dominant follicle. Administration of exogenous progesterone, estradiol, or GnRH have been utilized to turnover (progesterone and estradiol) or ovulate (GnRH) dominant follicles and to synchronize follicular waves in heifers and cows (see reviews by Bo et al., 1995; Diskin et al., 2002). Follicular turnover (atresia) of persistent follicles can be accomplished through the administration of progesterone. Progesterone as a single injection (Anderson and Day, 1994) or administered over a 24-hour period (McDowell et al., 1998) effectively regressed persistent follicles and initiated new follicular waves. Reduction of LH pulse frequency and amplitude following the administration of exogenous progesterone may be the mechanism by which persistent follicles are induced to undergo atresia (McDowell et al., 1998).

Estradiol benzoate has also been used to induce atresia of dominant follicles and to initiate a new follicular wave approximately 4.5 days after injection (Burke et al., 2000). When treatment with progesterone and estradiol were combined the dominant follicle stopped growing within 24 hours and became atretic resulting in the initiation of a new follicular wave 4 to 5 days after treatment (Burke et al., 1999). A single injection of a GnRH agonist is capable of ovulating dominant ($\geq$ 10 mm) but not subordinate follicles (Figure 7; Ryan et al., 1998). Following GnRH administration, a new follicular wave was initiated approximately 1.6 days later (Roche et al., 1999) and selection occurred 3 to 4 days later (Twagiramungu et al., 1995). However, the ability of a single injection of GnRH to induce ovulation and initiate a new follicular wave is dependent on the stage of follicular development (Geary et al., 2000; Atkins et al., 2005).

Management Considerations for Selection of Heifers and Cows for Synchronization of Estrus

The success of an estrous synchronization program is largely based on understanding the bovine estrous cycle, the biological actions of estrous synchronization products (progestins, $PGF_{2\alpha}$, and GnRH), and the selection of heifers and cows that have a high likelihood of responding appropriately to the preceding products. Below are listed a few management tips for identifying heifers and cows that will be good candidates for an estrous synchronization program and likely respond appropriately.

Heifers. Heifers need to reach puberty prior to estrous synchronization to increase the likelihood of responding to a synchronization program. Furthermore, a 21% increase in fertility is experienced at a heifer's third estrus compared to her pubertal estrus (Byerley et al., 1987). Age at puberty is affected by a variety of factors, including genotype, body weight, nutrition, social environment, and season. Reproductive tract scores (RTS) provide an estimate of reproductive maturity in heifers and help predict their response to an estrous synchronization protocol. Heifers are assigned a RTS score ranging from one (immature) to four and five (cycling) based on rectal palpation or ultrasound of the uterus and ovaries. Qualified personnel should assess the RTS for heifers two weeks prior to synchronization or six to eight weeks prior to breeding. Heifers should have a minimum RTS score of two to be considered for breeding and at least 50% of the heifers should score a four or five in order to achieve a high response to synchronization.

Furthermore, replacement heifers should not receive growth promoting implants since implants may impair normal development of reproductive organs in growing heifers. At weaning, older heifers should be selected as potential replacement females and each heifer should attain 65% of their mature body weight before breeding and 85% prior to first calving. Feeding heifers separately from cows will assist heifers in attaining a targeted rate of gain.

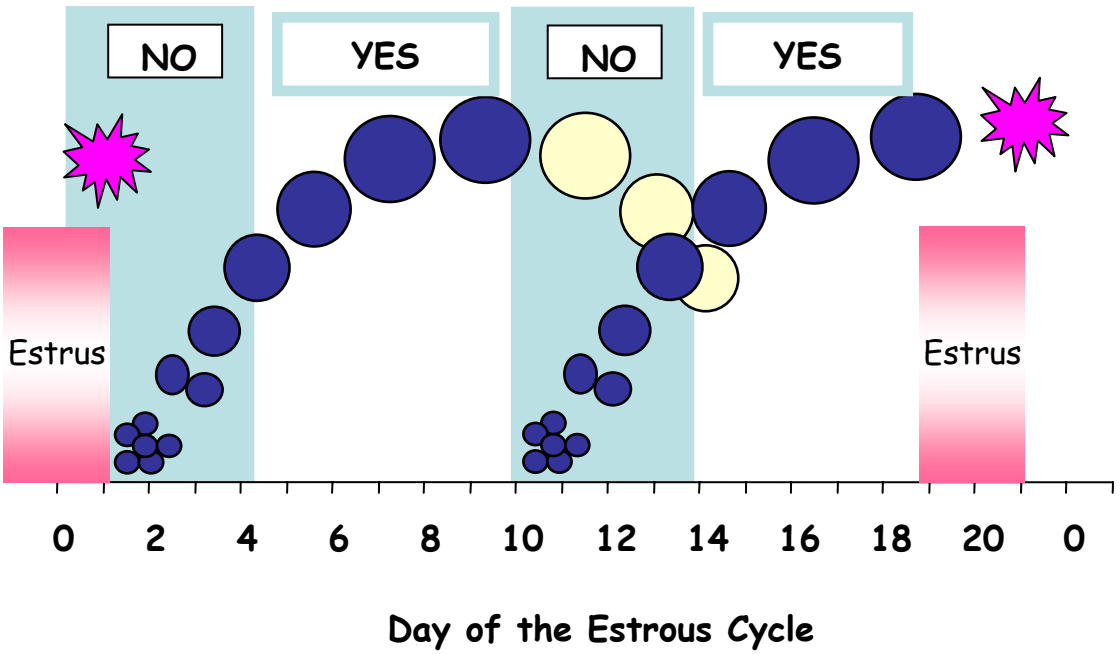

Figure 7 Injection of GnRH will induce ovulation of a dominant follicle (≥ 10 mm in diameter). Circles represent follicle development and atresia (light circles) during a wave. The above figure represents a "two-wave cow" and the shaded areas indicate when during a follicular wave follicles will ovulate (Yes) or not ovulate (No) in response to a single injection of GnRH (Modified from Kojima and Patterson, 2003).

Postpartum Cows: In postpartum cows, the response to an estrous synchronization program is primarily dependent upon cow body condition and days postpartum. Body condition score (BCS) is a subjective measurement of an animal's fat reserves and ranges from extremely thin (1) to obese (9). Cows should have a body condition score of 5 or greater at calving to be considered for an AI and estrous synchronization program. Cows that are too thin at calving are likely to have poor reproductive performance and are not good candidates for AI. In general, it takes 80 to 100 lbs to increase one BCS (i.e. 4 to 5). If possible, feed thin cows separately from well conditioned cows in order to promote a steady pattern of feed intake to attain the desired BCS.

The average number of days post partum for cows at the start of an estrous synchronization program should be > 40 days. Increased energy requirements associated with lactation can result in a delay in the interval from calving to first estrus. A longer recovery period between calving and the beginning of the breeding season corresponds to a larger proportion of cows cycling at the start of the breeding season.

Summary

Understanding the basic principles of the bovine estrous cycle and how estrous synchronization products affect the cycle is essential when choosing the best protocol for heifers or cows and for determining what went wrong when pregnancy rates following a

synchronized estrus are less than expected. Three general approaches that have been used to develop estrous synchronization protocols include the following: 1) Inhibit ovulation following spontaneous corpus luteum regression (long-term progestin treatment), 2) Induction of corpus luteum regression ($PGF_{2\alpha}$ treatment), and 3) a combination of 1 and 2. Most of the protocols utilized today can be categorized under the third approach. The ability to synchronize bovine follicular waves through an injection of GnRH has added a new and important dimension to estrous synchronization and has made fixed-time AI in cows a viable option. Many of the current protocols are able to synchronize the growth of a dominant follicle in addition to the time of corpus luteum regression.

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NOTES

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HISTORY, EFFICACY AND UTILIZATION OF PROSTAGLANDIN $F_2\alpha$ FOR ESTROUS SYNCHRONIZATION

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General History of Prostaglandins

In 1930 Kurzroc and Lieb reported the human uterus would either contract or relax upon instillation of fresh human semen. M.W. Goldblatt (1933) and von Euler (1934) reported strong smooth-muscle stimulating activity of human seminal plasma. Von Euler (1935) reported strong smooth-muscle stimulating activity of seminal fluid from the monkey, sheep, and goat and in extracts of the vesicular glands of male sheep but not in a number of other species. Von Euler prepared lipid extracts of sheep vesicular glands and found the strong smooth-muscle stimulating activity to be associated with a fraction containing lipid-soluble acids. The active factor was named prostaglandin. The new names prostaglandin and progesterone were published on the same page.

Research on the prostaglandins did not proceed until 1963, in contrast to the extensive research between 1935 and 1963 with progesterone and progestogens, especially for control/management of reproductive cycles of numerous mammals, especially the human. An important contributor to renewed interest in and collaborative support for research with prostaglandins resulted from the friendship developed as graduate students at The Ohio State University between Dr. David Weisblatt, Vice-President of Research at The Upjohn Company and Professor Sune Bergstrom of the Karolinska Institute in Stockholm, Sweden. The collaboration between Karolinska scientists primarily addressing chemical structure identification, metabolism and pharmacology of the prostaglandins and Upjohn scientists addressing production of usable quantities, biology and pharmacology of the prostaglandins allowed research to proceed rapidly. For example, the number of papers published in scientific literature was five by 1963, but about 63 by 1965; the publication rate thereafter approached two per day.

During the 1960s and 1970s the prostaglandin families were identified, characterized, and hundreds of analogs were synthesized. Early production of prostaglandins at The Upjohn Company depended on extracting harvested *Plexaura homamalla* (Caribbean sea whip) for substrate for further chemical modification to the desired specific prostaglandins. Subsequently, Corey (Harvard) chemical synthesis was established for prostaglandin production. Prostaglandins were termed ubiquitous since they were detected in or released from lung, thymus, brain, spinal cord, kidney, iris, umbilical cord, deciduas, fat, adrenals, stomach, intestines, nerves, menstrual fluid, amniotic fluid, seminal plasma, blood skeletal muscle, cardiac muscle, salivary glands, thyroid, pancreas, and uterus. Biological activity was described for cardiovascular, kidney and ureter, reproductive, gastrointestinal, respiratory, central nervous, and peripheral nervous systems.

History of Prostaglandin $F_2\alpha$ for Luteolysis and Relationship to the History of Progestogens for Cattle Estrus Synchronization

Perceived need for beef cattle estrous synchronization

Cattle estrous synchronization was perceived (1960) to meet an unmet need of beef cattle producers who desired to utilize artificial insemination (AI). During the 1950s frozen bovine semen was developed and AI to progeny tested bulls became recognized as effective to make more rapid genetic progress for milk yield and beef production. In the 1960s, for beef cattle, a major detriment to AI was the requirement for daily estrus detection and AI over 60 to 90 days or more. Thus, numerous companies, cited below, believed an orally active progestogen that could be delivered under farm and ranch conditions at an economically attractive price would both meet an unmet need in the beef industry and would generate income for the successful company. Based on both the paper by Ulberg, Christian and Casida (1951) that injected progesterone would block estrus and the understanding of reproductive biology of the bovine estrous cycle in 1960, progestogens, to block estrus for 18 days and then release the block, were the only potentially practical hormones available.

Numerous university and pharmaceutical company researchers were seeking use of progesterone and progestogens to synchronize estrus in cattle and other species during the 1960s. *Ovarian Regulatory Mechamisms* was a conference hosted by The Upjohn Company's Robert Zimbelman (animal health) and Gordon Duncan (human fertility research). Zimbelman received his PhD at the University of Wisconsin Madison in the laboratories of L. E. Casida and Duncan received his PhD at Iowa State University in the laboratories of R. M. Melampy. This conference was held at The Upjohn Company Conference Center, Brook Lodge, Augusta, MI in 1965 and the proceedings were published in the Journal of Reproduction and Fertility, Supplement No.1, 1966. This conference was one of a series of conferences held at Brook Lodge beginning in 1956 and continuing into the 1980s, several of which addressed reproductive biology. The topics of the 1965 Brook Lodge Conference and the presenters were:

- Introductory Note, A.S. Parkes
- Modification of ovarian activity in the bovine following injection of estrogen and gonadotropin, J.N. Wiltbank
- Effect of progestogens on ovarian and pituitary activity in the bovine, R.G. Zimbelman
- Pituitary-ovarian-uterine relationships in pigs, L.L. Anderson
- Luteotrophic and luteolytic mechanisms in bovine corpora lutea, W. Hansel
- The nature of the luteolytic process, I. Rothchild
- Luteal maintenance in hypophysectomized and hysterectomized sheep, C. Thibault
- Localization and sexual differentiation of the nervous structures which regulate ovulation, R.A. Gorski
- Steroidogenesis in the perfused bovine ovary, E.B. Romanoff

- Competitive studies of the action of luteinizing hormone upon ovarian steroidogenesis, D.T. Armstrong
- Studies on the mode of action of luteinizing hormone on steroidogenesis in the corpus luteum *in vitro*, J. Marsh and K. Savard
- Summation, R.O. Greep, A.V. Nalbandov, R.M. Melampy

Additional participants from academia who did not present papers were C.A. Barraclough, E.M. Bogdanove, L.E. Casida, B.N. Day, H.D. Hafs, Carl Hartman, K.A. Laurence, and M.B. Nikitovich-Winer. This 1965 Brook Lodge Conference featured the thought and research leaders in reproductive biology of domestic animals.

I interpret the 1965 Brook Lodge Conference as the scientific discussion that launched and/or reinforced existing fledgling cattle estrous synchronization progestogen development programs. During the 1960s, progestogens were THE orally active and potentially economically feasible hormones with promise to be developed for estrous synchronization of cattle. Companies actively seeking progestogens during the 1960s for use in estrous synchronization of cattle were The Upjohn Company, Elanco, Squibb, American Cyanamid, Searle and Syntex. The only progestogen to survive as an orally active progestogen available today for cattle estrous synchronization is MGA (melengestrol acetate). The Squibb product, norgestomet, eventually became available as SyncroMate-B.

Brook Lodge 1965 Conference; Impact on development of prostaglandins for cattle estrous synchronization

I interpret the 1965 Brook Lodge Conference as the scientific discussion that launched research and development of prostaglandins for both human and domestic animal use. Specifically, during the general discussion of Hansel's paper (listed above), John Babcock, The Upjohn Biochemical Research Division, is cited: "I wonder if anyone here has thought of the possible role of a family of agents known as prostaglandins, which have been studied extensively by Bergstrom. They have found a pronounced effect on smooth muscle, for one thing, and have found they may play a role in fertility because they are found in very high concentrations in the semen of some species. Whether or not release of prostaglandins from the uterus could have a luteolytic effect, I have no idea" (J. Reprod. Fertil. Suppl. No.1:47, 1965). Immediately following the 1965 Brook Lodge Conference, Bruce Pharriss, The Upjohn Company Fertility Research (Duncan's group) initiated research, in collaboration with scientists of Babcock's group, to investigate prostaglandins for luteolytic activity. Babcock and Pharriss chose $PGF_2\alpha$ as the prostaglandin to investigate and chose the pseudopregnant rat as the animal model to investigate luteolysis. Their report that $PGF_2\alpha$ was luteolytic in the pseudopregnant rat was not published until 1969 (19). An attendee at the 1965 Brook Lodge Conference shared Babcock's comment with a colleague in the United Kingdom who secured PGE_2, tested it for luteolytic activity, and, finding none, concluded prostaglandins were not luteolytic.

Development of prostaglandins for cattle estrous synchronization

From 1963 onward The Upjohn Company leadership invested extensively in prostaglandins for human potential products, and, until more effective synthesis strategies were developed, supply of prostaglandins was limited. Following the discovery by Pharriss and Wyngarden (1969) that $PGF_2\alpha$ was luteolytic, research for human fertility/parturition/abortion was underway and senior leadership chose not to allow research in cattle until 1971. At the same time, ICI of the United Kingdom had hired Mike Cooper to research and develop $PGF_2\alpha$ analogs for use in cattle. We initiated our $PGF_2\alpha$ research in cattle at The Upjohn Company using the 35-40 day confirmed pregnant (rectal palpation being the only method available in 1971) beef heifer as the model to investigate luteolysis. $PGF_2\alpha$ was reported to be luteolytic in the bovine in 1972 (Rowson et al., 1972; Lauderdale, 1972; Liehr et al., 1972). $PGF_2\alpha$ was reported to be luteolytic in equine (Douglas and Ginther, 1972) and ovine (Thorburn and Nicol, 1971, Goding et al., 1972) and potential uses to control reproductive cycles in domestic animals were described (Inskeep, 1973). Thus, in ten years, between 1963 and 1973, prostaglandin research was reinitiated and data were published stating $PGF_2\alpha$ and $PGF_2\alpha$ analogs were luteolytic in cattle and the potential existed for them to have practical value for estrous synchronization.

Research at The Upjohn Company was directed towards achieving approval for $PGF_2\alpha$ in the mare, a non-food animal, which would allow for more rapid approval through the Food and Drug Administration Center for Veterinary Medicine (FDACVM), followed by approvals in cattle and other species. $PGF_2\alpha$ was approved for 1) equine (Prostin F2 Alpha®;1 mL ampoule,1976), 2) 10 mL vial, (1977), 3) beef cattle and dairy heifer double injection program for estrous synchronization (Lutalyse, 1979), 4) 30 mL vial (1980), 5) beef cattle and dairy heifer single injection program for estrous synchronization (1981), 6) feedlot cattle abortion (1981), 7) lactating dairy cattle no-visible estrus (1983), 8) non-lactating cattle abortifacient (1983), 9) lactating dairy cattle pyometra treatment (1983), and 10) swine parturition (1983).

During the 1970s and 1980s, data were not available regarding follicular waves. Researchers investigating $PGF_2\alpha$ and its analogs recognized something other than the regression of the CL, was contributing to the variance in consistency both of return to estrus in a predictable 48 hours and of effective pregnancy rates in response to timed AI post-$PGF_2\alpha$ injection. Research of follicular waves in cattle now allows for more consistent pregnancy rates resulting from timed-AI protocols utilizing $PGF_2\alpha$ products, with or without progestogens, and gonadotropin releasing hormone.

Prostaglandin products

Because the market for $PGF_2\alpha$ products was perceived, and then documented, to be lucrative for companies, numerous $PGF_2\alpha$ products were approved and sold in various countries. Some of the products were Lutalyse/Dinolytic Pronalgon F (Upjohn), Estrumate/Planate and Equimate (ICI, with subsequent sale to numerous companies),

Prosolvin (Intervet), Bovilene (Fort Dodge), Iliren (Hoechst), Alfabedyl (Hoechst-Roussel), and numerous generics throughout the world.

Product indications

Control CL lifespan for cattle and equine; pregnancy termination for bovine, equine and porcine; parturition induction for bovine, porcine and equine; and treatment for mummified foetus, pyometra/endometritis/metritis, and luteal cysts in bovine.

Lauderdale's interpretation of the scientific literature for effectiveness of $PGF_2\alpha$ products used in cattle

Estrus synchronization → Effective
Early postpartum, in the absence of a CL (hasten involution) → Minimal to ineffective
Single injection 14 or more days postpartum (return to estrus, increased pregnancy) → Minimal to ineffective
Treatment of retained placenta → Minimal to ineffective
Treatment of metritis → Effective
Treatment of cystic ovarian follicles → Effective when the follicles are luteinized
Do $PGF_2\alpha$ products cause ovarian cysts → No

Original Programmed Breeding Programs Using $PGF_2\alpha$ (Lauderdale et al., 1977; Moody, 1977; Lauderdale, 1979)

Older and current technology allows for programmed breeding at the first synchronized estrus. Breeding management protocols under development should result in continuous programmed breeding management until 100% of the cattle are pregnant in the designated time interval.

Today we recognize effective programmed breeding requires synchronization of follicle waves, management of the CL lifespan, and induction of ovulation. Thus, selection of an effective programmed breeding program is dependant upon matching the components of follicle wave management, CL lifespan management, ovulation induction, labor management, and economic management consistent with the farm/ranch/dairy objectives. However, when $PGF_2\alpha$ and its analog products were developed, the component of follicular wave management was not recognized. Thus, all programs reported herein are the ones originally developed for $PGF_2\alpha$ and its analogs. Cattle must be in cycling estrous in order to achieve estrous synchronization and pregnancy. Additionally, with understanding of follicle waves, research documented the interval between Lutalyse injections should be increased from 11 (10-12) days (the original recommendation) to 14 days to achieve more precise estrus control and higher pregnancy rates. The original selection of 10 to 12 days between Lutalyse injections was based on an attempt to minimize the days between injections but achieve a sufficient interval to assure CL regression of both those CL not responsive to the first injection and those CL formed subsequent to regression of the CL after the first injection.

Definitions

Estrus Detection Rate = $\frac{\text{No. Detected in estrus x 100}}{\text{No. Assigned}}$

Estrus % was calculated for each interval of interest.

Conception Rate = $\frac{\text{No. Pregnant x 100}}{\text{No. Detected in Estrus and AI}}$

Conception Rate was calculated for first service only.

Pregnancy Rate = $\frac{\text{No. Pregnant x 100}}{\text{No. Assigned}}$

Pregnancy Rate was calculated for each interval of interest.

The pregnancy rate is the measure that provides the number of pregnant heifers/cows resulting from the breeding program and is the cumulative result of estrus detection rate and conception rate.

Figure 1 identifies the schedule for using either Double or Single Lutalyse injection programs.

Program Designation			Breeding Method					
LLAIE	L↓	L↓	AIE			AIE or Bull	AIE or Bull	
LLAI80	L↓	L↓		TAI		AIE or Bull	AIE or Bull	
LAIE		L↓	AIE			AIE or Bull	AIE or Bull	
AILAI			AIE		L↓	AIE	AIE or Bull	AIE or Bull
-14 to –12		-1	0	3	5	9	22	27
Days before Breeding Season			Days of Breeding Season					

Figure 1. Cattle Breeding Management with 5 mL Lutalyse sterile solution (**L↓**; 25 mg $PGF_2\alpha$/33.5 mg dinoprost tromethamine; IM). **AIE**: inseminated 6 to 13 hours after detected estrus. **TAI**: inseminated at about 77 to 80 h after the second injection of Lutalyse.

Dose Titration for Lutalyse® sterile solution for cattle

Beef cows (9 herds, 767 cows), beef heifers (9 herds, 448 heifers) and dairy heifers (3 herds, 243 heifers) were investigated to estimate the optimal dose for Lutalyse. Doses investigated were 0, 5, 15, 25 and 35 mg dinoprost intramuscularly at an 11 (10 to 12) day interval. Response variables were percent in estrus and pregnancy rate for days 2-5 post-second injection. Walker-Carmer statistical estimates for the optimal dose, based on estrus and pregnancy rates, were 25.7 mg and 22.8 mg for beef cows, 25.1 and 21.5 for beef heifers, and 26.4 and 30.2 for dairy heifers. Based on these data, FDA CVM approved a dose of 25 mg dinoprost as the dose for use in cattle. This dose was used in all subsequent studies to investigate the various breeding management programs with

Lutalyse. Papers can be found in the scientific literature reporting the dose should be something less than the FDA CVM approved dose of 25 mg dinoprost (5 mL Lutalyse). Additionally, rumors abound the dose is too little for big framed cattle or breed X. However, those papers consistently report data based on a single or minimal locations and minimal numbers of cattle. The dose of 25 mg dinoprost (5 mL Lutalyse) is the dose derived by a statistically valid process to consistently be effective across farms and ranches with various management styles and cattle types and sizes.

Double injection of Lutalyse® sterile solution breeding programs

Cattle were injected intramuscularly (IM) with 5 mL Lutalyse twice at an 11 (10-12) day interval. Cattle were artificially inseminated (Al) either at detected estrus (LLAIE) or at about 80 h (LLAI80) after the second injection (Fig. 1). For the studies represented by the data in the presentation, cattle of the control and LLAIE groups were observed for estrus twice daily and Al about 6 to 13 h after first observation of estrus. Cattle of the LLAI80 were Al at about 77 to 80 h after the second injection of Lutalyse and were rebred at any estrus detected 5 days or more after the 80 h AI. Dates of injections of Lutalyse were established such that the second injection would be administered the day prior to initiation of the normal breeding season within herd.

Beef cows. Beef cows from 24 herds with 1844 cows were investigated.

Estrus detection. Significantly ($P < 0.05$) greater percentages of cows were detected in estrus during the first 5 days of the AI season for the LLAIE cattle (47%) compared to Controls (11%). Fewer percents of LLAIE cattle (47%) were detected in estrus at least once during the first 5 days compared to Controls (66%) during the first 24 days (one estrous cycle) of the AI season, indicating the cows were just beginning to estrus cycle at the beginning of the breeding season.

Conception rate. First service conception rates were similar between Control and LLAIE cattle for both the first 5 days (68%, 61%) and days 1-24 (61%, 66%) of AI. These data reinforce previously reported data that conception rate was not altered significantly following use of $PGF_2\alpha$ (2, 3, 7).

Pregnancy rate. Pregnancy rates were greater for both LLAIE (34%) and LLAI80 (35%) cattle compared to Controls for 5 days (11%) and were slightly lower than Controls for 24 days (48%). These investigations did not identify a significant difference in pregnancy rate between cattle of LLAIE (5 days of AI at estrus, 34%) and LLAI80 (single timed AI, 35%). Pregnancy rates generally were similar between Control, and either LLAIE or LLAI80 cattle for days 1-24 (48% Control and 55%/49%) and 1-28 (52% Control and 61%/57%).

Beef heifers. Beef heifers from 22 herds with 1614 heifers were investigated.

Estrus detection. Significantly ($P < 0.05$) greater percentages of heifers were detected in estrus during the first 5 days of the AI season for the LLAIE cattle (66%) compared to

Controls (13%). Fewer percents of LLAIE cattle (66%) were detected in estrus at least once during the first 5 days compared to Controls (81%) during the first 24 days (one estrous cycle) of the AI season, indicating that not all heifers were estrous cycling at the beginning of the breeding season.

Conception rate. First service conception rates were similar between Control and LLAIE cattle for both the first 5 days (50%, 55%) and days 1-24 (58%, 54%) of AI. These data reinforce previously reported data that conception rate was not altered significantly following use of $PGF_2\alpha$ (2, 3, 7).

Pregnancy rate. Pregnancy rates were greater for both LLAIE (38%) and LLAI80 (36%) cattle compared to Controls for 5 days (9%) and were slightly lower than Controls for 24 days (53%). These investigations did not identify a significant difference in pregnancy rate between cattle of LLAIE (5 days of AI at estrus, 38%) and LLAI80 (single timed AI, 36%). Pregnancy rates generally were similar between Control, and either LLAIE or LLAI80 cattle for days 1-24 (53% Control and 56%/51%) and 1-28 (56% Control and 58%/50%).

For both beef cows and heifers, the 80 hr timed AI reported herein had a similar pregnancy rate to the cows bred at estrus for 5 days. However, the success of timed AI was highly variable among herds and within herds over time. The bases for this variation in response are the variation both in control of follicular waves and in the percent of cattle anestrus at the beginning and 14-days prior to the breeding season. In those groups of cattle where timed AI worked well, the incidence of anestrus or pre-puberty was very low and the cattle were in the stage of the estrus cycle where follicular waves were "similar" among the cohort of cattle treated. We now know, based on an understanding of follicle waves, that, to achieve consistently high pregnancy rates using timed AI, follicular waves must be synchronized/managed and the lifespan of the corpus luteum (CL) must be managed. Follicle waves can be managed through the use of GnRH and the CL lifespan can be managed by use of $PGF_2\alpha$. The results of these studies have been confirmed both by repeated research studies by numerous academicians and by use on-farm and on-ranch over the past 25 years.

Single injection of Lutalyse® sterile solution breeding programs

The AILAI cattle management system requires the observation of cattle for estrus and AI for 4 days, followed by injection of cattle not detected in estrus during those four days with 5 mL Lutalyse, IM, on the morning of day 5, followed by continued observation of cattle for estrus and AI accordingly on days 5 through 9, i.e. a 9-day AI season (Fig. 1). Breeding for the remainder of the breeding season can be by AI, bulls or some combination of AI and bulls. The LAIE cattle management system is IM injection of cattle with 5 mL Lutalyse on the day before initiation of the breeding season followed by observation of cattle for estrus and AI for 5 days (Fig. 1). Breeding for the remainder of the breeding season can be by AI, bulls or some combination of AI and bulls. For the data presented in support of the results derived from these breeding programs, within herd

comparisons were made between Control and LAIE cattle and between Control and AILAI cattle. In three additional herds, within herd comparisons were made among Control, LLAIE and LAIE cattle.

AILAI Beef Heifers. Beef heifers from ?? herds with ?? heifers were investigated.

Estrus detection. The percent cattle detected in estrus the first time for days 1 through 5 was similar between AILAI (25%) and Control (24%) beef heifers. The percent heifers detected in estrus the first time during days 1 through 9 was greater ($P < 0.01$) for AILAI than for Controls (64% vs 38%). First estrus detection rates for the first 24 days of breeding were similar between AILAI and Control cattle (77% vs 78%).

First service conception. Conception rates were not different between cattle assigned to AILAI and Control groups respectively for days 1 through 5 (62%, 62%), 1 through 9 (56%,53%), and 1 through 24 (59%, 57%).

Pregnancy rate. Pregnancy rate for days 1 through 5 was similar between AILAI and Control heifers (16% vs 15%). Pregnancy rates were greater ($P < 0.01$) for AILAI than for Control heifers for days 1 through 9 (45% vs 24%). Pregnancy rates were not different significantly between Control (55%) and AILAI (56%) heifers for days 1 through 24. Pregnancy rates for days 1 through 28 were 63% and 59% for AILAI and Control ($P < 0.16$) heifers.

The percentages of cattle detected in estrus the first time, first service conception rates and pregnancy rates should be similar between Controls and cattle assigned to the AILAI group for days 1 through 5 since the AILAI cattle would not have been injected with Lutalyse. That was the case for beef heifers.

AILAI Beef Cows

Pregnancy rate. Pregnancy rates for Control (N=638) and AILAI (N=637) cows respectively were 17% and 32% at 9 days and 57% and 70% at 32 days.

The data on enhanced pregnancy rates after 9 days of AI with the AILAI management system are consistent with data published previously (1, 4, 5). The greater pregnancy rate in the AILAI group for days 1 through 9 demonstrated the effectiveness of use of Lutalyse in that system of breeding management. The trend for more pregnancies in the AILAI group after 28 days of AI reinforces the conclusion that the AILAI management system was effective. The results of these studies have been confirmed both by repeated research studies by numerous academicians and by use on-farm and on-ranch over the past 25 years.

LAIE Beef Heifers. Beef heifers from ?? herds with ?? heifers were investigated. *Estrus detection.* The percent of heifers detected in estrus the first time during days 1 through 5 was greater for LAIE than for Controls (52% vs 28%, $P < 0.05$). The percent of heifers detected in estrus the first time during days 1 through 24 was similar between

LAIE and Controls (83% vs 82%). The percentage of Control heifers detected in estrus during the first 24 days of AI was 82. This value should be an over estimate of the percent of the herd having estrous cycles on the day of Lutalyse injection, since the Control heifers had 24 more days to initiate estrous cycles. Since $PGF_2\alpha$ has been shown to be ineffective in regressing the CL during days 1 through 4 or 5 after estrus and cattle have an 18 to 24 (x = 21) day estrus cycle, a single injection of $PGF_2\alpha$ would be expected to regress the CL and synchronize about 75% to 80% of a group of estrous cycling cattle. Calculation of the predicted estrus detection rates for cattle of this study would be as follows for the Lutalyse single injection program: 75% with responsive CL of 82% of estrous cycling heifers equals 62% expected (actual was 52% for LAIE heifers). Thus, the predicted and observed estrus detection rates of 62% and 52% for heifers appeared to be similar, which reinforces the conclusion that a single injection of Lutalyse yielded the predicted response.

First service conception rate. These were similar for heifers of the Control and LAIE groups, as would be expected (47%, 52%).

Pregnancy rate. Pregnancy rates for days 1 through 5 for LAIE and Control heifers were 28% and 12% ($P < 0.04$). Pregnancy rates for days 1 through 24 for LAIE and Control heifers were 55% and 49%. Pregnancy rates for days 1 through 28 for LAIE and Control heifers were 57% and 52%.

These data are similar to those reported previously relative to use of the LAIE management system (Inskeep, 1973; Lauderdale et al., 1974; Moody, 1979; Turman et al., 1975). The pregnancy rates for 5 days of breeding in the LAIE management system demonstrated that system to be effective. The results of these studies have been confirmed both by repeated research studies by numerous academicians and by use on-farm and on-ranch over the past 25 years.

Comparison of LAIE and LLAIE

Cattle of the LLAIE system compared to cattle of the LAIE system should have about a 20% to 25% greater estrus detection rate and pregnancy rate for breeding during the first 5 days after $PGF_2\alpha$ since $PGF_2\alpha$ is ineffective or less effective as a luteolytic agent when injected during the first five days after ovulation (Lauderdale, 1972). The observed percentage differences between LAIE and LLAIE heifers for first estrus were 23% and for pregnancy rate were 23%. Thus, the expected percentage differences of about 20% to 25% and the observed percentage differences of 23% and 23% were similar in this limited study.

MGA and Lutalyse

Ed Moody, Montana State University, collaborating with The Upjohn Company scientists, investigated MGA and Lutalyse to synchronize estrus in beef cattle in about 1977-1978 (9). For example, beef heifers were fed MGA at 1.0 mg/heifer daily (the estrus synchronization dose we were pursuing at that time) for either 4-days or 5-days

immediately prior to start of 19 days of AI followed by 26 days of bull breeding. Heifers fed MGA were fed for 4-days (T1, N=31, last day of feeding was 2-days before breeding start) or fed 5-days (T2, N=32, last day of feeding was 1-day before breeding start) and all MGA fed heifers were injected with Lutalyse 1-day before breeding started. Non-treated Control heifers (T3, N=33) were included in this study. Heifers were observed for estrus twice daily for the 19 days of AI.

First service AI conception rate. This was 61%, 44% and 58% for T1, T2 and T3, respectively.

Pregnancy rate. Pregnancy rates for T1, T2, T3 were 42%, 25%, 18% for five days of AI, were 65%, 47%, 61% for 19 days of AI, and were 90%, 88%, 85% for the 44 days (19 days of AI followed by 25 days with bulls).

Prostaglandin $F_{2\alpha}$ Product Comparisons

Rumors abound regarding relative effectiveness of various $PGF_{2\alpha}$ products. The $PGF_{2\alpha}$ products either contain the natural $PGF_{2\alpha}$ or various analogs of $PGF_{2\alpha}$. Analogs of $PGF_{2\alpha}$ were developed to obviate patents existing at the time of initial marketing or to increase "potency" and/or decrease side effects. Although active ingredients and their properties differ among the various $PGF_{2\alpha}$ products, each $PGF_{2\alpha}$ product induces luteolysis by triggering a cascade of endogenous events that ultimately lead to the regression of the corpus luteum. Each U.S. $PGF_{2\alpha}$ product has been approved by the Food and Drug Administration/Center for Veterinary Medicine (FDA/CVM); to be approved by FDA/CVM each product had to have sufficient data documenting efficacy for the label indication. Efficacy is based on dose, route of administration, species, and endpoints for label indication(s). Some U.S. $PGF_{2\alpha}$ products have more label claims than others simply due to the decisions of the various companies developing the $PGF_{2\alpha}$ products that the market did or did not justify the additional expense of securing said label claims.

One example (Figure 2) of a $PGF_{2\alpha}$ analog compared to $PGF_{2\alpha}$ is Estrumate, containing cloprostenol sodium, and Lutalyse, containing the natural $PGF_{2\alpha}$. The label intramuscular doses, based on extensive field studies with cattle, are 2 mL (0.5 mg) for Estrumate and 5 mL (25 mg) for Lutalyse.

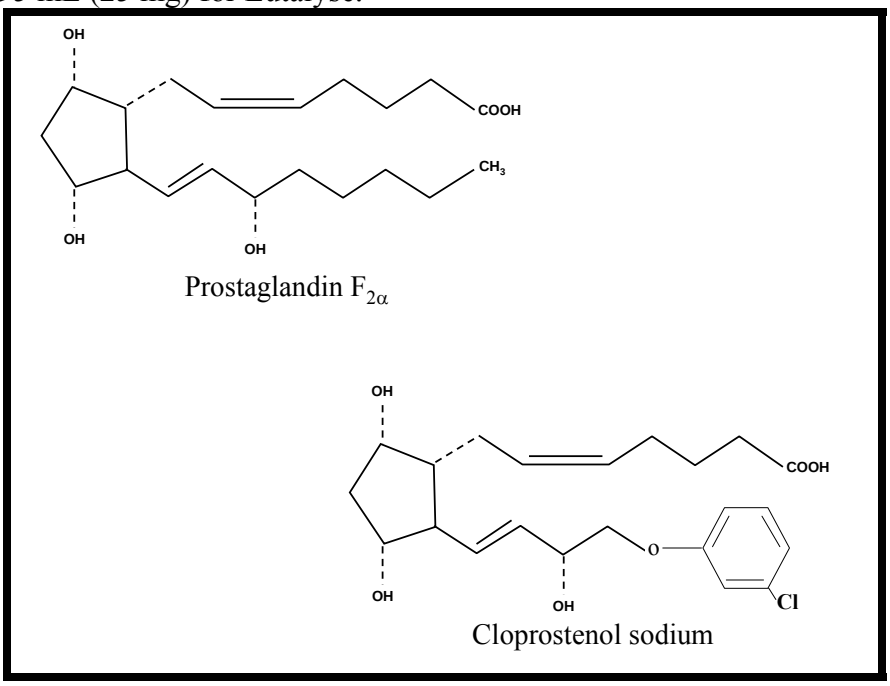

Figure 2. Chemical structures of $PGF_{2\alpha}$ (dinoprost) and a $PGF_{2\alpha}$ analog (cloprostenol).

Products containing $PGF_{2\alpha}$ analogs consistently require lower doses to regress the corpus luteum in cattle than products containing natural $PGF_{2\alpha}$. One rumor is $PGF_{2\alpha}$ products with $PGF_{2\alpha}$ analogs are more potent (lower dose) therefore more efficacious. There are hundreds of papers reporting use of $PGF_{2\alpha}$ products in cattle with response measured as return to estrus, conception rate and pregnancy rate. I interpret the scientific literature to support an interpretation of "no difference" among the FDA/CVM approved $PGF_{2\alpha}$ products used in cattle. I interpret anyone skilled in the art can select papers to show what we want; such as one $PGF_{2\alpha}$ product is better or worse than another. This can be accomplished since, either by chance or due to insufficient numbers of cattle on a study, a paper will report one $PGF_{2\alpha}$ product is numerically superior or inferior to another $PGF_{2\alpha}$ product, usually the differences are numerical but not statistically different, but the difference is interpreted to be real.

The $PGF_{2\alpha}$ and $PGF_{2\alpha}$ analogue products achieve efficacy through regression (luteolytic) of the corpus luteum (CL). Following CL regression, progesterone concentrations decrease to baseline in about 24 hours, which allows maturation of the dominant pre-ovulatory follicle that results in an increase in serum concentrations of estradiol-17β. Increased serum estradiol-17β concentration leads to the LH surge that induces ovulation. Increased serum estradiol-17β concentration stimulates the immune system in the uterus. These biological relationships are the bases for the label indications

of the various $PGF_{2\alpha}$ products, synchronization of estrus, treatment of uterine infections such as pyometra, and induction of abortion in pregnant cattle.

The following published papers address effectiveness of various $PGF_{2\alpha}$ products. I did not place the references for this section in "References" but retained the references within this section.

1. Comparison among dinoprost, cloprostenol and fenprostalene (Theriogenology 29:1193,1988, Guay, Rieger, Roberge). No difference in serum progesterone (P4) rate of decrease (all P4 at baseline by 24 hr after injection). No difference in ova/embryos collected between Days 6 and 8 of gestation.
2. Comparison among cloprostenol, alfaprostenol, prosolvin, and iliren (Theriogeno. 17:499, 1982, Schams and Karg). P4 decreased to baseline in 24 hr for each. Visual inspection of the P4 patterns suggested support of the author's conclusion of "no difference" among the $PGF_{2\alpha}$ products.
3. Comparison between dinoprost and fenprostalene (Theriogen. 28:523, 1987. Stotts et al). No difference in P4 profile following injection on either day 6 or day 11 of the estrous cycle.
4. Comparison among dinoprost, cloprostenol and fenprostalene (Theriogen. 34:667, 1990. Desaulniers, Guay, Vaillancourt) . Similar pattern of return to estrus. However, 5/10 fenprostalene cattle, but zero cattle for dinoprost and cloprostenol groups, had P4 greater than 1 ng/mL at 48 hr, suggesting slower P4 decline with fenprostalene. However, note the data of "1)" and "3)" above did not show such a difference.
5. Comparison between dinoprost and cloprostenol. The series of papers by Macmillan et al using either dinoprost of cloprostenol and measuring return to estrus/estrus synchrony, conception rate and pregnancy rate indicate to me "no difference" (An. Repro. Sci, 6:245, 1983/1984; NZ Vet. J. 31:110, 1983 and 43:53, 1983; Theriogen.18:245, 1982).
6. Comparison between dinoprost and cloprostenol (Theriogen. 21:1019, 1984. Donaldson). Estrus control similar, although the dose of dinoprost was 65mg in three doses. I grant Donaldson has published other papers criticising dinoprost vs cloprostenol for embryo transfer use.
7. Tiaprost. P4 decreased to baseline in about 24 hours, a pattern reported above for various $PGF_{2\alpha}$ products.
8. Alfaprostol. (Theriogen.24:737, 1985. Kiracofe, Keay, Odde). Pattern of return to estrus, day of estrous cycle response rate, conception rate and pregnancy rate patterns similar to those reported for various $PGF_{2\alpha}$ products.
9. Fenprostalene (Theriogen. 25:463, 1986. Herschler, Peltier, Duffy, Kushinsky). Patterns of P4 decrease and return to estrus similar to those reported for various $PGF_{2\alpha}$ products.
10. Comparison among dinoprost, cloprostenol and luprostiol (Theriogen. 33:943,1990. Plata et al). Estrus response (5-d synchrony) and pregnancy rates did not differ among the $PGF_{2\alpha}$ products.
11. Comparison between luprostiol and cloprostenol (J. Animal Sci. 67:2067, 1989. Godfrey et al). Brahman cattle. P4 declined but needed a dose of about 30mg luprostiol vs 0.5 mg cloprostenol and fertility apperaed depressed by that dose of luprostiol.

Peer-reviewed studies comparing the efficacy of Lutalyse and Estrumate to synchronize estrus in cattle are summarized in the following Table, courtesy of Fred Moreira.

Reference	Type[4]	N[5]	Estrus detection rate[1] (%)			Conception rate[2] (%)			Pregnancy rate[3] (%)		
			Lutalyse	Estrumate	*P*	Lutalyse	Estrumate	*P*	Lutalyse	Estrumate	*P*
Johnson, 1984	LDC	52	61.5	42.3	NS[6]	45.8	20.8	NS	54.2	29.2	NS
Seguin et al., 1985	NLDC	124	88.7	96.8	NS	60.0	64.3	NS	56.3	62.5	NS
	LDC	245	66.1	65.3	NS	51.2	50.6	NS	33.9	33.1	NS
Turner et al., 1987[7]	BC-BH	63	66.6	76.8	NS	50.2	44.1	NS	35.3	34.5	NS
Salverson et al., 2002	BH	1002	85.9	88.7	NS	66.5	67.5	NS	57.5	60.6	NS
Martineau, 2003	LDC-DH[8]	203	85.9	82.8	NS	33.7	41.8	NS	29.3	34.9	NS
	LDC-DH[9]	404	82.6	83.0	NS	38.6	46.6	NS	31.4	39.2	NS

[1] Percentage of animals detected in estrus relative to the total number of animals within each group.
[2] Percentage of animals that conceived relative to the number of animals inseminated.
[3] Percentage of animals that conceived relative to the total number of animals within each group.
[4] Type of cattle used in the study (LDC = lactating dairy cows; NLDC = non-lactating dairy cows; BC = beef cows; BH = beef heifers; DH = dairy heifers).
[5] Number of animals included in the experiment.
[6] NS = differences were not statistically significant.
[7] Pregnancy rates were calculated based on reported Least Square Means for estrus detection and conception rates.
[8] Includes only cows injected with LUTALYSE and ESTRUMATE intramuscularly.
[9] Includes both intramuscular and intravenous route of administration for LUTALYSE and ESTRUMATE.

Of the 217 prostaglandin papers published in the Journal of Animal Science, Journal of Dairy Science and Theriogenology, citations per $PGF_{2\alpha}$ product were 86% (186/217) for Lutalyse, 3% (7/217) for Estrumate, 4% (9/217) for all others, and 7% (15/217) no $PGF_{2\alpha}$ product identified (courtesy of Dr. Fred Moreira).

The scientific literature does not support a defendable interpretation that, when each $PGF_{2\alpha}$ product is used at the label dose, there are real differences among the $PGF_{2\alpha}$ products in efficacy. I propose technical service available per $PGF_{2\alpha}$ product makes the greatest significant difference among the $PGF_{2\alpha}$ products, assuming price to be competitive among the $PGF_{2\alpha}$ products.

Summary

This presentation provides data from studies conducted in commercial herds with various breeding management programs. The variety of breeding management programs available today gives the producer wide flexibility is selecting the program that best fits the breeding objectives for that herd. However, the large variety of breeding management programs also brings the potential for high confusion as to "what to do". I encourage us to remember the biology of the heifer/cow and attempt to match that biology with the breeding objectives for the herd. Thus, selection of the breeding management program for a herd might take into consideration some of the following:

- If puberty is of concern, progestogens, such as MGA and CIDR, where approved for use by Regulatory Authorities, are justified to increase the percent of heifers estrus cycling at the time of desired breeding initiation.
- If timed AI is of interest, control of both follicle waves and lifespan of the CL is required. Thus, $PGF_{2\alpha}$ or $PGF_{2\alpha}$ analog products and GnRH, with or without a progestogen, are required.
- If limited input is desired, one might consider
  - Single $PGF_{2\alpha}$ or $PGF_{2\alpha}$ analog products followed by AI at estrus for 5 days
  - Single GnRH followed by $PGF_{2\alpha}$ or $PGF_{2\alpha}$ analog products 7 days later followed by AI at estrus for 5 days
  -Double $PGF_{2\alpha}$ or $PGF_{2\alpha}$ analog products at 14 days followed by either AI at estrus for 5 days or AI at about 80 hours after $PGF_{2\alpha}$ or $PGF_{2\alpha}$ analog products, or a combination of estrus detection and breeding to "80 hours" with timed AI of those not bred.

Although not presented, data exist that, with breeding management programs that result in estrus detected over several days, such as is achieved with Double or Single $PGF_{2\alpha}$ or $PGF_{2\alpha}$ analog product breeding programs, cattle can be bred with bulls rather than by AI. However, bull management, rotation of bulls into breeding for a few days followed by rest, is essential for the full success of this breeding program.

The scientific literature does not support a defendable interpretation that, when each $PGF_{2\alpha}$ product is used at the label dose, there are real differences among the $PGF_{2\alpha}$ products in efficacy. I propose that technical service available per $PGF_{2\alpha}$ product makes the greatest significant difference among the $PGF_{2\alpha}$ products, assuming price to be competitive among the $PGF_{2\alpha}$ products.

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NOTES

Proceedings, Applied Reproductive Strategies in Beef Cattle
November 12 and 13, 2005, Texas A&M University, College Station

ESTRUS SYNCHRONIZATION SYSTEMS: GnRH

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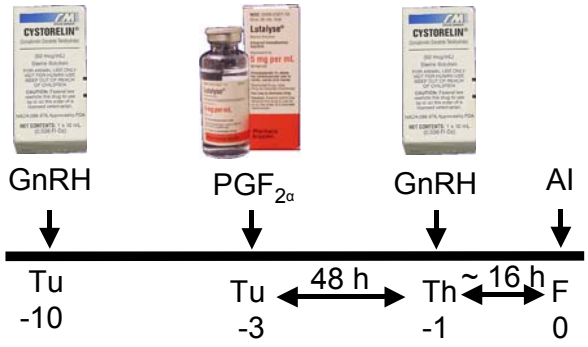
Introduction

Development of methods to manipulate the estrous cycle so that all cows are in estrus during a short, predefined period (synchronized estrus) while maintaining normal fertility has been a difficult goal to achieve; however, a number of valuable synchronization protocols have been created and are available to producers today. Although implementation of estrus synchronization and AI will improve the profitability of beef operations, no more than 3 to 5% of all beef operations in the U.S. utilize the technology (Patterson et. al., 2001). The major barriers to utilization of estrus synchronization and AI are time and labor (Kesler, 2003).

During the past 25 years, protocols have been developed that minimize time and labor, and yield excellent pregnancy rates. One of the most important steps to creating the wide variety of effective protocols that are available today began with the understanding of follicular waves and the development of the Ovsynch protocol (illustrated in Figure 1). Ovsynch was originally created for use in dairy cattle, however the basic elements (GnRH followed by $PGF_2\alpha$ seven days later) have as much value in beef cattle. Three protocols (Select Synch, CO-Synch, and Hybrid Synch) have emerged for use in beef cattle and will be discussed within this manuscript.

Figure 1. Ovsynch protocol



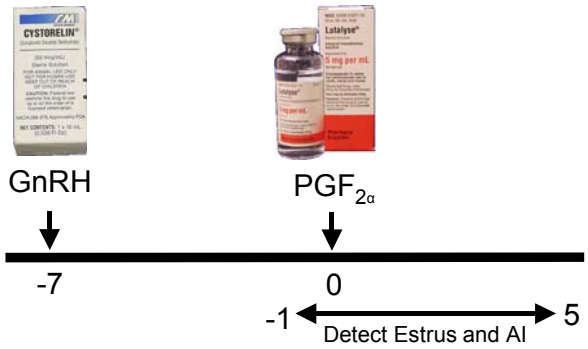
Select Synch

Select Synch, as well as all of the protocols discussed in this review, includes an injection of GnRH followed by $PGF_2\alpha$ seven days later. The initial injection of GnRH provokes a preovulatory-like LH surge (Pursley et al., 1995). Studies have demonstrated

that this single injection of GnRH induces ovulation in most cows, including >80% of late-calving anestrous cows suckling calves (Thompson et al., 1999). A new follicular wave is then initiated about two days after the GnRH-induced ovulation (Kojima and Patterson, 2003). There are a number of GnRH products available and all seem to have similar efficacy, assuming a full 100 mcg dose is administered. More variable responses, including decreased efficacy, have been reported when cows are administered a half dose of GnRH (John B. Hall, personal communications). Furthermore, 18 g needles that are 1.5 inches long are recommended and GnRH and $PGF_2\alpha$ should be injected intramuscularly in the neck.

Seven days after the injection of GnRH cows are administered an injection of $PGF_2\alpha$ to induce regression of corpora lutea, if present. Although 25-33% of the estrus-cycling cows will not have corpora lutea and do not need the $PGF_2\alpha$, it is not efficient to attempt to differentiate cows with corpora lutea from those without corpora lutea. Therefore, all cows should receive an injection of $PGF_2\alpha$ seven days after the GnRH injection. The protocol is illustrated in Figure 2.

Figure 2. SelectSynch protocol



Cows synchronized with the Select Synch protocol are bred based upon the detection of estrus. The majority of cows will exhibit estrus 36 to 72 hours after $PGF_2\alpha$ (Stevenson et. al., 2000). However, a small percentage will exhibit estrus outside this peak period (see Figure 3), including 8 to 10% that show estrus prior to the injection of $PGF_2\alpha$ (Geary et al., 2000). Furthermore, not all cows are detected in estrus—ranging from 7 to 61% in the published data. I recommend that estrus detection begin the day before injecting $PGF_2\alpha$ followed by 4 to 7 days of estrus detection—including the day $PGF_2\alpha$ is administered. Although the injection of GnRH may induce the first postpartum ovulation and hasten conception, fertility in cows in poor body condition will still be low (Stevenson et al., 2000; see Table 1).

Figure 3. Distribution of estrus after SelectSynch

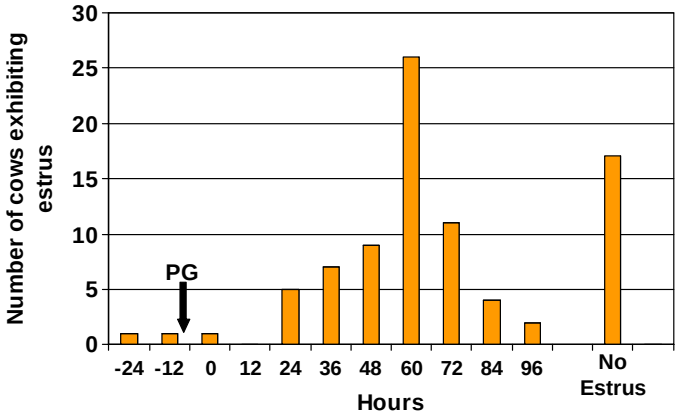


Table 1. Pregnancy rates in suckled beef cows after treatment with Select Synch

Body Condition	Select Synch
4.0 or less	28%
4.5	39%
5.0 or greater	50%

The Select Synch procedure was developed for operators who do not object to, or feel more comfortable with, breeding upon the detection of estrus. The Select Synch protocol has been effectively utilized with very encouraging results as reported in Table 2. As shown in Table 2, estrus detection rates and pregnancy rates are highly correlated ($r = .96$; $P < .01$). Low responses may be due to compromised estrus detection efficiency, postpartum anestrus, or a combination of both. However, it does illustrate the importance of estrus detection and of using this protocol only when one is fully committed to thorough monitoring of estrus.

Table 2. Estrus response rates and pregnancy rates in cows administered the Select Synch protocol

Study	Estrus Response	Pregnancy Rate
Kojima et al., 2000	69%	47%
DeJarnette et al., 2001a:		
experiment 1	93%	70%
experiment 2	78%	52%
Stevenson et al., 2000:		
experiment 1	59%	38%
experiment 3	63%	44%
Patterson et al., 2001	67%	45%
Constantaras et al., 2004	80%	65%

CO-Synch

The CO-Synch protocol utilizes the same strategy as Select Synch; however, it uses a single fixed time AI. The protocol is illustrated in Figure 4. No estrus detection is required with CO-Synch—a major attribute of this protocol. Like Select Synch, cows must be in good body condition as results are compromised in cows in poorer body condition, as illustrated in Table 3 (Lamb et al., 2001).

Figure 4. CO-Synch protocol

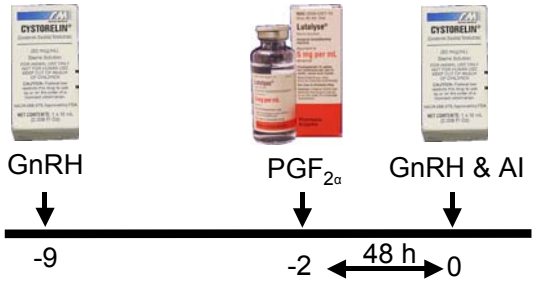


Table 3. Pregnancy rates in suckled beef cows after treatment with CO-Synch

Body Condition	Select Synch
4.5 or less	30%
4.5 to 5.0	41%
5.5 or greater	59%

The CO-Synch protocol has been used in a large number of diverse situations quite successfully. Table 4 is a summary of the available published data where CO-Synch was used. Overall, pregnancy rates have average 48%. The protocol is quite simple to employ as all injections and timed AI can be done at the same time of the day. However, details must be followed closely. In the study by Larson et al. (2004) cows were bred at 54 hours after the injection of $PGF_2\alpha$, by design in this case, and pregnancy rates were compromised.

Table 4. Pregnancy rates in cows administered the CO-Synch protocol

Study	**Pregnancy Rates**
Geary et al., 1998:	
cyclic cows	59%
anestrus cows	49%
Geary et al., 1998:	
location 1	49%
location 2	52%
location 3	46%
Stevenson et al., 2000	33%
Geary et al., 2001	49%
Geary et al., 2001	54%
Stevenson et al., 2003:	
experiment 1	61%
experiment 2	31%
Lamb et al., 2001:	
location 1	52%
location 2	54%
location 3	38%
location 4	53%
Perry et al., 2001	47%
Larson et al., 2004	43%
Constantaras et al., 2004	48%

Some have speculated that short-term calf removal, from the time of $PGF_2\alpha$ until AI is completed, may improve pregnancy rates. Geary and co-workers (2001) examined this concept and demonstrated an improvement in one experiment, but not another as illustrated in Table 5. Similar results were observed when short-term calf removal was used with Syncro-Mate B. It is important to note that in order to utilize short-term calf removal one must have excellent facilities.

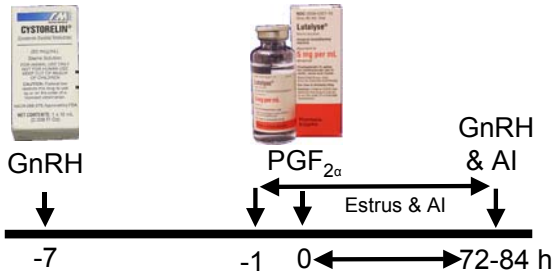
Table 5. Effect of short-term calf removal on pregnancy rates of cows synchronized with CO-Synch

Study	Pregnancy Rates
Geary et al., 2001:	
with calves	54%
calf removal	63%
Geary et al., 2001:	
with calves	49%
calf removal	46%

Hybrid Synch

Hybrid Synch, as the name implies, is a blend between Select Synch and CO-Synch. This procedure was created to optimize pregnancy rates in cows administered GnRH-$PGF_2\alpha$ protocol. Because the interval from $PGF_2\alpha$ to estrus is variable, as illustrated in Figure 3, it is impossible to select a single time that all cows have an excellent opportunity to conceive. Therefore, the insemination time for CO-Synch is the single time expected to achieve the highest pregnancy rate—not the optimum time when each individual has the best opportunity to conceive. In order for more cows to have an opportunity to conceive one may breed upon the detection of estrus for a period of time followed by a clean up timed AI—the Hybrid Synch protocol (illustrated in Figure 5). Upon examination of Figure 3, one will note that the highest percentage of cows in this study were in estrus at 60 hours after the $PGF_2\alpha$ injection.

Figure 5. HybridSynch protocol



Therefore, the ideal time for clean up timed AI for the majority of the cows is 72 hours. In the Hybrid Synch protocol it is recommended that the clean up timed AI be done at 72 to 84 hours after $PGF_2\alpha$. This clean up timed AI is only for cows not previously detected in estrus. Furthermore, cows detected in estrus do not need an injection of GnRH at insemination. However, cows at the clean up timed AI should be concurrently administered an injection of GnRH. This will improve the likelihood that ovulation will be synchronized with the insemination. Results from published data are summarized in Table 6.

Table 6. Pregnancy rates in cows administered the Hybrid Synch protocol

Study	Estrus Response	Pregnancy Rates
Stevenson et al., 2000	19%	34%
DeJarnette et al., 2001b:		
experiment 1	44%	44%
experiment 2	74%	47%
Larson et al., 2004		53%
DeJarnette et al., 2004:		
herd A-01	75%	51%
herd A-02	60%	44%
herd B-F-01	100%	71%
herd C-00	75%	67%
herd C-01	23%	23%

The results are variable (overall average of 48% [data in Table 6]) and don't appear considerably higher than for Select Synch (overall average of 52% [data in Table 2]) and CO-Synch (overall average of 48% [data in Table 4]); however, it will allow one to maximize the opportunity for obtaining the greatest overall pregnancy rates. Similar to results in Table 2 for Select Synch, the estrus response was correlated ($r = .90$; $P < .01$) to pregnancy rates. Again this suggests that poor estrus detection and/or postpartum anestrus compromised efficacy. Some have even suggested that if the estrus response before the timed AI is poor, following up with the timed AI should be reconsidered.

Select Synch + ReCycleSynch

Because not all cows are inseminated in the Select Synch protocol, cows not detected in estrus and inseminated may be resynchronized for a second breeding. This potentially reduces the time to conception and allows for utilization of AI. This procedure was used on a group of cows by administering CO-Synch beginning six days after the original injection of $PGF_2\alpha$ to cows that were not observed in estrus and inseminated. Because we started breeding the day before $PGF_2\alpha$ we had a 16-day breeding period. Pregnancy rate at the end of the Select Synch protocol was 65% (Constantaras et al., 2004). With the additional cows conceiving to the CO-Synch protocol, the 16 day AI breeding pregnancy rate was 78%. This is only a slight increase in drug cost as only the cows that were not inseminated after Select Synch were administered CO-Synch; however, there is a significant increase in time and labor.

Heifers

Early studies concluded that GnRH-based protocols with timed AI (Ovsynch and CO-Synch) should not be used in heifers. For example, Martinez et al. (2002) reported pregnancy rates of 39% in heifers synchronized with CO-Synch. This compares to a 68% pregnancy rate in heifers synchronized with a CIDR-based system in the same study (Martinez et al., 2002) and an average 56% pregnancy rate for heifers synchronized with an MGA-based system (14 days of MGA followed by $PGF_2\alpha$ 19 days after the last day of

MGA feeding; Kesler, 2003) in other studies. More recently, Select Synch has been successfully used in heifers with very good fertility. Lamb et al. (2004) conducted a multi-herd study: heifers were administered Select Synch, two injections of $PGF_2\alpha$, or the MGA-based system. A greater percentage of MGA treated heifers (83%) were detected in estrus during the targeted-breeding week than for Select Synch and $PGF_2\alpha$ treated heifers (74% and 75% respectively). Most of the heifers displayed estrus between 24 and 72 hours. The peak period for Select Synch treated heifers was between 24 and 48 hours after $PGF_2\alpha$, whereas the peak period for the MGA treated heifers was between 48 and 72 hours. Conception rates ranged from 63 to 68% and pregnancy rates ranged from 47% to 56% and were not different. Funston et al. (2004) also conducted a multi-herd study. They similarly demonstrated that the MGA-based protocol was more effective in synchronizing estrus; however, conception rates and overall AI pregnancy rates for the MGA-based protocol and Select Synch were similar. Combined, these data suggest that Select Synch will effectively synchronize estrus in heifers; however, attempting to time AI is not recommended at this time.

Follicular Dynamics

Research to further understand and/or improve the efficacy of these protocols continues. Follicular dynamics are of particular interest. The use of GnRH at the time of insemination results in a wide range of follicle sizes being ovulated (Perry et al., 2003). Lamb et al. (2001) demonstrated that pregnancy rates increased as follicular size at the time of second GnRH injection (for the CO-Synch protocol) increased to 16.0 to 17.9 mm and then dropped. Furthermore, Mussard et al. (2003) demonstrated that when embryos of similar quality were transferred into cows induced to ovulate small (< 12 mm) or large (> 12 mm) follicles, pregnancy rates were significantly higher in cows that ovulated with large follicles. Therefore, the goal in a timed AI protocol is to administer the second GnRH injection at a time when cows have large follicles, yet before spontaneous ovulation—a difficult goal to achieve.

Estrogens

It is important to point out that some scientists have reported that the use of estrogen—estradiol and estradiol benzoate—may improve synchronization efficacy; however, extensive multi-location studies do not exist. The consensus of many, including most of the scientists with reports at this workshop, agree that estradiol use should be suspended. This recommendation is based upon a study that reported a higher incidence of invasive breast cancers in women administered a postmenopausal estrogen/progestin product (Women's Health Initiative, 2002). Estrogens will certainly cause breast cancers to proliferate; however, is it a cause of breast cancer? The Women's Health Initiative study convinced the public, including a high percentage of physicians, that estrogens cause breast cancer. A smaller arm of the Women's Health Initiative (2002)—that did not receive significant publicity—was the study where estrogen alone was used in women with hysterectomies. In this study, there was no evidence that estrogen caused cancer (Nelson et al., 2002). However, there is considerable public concern and there are other demonstrated clinical implications of estrogen therapy. We do not need to further

concern the public with the safety of the product beef producers provide. Besides, estradiol and estradiol benzoate are not approved by FDA for this use. Hence, it is not an extra-label use—it is illegal to use estradiol or estradiol benzoate to synchronize estrus and ovulation. The only estrogen approved for use in cattle was estradiol cypionate (ECP®); however, because of the public concern with estrogens it is no longer commercially available.

Efficacy of Different GnRH Products

The efficacy of the specific GnRH product used with the Select Synch, CO-Synch, and Hybrid Synch protocols has been discussed. Much of the discussion was caused by a study published by Martinez et al., (2003). Martinez et al. (2003) reported that Cystorelin® provoked a greater LH surge than Fertagyl® and Factrel®. Similarly, Cystorelin® induced a higher ovulation rate; however, all products synchronized follicular wave emergence. GnRH is a decapeptide—a linear chain of ten amino acids. The base for Cystorelin®—and Fertagyl® (and Ovacyst™ another GnRH product not included in the Martinez study)—is diacetate, tetrahydrate. Therefore, Cystorelin®, Fertagyl®, and Ovacyst™ are chemically identical. Factrel® has a HCl base which should not alter bioactivity. If the GnRH products are chemically identical, then why did Martinez et al. (2003) observe differences? Being quite familiar with pharmaceutical manufacturing I realize that companies are permitted to include a wide range of active compound in the product. It is unknown if the company manufactures at the low or high end of this range. Hence, the results of Martinez et al. (2003) may only be a difference in active GnRH within the product. One must remember, the dose was selected based on the treatment of cystic ovarian disease—the clinical claim for GnRH products. This raises a previously mentioned point. One should use a full dose of GnRH as more variable responses, including decreased efficacy, has been reported when cows are administered a half dose of GnRH (John B. Hall, personal communications). Although all dominant follicles (≥ 10 mm) have the ability to ovulate in response to a GnRH-induced LH surge, Sartori et al. (2001) demonstrated that a larger dose of LH was required to induce ovulation of a 10 mm follicle compared to larger follicles. Certainly, this subject needs further study.

Implications

The purpose of this article is to review the GnRH-based estrus synchronization protocols. A succinct summary is provided in the following table (Table 7).

Table 7. GnRH/$PGF_2\alpha$-based estrus synchronization protocols used in beef cows

Protocol	Description
Select Synch	• The duration of the protocol is only one week; however, breeding should begin six days after initiating the protocol because a percentage of cows exhibit estrus before the injection of $PGF_2\alpha$. • This protocol requires minimal drug cost; however, considerable time is required for detection of estrus. • In order for this protocol to be successful, estrus detection must be emphasized. With emphasis on estrus detection, one can obtain excellent pregnancy rates if cows are in good body condition. • AI pregnancy rates may be improved if cows not detected in estrus are subsequently administered CO-Synch.
CO-Synch	• The duration of this system is nine days. • Because this is a timed AI protocol and all cows are inseminated 48 hours after the injection of $PGF_2\alpha$, it does not require the time and labor associated with detecting estrus. • At the time of AI, cows are also administered an injection of GnRH which increases the drug cost as compared to Select Synch; however, time and labor are minimized.
Hybrid Synch	• This is a blend of Select Synch and CO-Synch protocols and maximizes the opportunity for obtaining the greatest overall pregnancy rates. • Cows are bred upon the detection of estrus for the first 72-84 hours. Then any cow not detected in estrus is administered GnRH and inseminated. Drug costs are reduced as compared to CO-Synch as cows detected in estrus are not administered GnRH at AI. However, labor costs are increased as compared to CO-Synch.

Other scientists are summarizing results utilizing progestins (MGA- and CIDR-based systems) and can be found elsewhere in these proceedings. Although the progestin-based systems may have higher pregnancy rates in some situations, the GnRH-based systems without progestins have value. In fact, a supermarket of estrus synchronization protocols for producers with different needs exists today. Three of the protocols within this estrus synchronization supermarket are Select Synch, CO-Synch, and Hybrid Synch. These are systems minimizing drug costs compared to some others; however, cows must be in good body condition and postpartum anestrus may compromise efficacy.

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NOTES

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REVIEW OF ESTRUS SYNCHRONIZATION SYSTEMS: MGA®, [1]

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INTRODUCTION

Estrus synchronization and artificial insemination (AI) remain the most important and widely applicable reproductive biotechnologies available for cattle (Seidel, 1995). Although hormonal treatment of heifers and cows to group estrous cycles has been a commercial reality now for over 30 years, beef 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. In addition, early estrus synchronization programs failed to manage follicular waves, resulting in more days in the synchronized period, which ultimately precluded fixed-time artificial insemination with acceptable pregnancy rates. The development of convenient and economical protocols to synchronize estrus and ovulation to facilitate use of fixed-time AI with resulting high fertility should result in increased adoption of these important management practices (Patterson et al., 2003). Current research has focused on the development of methods that effectively synchronize estrus in postpartum beef cows and replacement beef heifers by decreasing the period of time over which estrus detection is required, thus facilitating the use of fixed timed AI.

Although tools are now available for beef producers to successfully utilize these procedures, transfer of the technology must assume a high priority. Transfer of this technology to beef producers in the U.S. will require an increase in technical support to facilitate successful use and adoption of these procedures, otherwise the products of our research and technology may be used more effectively in foreign countries (i.e., Brazil) whose beef products will ultimately compete with our own (Patterson et al., 2000).

Improving traits of major economic importance in beef cattle can be accomplished most rapidly through selection of genetically superior sires and widespread use of artificial insemination. Procedures that facilitate synchronization of estrus in estrous cycling females and induction of an ovulatory estrus in peripubertal heifers and anestrous postpartum cows will increase reproductive rates and expedite genetic progress. Estrus synchronization can be an effective means of increasing the proportion of females that become pregnant early in the breeding season resulting in shorter calving seasons

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and more uniform calf crops (Dziuk and Bellows, 1983). Females that conceived to a synchronized estrus calved earlier in the calving season and weaned calves that were on average 13 days older and 21 pounds heavier than calves from nonsynchronized females (Schafer et al., 1990).

Effective estrus synchronization programs offer the following advantages: 1) cows or heifers are in estrus at a predicted time which facilitates AI, embryo transfer, or other assisted reproductive techniques; 2) the time required for detection of estrus is reduced thus decreasing labor expense associated with estrus detection; 3) cattle will conceive earlier during the breeding period; 4) AI becomes more practical; and 5) calves will be older and heavier at weaning.

WHY BEEF PRODUCERS DO NOT USE EXISTING AND POTENTIAL TECHNOLOGIES. Beef producers cite several reasons for the lack of widespread use of AI to breed heifers and cows. These reasons include: lack of time and labor, available procedures are viewed as being too complicated or costly to implement, inadequate means to detect estrus, or inconvenience (NAHMS, 1998). Continuation of low adoption rates of these technologies in the U.S. will ultimately erode the competitive position of the U.S. cattle industry. Other countries are adopting new technologies for animal production more rapidly than the U.S. For example, growth in the use of AI in Brazil has outpaced that of the U.S. (ASBIA, 2004; NAAB, 2004; Table 1). Beef producers in Brazil artificially inseminate nearly 5 times more cows annually compared with U.S. producers. Given the current scenario, elite seedstock herds in the U.S. will soon provide a sizeable percentage of the germ plasm used worldwide. Unless, however, owners of commercial cowherds aggressively implement reproductive and genetic improvement, the U.S. will lose its competitive advantage in 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.

Table 1. Import and domestic beef semen sales in Brazil and the U.S. over 10 years.

Import and domestic beef semen sales (units sold)			
COUNTRY	1993	2003	% change
Brazil[a]	1,874,996	4,896,204	+161
United States[b]	1,025,116	906,923	-8

Export sales in the U.S. rose from 393,365 units in 1993 to 614,904 units in 2003 (+56%, NAAB, 2004). [a]ASBIA, 2004; [b]NAAB, 2004.

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 estrous 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 for AI performed on the basis of detected estrus:

PG: Prostaglandin $F_{2\alpha}$ (PG; Lutalyse®, Estrumate®, ProstaMate®, InSynch®, estroPLAN®).

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 (GnRH; Cystorelin®, Factrel®, Fertagyl®, OvaCyst®) followed in 7 days with an injection of PG.

MGA-GnRH-PG (MGA® Select): MGA is fed for 14 days, GnRH is administered 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.

Protocols for fixed-time AI:

MGA® Select: MGA is fed for 14 days, GnRH is administered 12 days after MGA withdrawal, and PG is administered 7 days after GnRH. Insemination is performed 72 hours after PG with GnRH administered at AI.

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. Insemination is performed 60 hours after PG with GnRH administered at AI.

CO-Synch + CIDR: GnRH is administered at CIDR insertion on day 0, followed 7 days later with CIDR removal, and PG. Insemination is performed 66 hours after CIDR removal and PG, with GnRH administered at AI.

Terms:

Estrous 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 became 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.

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 to 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 2 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 six distinct phases. The physiological basis for estrus synchronization followed the discovery that progesterone inhibited ovulation (Ulberg et al., 1951) and preovulatory follicular maturation (Nellor and Cole, 1956; Hansel et al., 1961; Lamond, 1964). 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 (Thimonier et al., 1975; Patterson et al., 2003). The Progesterone Phase 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 the Progesterone–Estrogen Phase. Prostaglandin $F_{2\alpha}$ and its analogs were reported in 1972 to be luteolytic in the bovine (Lauderdale, 1972; Rowson et al., 1972; Liehr et al., 1972; Lauderdale et al., 1974) and ushered in the PG Phase. Treatments that combined progestational agents with PG characterized the Progestogen-PG Phase. All of these protocols addressed control of the luteal phase of the estrous cycle since follicular waves were not recognized at the time.

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 (Fortune et al., 1988). 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 that precise control of estrous cycles requires the manipulation of both follicular waves and luteal lifespan (GnRH-PG Phase).

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 (≥ 10 mm; Garverick et al., 1980; Bao and Garverick, 1998; Sartori et al., 2001). 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). 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, however, 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). This information stimulated research in the Progestogen-GnRH-PG Phase.

SYNCHRONIZATION OF ESTRUS AND OVULATION WITH THE GNRH-PG-GNRH PROTOCOL

Administration of PG alone is commonly utilized to synchronize an ovulatory estrus in estrous 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 (Figure 1) 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 (Ovsynch; Pursely et al., 1995). Ovsynch was validated as a reliable means of synchronizing ovulation for fixed-time AI in lactating dairy cows (Pursley et al., 1995; Burke et al., 1996; Pursley et al., 1997a, b; Schmitt et al., 1996). 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 (Pursley et al., 1997a). Pregnancy rates among cows that were inseminated at a fixed time following Ovsynch ranged from 32 to 45% (Pursley et al., 1997b; 1998). The Ovsynch protocol, however, did not effectively synchronize estrus and ovulation in dairy heifers (35% pregnancy rate compared with 74% in PG controls; Pursley et al., 1997b).

Protocols for fixed-time insemination were recently tested in postpartum beef cows. Pregnancy rates for Ovsynch treated beef cows were compared with those of cows synchronized and inseminated at a fixed time following treatment with Syncro-Mate-B (Geary et al., 1998a). Calves in both treatment groups were removed from their dams for a period of 48 hours beginning either at the time of implant removal (Syncro-Mate-B) or at the time PG was administered (Ovsynch). Pregnancy rates following fixed-time AI after Ovsynch (54%) were higher than for Syncro-Mate-B (42%) treated cows. One should note that on the day following fixed-time insemination, cows were exposed to fertile bulls of the same breed; no attempt was made to determine progeny paternity. Additionally, we do not know the incidence of short cycles among cows that were anestrus prior to treatment and that perhaps returned to estrus prematurely and became pregnant to natural service.

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 (Figure 1). CO-Synch (Geary et al., 1998b) 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 (Geary et al., 2000) 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 4 days after GnRH injection and continue through 6 days 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).

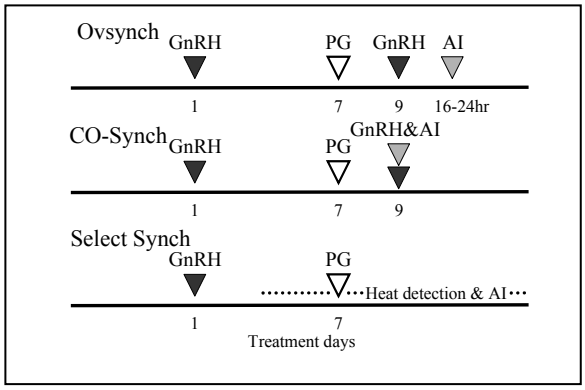

Figure 1. Methods currently being used to synchronize estrus and ovulation in postpartum beef cows using the GnRH-PG protocol: Ovsynch, CO-Synch and Select Synch.

MGA-BASED PROGRAMS

This manuscript reviews methods to control estrous cycles of beef cows or heifers using MGA in breeding programs involving artificial insemination. Four methods will be outlined for using the MGA program to facilitate estrus synchronization in beef 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. Melengestrol acetate is an orally active progestin. When consumed by cows or heifers on a daily basis, MGA will suppress estrus and prevent ovulation (Imwalle et al., 2002). Melengestrol acetate 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. Melengestrol acetate is fed at a rate of 0.5 mg/animal/day in a single daily feeding. 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 estrous response during the synchronized period. Therefore, adequate bunk space (60 linear cm/head) must be available so that all animals consume feed simultaneously (Patterson et al., 2003).

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 for natural service because of 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 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).

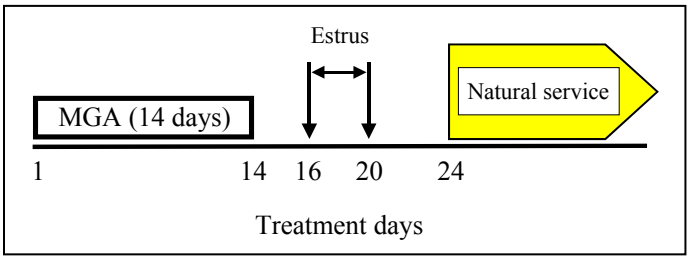

Figure 2. MGA and natural service (adapted from Patterson et al., 2000b).

This system works effectively, however careful consideration of bull to female ratios is advised. 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.

METHOD 2: MGA + PROSTAGLANDIN

This method of estrus synchronization involves the combination of MGA with prostaglandin $F_{2\alpha}$. 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 PG injection, which shortens the synchronized period and maximizes conception rate (Figure 3). 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; Deutscher, 2000; Lamb et al., 2000). Five available PG products for synchronization of estrus in cattle can be used after the MGA treatment: Lutalyse®, ProstaMate®, InSynch®, Estrumate®, or estroPLAN®. Label-approved dosages differ with each of these products; carefully read and follow directions for proper administration before their use.

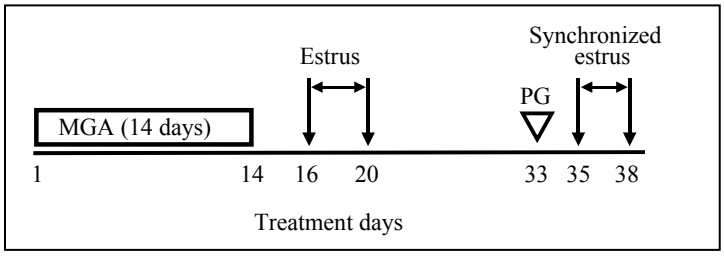

Figure 3. The MGA-PG protocol (adapted from Brown et al., 1988; Deutscher, 2000; Lamb et al., 2000).

Management related considerations to long-term feeding of MGA to heifers. Long-term feeding of MGA to beef heifers and associated effects on fertility may be a concern in specific production systems. It is not uncommon for heifers to be placed on MGA for extended periods of time and subsequently exposed for breeding after placement in backgrounding programs that necessitate long-term MGA administration. Zimbelman et al. (1970) reported no negative effect of either long-term or repeated intervals of feeding MGA to beef cows and heifers, other than the expected reduced conception rate when cattle were bred at the synchronized estrus 3 to 7 days after the last day of MGA feeding. Patterson et al. (1993) designed a study (Figure 4) to compare estrous response and fertility during synchronized estrous periods among beef heifers that were fed MGA for 87 days (long-term, LT) or 14 days (short-term, ST) prior to PG. Heifers were stratified by age and weight to LT- or ST-MGA treatments (Table 2), and received 0.5 mg MGA per head per day for 87 or 14 days, respectively. Heifers in each group were administered PG 17 days after MGA withdrawal. Heifers in both groups that failed to exhibit estrus within 6 days after the first injection of PG, were administered a second injection of PG 11 days later (Figure 4).

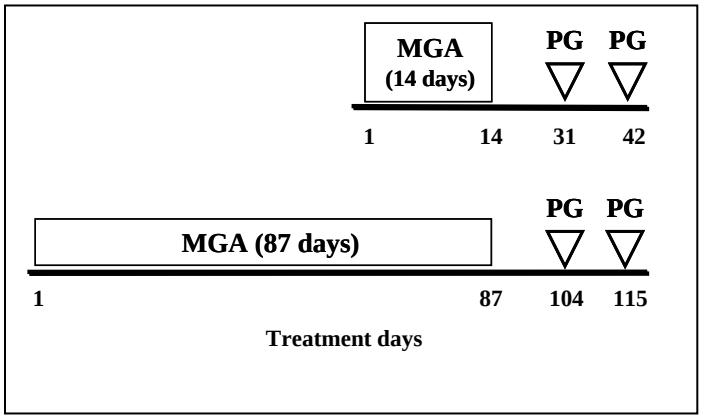

Figure 4. Comparison of short-term and long-term MGA treatments.

Transrectal ultrasonography was used to examine ovaries of all heifers at the end of treatment with MGA and at the time PG was administered. Heifers that failed to exhibit estrus after the first injection of PG were re-examined prior to the second PG injection. All heifers were exposed for natural-service for an additional 45 d after the AI period. More ST-treated heifers exhibited estrus after the first injection of PG than LT-treated heifers (Table 3; $P < 0.05$). Total response after the two injections of PG, however, did not differ between treatments. Furthermore, there were no significant differences between treatments in synchronized conception or pregnancy rates, or pregnancy rates at the end of the breeding period

(Table 3). A higher incidence of luteinized follicular cysts (Table 4) was observed among heifers in the LT-treatment compared with heifers in the ST-treatment [LT, 11/30 (37%); ST, 0/31 (0%)]. This observation may explain differences in estrous response between treatments following the first injection of PG. These data indicate that long-term feeding of MGA may result in a higher than normal incidence of luteinized follicular cysts and an associated reduction in estrous response after PG. The data indicate, however, that re-injection with PG resulted in satisfactory breeding performance among heifers that were fed MGA for extended periods of time.

Table 2. Ages and weights of heifers at the time PG was administered.

Treatment	No. of heifers	Age, d	Weight, kg
Short-term, 14 d	31	427	393
Long-term, 87 d	30	423	387

[1]Adapted from Patterson et al., 2003.

Table 3. Estrous response and fertility of heifers treated long-term or short-term with MGA.

Response variable	Short-term MGA, 14 d			Long-term MGA, 87 d		
	1st PG[a]	2nd PG[a]	Total	1st PG[a]	2nd PG[a]	Total
Estrus response	24/31 (77%[b])	4/7 57%)	28/31 (90%)	16/30 (53%[c])	10/14 (71%)	26/30 (87%)
Synchronized conception	15/24 (63%)	3/4 (75%)	18/28 (64%)	12/16 (75%)	6/10 (60%)	18/26 (69%)
Synchronized pregnancy	--------		18/31 (58%)	--------		18/30 (60%)
Final pregnancy	--------		28/31 (90%)	--------		27/30 (90%)

[a]1st PG refers to animals that responded to PG administered 17 days after MGA withdrawal. 2nd PG refers to animals that failed to respond to the first injection of PG that were reinjected 11 days later.

[b, c]Percentages within row and between treatments with unlike superscripts differ ($P < 0.05$; Adapted from Patterson et al., 2003).

Table 4. Ovarian morphology of heifers treated long-term or short-term with MGA.

Treatment	Normal		Abnormal[a]	
Short-term	31/31	(100%)	0/31	(0%)
Long-term	19/30	(63%)	11/30	(37%)

[a]Abnormal = presence of luteinized follicular cysts, 20-45 mm diameter (Adapted from Patterson et al., 2003).

METHOD 3: MGA® SELECT

The MGA® Select treatment (Wood et al., 2001; Figure 5) is useful in maximizing estrous response and reproductive performance in postpartum beef cows. The MGA® Select protocol involves feeding MGA for 14 days followed by an injection of GnRH 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.

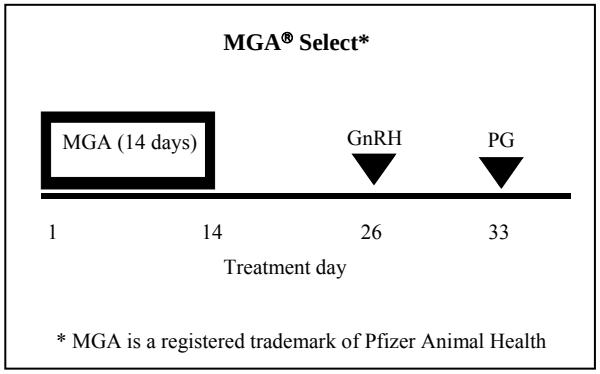

Figure 5. The MGA® Select protocol (Wood et al., 2001). MGA is fed for a period of 14 days followed in 12 days (day 26) by an injection of GnRH, and PG 19 days after MGA withdrawal (day 33).

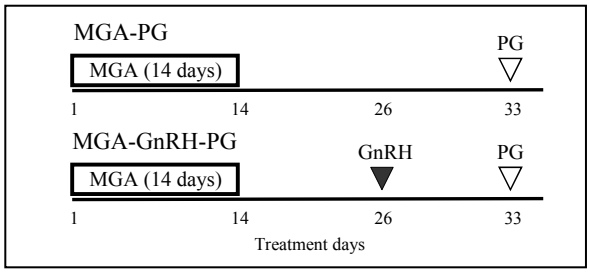

Figure 6. Cows were fed MGA for 14 days; 19 days after MGA withdrawal PG was administered to all cows. GnRH was administered to ½ of the cows 7 days prior to PG (Patterson et al., 2002).

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., 2002; Figure 6).

The following tables provide a summary of the results from the study conducted during the 2001 breeding season. Table 5 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 estrous cycling prior to the time treatment with MGA began. Estrous cyclicity status was determined based on two blood samples for progesterone obtained 10 days before and on the first day of MGA.

Table 5. Number of cows within age group per treatment, days postpartum, body condition and estrous cyclicity status at the time treatment with MGA began[1] (Patterson et al., 2002).

Treatment	Age group (yrs)	No.of cows	Days postpartum	Body condition score	Estrous cycling (%)
MGA-PG	2, 3 & 4	52	47	5.2	35
	5+	48	39	5.2	15
	Total	100	44	5.2	40
MGA Select	2, 3 & 4	53	47	5.3	38
	5+	48	40	5.3	13
	Total	101	44	5.3	53

[1]Average 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. Estrous 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 6 provides a summary of estrous response, synchronized conception and pregnancy, and final pregnancy rates for cows assigned to the two treatments. Estrous 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 6. Estrous response, synchronized conception and pregnancy rate, and final pregnancy rate at the end of the breeding period (Patterson et al., 2002). [a,b]Percentages within column and category with unlike superscripts are different ($P<0.05$).

Treatment	Age group (yrs)	Estrous response (no.)	(%)	Synchronized conception rate (no.)	(%)	Synchronized pregnancy rate (no.)	(%)	Final pregnancy (no.)	(%)
MGA-PG	2, 3 & 4	44/52	85	36/44	82	36/52	69	49/52	94
	5+	32/48	67	22/32	69	22/48	46[a]	48/48	100
	Total	76/100	76[a]	58/76	76	58/100	58	97/100	97
MGA Select	2, 3 & 4	46/53	87	33/46	72	33/53	62	51/53	96
	5+	42/48	88	34/42	81	34/48	71[b]	47/48	98
	Total	88/101	87[b]	67/88	76	67/101	66	98/101	97

METHOD 4: 7-11 SYNCH

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 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).

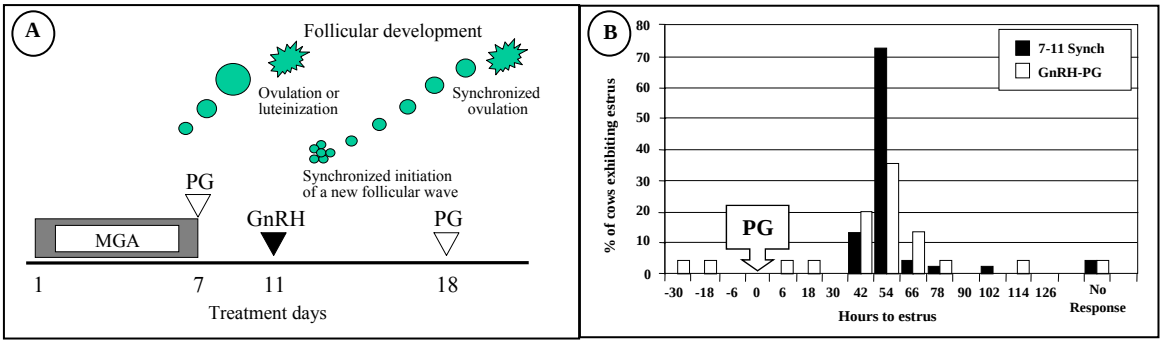

Figure 7A. Illustration of the treatment schedule and events associated with the 7-11 Synch protocol (Kojima et al., 2000). **Figure 7B.** Estrous response of cows treated with the 7-11 Synch or GnRH-PG protocols (Kojima et al., 2000).

ADDITIONAL CONSIDERATIONS. An additional consideration for Methods 2, 3, and 4 (MGA-PG, MGA Select, and 7-11 Synch) 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 (Federal Register, 1997; Kojima et al., 2001; Patterson et al., 1989; Wood-Follis et al., 2004; Zimbelman, 1963; Zimbelman and Smith, 1966).

USING MGA-BASED PROTOCOLS TO SYNCHRONIZE OVULATION PRIOR TO FIXED-TIME AI

Control of the follicular and luteal phase of the estrous cycle and induction of estrous cyclicity in anestrous cows is essential to the development of estrus synchronization protocols that facilitate fixed-time AI (Perry et al., 2002). Beef producers face uncertainty in knowing the percentage of cows that are anestrus in their herds, and which treatment or combination of treatments can be expected to provide the greatest likelihood of pregnancy following administration. The significance of progestin pre-treatment followed by administration of the GnRH-PG protocol and associated effects related to follicular development and subsequent fertility were demonstrated in previous experiments (Perry et.al., 2002; Kojima et al., 2002; Kojima et al., 2003a,b; Stegner et al., 2004a; Stevenson et. al., 2003). Previous research from our laboratory led to the development of the MGA Select and 7-11 Synch protocols. Both protocols effectively synchronize estrus in mixed populations of estrous cycling and anestrous postpartum beef cows (MGA Select, Wood et al., 2001; 7-11 Synch, Kojima et al., 2000). The two

protocols differ in length of treatment (MGA Select - 33 days; 7-11 Synch - 18 days) as well as length of the interval to estrus and resulting synchrony of estrus (Figure 8); however, there were no differences reported in pregnancy rates between these protocols among cows inseminated on the basis of observed estrus (Kojima et al., 2000; Patterson et al., 2002; Wood et al., 2001; Stegner et al., 2004b).

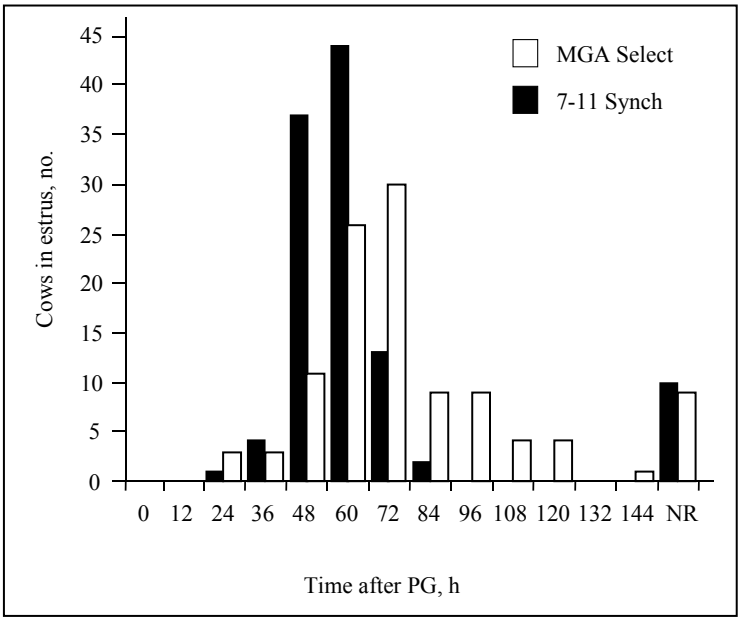

Figure 8. Distribution of estrus for MGA Select and 7-11 Synch treated cows. Non-responders (NR) refer to the number of cows that failed to exhibit estrus during the synchronized period (0 to 144 hous). Adapted from Stegner et al. (2004b).

The optimum and/or appropriate time to perform artificial insemination at fixed times following administration of these two protocols was reported (Kojima et al., 2003a; Perry et al., 2002; Stegner et al., 2004b); however, a direct comparison of the protocols to evaluate their efficacy for fixed-time AI was not made until recently (Bader et al., 2005). The MGA Select protocol provides an established synchrony of estrus and improves total herd estrous response, particularly among herds with high rates of anestrus (Patterson et al., 2002). Peak estrous response among cows assigned to the MGA Select protocol typically occurs 72 hours after PG (Figure 8; Patterson et al., 2002; Stegner et al., 2004a). Pregnancy rates were optimized for cows assigned to the MGA Select protocol when fixed-time AI was performed at 72 hours after PG (Perry et al., 2002; Stegner et al., 2004c), but were reduced when AI was performed at 48 or 80 hours after PG (Stevenson et al., 2003; Stegner et al., 2004c). The 7-11 Synch protocol (Kojima et al., 2000) improves synchrony of estrus over other protocols (Select-Synch, MGA Select) and peak estrous response typically occurs 56 hours after PG (Figure 8; Kojima et al., 2000; Stegner et al., 2004b). Pregnancy rates resulting from fixed-time AI after administration of the 7-11 Synch protocol were optimized when AI was performed 60 hours after PG (Kojima et al., 2003a).

Bader et al. (2005) compared the MGA Select and 7-11 Synch protocols used in conjunction with fixed-timed artificial insemination (Figure 9). The study was conducted at three locations with cows from the University of Missouri Experiment Station. Table 7 summarizes pregnancy rates resulting from fixed-time AI. There was no effect of treatment ($P = 0.25$), technician ($P = 0.81$), or sire ($P = 0.94$) on pregnancy rates resulting

from fixed-time AI. Table 8 summarizes pregnancy rates resulting from fixed-time AI on the basis of estrous cyclicity of cows prior to the initiation of treatment. Pretreatment estrous cyclicity did not influence (P = 0.12) pregnancy rates resulting from fixed-time AI. Furthermore, pregnancy rates resulting from fixed-time AI did not differ (7-11 Synch, P = 0.12; MGA Select, P = 0.50; Table 8) between cows that were estrous cycling or anestrus prior to initiation of the MGA Select and 7-11 Synch protocols.

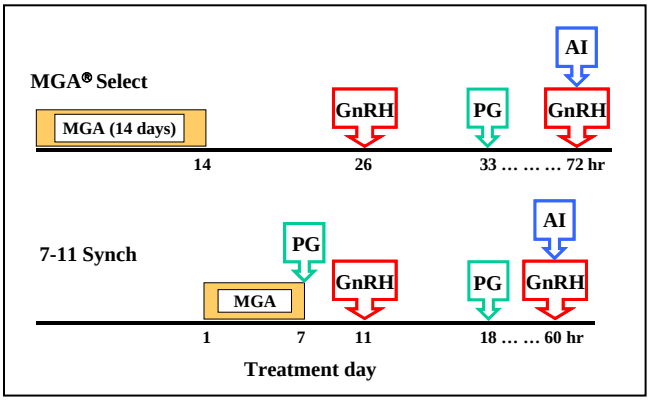

Figure 9. Comparison of the MGA Select and 7-11 Synch protocols in conjunction with fixed-time AI. From Bader et al. (2005).

Pregnancy rates resulting from fixed-time AI utilizing the MGA Select and 7-11 Synch protocols involved in this study are consistent with previously published reports [(MGA Select; Perry et al., 2002; Stegner et al., 2004c); (7-11 Synch; Kojima et al., 2002; Kojima et al., 2003a; Kojima et al., 2003b)]. Furthermore, pregnancy rates resulting from fixed-time AI in this study compare favorably with pregnancy rates after cows were inseminated on the basis of detected estrus using the same protocols to synchronize estrus (Kojima et al., 2000; Patterson et al., 2002; Stegner et al., 2004b).

Table 7. Pregnancy rates after fixed-time artificial insemination and at the end of the breeding season.

		Pregnancy rate to fixed-time AI[a]		Pregnancy rate at the end of breeding season[b]	
Location	Treatment	No.	(%)	No.	(%)
1	7-11 Synch[c]	64/104	(62)	98/104	(94)
	MGA Select[c]	68/104	(65)	102/104	(98)
2	7-11 Synch	34/60	(57)	57/59	(97)
	MGA Select	43/62	(69)	60/62	(97)
3	7-11 Synch	30/45	(67)	43/45	(96)
	MGA Select	31/47	(66)	42/47	(89)
Combined	7-11 Synch	128/209	(61)	198/208	(95)
Combined	MGA Select	142/213	(67)	204/213	(96)

[a,b]Fixed-time AI pregnancy rate determined by transrectal ultrasonography 40 to 50 d after AI and final pregnancy rate determined by ultrasonography 45 d after the end of breeding season (From Bader et al., 2005).

Table 8. Pregnancy rates after fixed-time AI based on estrous cyclicity prior to initiation of treatments.

Location	7-11 Synch				MGA Select			
	Estrous cycling		Anestrus		Estrous cycling		Anestrus	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
1	24/34	(71)	40/70	(57)	20/30	(67)	48/74	(65)
2	9/15	(60)	25/45	(56)	12/16	(75)	31/46	(67)
3	8/10	(80)	22/35	(63)	6/8	(75)	25/39	(64)
Combined	41/59	(69)	87/150	(58)	38/54	(70)	104/159	(65)

From Bader et al. (2005).

Perry et al. (2005) reported differences in late embryonic/fetal mortality following fixed-time AI among cows assigned to a CO-Synch protocol. Late embryonic/fetal mortality occurred at higher rates among cows that were induced to ovulate follicles ≤ 11 mm in diameter. Follicles induced to ovulate in this smaller range (≤ 11 mm) were characterized as being less physiologically mature at the time of ovulation, which may subsequently result in reduced oocyte and/or luteal competence. When cows were detected in standing estrus however, follicle size did not affect pregnancy rates or late embryonic mortality (Perry et al., 2005). The authors suggested that oocyte and luteal competence may be more dependent on steroidogenic capacity of the follicles from which they were ovulated than follicle size (Perry et al., 2005). A key observation from the preceding study suggests that follicular competence is important for both the establishment and maintenance of pregnancy. Vasconcelos et al. (2001) observed reduced peak concentrations of circulating estradiol (E_2), decreased size of the corpus luteum, decreased circulating concentrations of progesterone, and lower pregnancy rates to AI when dairy cows were induced to ovulate smaller sized follicles (≤ 14 mm).
Premature ovulation of a dominant follicle results in decreased ovulatory size, reduced luteal function, and compromised pregnancy rates compared to animals induced to ovulate larger, more mature dominant follicles (Mussard et al., 2003). The potential advantage in using either of these protocols (MGA Select or 7-11 Synch) to synchronize estrus prior to fixed-time AI is that mean follicle diameter at the time ovulation is induced (Kojima et al., 2002; Perry et al., 2002; Kojima et al., 2003a, b; Stegner et al., 2004a) exceeds the range described by Perry et al. (2005) and potentially minimizes problems with late embryonic/fetal mortality described by Perry et al. (2005) and Mussard et al. (2003).

Although presence of luteal tissue at PG affected subsequent pregnancy rate to fixed-time AI, the actual concentration of progesterone (P_4) at PG was not important in determining subsequent pregnancy. The difference between treatments in serum concentrations of P_4 at PG stems from the difference in hormonal environments between the two treatments under which the dominant follicle develops (Stegner et al., 2004a.). MGA Select treated cows have higher concentrations of serum P_4 and lower E_2 during the growth phase of the dominant follicle, than cows treated with 7-11 Synch (Stegner et al., 2004a). This hormonal milieu is similar to the mid-luteal phase of the estrous cycle while, 7-11 Synch cows develop a dominant follicle under higher E_2 and lower P_4

concentrations similar to the early luteal phase. Pregnancy rates based on pre-treatment estrous cyclicity status (estrous cycling versus anestrus) did not differ between treatments or among locations, which points to the efficacy of both protocols in successfully synchronizing estrus prior to fixed-time AI in mixed populations of estrous cycling and anestrous cows.

HOW DO MGA- AND CIDR-BASED PROTOCOLS COMPARE?

Substituting EAZI-BREED CIDR inserts for MGA in the MGA Select protocol in beef heifers. We recently designed a study to compare estrous response, timing of AI and pregnancy rate resulting from AI among beef heifers that were presynchronized with MGA or CIDR inserts prior to GnRH and PG (Kojima et al., 2004; Figure 10). Heifers (n = 353) at three locations (location 1, n = 154; 2, n = 113; and 3, n = 85) were randomly assigned to one of two treatments by age and weight. The MGA Select-treated heifers (MGA; n = 175) were fed MGA (0.5 mg/head/day) for 14 days, GnRH (100 µg i.m. Cystorelin) was injected 12 days after MGA withdrawal, and PG (25 mg i.m. Lutalyse) was administered 7 days after GnRH. The CIDR treated heifers (CIDR; n = 177) had CIDRs inserted for 14 days, GnRH was injected 9 days after CIDR removal, and PG was administered 7 days after GnRH. CIDR-treated heifers received carrier without MGA on days that coincided with MGA feeding.

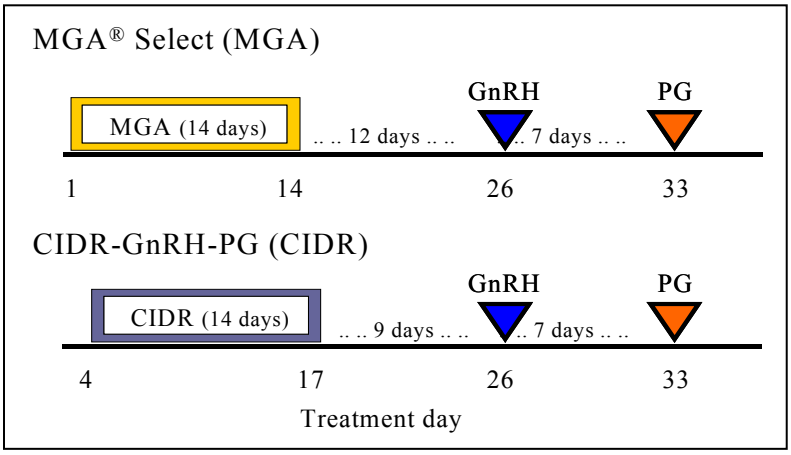

Figure 10. Substituting CIDR inserts for MGA in the MGA Select protocol in beef heifers. From Kojima et al. (2004).

Heifers were monitored for signs of behavioral estrus beginning the day PG was administered. AI was performed approximately 12 hours after onset of estrus and recorded as day of AI (Day 0 = PG). Pregnancy rate to AI was determined by ultrasonography 40 days after AI. Estrous response did not differ ($P > 0.10$) between treatments. Peak AI occurred on day 3 for heifers in both treatments (CIDR 122/177, 69%; MGA 93/175, 53%), and distribution of AI was more highly synchronized ($P < 0.05$) among CIDR- than MGA-treated heifers. Pregnancy rate to AI was greater ($P < 0.01$) in CIDR- (112/177, 63%) than MGA-treated heifers (83/175, 47%), however, final pregnancy rate did not differ ($P > 0.10$) between treatments (Table 9). In summary, replacing feeding of MGA with CIDR inserts improved synchrony of estrus and pregnancy rate resulting from AI in replacement beef heifers (Kojima et al., 2004).

Table 9. Estrous response, AI pregnancy, and final pregnancy rates.

	Estrous response	AI pregnancy rate	Final pregnancy rate
CIDR	154/177 (87 %)	112/177 (63 %)[a]	164/177 (93 %)
MGA	147/175 (84 %)	83/175 (47 %)[b]	159/175 (91 %)
Total	301/352 (86 %)	195/352 (55 %)	323/352 (92 %)
Difference	+ 3 %	[a,b] P = 0.01 + 16 %	+ 2 %

From Kojima et al. (2004).

HOW DO MGA SELECT AND CO-SYNCH + CIDR COMPARE IN SYNCHRONIZING OVULATION PRIOR TO FIXED-TIME AI IN POSTPARTUM BEEF COWS?

Previous research in our laboratory demonstrated the efficacy of using the MGA Select protocol to synchronize estrus and ovulation prior to fixed-time AI that was performed 72 h after PG (Perry et al., 2002; Stegner et al., 2004c; Bader et al., 2005). Other research showed an improvement in pregnancy rates resulting from fixed-time AI after treatment with the Co-Synch + CIDR protocol when insemination was performed 66 h as opposed to 48, or 54 h following CIDR removal and PG administration (Lamb et al., 2001; Bremer et al., 2004; Larson et al., 2004). Schafer (2005) designed a study to compare pregnancy rates resulting from fixed-time AI among cows assigned to the MGA Select and CO-Synch + CIDR protocols (Figure 11).

Crossbred, lactating, beef cows (n = 650) at four locations (n = 210; n = 158; n = 88; n = 194) were assigned within age group by calving date (days postpartum, **DPP)** and body condition score (**BCS**; 1 to 9 scale, 1 = emaciated, and 9 = obese) to one of two treatments (Table 10) during the spring 2004 breeding season (Schafer, 2005). Cows assigned to the MGA Select treatment (MGA Select; n = 327) were fed melengestrol acetate for 14 d, GnRH was injected on d 26, and **PG** was injected on d 33. CO-Synch + CIDR treated cows (CO-Synch + CIDR; n = 323) were fed carrier for 14 d, were injected with GnRH and equipped with an EAZI-BREED™ Controlled Internal Drug Release® insert (**CIDR**) 12 d after carrier removal, and PG was injected and CIDR were removed on d 33. Artificial insemination was performed at 72 h after PG for cows assigned to the MGA Select treatment, and at 66 h after PG administration for cows assigned to the CO-Synch + CIDR treatment (Figure 11). Time of PG administration and AI were recorded for each cow. All cows were injected with GnRH at the time of insemination, and AI was performed by one of three experienced technicians. Three AI sires were used at location 1, and one sire was used at locations 2, 3, and 4. One of the sires used at location 1 was the same sire used at locations 3 and 4. The AI sire and technician were assigned

to cows within each treatment by cow age, calving date, and BCS. Cows were exposed to fertile bulls for natural service 14 d after AI for a 60 day natural service period at Locations 1, 3, and 4 and for a 45 day natural service period at Location 2.

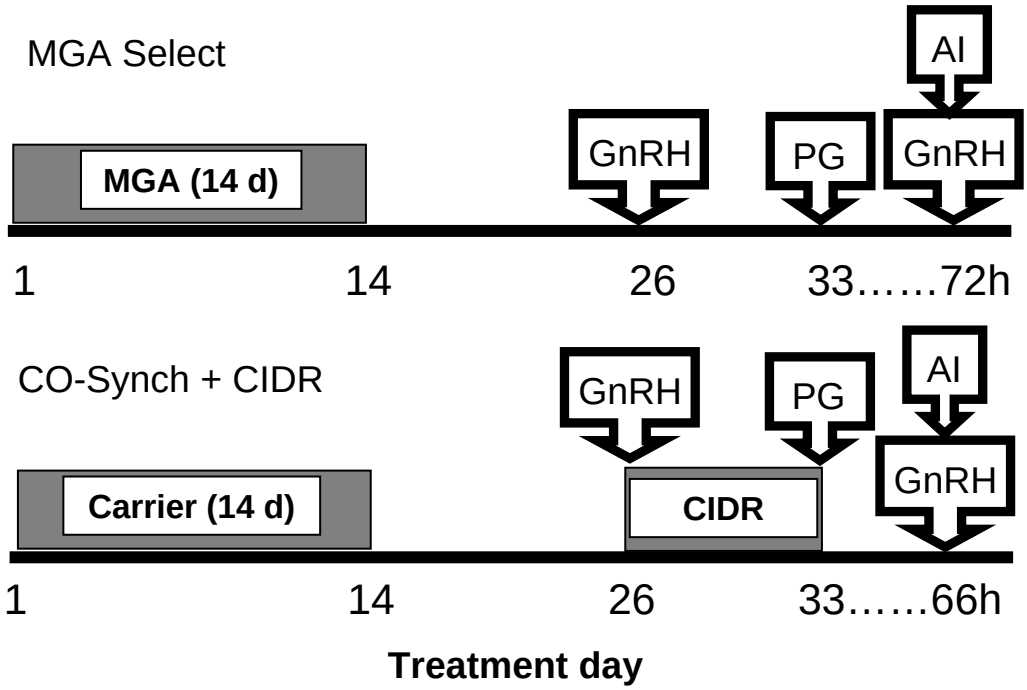

Figure 11. Treatment schedule for cows assigned to the MGA Select and Co-Synch + CIDR protocols. Cows assigned to the MGA Select protocol were fed melengestrol acetate (MGA; 0.5 mg•hd^{-1}•d^{-1}) for 14 d, GnRH was administered 12 d after MGA withdrawal, and PG was administered 7 d after GnRH. Cows were inseminated 72 h after d 33 PG with an injection of GnRH at AI. Cows assigned to the CO-Synch + CIDR protocol were fed carrier for 14 d, on d 26 cows were injected with GnRH and equipped with an EAZI-BREED^{TM} CIDR insert (CIDR), 7 d later CIDRs were removed and PG was administered. Cows were inseminated 66 h after d 33 PG with an injection of GnRH at AI. From Schafer (2005).

The number of cows at each location, age, days postpartum, BCS, and estrous cycling status of cows before the initiation of treatments are shown in Table 10. There were no differences between treatments at the respective locations for age, days postpartum, BCS, or estrous cyclicity status at the initiation of treatment; however, there were differences among locations (Table 10). There was no effect of treatment (P = 0.20), technician (P = 0.63), or sire (P = 0.11) on pregnancy rates resulting from fixed-time AI (Table 11). In addition, pre-treatment estrous cyclicity before the initiation of the MGA Select or CO-Synch + CIDR protocols, did not affect (MGA Select, P = 0.39; CO-Synch + CIDR, P = 0.31; Table 12) pregnancy rates resulting from fixed-time AI. Final pregnancy rates at the end of the breeding season did not differ (P = 0.25) between treatments (Table 11).

Table 10. Number of cows at each location, days postpartum, body condition score, and estrous cycling status for cows before initiation of each treatment (mean ± SE). From Schafer (2005).

Treatment	No.	Age, yr	Time postpartum, d[a]	BCS[b]	Cows with elevated progesterone[c]	
					Proportion	%
Location 1						
MGA Select[d]	106	5.3 ± 0.3	46.4 ± 1.4	5.6 ± 0.06	62/106	58
CO-Synch + CIDR[d]	104	5.4 ± 0.3	45.9 ± 1.4	5.7 ± 0.06	50/104	48
Combined	210	5.3 ± 0.2	46.1 ± 1.0[x]	5.7 ± 0.04[x]	112/210	53[x]
Location 2						
MGA Select[d]	80	5.7 ± 0.3	32.7 ± 1.6	6.1 ± 0.07	29/80	36
CO-Synch + CIDR[d]	78	5.7 ± 0.3	32.4 ± 1.6	6.0 ± 0.07	34/78	44
Combined	158	5.7 ± 0.2	32.5 ± 1.1[y]	6.0 ± 0.05[y]	63/158	40[y]
Location 3						
MGA Select[d]	45	5.5 ± 0.4	44.6 ± 2.1	5.2 ± 0.10	16/45	36
CO-Synch + CIDR[d]	43	5.4 ± 0.4	44.1 ± 2.1	5.3 ± 0.10	15/43	35
Combined	88	5.5 ± 0.3	44.4 ± 1.5[xz]	5.3 ± 0.07[z]	31/88	35[y]
Location 4						
MGA Select[d]	96	5.2 ± 0.3	43.8 ± 1.4	5.3 ± 0.07	78/96	81
CO-Synch + CIDR[d]	98	5.3 ± 0.3	41.7 ± 1.4	5.3 ± 0.07	78/98	80
Combined	194	5.2 ± 0.2	42.8 ± 1.0[z]	5.3 ± 0.05[z]	156/194	80[z]
Combined						
MGA Select	327	5.4 ± 0.2	41.9 ± 0.8	5.5 ± 0.03	185/327	57
Combined						
CO-Synch + CIDR	323	5.4 ± 0.2	41.0 ± 0.8	5.6 ± 0.03	177/323	55

[a]Number of days postpartum at the initiation of melengestrol acetate (MGA) feeding for MGA Select-treated cows and carrier feeding for CO-Synch + CIDR-treated cows.

[b]Body condition scores of cows at the time of the first blood sample before initiation of treatments (1 to 9 scale, where 1 = emaciated, and 9 = obese).

[c]Estrous cyclicity = the percentage of cows with elevated (≥ 0.5 ng/mL) concentrations of progesterone in serum before treatment. Cows were considered to be estrous cycling if progesterone was elevated in either of two blood samples collected 8 and 1 d prior to treatment.

[d]See Figure 11 for description of protocols.

[x,y,z]Means with at least one superscript in common within columns and between locations are not different, $P > 0.05$.

Table 11. Pregnancy rates after fixed-time artificial insemination and at the end of the breeding season. From Schafer (2005).

	Pregnancy rate to fixed-time AI[a]		Pregnancy rate at end of breeding season[b]	
Item	Proportion	%	Proportion	%
Location 1				
MGA Select[c]	70/106	66	99/106	93
CO-Synch + CIDR[c]	67/104	64	99/104	95
Location 2				
MGA Select	53/80	66	77/80	96[d]
CO-Synch + CIDR	56/78	72	76/78	97[d]
Location 3				
MGA Select	26/45	58	42/45	93
CO-Synch + CIDR	29/43	67	42/43	98
Location 4				
MGA Select	52/96	54	87/96	91
CO-Synch + CIDR	62/98	63	91/98	93
Combined				
MGA Select	201/327	61	305/327	93
Combined				
CO-Synch + CIDR	214/323	66	308/323	95

[a]Pregnancy rate to fixed-time AI determined by ultrasound 40 to 45 d after AI.
[b]Pregnancy rate determined 50 to 60 d after the end of the breeding season.
[c]See Figure 11 for a description of protocols.
[d]Pregnancy rate at the after 45 d breeding season.

Table 12. Pregnancy rates after fixed-time artificial insemination based on estrous cyclicity before initiation of treatments. From Schafer (2005).

	MGA Select[a]				CO-Synch + CIDR[a]			
	Estrous cycling[b]		Anestrus[b]		Estrous cycling		Anestrus	
Location	Proportion	%	Proportion	%	Proportion	%	Proportion	%
1	38/62	61	32/44	73	30/50	60	37/54	69
2	20/29	69	33/51	65	25/34	74	31/44	70
3	11/16	69	15/29	52	8/15	53	21/28	75
4	41/78	53	11/18	61	50/78	64	12/20	60
Combined	110/185	59	91/142	64	113/177	64	101/146	69

[a]See Figure 11 for a description of protocols.
[b]See Table 10 for a description of estrous cyclicity.

The MGA Select protocol results in a consistent synchrony of estrus with the peak estrous response typically occurring 72 h after the administration of PG (Patterson et al., 2002; Stegner et al., 2004a). Furthermore, pregnancy rates following administration of the MGA Select protocol and resulting from fixed-time AI have consistently run ≥ 60%, when AI was performed 72 h after PG (Perry et al., 2002; Stegner et al., 2004c; Bader et al., 2005). The pregnancy rates resulting from fixed-time AI reported in this study following treatment with the MGA Select estrus synchronization protocol are consistent with other published data when insemination was performed 72 h after PG (Perry et al., 2002; Stegner et al., 2004c; Bader et al., 2005).

The CO-Synch + CIDR protocol with fixed-time AI performed 60 h after PG resulted in comparable pregnancy rates when compared to CIDR-based protocols that involve estrus detection and AI up to 84 h after PG followed by fixed-time insemination of non-responders at 84 h (Larson et al., 2004). Other studies reported pregnancy rates to the CO-Synch + CIDR estrus synchronization protocol were optimized when insemination was performed at 66 h after PG compared to AI performed at 48 or 54 h (Bremer et al., 2004). Consideration of these various studies led to the decision to inseminate cows at 66 h following administration of the CO-Synch + CIDR protocol in the study by Schafer (2005). The results reported by Schafer (2005) are comparable to the study by Bremer et al. (2004), and support the concept that there is a critical window of time over which insemination should be performed following administration of the CO-Synch + CIDR protocol.

Successful application of these protocols requires careful consideration of the advantages and disadvantages that accompany their administration. Based on these data both protocols appear to work effectively in mixed-populations of estrous cycling and

anestrous cows, despite differences recently reported by Perry et al. (2004). The fertility after treatment was shown to produce pregnancy rates resulting from fixed-time AI consistently ranging from 54 to 72%. The CO-Synch + CIDR protocol may have broader application in comparison to the MGA Select protocol due to shorter treatment duration (< 10 d vs. 36 d), especially in herds with more widespread calving periods. Successful results with either protocol require proper application of each step of the respective treatment. The consistent results that were obtained with the CO-Synch + CIDR protocol may be due to more precise control of progestin treatment among cows that received CIDR inserts compared to more variable MGA intake patterns among cows assigned to the MGA Select protocol.

These results indicate that estrus synchronization with the MGA Select and CO-Synch + CIDR protocols produce comparable pregnancy rates to fixed-time AI when inseminations were performed at 72 and 66 h after PG, respectively. The results reported here present beef producers a choice and means for expediting genetic improvement and reproductive management.

MANAGEMENT CONSIDERATIONS RELATED TO ESTRUS SYNCHRONIZATION AND FIXED-TIME AI

Stegner et al. (2004b) discussed the advantages and disadvantages related to practical application and successful administration of the MGA Select and 7-11 Synch protocols. The advantages shown here and reported in other studies include the following: 1) MGA is economical to use (approximately $0.02 per animal daily to feed); 2) each protocol works effectively in mixed populations of beef cows that were estrous cycling or anestrus at the time treatments are imposed; and 3) pregnancy rates resulting from insemination performed on the basis of detected estrus or at predetermined fixed times are comparable and highly acceptable.

Stegner et al. (2004b) noted, however, that the feasibility of feeding MGA to cattle on pasture is limiting in some production systems and is viewed as a disadvantage. Furthermore, the MGA Select protocol requires feeding and management of cows for 33 d, whereas the 7-11 Synch protocol involves an 18 d period. Conversely, the 7-11 Synch protocol requires that animals be handled four times, including AI, compared to the MGA Select protocol, which requires three handlings.

The calving distribution is illustrated in Figure 12 for cows that were assigned to the MGA Select and 7-11 Synch protocols and inseminated on the basis of detected estrus from the study by Stegner et al. (2004b). A high proportion of calves were delivered within the first 15 and cumulative 30 days of the calving season for each protocol, with no differences between treatments. The cumulative number of cows that calved within the first 30 days of the calving period was 93% and 89% for the MGA Select and 7-11 Synch groups, respectively. The calving distribution of cows assigned to each of these protocols must be carefully considered. One of the obvious benefits of estrus synchronization is a shortened calving season that results in more uniform calves at weaning (Dziuk and Bellows, 1983). Reduced length of the calving season translates into a greater number of days for postpartum recovery of the cow to occur prior to the subsequent breeding season. Herd owners must be aware of the risks associated with a

concentrated calving period, including inclement weather or disease outbreaks, which separately or together may result in a decrease in the number of calves weaned.

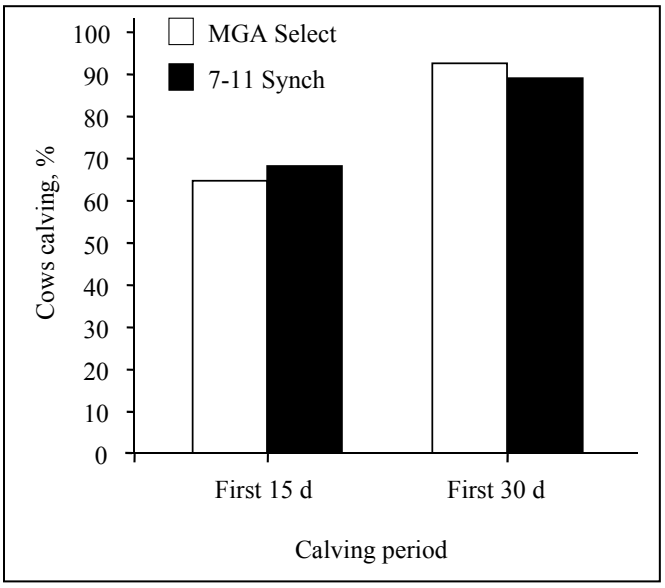

Figure 12. Cumulative calving distribution during the first 15 and 30 days of the calving season for MGA Select and 7-11 Synch- treated cows. [93% of MGA Select and 89% of 7-11 Synch treated cows calved within 30 days from the onset of the calving period]. From Stegner et al. (2004b).

These data support the use of estrus synchronization not only as a means of facilitating more rapid genetic improvement of beef herds, but perhaps, more importantly, as a powerful reproductive management tool. Profitability may be increased by reducing the extent to which labor is required during the calving period, and increasing the pounds of calf weaned that result from a more concentrated calving distribution and a resulting increase in the age of calves at weaning.

More recently, calving dates for cows that conceived on the same day to fixed-time AI were recorded to address concerns that pertain to the subsequent calving period (Bader et al., 2005). Calf birth dates were recorded for cows that conceived to fixed-time AI (Figure 13) at each location involved in the study by Bader et al. (2005). The resulting calving distribution for cows that conceived to the respective sires at each of the locations in the two treatments is illustrated in Figure 13. Calving distribution patterns differed among individual sires (Table 13; $P < 0.05$). Calving distribution among cows that conceived to fixed-time AI for Location 1 (sires A and B) was 21 and 16 days, respectively. Distributions for Location 2 (sires C and D) were 16 and 20 days, respectively. The calving distribution among cows at location 3 (sire E), was 18 days. Sire B at Location 1 and sire E at Location 3 was the same sire. Cows that conceived on the same day gave birth to calves over a 16 to 21 day period, dependent upon the respective sire.

Calving distribution patterns for cows involved in the study by Schafer (2005) are illustrated in Figure 14. These data also represent calving profiles among cows that became pregnant on the same day using semen from single sires as indicated by the respective panels. These distributions indicate that successful use of fixed-time AI will not result in an overwhelming number of cows calving on the same day(s). This furthermore suggests that current management practices will not need to be greatly

altered to accommodate the early portion of the calving season. Conversely, these data demonstrate that successful application of estrus synchronization protocols that facilitate fixed-time AI support improvements in whole-herd reproductive management and expanded use of improved genetics.

Table 13. Comparison of gestation lengths (Mean ± SE) among AI sires and locations.

Location	Sire	Gestation length, days	Range, days
1	A	283.5 ± 0.5	272 - 292
	B[a]	282.1 ± 0.5	275 - 290
2	C	282.9± 0.8	274 - 289
	D	284.1 ± 0.6	275 - 294
3	E[a]	282.0 ± 0.5	274 - 291

[a]Sire B at location 1 and sire E at location 3 are the same sire.
From Bader et al. (2005).

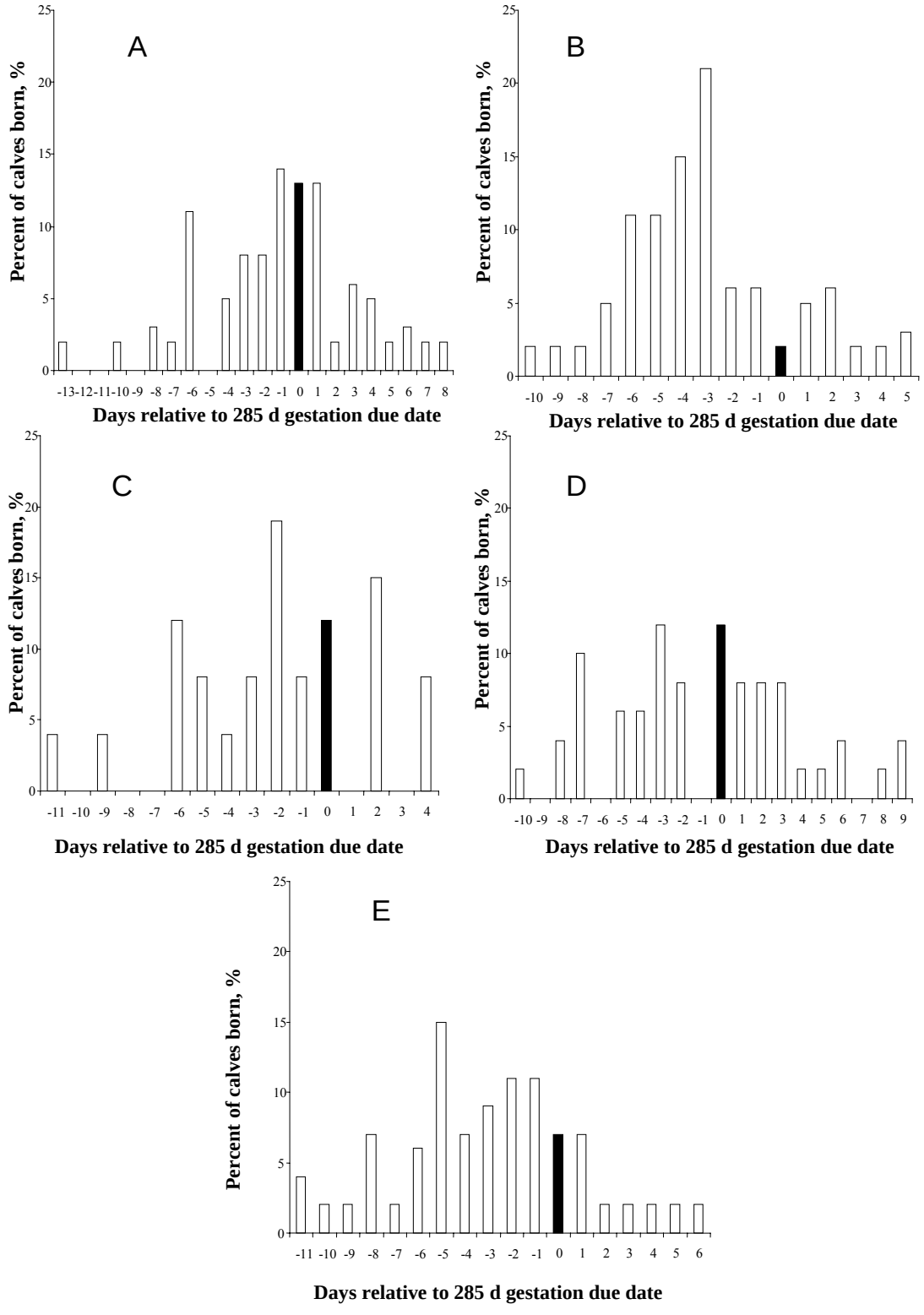

Figure 13. Calving distribution patterns at the respective locations for cows that conceived to fixed-time AI Calving dates among cows that conceived on the same day to the respective sires (A, B, C, D, and E) were 21, 16, 16, 20, and 18 days. Sire B at Location 1 and sire E at Location 3 were the same sire. The shaded bar in each graph represents an anticipated 285 day gestation due date. From Bader et al. (2005).

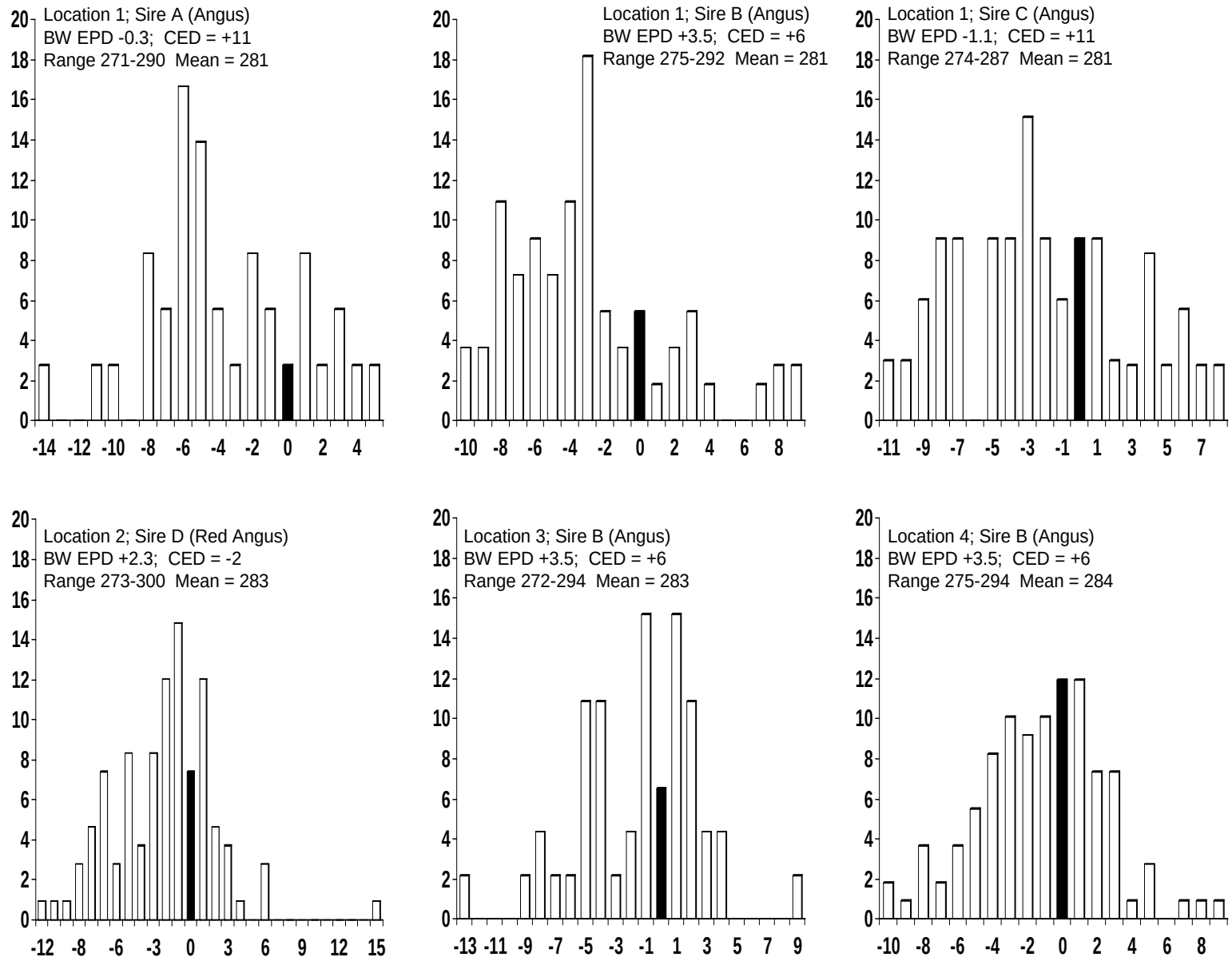

Figure 14. Calving distributions recorded for cows that conceived to fixed-time AI (Schafer, 2005). The shaded bar in each graph represents an anticipated 285 day gestation due date.

CONSIDER THE IMPACT OF ESTRUS SYNCHRONIZATION ON CALVING DISTRIBUTION

Economic considerations related to use of estrus synchronization and choice of the various protocols to use in beef heifers and cows was reviewed by Johnson and Jones (2004). Hughes (2005) reported that opportunities to increase profits for cow-calf operations lie in managing females from the later calving intervals forward toward the first and second 21-day calving intervals. Hughes (2005) reports that added pounds are the economic reward to tightening up the calving interval. The CHAPS benchmark values utilize IRM-SPA guidelines for operating high production herds. These guidelines suggest that 61% of the calves within a herd should be born by day 21 of the calving period, 85% by day 42, and 94% by day 63. Hughes (2005) goes on to say that today's high market prices are generating big economic rewards to intensified management, but more specifically "management as usual" may be what is amiss for many cow calf producers.

Figure 15 illustrates the cumulative calving percentages for the University of Missouri Thompson farm over a 10-year period. The graph compares the percentages of calves born during years when only natural service was used, followed by estrus synchronization and AI performed on the basis of observed heat, and finally fixed-time AI. The graph illustrates the respective distributions on the basis of days in the calving season. Notice the increased percentage of calves born early in the calving period during years when AI was performed on the basis of observed heat or at predetermined fixed times in comparison to years in which only natural service was practiced.

Figure 16 illustrates the combined calving data for 3 of the 4 locations in the study by Schafer (2005). Data from the fourth location was not included in the summary since cows that failed to conceive to AI were sold prior to the calving period. It is interesting to note that in comparison to the recommendation by Hughes (2005), 64% of the cows in this study had calved by day 15, 70% by day 21, 77% by day 30, and 91% by day 42. The economic reward from improvements in calf weaning weight that result from an increase in calf age at weaning, in many cases may offset the cost of implementing estrus synchronization in beef herds.

Finally, Figure 17 illustrates the calving profile for cows at the University of Missouri Forage Systems Research Center in Linnueus, MO, over a two year period. This herd maintains a 45-day breeding season, and until the spring of 2004, estrus synchronization and AI were not utilized. Figure 17 illustrates the calving profile of cows that calved during the spring of 2004 as a result of natural service during the 2003 breeding season. Figure 17 also illustrates the calving profile for cows that calved during the spring of 2005 as a result of fixed time AI performed during the 2004 breeding season (Schafer, 2005). This herd has been intensively managed over the years to breed successfully in a 45 day period with natural service. Notice, however, the increased percentage of cows that calved early in the calving period as a result of fixed-time AI performed during the previous year's breeding season. Estrus synchronization at this location in one year resulted in an increase of 7 days postpartum among cows at the start of the breeding period, which translates into an increase in calf age at weaning of seven calf days.

These figures (Figures 15, 16, 17) collectively demonstrate that estrus synchronization can be used effectively to influence calving distribution patterns during the subsequent calving period, which in turn impacts the economics of herds at weaning time.

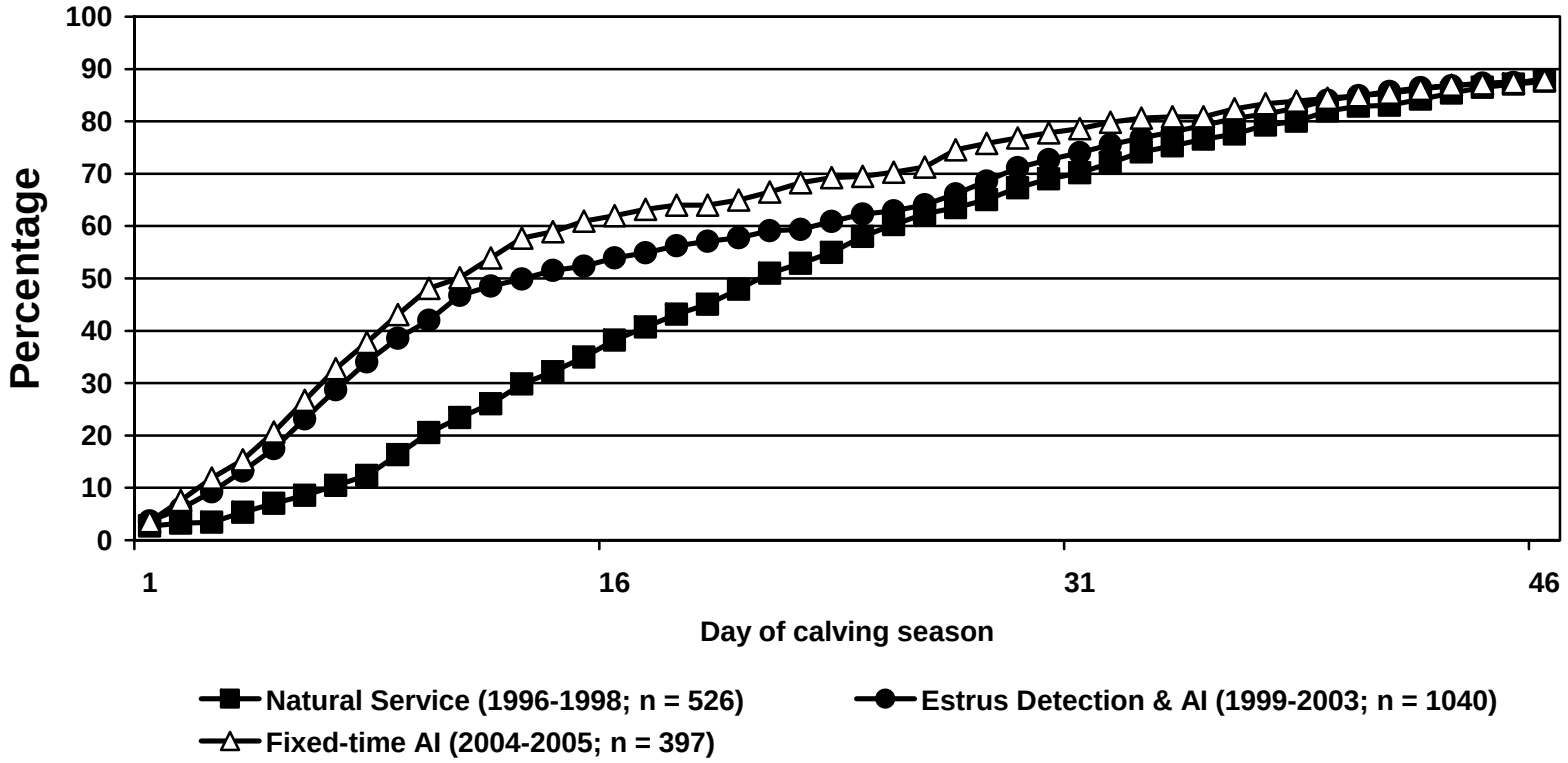

Figure 15. Cumulative calf crop for cows at the University of Missouri Thompson Farm combining years involving natural service, estrus synchronization and AI performed on the basis of observed heat, and fixed-time AI (Schafer and Patterson,unpublished data).

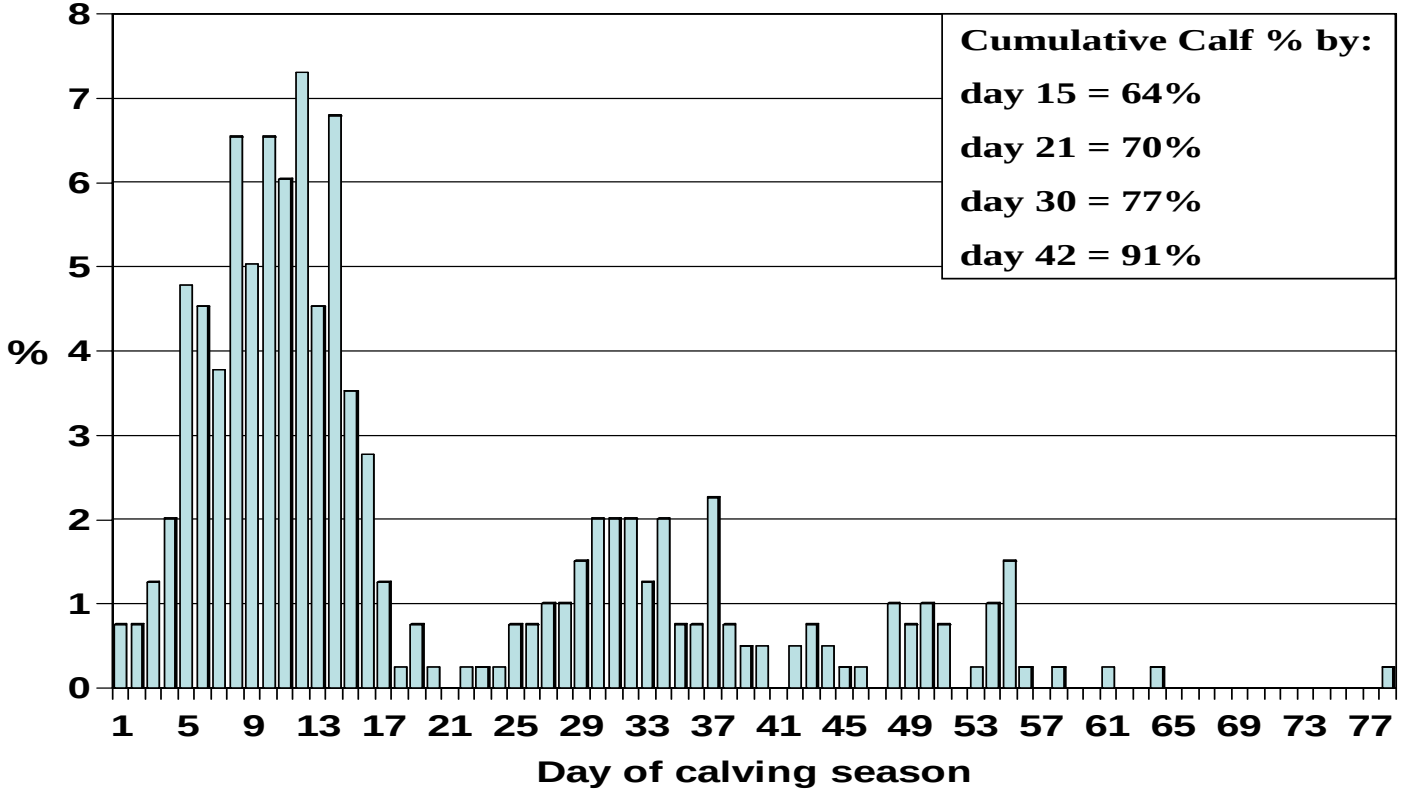

Figure 16. Calving distributions combined for 3 of the 4 locations in the study by Schafer (2005).

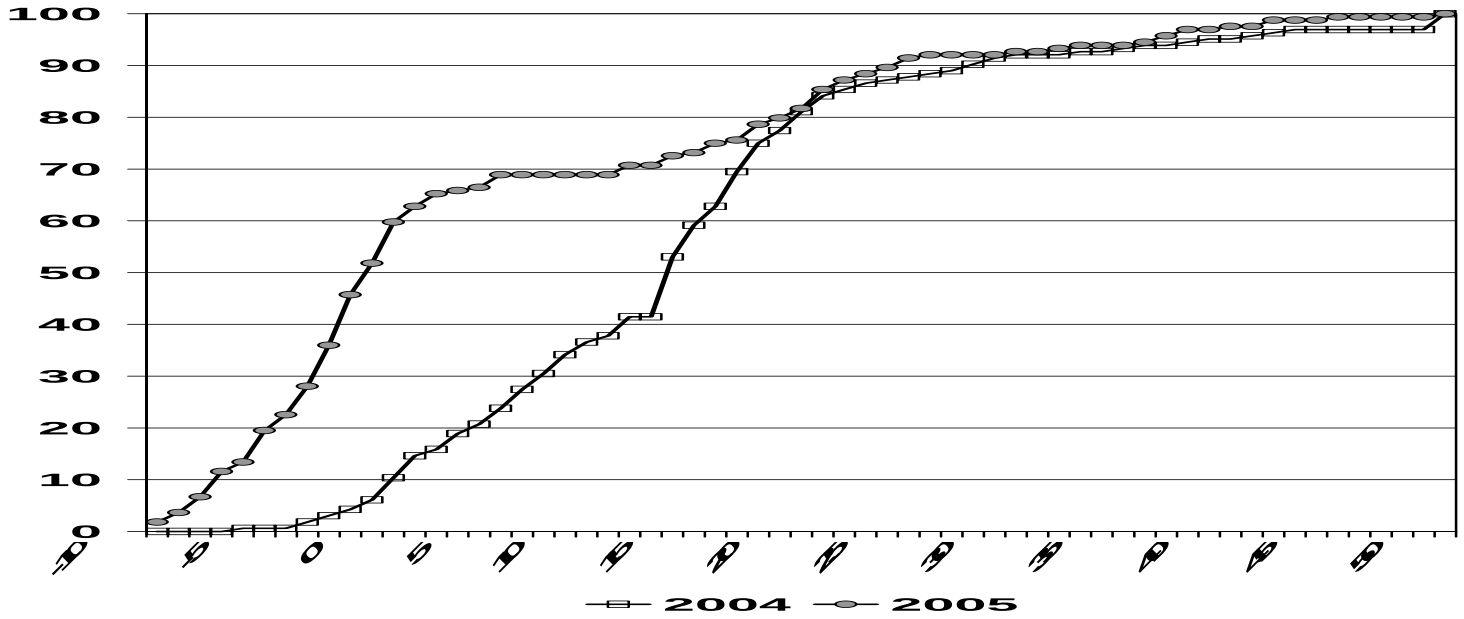

Figure 17. Calving profiles for cows at the University of Missouri Forage Systems Research Center in Linnueus, MO, over a two year period. This herd maintains a 45 day breeding season and until the spring of 2004 estrus synchronization and AI had not been utilized. The figure illustrates the calving profiles of cows that calved during the spring of 2004 as a result of natural service during the 2003 breeding season, and calving profiles for cows that calved during the spring of 2005 as a result of fixed time AI performed during the 2004 breeding season (Schafer, 2005).

SUMMARY AND CONCLUSIONS

Expanded use of AI and/or adoption of emerging reproductive technologies for beef cows and heifers require precise methods of estrous cycle control. Effective control of the estrous cycle requires the synchronization of both luteal and follicular functions. Efforts to develop more effective estrus synchronization protocols have focused 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 cows treated with the preceding protocols is that 5 to 15% of estrous cycling cows show estrus on or before PG injection. New protocols for inducing and synchronizing a fertile estrus in postpartum beef cows and replacement beef heifers in which progestins are used sequentially with the GnRH-PG protocol provide new opportunities for beef producers to synchronize estrus and ovulation and facilitate fixed-time AI.

Table 14 provides a summary of the various estrus synchronization protocols for use in postpartum beef cows. The table includes estrous 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 14 provide evidence to support the sequential approach to estrus synchronization in postpartum beef cows we describe.

These data suggest that new methods of inducing and synchronizing estrus for postpartum beef cows and replacement beef heifers now create the opportunity to significantly expand the use of AI in the U.S. cowherd.

Table 14. Comparison of estrous response and fertility in postpartum beef cows after treatment with various estrus synchronization protocols.

Treatment	Estrous response		Synchronized pregnancy rate	
AI based on detected estrus				
2 shot PG	241/422	57%	147/422	35%
Select Synch	353/528	67%	237/528	45%
MGA-PG 14-17 d	305/408	75%	220/408	54%
MGA-2 shot PG	327/348	93%	243/348	70%
MGA-PG 14-19 d	161/206	78%	130/206	63%
MGA® Select	275/313	88%	195/313	62%
7-11 Synch	142/155	92%	101/155	65%
AI performed at predetermined fixed times with no estrus detection				
MGA® Select	Fixed-time AI @ 72 hr		482/763	63%
7-11 Synch	Fixed-time AI @ 60 hr		446/728	61%
CO-Synch + CIDR	Fixed-time AI @ 66 hr		591/912	65%

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NOTES

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REVIEW OF ESTRUS SYNCHRONIZATION SYSTEMS:CIDR

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Introduction

The CIDR is an intravaginal progesterone insert, used in conjunction with other hormones to synchronize estrous in beef and dairy cows and heifers. The CIDR was developed in New Zealand and has been used for several years to advance the first pubertal estrus in heifers and the first postpartum estrus in cows. The CIDR is a "T" shaped device with flexible wings that collapse to form a rod that can be inserted into the vagina with an applicator. On the end opposite to the wings of the insert a tail is attached to facilitate removal with ease. The backbone of the CIDR is a nylon spine covered by progesterone (1.38g) impregnated silicone skin. Upon insertion blood progesterone concentrations rise rapidly, with maximal concentrations reached within an hour after insertion. Progesterone concentrations are maintained at a relatively constant level during the seven days the insert is in the vagina. Upon removal of the insert, progesterone concentrations are quickly eliminated.

Retention rate of the CIDR during a seven-day period exceeds 97%. In some cases, vaginal irritation occurs resulting in clear, cloudy or yellow mucus when the CIDR is removed. Cases of mucus are normal and does not have an impact on effectiveness of the CIDR. Caution should be taken when handling CIDRs. Individuals handling 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 treated females. The inserts are developed for a one-time use only. Multiple use may increase the incidence of vaginal infections.

CIDR/$PGF_{2\alpha}$ Protocols for Cows

During the seven days of CIDR insertion, progesterone diffusion from the CIDR does not affect spontaneous luteolysis. Assuming all 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 indicates that only about 90% of corpora lutea in 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 beings 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.

Table 1. Fertility rates in suckled beef cows treated with estrous synchronization protocols containing progestins.

Reference and treatment description	No. of cows	Conception rate[a], %	Pregnancy rate[b], %
Stevenson et al., 2000			
Exp. 1			
Select Synch	289	115/175 (66)	115/289 (38)
Select Synch + Norgestomet	289	123/208 (59)	123/289 (42)
2 × $PGF_{2\alpha}$	294	86/142 (61)	86/294 (28)
Dejarnette et al., 2001			
Exp. 2			
Select Synch	77	40/60 (67)	40/77 (52)
Select Synch + MGA from d -7 to -1	73	43/61 (72)	43/73 (60)
Lamb et al., 2001			
CO-Synch	287	-	138/287 (48)
CO-Synch + CIDR from d –7 to 0	273	-	160/273 (59)
Larson et al.., 2004a			
CIDR/$PGF_{2\alpha}$ *(PG on d 0) - anestrous*	147	-	74/147 (50)
CIDR/$PGF_{2\alpha}$ *(PG on d 0) - cyclic*	296	-	159/296 (54)
CO-Synch - anestrous	156	-	59/156 (38)
CO-Synch - cyclic	330	-	145/330 (44)
CO-Synch + CIDR - anestrous	180	-	85/180 (47)
CO-Synch + CIDR - cyclic	294	-	169/294 (57)
Hybrid Synch - anestrous	143	-	60/143 (42)
Hybrid Synch - cyclic	308	-	182/308 (59)
Hybrid Synch*+CIDR - anestrous*	136	-	72/136 (53)
Hybrid Synch*+CIDR - cyclic*	306	-	180/306 (59)
Lucy et al., 2001			
Control *- anestrous*	151	6/16 (38)	6/151 (4)
Control *- cyclic*	134	15/26 (58)	15/134 (11)
$PGF_{2\alpha}$ *- anestrous*	154	17/30 (57)	17/154 (11)
$PGF_{2\alpha}$ *- cyclic*	129	44/63 (70)	44/129 (34)
CIDR/$PGF_{2\alpha}$ *(PG on d –1) - anestrous*	141	36/63 (57)	36/141 (26)
CIDR/$PGF_{2\alpha}$ *(PG on d –1) - cyclic*	140	64/101 (63)	64/140 (46)

[a] Percentage of cows pregnant exposed to AI.
[b] Percentage of cows pregnant of all cows treated.

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 cyclicity. When suckled beef cows were assigned randomly in replicates to one of three groups (Lucy et al., 2001): 1) untreated controls, 2) a single intramuscular (IM) injection of 25 mg $PGF_{2\alpha}$ ($PGF_{2\alpha}$ alone), or 3) administration of a CIDR insert for 7 d with an IM administration of $PGF_{2\alpha}$ on day 6 of the 7 d CIDR insert administration period (CIDR + $PGF_{2\alpha}$) no differences were detected between the CIDR + $PGF_{2\alpha}$ treatment group and either the $PGF_{2\alpha}$ alone or control groups for first-service CR

for either the first 3 d of AI or the entire 31 d of AI. More cows were pregnant after either 3 d or 7 d of AI in the CIDR + $PGF_{2\alpha}$ group than in either the $PGF_{2\alpha}$ alone or the control group. No differences were detected in PR to first services during the 31 d AI period between the CIDR + $PGF_{2\alpha}$ and either the $PGF_{2\alpha}$ alone or the control group. Therefore, insertion of the CIDR increased the synchronization rates within the first 3 d following $PGF_{2\alpha}$, resulting in enhanced pregnancy rates. A drawback of the current protocol is that $PGF_{2\alpha}$ was administered on d 6 after CIDR insertion (a day before CIDR removal). For beef producers this tends to be impractical, because the cows need to be handled a minimum of four times including an AI. Therefore, a more practical modification of this protocol is to inject $PGF_{2\alpha}$ the on the day of CIDR removal.

Advances in Protocols Using the CIDR for Cows

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. Inclusion of the CIDR in the CO-Synch procedure appears to be the most researched alternative method for synchronizing beef cows. We (Lamb et al., 2001) published data in which the CIDR was included in the CO-Synch estrous synchronization procedure (Table 1). The CIDR was inserted at the time of the first injection of GnRH and removed at the time of the injection of $PGF_{2\alpha}$. 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 $PGF_{2\alpha}$ treatment, which may explain why there was not a positive effect at each location. Along with parity, days postpartum, calf removal, and cow body condition (Table 2) 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.

In a more recent study involving 14 locations in 7 states we (Larson et al., 2004) evaluated both fixed-time AI protocols and detection of estrus protocols with a clean-up AI. These protocols were compared to GnRH/ $PGF_{2\alpha}$ protocols. Although the location accounted for the greatest variation in overall pregnancy rates the Hybrid- Synch + CIDR protocol (Figure 1) was the protocol that most consistently yielded the greatest pregnancy rates within each location. However, the CO-Synch protocol (Figure 1) was an effective Fixed-time AI protocol that yielded pregnancy rates of 54%.

Table 2. Pregnancy rates in suckled beef cows after treatment with Cosynch or Cosynch+CIDR (Lamb et al., 2001)

Item	Treatment[a]		
	Cosynch	Cosynch+P	Overall
	---------- no. (%) ----------		
Body condition[b]			
≤ 4.5	12/40 (30)	11/36 (31)	23/76[x] (30)
4.5 to 5.5	30/74 (41)	40/80 (50)	70/154[y] (45)
≥ 5.5	19/32 (59)	11/13 (85)	31/45[z] (69)
Days postpartum			
≤ 50	23/60 (38)	27/58 (47)	50/118[x] (42)
51-60	25/62 (47)	36/54 (67)	61/116[y] (53)
61-70	28/49 (62)	25/44 (57)	53/93[y] (57)
71-80	18/41 (44)	30/45 (67)	48/86[y] (56)
> 80	44/75 (59)	42/72 (58)	86/147[y] (59)
Parity[c]			
Multiparous	61/138 (44)	79/132 (60)	140/270 (52)
Primiparous	25/50 (50)	20/45 (44)	45/95 (47)

[a] See experimental design for treatments in Figure 1.
[b] Body condition scores from IL and MN only.
[c] Parity data from KS and MN only.
[xyz]Percentages within an item and column lacking a common superscript letter differ (P < .05).

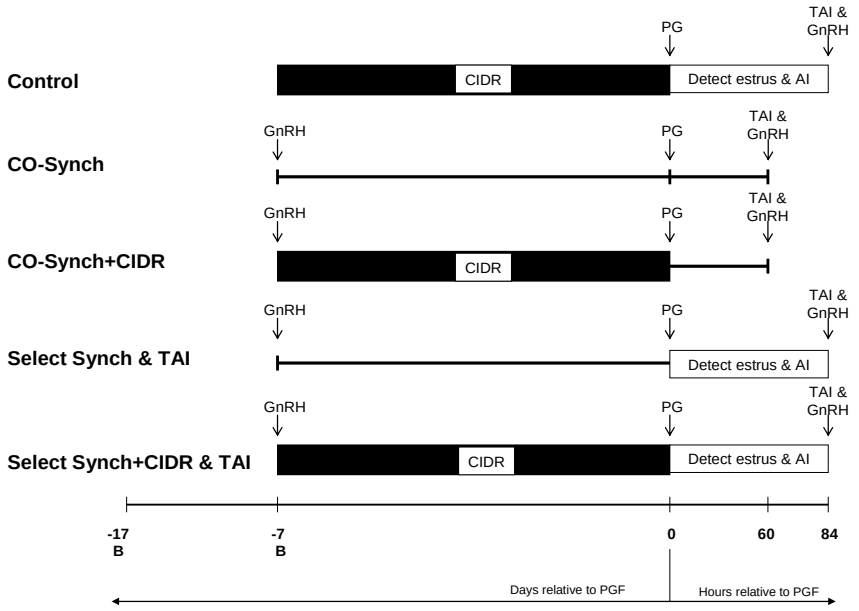

Figure 1. Estrous synchronization protocols using a CIDR (Larson et al., 2005).

Interestingly, the distribution of estrus among the Control, Select Synch & TAI, and the Select Synch + CIDR & TAI protocols was similar (Figure 2) as was the average interval from $PGF_{2\alpha}$ to estrus or AI was similar to among all three treatments (Figure 3). Since the estrus response was greater in the Hybrid Synch+CIDR protocol overall pregnancy rates were greater.

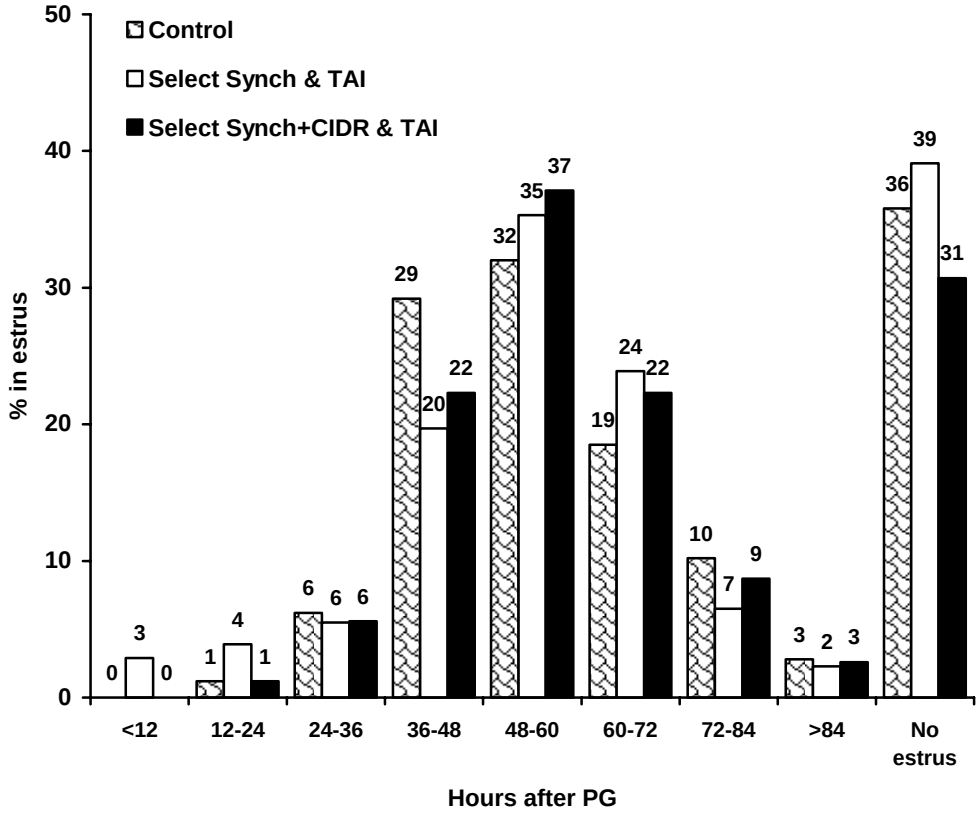

Figure 2. Percentage of cows treated with Control, Select Synch & TAI, Select Synch + CIDR & TAI that were observed in estrus, separated by hours from PG injection to AI (Larson et al., 2004a).

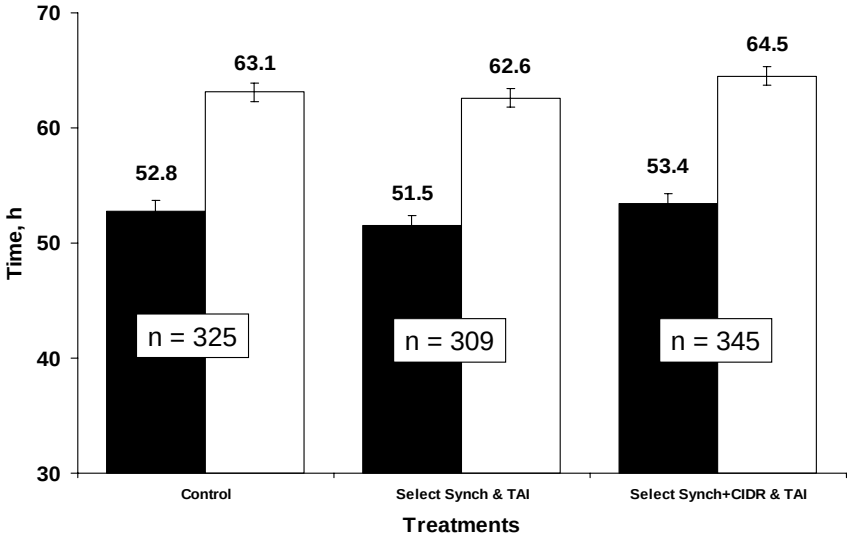

Figure 3. Time from PG injection to estrus (black bar) and time from PG injection to AI (white bar) for those cows exhibiting estrus in Control, Select Synch & TAI, Select Synch + CIDR & TAI treatments (Larson et al., 2005).

Calving data during the subsequent calving season was also assessed. Of the 1,752 calvings, 994 calves (56.7%) were the result of AI after estrus synchronization. Average duration of gestation among all AI sired calves was 281.9 ± 5.2 d (× ± SD), and the range was 258 to 296 d. Duration of gestation was similar among treatments, but a location effect ($P < 0.0001$) was detected, which may have included breed, sire and management differences. Period of gestation was greater ($P < 0.001$) for male (282.9 ± 0.2 d) than female calves (280.9 ± 0.2 d), and single calves were carried 3.0 d longer ($P < 0.05$) than multiple calves.

For those cows from which calving data was recorded, the average interval from the $PGF_{2\alpha}$ injection (Day 0 of the study) to calving among all cows was 297.3 ± 17.7 d (× ± SD) with a range of 258 to 373 d (Figure 4). Although average calving interval was similar among treatments, a ($P < 0.001$) location effect was detected.

At calving, gender was recorded in 1,490 calves, with 770 (52.2%) male calves compared with 704 females. In addition, 15 sets of twins and a single set of triplets were recorded. Gender ratio of calves that conceived to AI at estrus synchronization favored ($P < 0.01$) bulls (i.e., 52.7% of 841 calves born were male). Similarly, of the 635 calves that conceived to clean-up bulls, 51.7% were male. No difference was detected in gender ratio for AI compared with natural-sired calves. Multiple birth rate for AI-sired calves [1.1% (9 of 850)] was similar to that of calves sired by clean-up bulls [0.9% (6 of 641)].

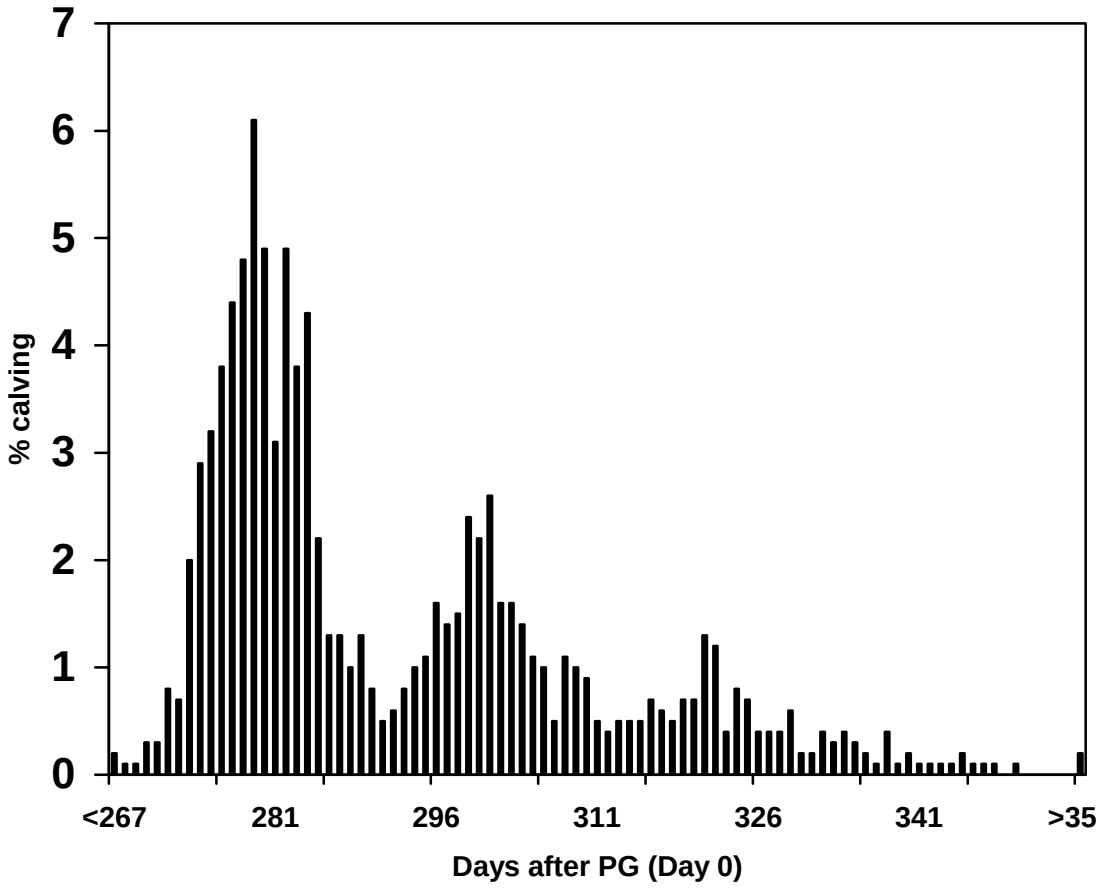

Figure 4. Distribution of calving during the subsequent calving season after synchronization of estrous with GnRH, $PGF_{2\alpha}$, and (or) a CIDR.

CIDR/$PGF_{2\alpha}$ Protocols for Heifers

As with cows, beef heifers have 21-day estrous cycles and respond to the CIDR in a similar fashion to cows, resulting in a majority of heifers that should be synchronized using the FDA approved CIDR/$PGF_{2\alpha}$ protocol. Heifers tend to be an easier population of females to synchronize for estrus, because they are not nursing calves, tend to express estrus well, and most of the heifers usually are cycling, and can be maintained in areas where they can be fed allowing them to respond 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.

First attempts focused at synchronizing estrus in heifers with a CIDR and $PGF_{2\alpha}$. The study by Lucy et al., (2001; Table 2) demonstrates the pregnancy rates of heifers synchronized with the FDA approved CIDR/ $PGF_{2\alpha}$ protocol. As in cows, the CIDR/$PGF_{2\alpha}$ protocol yielded greater pregnancy rates in heifers than for heifers that were untreated or for heifers treated with $PGF_{2\alpha}$ alone. Therefore, insertion of the CIDR increased the synchronization rates within the first 3 d following $PGF_{2\alpha}$, resulting in enhanced pregnancy rates. Again, the drawback of the current protocol is that $PGF_{2\alpha}$ was administered on d 6 after CIDR insertion, which requires an additional day of handling the heifers. Therefore, consideration should be to inject $PGF_{2\alpha}$ the on the day of CIDR removal.

The CIDR + $PGF_{2\alpha}$ treatment reduced the interval to first estrus (2 d) compared with either the control (15 d) or $PGF_{2\alpha}$ alone (16 d) treatments (Table 3). Similarly, for heifers that were prepubertal when the study was initiated the CIDR + $PGF_{2\alpha}$ shortened the interval to first estrus (14 d) compared to control (27 d) and $PGF_{2\alpha}$ alone (31 d). The CIDR + $PGF_{2\alpha}$ treatment improved the synchrony of estrus compared with the $PGF_{2\alpha}$ alone, with 60% vs. 25%, of heifers in estrus over 3 d after CIDR inserts were removed.

Table 3. Interval to estrus, synchrony of estrus and fertility of beef heifers following treatment with $PGF_{2\alpha}$ or CIDR and an injection of $PGF_{2\alpha}$ (Lucy et al., 2001).

Criterion	Untreated controls	$PGF_{2\alpha}$[1]	CIDR/$PGF_{2\alpha}$[2]
Interval[3] to estrus, d (n)			
All heifers	15*	16*	2
Anestrous heifers[5]	27**	31**	14
Estrus d 1-3, %	12**	25**	60
FSCR[4], % (n)			
D 1-3	57	52	60
D 1-31	58	52	58
FSPR[5], % (n)			
D 1-3	7**	14**	36
D 1-7	14**	18**	38
D 1-31	42	36*	47

[1]25 mg prostaglandin $F_{2\alpha}$

[2]CIDR insert administered intravaginally for 7 days with $PGF_{2\alpha}$ administered on day 6.

[3] Median interval in days from removal of CIDR inserts.

[4] First-service conception rate (number of heifers).

[5] First-service pregnancy rate (number of heifers).

* Different from CIDR/$PGF_{2\alpha}$, $P < 0.05$.

** Different from CIDR/$PGF_{2\alpha}$, $P \leq 0.01$.

Advances in Protocols Using the CIDR for Heifers

Although excellent pregnancy rates can be achieved with the MGA/$PGF_{2\alpha}$ protocol and acceptable pregnancy rates can be achieved with the CIDR/$PGF_{2\alpha}$ protocol, no system short duration system has managed to successfully synchronize estrus in replacement beef heifers that consistently yields pregnancy rates that match the MGA/$PGF_{2\alpha}$ protocol. In addition, there has not been a no reliable fixed-time AI protocol exists for synchronizing estrus in beef heifers. Therefore, in a more recent study involving 12 locations in 8 states we (Larson et al., 2004b) focused on developing a study to determine whether: 1) a TAI protocol could yield fertility similar to a protocol requiring detection of estrus; and 2) an injection of GnRH at CIDR insertion enhances pregnancy rates.

To evaluate our objectives, estrus in beef heifers was synchronized and artificial insemination occurred after four treatments: 1) CIDR/$PGF_{2\alpha}$; 2) Hybrid Synch+CIDR; 3) CO-Synch+CIDR; and 4) CIDR/$PGF_{2\alpha}$ + TAI. The percentage of heifers cycling at the initiation of estrous synchronization was 91.0%. Percentages of cycling heifers among locations ranged from 78 to 100%. Overall pregnancy rates were at days 30 to 35 after AI ranged from 38 to 74%. Although no differences in pregnancy rates were detected among treatments, heifers that were inseminated in the estrus-detection treatments had greater pregnancy rates than heifers in the fixed-time AI treatments (56 vs. 51%, respectively). However, the the CO-Synch+CIDR treatment provides a reliable fixed-time AI protocol for beef producers (Figure 5).

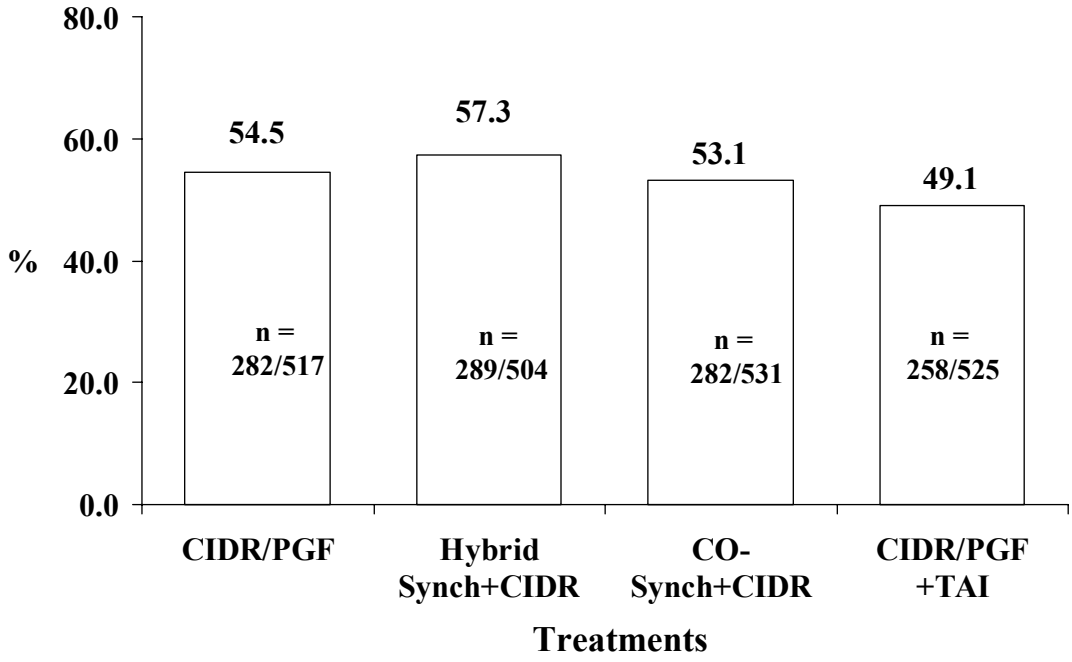

Figure 5. First service pregnancy rates in heifers after receiving one of four CIDR treatments (Larson et al., 2004).

For the two estrus-detection protocols, CIDR/$PGF_{2\alpha}$ and Hybrid Synch+CIDR, pregnancy rates for heifers detected in estrus before 84 hr were 44.6 and 45.0%, respectively. Therefore, the clean-up TAI at 84 hr enhanced pregnancy rates by 9.9 and 12.3 percentage points for CIDR/$PGF_{2\alpha}$ and Hybrid Synch+CIDR protocols, respectively. These results indicate that TAI after a period of estrus detection enhances the potential for improving pregnancy rates to exceed those of estrus detection alone (Figure 6).

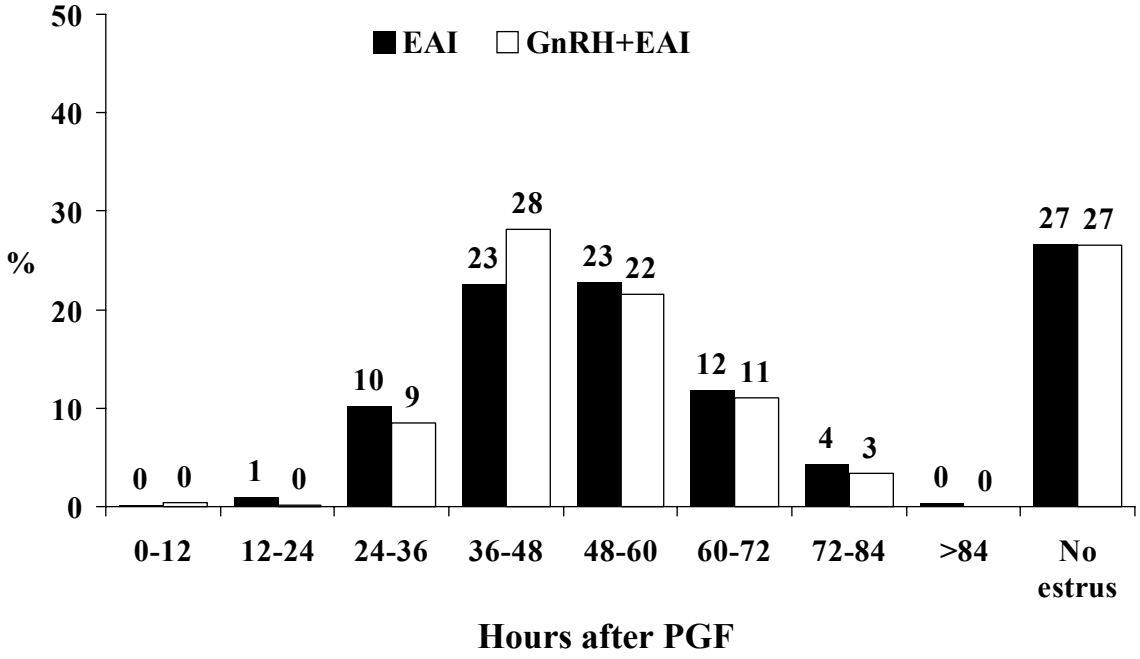

Figure 6. Percentage of heifers treated with CIDR/ $PGF_{2\alpha}$, or Hybrid Synch+CIDR that were observed in estrus, separated by hours from PG injection to AI (Larson et al., 2004b).

The time from PG injection to detection of estrus and AI for those heifers exhibiting estrus was similar among CIDR/$PGF_{2\alpha}$ (49.9 and 61.7 hr, respectively) and Hybrid Synch+CIDR (49.8 and 61.3 hr, respectively). These results demonstrate that estrus in heifers can be synchronized effectively with GnRH, PG, and a CIDR. The hybrid Synch+CIDR treatment most frequently produced the greatest pregnancy rates and provided a reliable alternative to an MGA/$PGF_{2\alpha}$ protocol.

Summary

To achieve optimal pregnancy rates with CIDR based estrous 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 and replacement beef heifers with a CIDR and GnRH will yield industry accepted pregnancy rates. Results of the most recent CIDR based studies indicate that for a fixed-timed AI

protocol the CO-Synch+CIDR protocol yields the most impressive pregnancy rates for a fixed-time AI protocol, whereas the Hybrid Synch+CIDR treatment yields the best overall pregnancy rates. Similarly, heifers can be synchronized effectively with GnRH, PG, and a CIDR. The Select Synch+CIDR protocol most frequently yields the greatest pregnancy rates and provides a reliable alternative to an MGA/$PGF_{2\alpha}$. In addition, a fixed-time AI CIDR-based estrous synchronization protocol has been developed to inseminate both suckled beef cows and replacement heifers with acceptable pregnancy rates.

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NOTES

Proceedings, Applied Reproductive Strategies in Beef Cattle
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REPRODUCTION OF *BOS INDICUS* BREEDS AND CROSSES

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Introduction

The most numerous *Bos indicus* breed in the United States is the Brahman. The Brahman breed was developed in the Gulf Coast States by upgrading native United States cattle with various breeds of *Bos indicus* cattle from India (Phillips, 1963; Yturria, 1973). Breeders did not control the proportions of different *Bos indicus* breeds used in developing the Brahman. Even with this genetically diverse beginning, the adaptation of this new *Bos indicus* breed to the Gulf Coastal environment has led to its use in crossbreeding and development of other Brahman-influenced breeds. The adaptive traits of the Brahman and its crosses that account for their use in beef production systems include: tolerance of internal and external parasites; tolerance of high solar energy, high ambient temperature and humidity; and the ability to utilize high fiber forages (Koger, 1963). The positive influence of *Bos indicus* breeding on beef production is well documented (Rhoad, 1955; Cartwright and Fitzhugh, 1972; Koger, 1973). Reproduction has generally been reported to be lower in *Bos indicus* compared with *Bos taurus* breeds (Kincaid, 1957; Warnick, 1963; Reynolds, 1967; Temple, 1967; Plasse, 1973). Research results explaining some of the reasons for these results will be presented in this manuscript.

Age at Puberty: Heifers

Emphasis on heifers calving at 2 years of age has made early maturity an important economic trait. *Bos indicus* heifers reach puberty at older ages than *Bos taurus* heifers (Table 1). *Bos indicus* and *Bos indicus* composite breeds mature later than *Bos taurus* breeds (Warnick et al., 1956; Luktuke and Subramanian, 1961; Temple et al., 1961; Reynolds et al., 1963; Reynolds, 1967; Plasse et al., 1968a). *Bos indicus*, but not *Bos indicus* x European, heifers reach puberty at too old of an age to calve at 2 years of age.

Table 1. Age at puberty in heifers

Breed	Age (days)	Source
British breeds	436	Reynolds (1967)
Angus	459	Chase et al. (1997b)
Hereford	413	Chase et al. (1997b)
Hereford x *Bos taurus*	358	Lammoglia et al. (2000)
Limousin x *Bos taurus*	379	Lammoglia et al. (2000)
Piedmontese x *Bos taurus*	338	Lammoglia et al. (2000)
Romosinuano	427	Chase et al. (1977b)
Senepol	481	Chase et al. (1997b)
Hereford x Senepol	384	Chase et al. (1997b)
Senepol x Hereford	427	Chase et al. (1997b)
Senepol x Angus	475	Chase et al. (1997b)
Tuli x Angus	466	Chase et al. (1997b)
Brangus	528	Reynolds (1967)
Brahman x *Bos taurus*	438	Reynolds (1967)
Brahman x Angus	478	Chase et al. (1997b)
Brahman	690	Reynolds (1967)
Brahman	592	Chase et al. (1997b)

Gestation Length

Cows of different breeds have different gestation lengths. There is a disadvantage for breeds with longer gestation periods when they are expected to maintain a 365 day calving interval. The *Bos indicus* breeds derived from India have gestation lengths about 10 days longer than *Bos taurus* breeds (Table 2). To maintain a yearly calving interval, the *Bos indicus* cow must rebreed within 73 days after calving whereas the *Bos taurus* cow must rebreed within 83 days after calving. Composite breeds that include *Bos indicus* breeding are intermediate between *Bos taurus* and *Bos indicus* with regard to gestation length. The only *Bos indicus* cattle with gestation lengths similar to *Bos taurus* (Table 2) are the small African Zebu breeds which lack the productivity of the Indian breeds.

Table 2. Gestation length

Breed	Gestation length (days)	Source
Bos taurus	282	Lush (1945)
Brahman	293	Plasse et al. (1968b)
Brangus	286	Reynolds (1967)
Nelore	291	Veiga et al. (1946)
Nelore and Guzerat	293	Haines (1961)
Nelore, Gir and Guzerat	292	Briquet and DeAbrea (1949)
Afrikander	295	Joubert and Bonsma (1959)
Afrikander	295	VanGraan and Joubert (1961)
African Zebu	283	Hutchison and Macfarlane (1958)
Ethiopian Zebu	283	Mukasa-Mugerwa and Tegegne (1989)

Postcalving Fertility

The principal reason that *Bos indicus* or *Bos indicus* crossbred cows are not pregnant at the end of the breeding season is that they do not come into estrus during the breeding season (Reynolds, 1967). Reynolds (1967) found that average intervals from calving to estrus were shortest in Angus, intermediate in Brangus and longest in Brahman cows (Table 3).

More recent reports (Stahringer et al., 1999; Webb et al., 2001; Strauch et al., 2003) indicate that the interval from calving to estrus is similar in the Brahman compared with *Bos taurus* breeds. With intervals less than 60 days there does not appear to be a longer interval from calving to estrus in the *Bos indicus* cow compared with *Bos taurus* cows. The greatest proportion of *Bos indicus* cows can return to estrus after calving quickly enough to rebreed to calve on an annual basis. Reports in the literature spanning over 30 years show that Brahman cows can have between 61 and 65 day intervals from calving to conception (Plasse et al., 1968c; Stahringer et al., 1999). First service conception rates in postpartum Brahman cows were from 50 to 73% in one report (Webb et al., 2001) and from 40 to 68% in another report (Strauch et al., 2003). From these results there seems to be little evidence of reduced postcalving fertility, at least in current Brahman genetics.

Table 3. Interval from calving to estrus

Breed	Interval (days)	Source
Hereford	59	Warnick (1955)
Angus	63	Reynolds (1967)
Brangus	74	Reynolds (1967)
Brahman	79	Reynolds (1967)
Brahman	45	DeFries et al. (1998)
Brahman	48	Stahringer et al. (1999)
Brahman	54	Webb (2001)
Brahman	59	Strauch et al. (2003)

Endocrine Controlled Reproductive Traits: Cows and Heifers

Estrogen induces estrus behavior in cattle (Short et al., 1973) and is the primary stimulus for the preovulatory LH surge (Henricks et al., 1971; Christensen et al., 1974). The duration of standing estrus is shorter in *Bos indicus* cattle compared with *Bos taurus* cattle (Anderson, 1936; De Alba et al., 1961; Plasse et al., 1970; Rhodes and Randel, 1978).

Ovariectomized Brahman cows have been reported to be less responsive to exogenous estrogen than ovariectomized Brahman x Hereford or Hereford cows (Rhodes and Randel, 1978). Ovariectomized Brahman cows did not accept heterosexual mounting at any estrogen dose and a lower proportion of Brahman x Hereford cows accepted heterosexual mount at the 1 mg dose than did the ovariectomized Hereford cows (Table

4). When homosexual behavior was used as the measurement for behavioral estrus a lower response was reported for the ovariectomized Brahman compared with the ovariectomized Brahman x Hereford or Hereford cows (Table 5). Duration of estrogen induced estrus was shorter in the ovariectomized Brahman and Brahman x Hereford cows than in the ovariectomized Hereford cows (Table 6). The differential response to estrogen may be clearer when the time from estrogen stimulus to behavioral estrus is compared between breedtypes. The time from estrogen to estrus was longest in the ovariectomized Brahman, intermediate in Brahman x Hereford and shortest in Hereford cows (Table 6).

Table 4. Proportion of ovariectomized cows accepting heterosexual mount after injection of estrogen

Breed	Cows showing estrus (%)			
	Estradiol-17ß dose			
	1 mg	2 mg	4 mg	8 mg
Brahman	0**	0**	0**	0**
Brahman x Hereford	33†	100	100	100
Hereford	83	100	100	100

From: Rhodes and Randel (1978)
†P < 0.10.
**P < 0.005.

Table 5. Proportion of ovariectomized cows accepting homosexual mount after injection of estrogen

Breed	Cows showing estrus (%)			
	Estradiol-17ß dose			
	1 mg	2 mg	4 mg	8 mg
Brahman	66*	83*	66*	83*
Brahman x Hereford	83	100	100	100
Hereford	83	100	100	100

From: Rhodes and Randel (1978).
*P < 0.05.

Table 6. Response of ovariectomized cows to estrogen injection

Breed	Duration of estrus (hours)	Time to estrus (hours)
Brahman	8.2	20.6
Brahman x Hereford	8.4	12.9
Hereford	12.3	9.9

From: Rhodes and Randel (1978).

The greatest concentrations of circulating estrogen before estrus (Randel, 1980) in estrous cycling heifers occur nearest estrus in Hereford, intermediate in Brahman x Hereford and furthest from estrus in Brahman (Table 7). The elapsed time between endogenous estrogen and onset of estrus in estrous cycling heifers is remarkably similar to the elapsed times from estrogen stimulus to behavioral estrus in ovariectomized cows

(Tables 6 and 7). These results show that *Bos indicus* and *Bos taurus* cows have different responses to estrogen. *Bos indicus* cows have a shorter, less intense estrus which occurs later after the estrogen stimulus than in *Bos taurus* cows. *Bos indicus* x *Bos taurus* crosses are intermediate to the parent breeds for these physiological parameters.

Luteinizing hormone (LH) is responsible for ovulation in cattle and the preovulatory LH surge occurs 20 to 22 hours before ovulation (Schams and Karg, 1969) or 3 to 6 hours after the onset of estrus (Henricks et al., 1970) in *Bos taurus* cattle. Gonadotropin releasing hormone (GnRH) is the hormone responsible for pituitary release of LH in cattle (Convey, 1973).

Griffin and Randel (1978) challenged ovariectomized Brahman and Hereford cows with 500 µg injections of GnRH and all cows responded by increasing circulating concentrations of LH within 15 minutes (Figure 1). Mean concentrations of LH were lower ($P < 0.005$) in ovariectomized Brahman (34 ± 4 ng/ml) than in ovariectomized Hereford (67 ± 20 ng/ml) cows. Peak LH concentrations were lower ($P < 0.005$) in ovariectomized Brahman (95 ± 7 ng/ml) than in ovariectomized Hereford (185 ± 68 ng/ml) cows. These results, that *Bos indicus* females show a smaller pituitary response than *Bos taurus* females, are similar to those reported (Godfrey et al., 1990b) for *Bos indicus* and *Bos taurus* bulls. From these results it seems appropriate to assume that the *Bos indicus* pituitary gland secretes less LH when given a measured dose of GnRH than does the *Bos taurus* pituitary gland, regardless of sex.

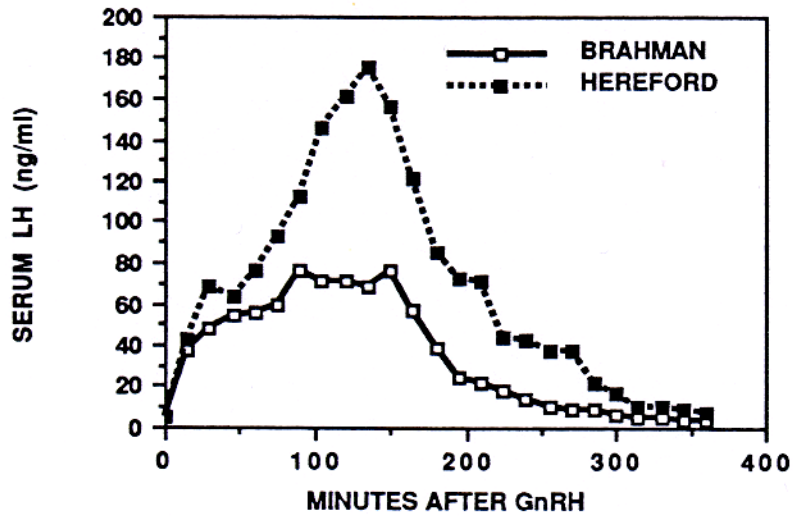

Figure 1. Response of ovariectomized cows to GnRH.

Comparative data evaluating the preovulatory LH surge in Brahman, Brahman x Hereford and Hereford heifers have been reported for estrous synchronized heifers (Randel, 1976) and normal estrous cycling heifers (Randel and Moseley, 1977). In estrous synchronized heifers (Figure 2) and normal estrous cycling heifers (Figure 3), the Brahman heifers had the smallest preovulatory LH surge with the Brahman x Hereford heifers and Hereford heifers having the larger LH surges. In both experiments, the time

from estrus to the preovulatory LH peak was shorter in the Brahman heifers than in the Hereford heifers (Table 8).

Table 7. Time of peak circulating estrogen before estrous in estrous cycling heifers

Breed	Time (hours)
Brahman	24[a]
Brahman x Hereford	16[b]
Hereford	8[c]

From: Randel (1980).
[a,b,c]Different superscript differ $P < 0.05$.

Table 8. Timing of the preovulatory LH surge

	Time from estrus to peak LH (mean hours ± SE)	
Breed	Randel (1976)	Randel and Moseley (1977)
Brahman	0.4 ± 3.4	2.0 ± 1.3
Brahman x Hereford	6.8 ± 2.1	3.0 ± 1.3
Hereford	5.3 ± 1.3	6.5 ± 1.8

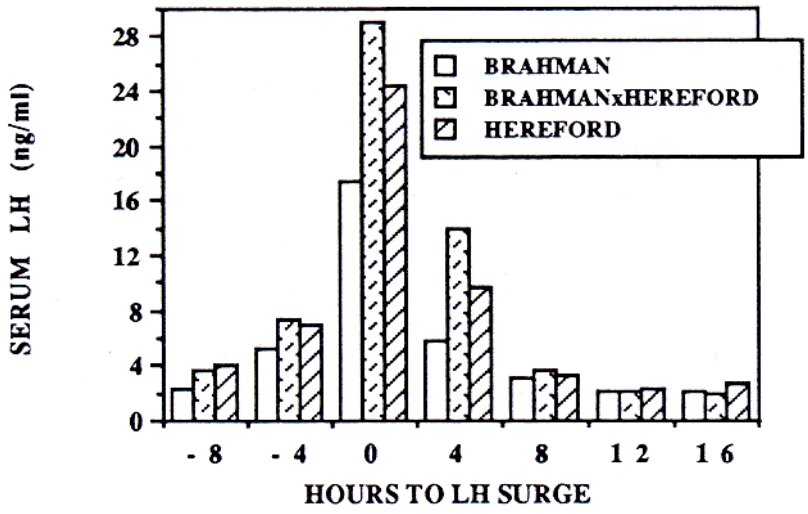

Figure 2. The preovulatory LH surge in estrous synchronized heifers.

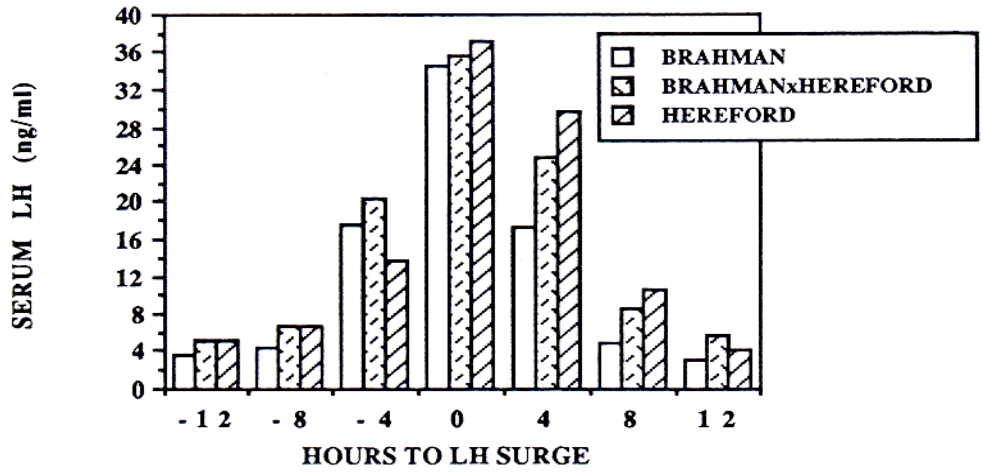

Figure 3. The preovulatory LH surge in estrous cycling heifers.

Estrogen has been reported to be the primary stimulus for hypothalamic release of GnRH which in turn stimulates pituitary release of LH in cattle (Henricks et al., 1971; Hobson and Hansel, 1972; Short et al., 1973; Christensen et al., 1974). Rhodes et al. (1978) found that elapsed time from estrogen injection to peak LH concentration was longest in ovariectomized Brahman, intermediate in Brahman x Hereford and shortest in Hereford cows (Table 9). Ovariectomized Brahman cows had the smallest area under the LH release curve, Brahman x Hereford were intermediate and Hereford cows released the greatest amount of LH (Table 9).

Bos indicus cows have a smaller preovulatory LH surge, a smaller estrogen or GnRH releasable pool of pituitary LH and are slower to respond to an estrogen stimulus with GnRH secretion from the hypothalamus compared with *Bos taurus* cattle.

The timing of physiological events leading to ovulation has been reported for Brahman, Brahman x Hereford and Hereford heifers (Randel, 1976). Brahman heifers ovulated earlier after the onset of estrus than did the Brahman x Hereford or Hereford heifers (Table 10). The interval from the LH surge to ovulation did not differ between the breeds. Ovulation times following the onset of estrus have been reported for grade Brahman heifers in Florida (25.6 hours; Plasse et al., 1970), for Brahman heifers in Venezuela (20.6 hours; Troconiz, 1976) and for *Bos taurus* heifers in Montana (33.2 hours; Randel et al., 1973). *Bos indicus* females ovulate 8-10 hours earlier after the onset of estrus than *Bos taurus* females. The primary difference between *Bos indicus* and *Bos taurus* females is that the *Bos indicus* female takes longer from the peak in estrogen to onset of behavioral estrus and then a shorter time from estrus onset to ovulation than the *Bos taurus* female.

Detection of corpora lutea (CL) by rectal palpation is more difficult in *Bos indicus* females than in *Bos taurus* females (Plasse et al., 1968a). Brahman heifers have smaller CL than Brahman x Hereford or Hereford heifers (Irvin et al., 1978) and CL from Brahman cows are smaller than in Angus cows (Segerson et al., 1984; Table 11). Progesterone content of CL from Brahman heifers, Brahman x Hereford heifers and

Brahman cows has been reported to be lower than Hereford heifers (Irvin et al., 1978) or Angus cows (Segerson et al., 1984; Table 12). Conversely, Segerson et al. (1984) found that Brahman cows had greater ovarian and stromal weights on day 17 after estrus compared with Angus cows (Table 13). Brahman cows also had greater numbers of small (< 5 mm) follicles and more follicular fluid but smaller numbers of large (> 5 mm) compared to Angus cows (Table 14).

As *Bos indicus* cows have smaller CL and both *Bos indicus* and *Bos indicus* x *Bos taurus* cows have less progesterone in the CL than *Bos taurus* cows, it is not surprising that both have lower circulating concentrations of progesterone from day 2 through 11 after estrus than *Bos taurus* (Randel, 1977; Figure 4). Segerson et al. (1984) also reported that Brahman cows had lower serum progesterone from day 7 through 17 after estrus than Angus cows (Figure 5).

Table 9. LH response to estrogen injection in ovariectomized cows

Breed	Time to peak LH (mean hours ± SE)	Area under the LH curve (mean ± SE)
Brahman	27.8 ± 2.0[a]	6.0 ± 2.8[a]
Brahman x Hereford	23.8 ± 0.9[b]	11.1 ± 2.1[b]
Hereford	22.1 ± 1.0[c]	25.1 ± 7.4[c]

From: Rhodes et al. (1978).
[a,b,c]Means in columns with different superscripts differ P < 0.05.

Table 10. Timing of physiological events leading to ovulation (mean hours ± SE)

Breed	Estrus to LH surge	LH surge to ovulation	Estrus to ovulation
Brahman	0.4 ± 3.4	18.5 ± 3.1	18.9 ± 2.2[a]
Brahman x Hereford	6.8 ± 2.1	22.2 ± 2.6	29.0 ± 1.3[b]
Hereford	5.3 ± 1.3	23.3 ± 2.1	28.6 ± 1.5[c]

From: Randel (1976).
[a,b,c]Means in columns with different superscripts differ P < 0.05.

Table 11. Corpus luteum weight (mean g ± SE)

	Day of the estrous cycle		
Group	8	13	17
Brahman heifers[a]	2.5 ± 0.1[c]	2.7 ± 0.1[c]	--
Brahman x Hereford heifers[a]	4.6 ± 0.4[d]	3.8 ± 0.3[d]	--
Hereford heifers[a]	4.0 ± 0.4[d]	3.6 ± 0.3[d]	--
Brahman cows[b]	--	--	2.4 ± 0.1[c]
Angus cows[b]	--	--	4.1 ± 0.3[d]

[a]From: Irvin et al. (1978).
[b]From: Segerson et al. (1984).
[c,d]Means in columns with different superscripts differ P < 0.05.

Table 12. Progesterone content of corpora lutea (mean ± SE)

Group	µg/CL	Source
Brahman heifers	216.9 ± 45.0	Irvin et al. (1978)
Brahman x Hereford heifers	217.7 ± 35.3	Irvin et al. (1978)
Hereford heifers	334.6 ± 87.8	Irvin et al. (1978)
Brahman cows	190.8 ± 28.9	Segerson et al. (1984)
Angus cows	266.3 ± 23.9	Segerson et al. (1984)

Table 13. Ovarian and stromal weights on day 17 after estrus (mean ± SE)

	Ovarian weight (g)		Stromal weight (g)	
Breed	Active[a]	Inactive[b]	Active	Inactive
Angus	9.2 ± 0.4	4.6 ± 0.3[c]	3.9 ± 0.3[c]	3.6 ± 0.3[c]
Brahman	11.0 ± 1.1	7.9 ± 0.9[d]	6.8 ± 0.9[d]	6.2 ± 0.7[d]

From: Segerson et al. (1984)
[a]Active ovary contains CL.
[b]Inactive ovary does not contain CL.
[c,d]Means in columns with different superscripts differ $P < 0.01$.

Table 14. Ovarian follicular characteristics on day 17 after estrus (mean ± SE)

	Number of follicles			
Breed	Small (< 5 mm)		Large (> 5 mm)	
	Active[a]	Inactive[b]	Active	Inactive
Angus	22.3 ± 3.4	20.2 ± 0.3	2.3 ± 0.5	1.8 ± 0.5
Brahman	40.8 ± 5.6	37.1 ± 5.3	1.2 ± 0.3	0.9 ± 0.2

From: Segerson et al. (1984).
[a]Active ovary contains CL.
[b]Inactive ovary does not contain CL.

Influence of Season

The *Bos indicus* and *Bos indicus* crossbred cows are long day breeders. There are numerous reports in the literature that as day length decreases reproductive function decreases in *Bos indicus* cattle (Anderson, 1944; Tomar, 1966; Jochle, 1972; Randel, 1984). *Bos indicus* tend to become anestrus during unfavorable seasons (Dale et al., 1959; Tomar, 1966; Plasse et al., 1968a). The frequency of estrus without ovulation also increased in *Bos indicus* females during unfavorable seasons (Luktuke and Subramanian, 1961; Plasse et al., 1970).

Research from our laboratory (Stahringer et al., 1990) supports the literature. Brahman heifers which were experiencing normal estrous cycles in October experienced a relatively high proportion (88%) of either anestrus or abnormal estrus cycles in November, December, January and February before returning to normal estrous cyclicity in March (Figure 6). Serum progesterone concentrations were lower ($P < 0.001$) in heifers during the months of November, December, January and February than in either October or March

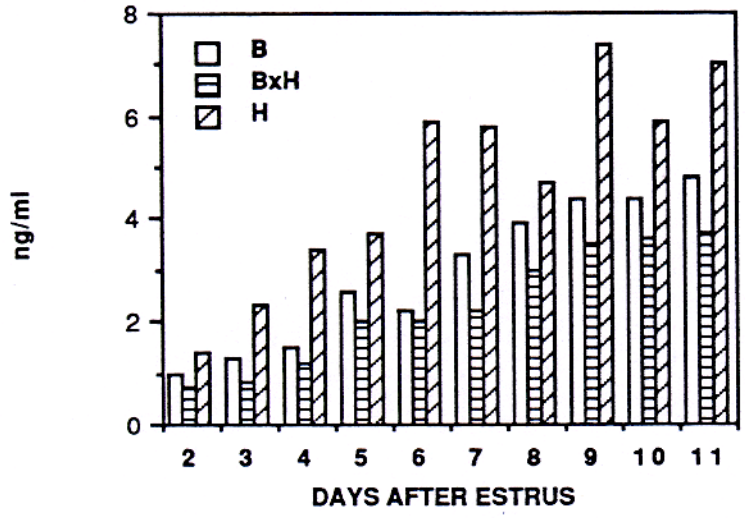

Figure 4. Serum progesterone.

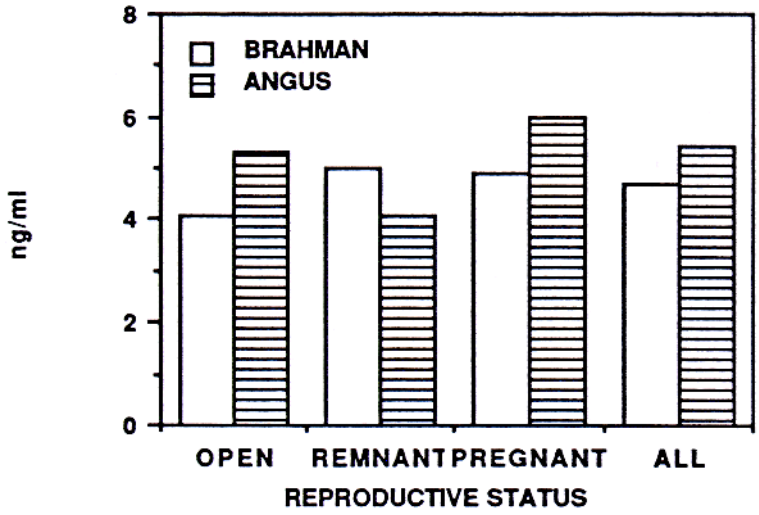

Figure 5. Serum progesterone in mature cows 7-17 days after breeding.

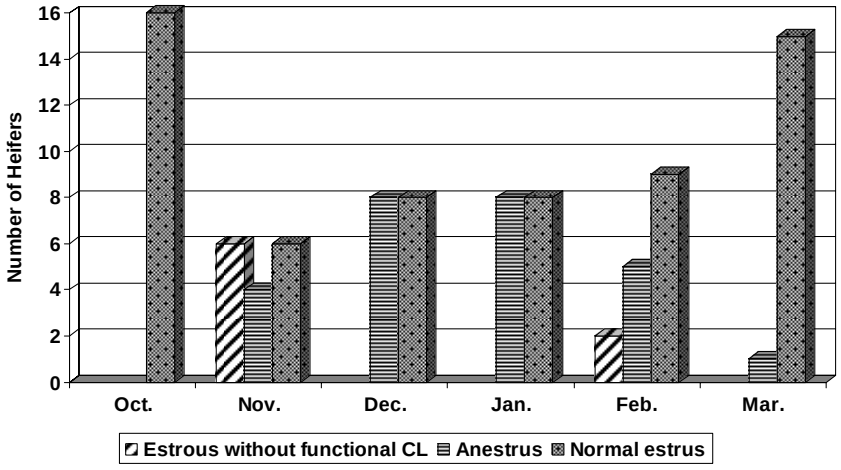

Figure 6. Distribution of the occurrence of normal estrus, estrus without formation of functional CL and anestrus by month in Brahman heifers.

Jochle (1972) reported that conception rates in Brahman cattle were higher during the summer months. Data from our laboratory (Neuendorff et al., 1984) show that Brahman females have higher ($P < 0.005$) first service conception rates in the summer (61%) compared with the late fall (36%). This experiment reported results from artificial insemination so that there were no seasonal influences due to the males involved.

Production of gametes is also affected by season in the female. Bastidas and Randel (1987) reported that the number of transferable embryos produced per Brahman donor cow was greatest in the fall and lowest in the winter (Figure 7). *Bos taurus* breeds have not been reported to be affected by season in production of transferable embryos (Massey and Oden, 1984) yet in this report the authors found that Brahman cows produced the greatest number of embryos in the spring season. Bastidas and Randel (1987) found that pregnancy rates per Brahman donor cow were lower in the winter months (Figure 8). The combination of reduced production of transferable embryos and lower pregnancy rates in the winter months resulted in fewer pregnant recipient cows per Brahman donor cow (Figure 9).

Not all of the fertility data are negative for the *Bos indicus* cattle. High ambient environmental temperatures decrease pregnancy rates in *Bos taurus* cattle (Biggers et al., 1987; Dunlap and Vincent, 1971; Ingraham et al., 1974; Putney et al., 1988; Putney et al., 1989a; Ryan et al., 1992). *Bos taurus* heifers subjected to heat stress during the later stages of oocyte maturation produced fewer embryos to superovulation and had a greater proportion of embryos with retarded development (Putney et al., 1989b).

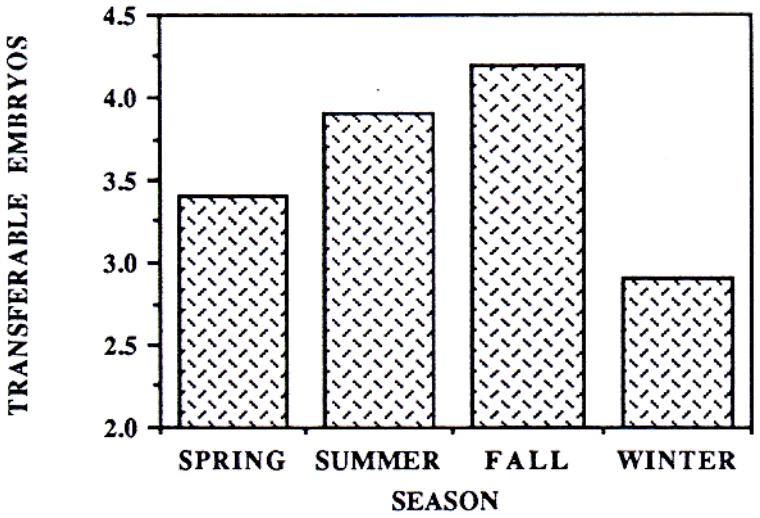

Figure 7. Transferable embryos per Brahman donor cow.

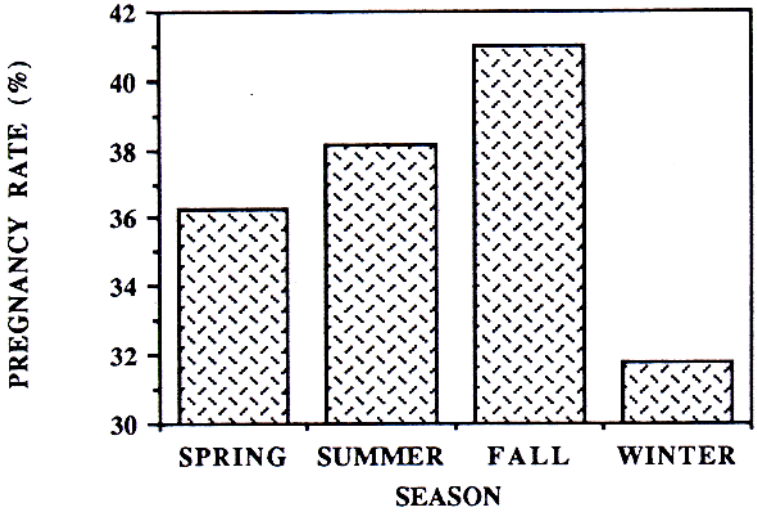

Figure 8. Pregnancy rate per Brahman donor cow.

An experiment was carried out to determine the effects of environmental temperature and humidity on both Brahman and Holstein oocytes (Rocha et al., 1998). Brahman and Holstein donor cows were treated with follicle stimulating hormone and oocytes were harvested in August and in January. When these oocytes collected in August were fertilized in vitro and incubated through developmental stages allowing for transfer to recipient cows, none of the oocytes from Holstein cows (Table 15) resulted in transferable embryos but reasonable proportions of Brahman oocytes developed into transferable embryos (Table 16). Brahman cows responded to super stimulation with production of similar numbers and quality of oocytes as in the summer months. It appears that if a Brahman cow is estrous cycling during the winter she produces high quality oocytes capable of being fertilized and developing into a normal embryo.

In an experiment evaluating the effect of breed and season (Rhodes et al., 1982), Brahman heifers had smaller corpora lutea than *Bos taurus* heifers (Table 17). Both *Bos indicus* and *Bos taurus* corpora lutea that developed in the winter had higher concentrations and content of progesterone than those from the summer. When luteal cells from these corpora lutea were incubated with luteinizing hormone in a culture system, the luteal cells from the Brahman heifers produced less progesterone and were less responsive to luteinizing hormone than cells from *Bos taurus* heifers and luteal cells collected in the winter were less responsive than those collected in the summer (Figure 10).

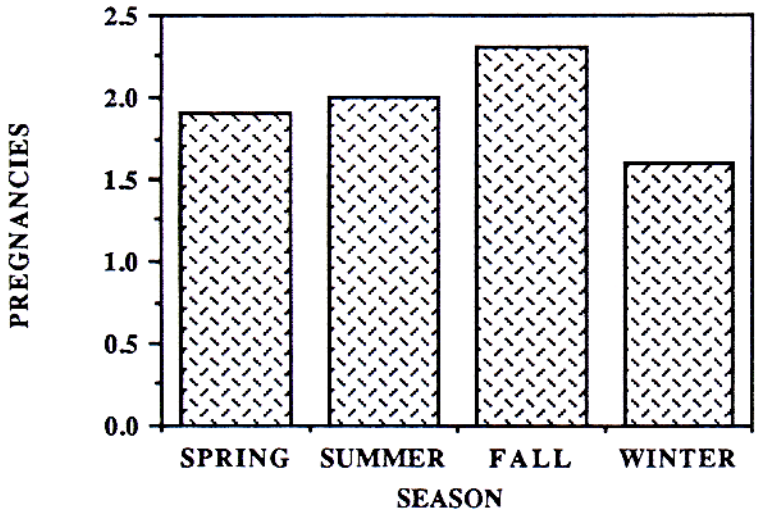

Figure 9. Pregnant recipient cows per Brahman donor cow.

A possible explanation for some of the seasonal influences found in *Bos indicus* cattle may be that pituitary function is altered during the winter. Brahman cows have a lower preovulatory luteinizing hormone surge during the winter compared with the spring or summer periods (Harrison et al., 1982; Figure 11).

It is clear from these experiments that season moderates endocrine function to a greater extent in *Bos indicus* cattle than in *Bos taurus* cattle resulting in suppressed reproductive function in *Bos indicus* cattle during the winter months.

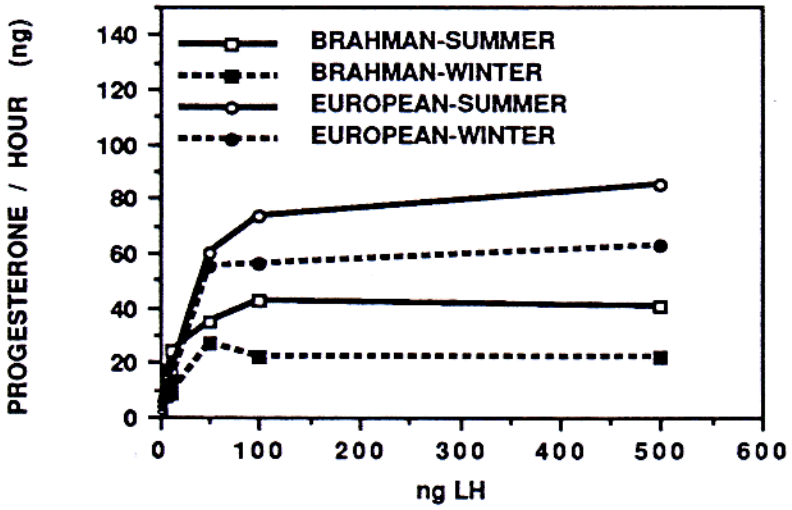

Figure 10. Progesterone secretion by cultured luteal cells.

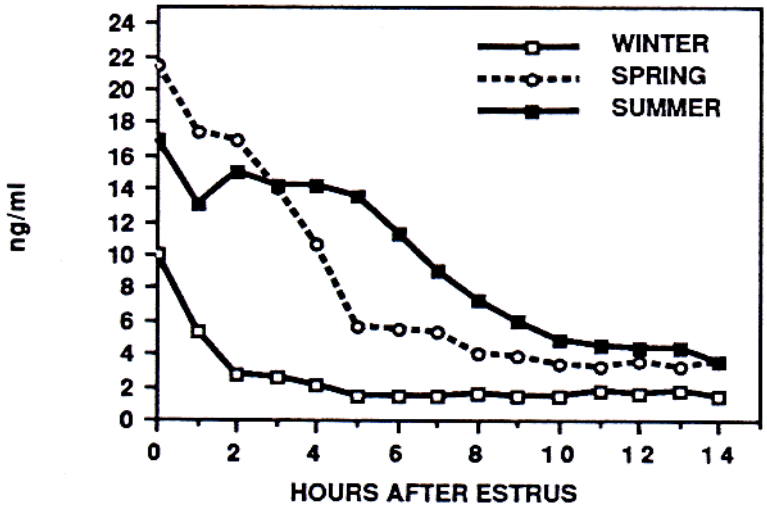

Figure 11. Serum LH in Brahman cows.

Table 15. Percentage of normal oocytes collected from Holstein cows and embryo development from the 2 cell to the blastocyst stage

Season	Total Number of Oocytes	Percentage of Normal Oocytes	Percentage of Oocytes Developing To[a]			
			≥ 2-cell (48 h)	≥ 8-cell (96 h)	Morula (144 h)	Blastocyst (192 h)
Cool	67	80.0±19.1[b]	59.8±11.7	44.4±12.7[d]	34.2±12.7[d]	29.0±14.8[d]
Hot	28	24.6±6.3[c]	52.3±10.6	1.1±4.8[e]	0[e]	0[e]

From: Rocha et al. (1998).

[a]Numbers in parentheses indicate the number of hours post insemination. Normal and abnormal oocytes were fertilized.

Means in the same column with different superscripts differ: [bc]P = 0.01; [de]P ≤ 0.003.

Table 16. Percentage of normal oocytes collected from Brahman cows and embryo development from the 2 cell to the blastocyst stage

Season	Total Number of Oocytes	Percentage of Normal Oocytes	Percentage of Oocytes Developing to[a]			
			≥ 2-cell (48 h)	≥ 8-cell (96 h)	Morula (144 h)	Blastocyst (192 h)
Cool	83	83.3±17.4	83.1±10.7	71.3±11.6	55.5±12.2	52.3±13.5
Hot	89	77.0±6.3	79.3±10.6	69.9±4.8	58.1±4.8	41.3±7.2

From: Rocha et al. (1998).
[a]Numbers in parentheses indicate the number of hours post insemination. Normal and abnormal oocytes were fertilized.

Table 17. Effect of breed and season on CL weight, Progesterone (P_4) concentration and progesterone content[a]

Measurement	Brahman		Hereford x Holstein	
	Summer	Winter	Summer	Winter
Weight (g)	2.74 ± 0.10^{b}	3.01 ± 0.29^{b}	4.58 ± 0.44^{c}	5.11 ± 0.49^{c}
P_4 concentration (µg/g)	30.8 ± 2.8^{d}	52.6 ± 7.8^{e}	39.0 ± 7.1^{f}	40.4 ± 1.9^{f}
P_4 content (µg/CL)	104.0 ± 5.3^{g}	153.2 ± 35.9^{h}	174.1 ± 35.9^{i}	201.9 ± 9.5^{j}

[a]From Rhodes et al. (1982).
[b,c]Means within a row with different superscripts differ ($P < 0.001$).
[d,e,f]Means within a row with different superscripts differ ($P < 0.10$).
[g,h,i,j,k]Means within a row with different superscripts differ ($P < 0.01$).

SUMMARY

Reproductive performance differs in subtle ways in *Bos indicus* cattle compared with *Bos taurus* cattle. Developmental differences are apparent during the pubertal process in both males and females. Reproductive endocrinology is similar between *Bos indicus* and *Bos taurus* in that the mechanisms are the same yet nuances of timing are different around estrus and ovulation in the female. Reproductive efficiency, as measured by first service conception rates, is similar in *Bos indicus* and *Bos taurus* cattle. In fact, summer reproductive efficiency may be superior in *Bos indicus* compared with *Bos taurus.* During the winter months *Bos indicus* cattle have marked decreases in reproductive efficiency compared with *Bos taurus*. In most reproductive traits *Bos indicus* x *Bos taurus* animals are superior to the mean of the parents or not different from the superior parent breedtype. The subtle differences between *Bos taurus* and *Bos indicus* cattle must be taken into consideration when designing treatments targeting reproductive function.

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NOTES

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SYNCHRONIZATION OF *BOS INDICUS*-INFLUENCED CATTLE FOR TIMED ARTIFICIAL INSEMINATION

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Introduction

Developing successful methods for synchronizing estrus and ovulation in cattle has been a major research interest for at least the last 35 yr. Early objectives focused on the control of the corpus luteum (CL) and artificial insemination (AI) relative to estrus. Ultimately, the goal has been to achieve precise synchronization of ovulation so that cattle can be inseminated without regard to estrus. However, for most treatments developed before 1995, variation in intervals from CL regression to ovulation has resulted in highly variable timed AI (TAI) conception rates (Odde, 1990).

In recent years, owing to a better understanding of ovarian follicular dynamics, treatments have been designed to control both the CL and the timing of follicular wave emergence (Pursley et al., 1995). Most recently, the CO-Synch protocol (COS; combination of GnRH and prostaglandin F2α; PGF) has been combined with an exogenous source of progesterone, the controlled internal drug release device (CIDR), to produce timed AI (TAI) pregnancy rates that are consistently greater than 50% in *Bos taurus* females (Lamb et al., 2001; Larson et al., 2004 a, b). Thus, the CO-Synch + CIDR protocol appears to offer much greater potential for achieving highly-successful TAI in beef cattle than in the past. However, in the southern regions of the U.S and in many other locations around the world where the environment is predominantly subtropical to tropical, the need to use cows with *Bos indicus*-influenced genetics may create additional challenges. Important deficiencies in conception rates have been reported when synchronization and TAI have been employed in these types of cattle (Hiers et al., 2003), and results using the CO-Synch + CIDR protocol have been particularly disappointing (Saldarriaga et al., 2005 a, b).

The objective of this review is to summarize results of TAI programs in *Bos taurus x Bos indicus* cattle in South Texas. This will be approached by first reviewing the performance of older progestin-based protocols (Syncro-Mate-B; SMB) that were established in the 1970's (Spitzer et al. 1978) and in conjunction with different methods of calf manipulation. This will be followed by a review of recent TAI results using the more-recently developed techniques, Ovsynch, CO-Synch and Co-Synch + CIDR.

Timed AI Fertility in Brahman-Influenced Cattle: Historical Perspectives from South Texas

Conception and Pregnancy Rates in Cows Synchronized with Syncro-Mate-B: Synchronized Breeding with TAI vs Natural Service

Table 1 summarizes historical data that provides a perspective of typical TAI conception rates in Brahman-influenced cows synchronized with SMB (Williams et al., 1987; Williams, 1988). In the first study, TAI was compared to the use of bulls for synchronized breeding. Cows were stratified by BW, body condition score (BCS), age and d postpartum and either left untreated (**Control**) or synchronized with SMB followed by TAI at 48-54 h (**SMB-AI**) or turned with fertile bulls for natural service in confinement at a bull:cow ratio of 1:15 to 1:20 (**SMB-NS**) in pens measuring 25.6 x 9.6 m . Calves were removed from cows in all groups for 48 h beginning at the time of SMB implant removal. Based on serum progesterone concentrations determined in blood samples collected before and at the start of synchronization, only 49% of all cows in these studies were cycling at the time of treatment onset. Cows averaged 71 d postpartum at the time of entry into the synchronization program. Both TAI (Day 2) and d-30 pregnancy rates favored SMB-AI over both SMB-NS and non-synchronized controls (**Table 2**). Average d of birth of calves also favored SMB-AI by 5 d over controls and by 11 d over SMB-NS.

Table 1. Effect of synchronization treatments and TAI or natural service on cumulative pregnancy rates in suckled cows on d 2, 30 and 90 of the breeding season [Adapted from Williams et al., (1987) and Williams (1988) with permission]

		Cumulative Pregnancy (%)		
Treatment	No. Cows	Day 2	Day 30	Day 90
Control	97	3.0[a]	57.7 [a]	85.6
SMB-AI	98	38.8[b]	74.5 [b, c]	84.0
SMB-NS	95	29.5[b]	64.2 [d]	85.3

[a,b] $P < 0.05$
[c, d] $P < 0.10$

Bulls used in these studies, both for NS during the synchronization period and for clean–up, were Brahman-influenced (Beefmaster, Simbrah). For a complete summary of the physiology and behavior of bulls associated with this work, the reader is referred to Williams (1988). However, in brief, bulls in the SMB-NS group averaged 23.6 services during 33 h of cow exposure, with a range of 11-41 services. The average interval between services over the entire breeding period was 54.4 min. Approximately 80-90% of this activity occurred between 24 and 36 h after implant removal. One bull was observed to service a female approximately every 15 min from 1800 to 2400 h. Many females were serviced multiple times (3-10 times), and some were not serviced at all. Therefore, the average number and percentage of estrus females serviced per bull (8.6; 72%) was markedly less than the average

number of estrus females available, and represented slightly more than half of all females in each pen. Although conception rate (number pregnant/number serviced) for SMB-NS was acceptable (57.3%), total pregnancy rate (number pregnant/number available) was low (32.6%).

Table 2. Effect of synchronization treatments on average d of birth of calves during the calving season in mature Brahman x Hereford, F_1 cows (From Williams et al., 1987)

Treatment	No. calves	Average d of birth
Control	97	$36.2^{b,c}$
SMB-AI	98	$31.0^{a,c}$
SMB-NS	95	42.9^{d}

[a,b] $P < 0.10$
[c,d] $P < 0.05$

Effects of Temporary Calf Removal and Alien Cohabitation for Timed AI of Cows Synchronized with SMB

Synchronization protocols developed in the 1970's, such as SMB, have historically benefited when cows were temporarily weaned (usually 48 h) before targeted insemination. Temporary weaning increases both the synchrony of estrus and conception rates at TAI. However, of all the procedures involved in estrus synchronization and AI, the process of temporary weaning seems to create the greatest concern for cattlemen. This aversion to temporary weaning is not without some degree of justification, since stress and weather conditions can result in morbidity in some groups of calves. Based on studies conducted at Beeville and in other laboratories (see reviews, Williams and Griffith, 1992; 1995), we know that calf association and suckling by a calf other than a cow's own calf does not inhibit estrus and ovulation. It appears that the maternal bond must be present in order for a calf to suppress ovarian and sexual activity. Based on this information, we proposed that sets of calves could be effectively switched among groups of cows kept temporarily in pens for estrus synchronization. We proposed that 1) this process would not attenuate the positive effects of temporary weaning of "own" calves, and 2) could result in some degree of suckling and/or nurturing of alien calves by the caretaker group. Therefore, objectives were to determine the effect of alien cohabitation vs temporary weaning in conjunction with SMB treatment on estrus synchronization, conception to timed AI, behavioral characteristics of cows and calves, and weight changes of calves. A control group (SMB-S) was included in which synchronized cows were maintained with their own calves throughout.

Procedures. Two-hundred sixty-eight mature, predominantly Brahman x Hereford cows were used. Cows at Beeville were maintained on tame pastures (Coastal bermudagrass and kleingrass, except during the synchronization/AI process. Cows at the La Copita Research Area, Alice, TX, were maintained on open range except during the synchronization/AI process. Cows at each location and each year were stratified by age, BW, body condition score, and date of calving, then assigned randomly to one of three

groups: 1) **SMB-W;** standard SMB treatment plus 48-h calf removal, 2) **SMB-A**; Standard SMB treatment plus alien cohabitation 3) **SMB-S**; standard SMB treatment and ad libitum suckling by the cows' own calves.

SMB treatments were begun 12 d prior to targeted AI. Calves were removed from cows for 48 h in the SMB-W group at the time of implant removal. Calves weaned from SMB-W cows were placed with SMB-A cows to serve as "aliens" for the 48-h period. Calves removed from SMB-A cows were placed in separate pens 25 to 50 yards away. They were fed hay and creep feed and provided with water during this period. Cows were maintained in dry-lot and provided free access to hay and water, and were fed 3 lb (2.36 kg) of a supplemental concentrate daily during the 48-h period. Cows were inseminated at 48 h after SMB implant removal with semen from three different bulls and three AI technicians distributed evenly among groups at each trial. All calves were returned to their own dams immediately after AI. Pairs were then returned to pasture. Three d after AI, Angus or Red Angus clean-up bulls of known fertility were placed with the cows at a bull:cow ratio of approximately 1:25 for 90 d. Forty-five to 50 d after AI, cows were examined for pregnancy by palpation and transrectal ultrasound to determine conception at TAI and during the subsequent 3-wk period. Cows were palpated again approximately 45 d after bulls were removed to determine final pregnancy rates.

Results. **Table 3** summarizes the number and percentage of SMB-A cows that allowed suckling, number and percentage of calves allowed to suckle, and average total suckling time per calf for the 48-h period. Approximately 30% of cows allowed some suckling during both years. However, only 24 and 43% of alien calves in yr 1 and 2, respectively, suckled for 15 min or more during the 2-d period. There was no advantage to SMB-A vs SMB-W with regard to calf weight loss during the temporary weaning/alien cohabitation period (data not shown).

Table 3. Behavioral characteristics of temporarily weaned cows and alien calves maintained together for the 48-h weaning period during trials at Beeville

Year	No. Cows allowing suckling ≥ 5 min. (%)	No. Diff. Calves suckling ≥ 5 min. (%)	No. Calves allowed to suckle ≥ 15 min. (%)	Average attempts/ calf	Average total suckling/calf, min.
1	8/24 (33)	13/29 (44.8)	7/29 (24)	9	14.7
2	12/41 (29)	25/41 (61.0)	18/41 (43.9)	13.1	24.0

Reproductive performance is summarized in **Table 4**. Both SMB-W and SMB-A groups had higher timed AI conception rates than SMB-S groups both years and at both locations, with one exception. During yr 2 at Beeville, conception rates to timed AI in SMB-S were unexpectedly high and similar to SMB-W. Overall, a greater number of cows in the SMB-W and SMB-A groups became pregnant at timed-AI and cumulative pregnancy rates after 3 wk of breeding favored these groups over SMB-S. Timed AI conception in SMB-W

and SMB-A was 15% greater than in SMB-S.

Table 4. Conception rates to TAI and cumulative pregnancy rates in SMB-S, SMB-W, and SMB-A cows over a 2-yr period at Beeville and La Copita

		Percent Pregnant		
Group	No. Cows	Timed AI	3 wk	90 d
SMB-S	88	40.9[b]	80.6	92.0
SMB-A	90	55.5[a]	86.6	92.2
SMB-W	90	54.4[a]	87.7	95.5

[a,b] $P < 0.05$
SMB-S = SMB suckled
SMB-A = SMB alien cohabitation
SMB-W = SMB and 48-h calf removal

Comparison of Ovsynch to Syncro-Mate-B and Norgestomet-Prostaglandin

The three most important factors affecting the relative value of a synchronization protocol is the number of times cattle must be worked, the cost of hormones, and pregnancy rates. For seedstock, a wider array of protocols, including those that utilize estrus detection, can be profitably exploited. However, for commercial cows, we believe that TAI must be possible with conception rates consistently of 50% or greater. Our long-term timed AI conception rate using SMB in mature, *Bos indicus x Bos taurus* cows in combination with 48-h calf removal has averaged about 48%, but has ranged from 30 to 60%. Hence, although the SMB protocol did not allow us to consistently achieve 50% TAI conception rates, dissatisfaction with the protocol probably resided more with the variation in results than with the average. Moreover, SMB has been removed from the U. S. market, further predicating a need for alternatives.

A protocol that controlled both CL function and follicular wave emergence using a combination of GnRH and PG (Ovsynch) was first introduced in dairy cattle in the mid 1990's (Pursley et al., 1995). When applied to beef cattle, conception rates using Ovsynch and TAI were reported to be greater than with SMB in cycling *Bos taurus* cattle (Geary et al., 1998). Much earlier, another approach had been to combine a progestin (SMB implant; norgestomet) with an injection of PG 2 d before implant removal (termed herein as NP; Hansel and Beal, 1979) to achieve better control of the CL. This protocol does not address synchronization of follicular wave emergence, but was used successfully in dairy heifers, particularly in combination with estrus detection and was later used in beef cattle as well (Beal et al., 1984). Neither of these methods represented a commercially-available package, but could be utilized by combining various parts of other commercially-available treatments. Objectives of the study summarized below were to compare the relative efficacies of SMB, Ovsynch and NP to synchronize estrus and ovulation for TAI in *Bos indicus x Bos taurus* beef cows.

Procedures. In Experiment 1, 273 Brahman x Hereford (F_1) cows at 3 locations were stratified by BW, body condition score (BCS), age, and d postpartum, and assigned randomly to one of three treatment groups: 1) **SMB**; SYNCRO-MATE-B, 2) **NP**; Norgestomet-prostaglandin, and 3) **Ovsynch**. The management approach for Experiment 1 required that cows have a minimum BCS of 5 and be at least 36 d postpartum at treatment onset. In Experiment 2, a total of 286 pubertal beef heifers were stratified by weight and BCS and allocated randomly to the three treatments. Heifers were predominantly Brahman crossbred or composites (n = 239; Brahman x Hereford, F_1; Santa Cruz, and Santa Gertrudis) with a smaller proportion (n = 42) of Hereford heifers used. Syncro-Mate-B treatment consisted of the standard 9-d norgestomet ear implant plus an estradiol valerate/norgestomet injection on d 0. NP females were implanted with the same 9-d norgestomet implant as in SMB, but received 25 mg prostaglandin $F2_\alpha$ i.m. 2 d before implant removal and did not receive the norgestomet-estradiol valerate injection at the time of implantation. OvSynch consisted of 100 μg GnRH i.m. on d 1, 25 mg PG i.m. on d 8, and a second GnRH injection on d 10. Beginning on d 9, calves were removed for 48 h in suckled cows. Cattle in both experiments were inseminated 48-54 h after implant removal (SMB; NP) and at 12-15 h after the second GnRH injection (Ovsynch), with the exception of a small group of cattle in the first trials. Those cattle were inseminated 18-24 h after the second GnRH injection, but this did not influence conception rates.

Results. Overall mean (± SEM) BW and BCS of heifers was 350 ± 2.8 kg and 5.5 ± .03, respectively and did not differ among groups. In this experiment, all heifers had a minimum BCS of 5.0 and were confirmed pubertal based on determination of twice weekly serum progesterone concentrations. The timed AI conception rate was greatest ($P < 0.056$) in NP-treated heifers compared to SMB- and Ovsynch-treated heifers (**Table 5**). During yr-3 of our study, 52 heifers that were allotted to the OvSynch/TAI treatment were also observed for estrus throughout the 9-d treatment period. Fifteen of 52 (28.9%) exhibited a natural estrus during the treatment period. We inseminated these heifers at the natural estrus, with 8 of 15 (53.3%) conceiving. Timed AI conception rate for these heifers was considered to be 0. When conception data for OvSynch heifers inseminated after a detected estrus during the synchronization period was combined with that obtained in the balance of the Ovsynch heifers at timed AI, conception rates increased from 42.4% (timed AI alone) to 57.7% (TAI + insemination at estrus), comparable to those achieved with NP (57.7%).

Overall mean (± SEM) BW, BCS, and d postpartum for cows was 554 ± 3.5 kg, 5.9 ± .06, and 61.4 ± .8 d. Timed AI conception rates did not differ among SMB, NP, and Ovsynch, groups (**Table 5**). However, there was a tendency for NP-treated cows to have a lower ($P < 0.13$) overall conception rate than those treated with SMB or OvSynch. When cows in each treatment were categorized into late (36-59 PP, n = 126), middle (60-79 d PP, n = 116), and early (80-99 d PP, n = 31) calving groups, late calving cows in the NP treatment had a lower ($P < 0.05$) conception rate than SMB and OvSynch. Since more cows would be expected to be anovulatory in the late-calving group, we can speculate that the lack of estradiol or GnRH treatment in the NP protocol resulted in a lower induction of ovulation in anovulatory cows compared to the other treatments.

Table 5. Timed AI conception rates in nulliparous heifers and suckled cows treated with Syncro-Mate-B (SMB), norgestomet-prostaglandin (NP) or Ovsynch (adapted with permission from Williams et al., 2002)

Age	Treatment	No.	TAI conception rate, %
Heifers	SMB	99	40.4
	NP	95	54.7*
	Ovsynch	92	39.1
Cows	SMB	91	45.1
	NP	90	31.1
	Ovsynch	92	42.4

* P < 0. 056
Calves were removed from all cows for 48 h beginning on d 9

Synchronization and TAI Conception Rates in *Bos taurus* x *Bos indicus* Cattle using GnRH, PGF and CIDR Combinations

Timed AI in Brahman-Influenced Cattle using CO-Synch + CIDR: Field Trials

Recently the CO-Synch protocol (Geary and Whittier, 1998), which involves the combined use of GnRH and PGF, has been coupled with an exogenous source of progesterone, the CIDR. This combination (Co-Synch + CIDR) appears capable of producing TAI conception rates that average consistently above 50% (Lamb et al., 2001, Larson et al., 2004 a, b) in *Bos taurus* females, which are greater than those reported previously using other traditional methods (Stevenson et al., 2003a). Improved outcomes have been linked in part to the ability of exogenous progesterone to induce ovulation in a high proportion of anestrous cows (Stevenson et al., 2000) and to reduce the occurrence of estrus before TAI (DeJarnette et al., 2001; Martinez et al., 2002). However, in environments that are predominantly subtropical to tropical, the need to utilize *Bos indicus*-influenced females may reduce the efficiency of synchronization and TAI conception rates compared to *Bos taurus* females (Lemaster et al., 2001; Hiers et al., 2003). Although not well-characterized, this may occur due to increased excitability and stress in *Bos indicus*-influenced cattle when subjected to intense management and (or) differences in timing of ovarian events. Reports specifically evaluating the CO-Synch + CIDR for TAI in *Bos indicus*-influenced cattle are limited.

Objectives of studies reported herein were to 1) evaluate the use of the CO-Synch + CIDR protocol for synchronization of ovulation and TAI in *Bos indicus*-influenced cattle, 2) compare cumulative pregnancy rates after CO-Synch + CIDR synchronization and TAI to those in a traditional management (TM) scheme 3) evaluate specific ovarian, hormonal, and estrual events associated with the use of CO-Synch + CIDR and related protocols to identify aspects of the system that may contribute to reductions or improvements in efficiency of the

protocol in *Bos indicus*-influenced cattle.

Procedures. All cattle in this experiment were required to have a minimum BCS of 4.8 (1-9 scale), and if suckled, be at least 50 d postpartum. Cows were stratified by parity and BCS at each location, and assigned randomly in groups of not less than 25 to either a **TM** control or a synchronized, TAI group (**CO-Synch + CIDR**). The regimen included the insertion of a CIDR (Pfizer Animal Health, New York, NY) and an injection of GnRH (GnRH-1; 100 µg Cystorelin, Merial, Iselin, NJ) on d 0, removal of the CIDR and injection of PGF (25 mg Lutalyse; Pfizer Animal Health, New York, NY) on d 7, and an injection of GnRH (100 µg GnRH-2) and TAI at 48 h after PGF and CIDR removal (d 9). Cows in TM were managed as normal for each location, with both groups placed with fertile bulls for at least 60 d beginning 5 to 7 d after TAI. Pregnancy rates to TAI were determined in both groups by transrectal ultrasonography 30 d after TAI in the CO-Synch + CIDR group. Final pregnancy rates were assessed by palpation per rectum 45 d after the end of the breeding season. Control (TM) cattle were not available at all locations for management comparisons to CO-Synch + CIDR. Therefore, while there were 266 cows and heifers synchronized for TAI, only 170 were managed with a contemporary set of TM females (n = 165) for comparison.

Results. Timed AI pregnancy rates in all females synchronized with CO-Synch + CIDR are summarized in **Table 6**. Pregnancy rates using the CO-Synch + CIDR protocol and TAI at 48 h after CIDR removal averaged about 39%. Pregnancy rates were not affected by location (n = 4), yr (n = 2), BCS, d postpartum, parity, sire or AI technician. **Table 7** summarizes cumulative pregnancy rates after 30 and 60 d of breeding (TAI and/or natural service) for the CO-Synch + CIDR group and the contemporary control groups (TM). Overall, cumulative pregnancy rates were greater ($P < 0.05$) in synchronized cows at 30 and 60 d of the breeding season than in the TM group.

Table 6. Timed AI (TAI) pregnancy rates in nulliparous heifers, postpartum primiparous heifers, and pluriparous cows synchronized with CO-Synch + CIDR

Source	N	TAI Pregnancy Rate, %
Nulliparous	89	39.3
Primiparous	34	35.3
Pluriparous	143	39.9
Total	266	39.1

Table 7. Cumulative pregnancy rates after 30 and 60 d of breeding in nulliparous heifers, primiparous heifers, and pluriparous suckled cows synchronized with CO-Synch + CIDR followed by timed AI (TAI) or managed using traditional methods (TM)

		Cumulative Pregnancy Rate[a], %		
Source	Treatment	N	30 Days	60 Days
Nulliparous	CO-Synch + CIDR	62	75.8	95.2
	TM	71	71.8	88.7
Primiparous	CO-Synch + CIDR	34	67.6	100.0
	TM	28	60.7	89.3
Pluriparous	CO-Synch + CIDR	74	75.7[b]	94.6
	TM	66	51.5[c]	90.9
Total	CO-Synch + CIDR	170	74.1[b]	95.9[b]
	TM	165	61.8[c]	89.7[c]

[b,c] Percentages in columns with uncommon superscripts differ $P < 0.05$.

Follicular, Luteal and Hormonal Characteristics of CO-Synch and CO-Synch + CIDR Synchronization

Timed artificial insemination at 48 h after CIDR removal with CO-Synch + CIDR synchronization failed to produce acceptable (≥ 50%) TAI conception rates in *Bos indicus* influenced cattle. We hypothesize that CO-Synch + CIDR produced such results due to failure of one or more aspects of the procedure, which could potentially include: failure to 1) optimize the frequency of ovulation or regression of follicles on d 0; 2) cause optimally timed emergence of a new follicular wave between days 1 to 4; 3) efficiently regress the CL at the time of PGF; 4) produce an optimally-receptive preovulatory follicle at the time of the second GnRH injection.

Procedures. To gain further insight into ovarian and hormonal events associated with CO-Synch + CIDR synchronization in *Bos indicus*-influenced cattle, 100 postpartum Brahman x Hereford (F_1) cows were divided into four replicates of 25 females each. Criteria for inclusion in the study and stratification procedures were similar to the field trials discussed above. Cattle were placed in pens measuring 25.6 x 9.6 m 8 d before the onset of treatments, with five cow-calf pairs per pen, and fed according to National Research Council (NRC) recommendations for lactating beef cows. Half of the cows within each replicate (n = 12-13) received the CO-Synch + CIDR treatment and half the CO-Synch treatment alone without the CIDR. The CO-Synch + CIDR treatment was as described previously in Experiment 1.

Transrectal ultrasonography was performed every other d from d -8 to d 0, and then daily from d 0 until ovulation or d 12, whichever occurred first. Blood samples were collected via puncture of a coccygeal tail vessel following the same schedule as for transrectal ultrasonography. Serum was assayed by RIA for progesterone. Concentrations of

LH were also determined in blood samples collected during the first replicate at 0, 30, 60 and 120 min relative to GnRH injections on d 0 (GnRH-1) and 9 (GnRH-2). Cows were observed for estrus 3x daily from d 0 until ovulation or d 12, whichever occurred first, with the aid of androgenized cows. On d 12, all cows were returned to their pasture with clean-up bulls for a 60-d breeding period. Pregnancy determination was performed by transrectal ultrasonography at 30-32 d post AI, and re-confirmed by palpation per rectum 45 d after bulls were removed.

Results. Mean (± SEM) age, BCS, BW, and d postpartum were 8.8 ± 0.3 yr, 5.3 ± 0.07 (range 4-8), 543 ± 7.4 kg, and 77 ± 0.66 d, respectively. Ovarian and reproductive variables are summarized in **Table 8**. No differences in the major ovarian and reproductive endpoints were observed between CO-Synch + CIDR and CO-Synch. Therefore, data for both treatments are presented as pooled means. Data are also presented relative to cyclic status at the onset of treatments. The number of non-cyclic cows ovulating after GnRH-1 was greater ($P < 0.01$) than for cyclic cows. The number ovulating in response to GnRH-2 also differed between cyclic and non-cyclic cows; however, in this case, cyclic cows had the greater ($P < 0.05$) response. Mean follicular diameters are presented in **Table 9**. Non-cyclic cows had greater ($P < 0.05$) mean follicular size at PGF than cyclic cows, and therefore a greater ($P < 0.05$) follicular growth rate. Follicular sizes were not different at the subsequent stages.

Data were also summarized relative to presence or absence of ovulation after GnRH-1 to evaluate their effects on subsequent ovarian responses (**Table 10**). More ($P < 0.01$) cows that ovulated after GnRH-1 developed a synchronized follicular wave compared to cows that did not ovulate. Moreover, there was a trend ($P = 0.15$) for ovulation rates after GnRH-2 to be greater in cows that ovulated in response to GnRH-1 than cows that did not. Also, ovulation and TAI pregnancy rates after GnRH-2 were increased ($P < 0.01$) in cows that developed a synchronized follicular wave after GnRH-1 compared to cows that did not develop a new wave (**Table 11**).

Mean serum concentrations of progesterone are illustrated in **Figure 1**. As expected, concentrations of progesterone from d -8 to 0 relative to GnRH-1 differed between cyclic and non cyclic cows. After CIDR insertion (d 0), serum progesterone increased ($P < 0.001$) acutely for both cyclic and non-cyclic cows that received the CO-Synch + CIDR treatment. Serum concentrations of progesterone on d 1 were highest ($P < 0.05$) for cyclic cows receiving CO-Synch + CIDR compared to all other groups. Mean concentrations of progesterone did not differ between cyclic cows treated with CO-Synch + CIDR and non-cyclic cows treated with CO-Synch + CIDR. Mean concentrations of progesterone were lowest ($P < 0.01$) for the non-cyclic CO-Synch group compared to all others, and mean serum concentrations of progesterone never exceeded 1 ng/ml during the treatment period. After injection of PGF and CIDR removal (d 7), progesterone decreased below 1 ng/ml within 24 h in all groups and remained low until d 12 when mean progesterone exhibited a slight increase ($P = 0.09$) in cyclic, CO-Synch treated cows. The latter was caused by two cows that ovulated asynchronously before d 9.

Table 8. Ovarian and reproductive outcomes in postpartum suckled cows synchronized with CO-Synch and CO-Synch + CIDR and for cycling and non-cycling cows (Treatments did not differ; therefore, data are presented as pooled means.)

Variable	All Cows	Ovarian Status	
		Cycling	Non-cycling
No. Cows	100	78	22
Estrous cycling, %	78	-	-
Response to GnRH-1, %			
Ovulating	40	33[c]	64[d]
Follicle regression	39	40	36
Not responding	21	27[c]	0[d]
New follicular wave after GnRH-1, %			
Synchronized[a]	60	56	73
Not synchronized[b]	31	35	18
No emergence	9	9	9
Day of emergence	2.5 ± 0.12	2.4 ± 0.15	2.75 ± 0.23
CL regression, % (No.)	92 (75/81)	91(61/67)	100(14/14)
Ovulatory Response to GnRH-2, %			
0-24 h after TAI	15	14.1	36.3
24-48 h after TAI	57	62.8	18.2
Total	72	76.9[c]	54.5[d]
TAI pregnancy, %			
Ovulation 0-24 h after AI	9	10.3	4.5
Ovulation 24-48 h after AI	24	23	27.3
Total	33	33.3	31.8

[a] Cows that developed a follicular wave from d 1 to d 4 after GnRH-1.
[b] Cows that developed a follicular wave before day 1 and after day 4.
[c,d] Percentages within row with uncommon superscripts letters differ ($P < 0.01$).

Table 9. Mean follicular diameters in postpartum suckled cows synchronized with CO-Synch and CO-Synch + CIDR at different stages of the experiment (Treatments did not differ; therefore, data are presented as pooled means.)

Variable	All Cows	Ovarian Status	
		Cyclic	Anestrous
Diameter of the largest Follicle, mm (range)			
GnRH-1	9.6 ± 0.2 (4.0 - 12.95)	9.4 ± 0.2 (4.0 - 12.95)	10.2 ± 0.3 (6.8 - 12.3)
PGF	9.8 ± 0.2 (6.3 - 15.4)	9.6 ± 0.2[a] (6.3 – 13.9)	10.5 ± 0.2[b] (7.0 - 15.4)
GnRH-2	11.1 ± 0.2 (6.0 - 15.4)	11 ± 0.3 (6.0 – 15.4)	11.4 ± 0.5 (7.5 – 14.5)
Before ovulation	11.6 ± 0.2 (8.1-15.4)	11.4 ± 0.2 (8.1 – 15.4)	12.2 ± 0.5 (9.1 – 14.7)
Follicular growth rate, mm/day	1.4 ± 0.06	1.3 ±0.07[a]	1.7 ± 0.1[b]

[a,b] Percentages within row with uncommon superscripts letters differ ($P < 0.05$).

Table 10. Effects of the response to the first GnRH injection (GnRH-1) on subsequent ovarian and reproductive outcomes in cows synchronized with CO-Synch and CO-Synch + CIDR (Treatments did not differ; therefore, data are presented as pooled means.)

Variable	Ovulatory Response to GnRH-1	
	Ovulating	Not Ovulating
	No. (%)	No. (%)
No of cows	40	60
Synchronized follicular wave		
Yes	35 (88)[a]	25 (42)[b]
No	5 (12)	35 (58)
Ovulated after GnRH-2		
Yes	32 (80)	40 (67)
No	8 (20)	20 (33)
TAI pregnancy	15 (37)	18 (30)

[a,b] Percentages within rows with uncommon superscripts differ ($P < 0.01$).

Table 11. Effects of synchronized follicular wave emergence after GnRH-1 on subsequent ovarian and reproductive outcomes in cows synchronized with CO-Synch and CO-Synch + CIDR (Treatments did not differ; therefore, data are presented as pooled means.)

	Occurrence of Synchronized Follicular Wave after GnRH-1	
	Yes	No
Variable	No. (%)	No. (%)
No of cows	60	40
Ovulation after GnRH-2		
Yes	51 (85)[a]	21 (52)[b]
No	9 (15)	19 (48)
TAI pregnancy	26 (43)[a]	7 (17)[b]

[a,b] Percentage within row with uncommon superscripts letters differ (P < 0.01).

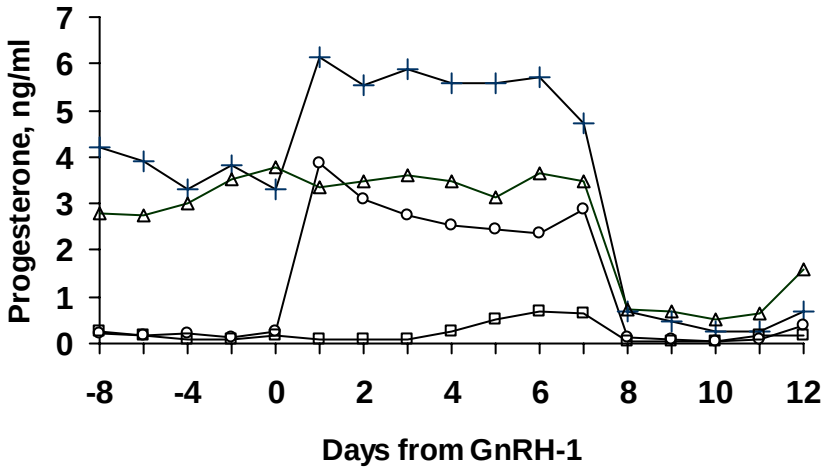

Figure 1. Concentrations of progesterone in serum of cycling (+; n = 39) and non-cycling (○; n = 11) cows treated with CO-Synch + CIDR, and cycling (Δ; n = 39) and non-cycling (□; n = 11) cows treated with CO-Synch only

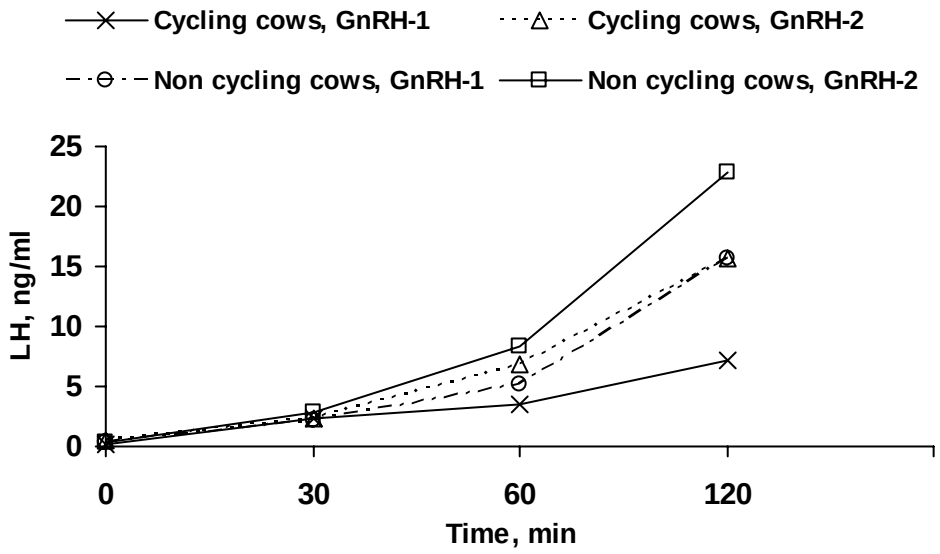

Figure 2. Mean serum concentrations of LH after GnRH-1 in cows that were cycling (n = 15) and not cycling (n = 10) before treatment onset, and in cyclic (n = 14) and non-cyclic (n = 9) cows after GnRH-2. Cows not cycling before treatment onset had greater ($P < 0.05$) induced release of LH after GnRH-1 than cycling cows, but not after GnRH-2 (cycling status x time, $P < 0.05$).

Release of LH induced by GnRH was considered to have occurred when an increment in the concentration of LH of at least 2 SD above the baseline was observed. Two cows had an endogenous LH surge before GnRH-2 and were excluded from further analysis in relation to this variable. The latter conclusion was based on the fact that concentrations of LH during the sampling period were in a declining mode. All other cows (n = 23) in replicate 1 exhibited increases ($P < 0.01$; **Figure 2**) in LH after both GnRH-1 and 2. Magnitude of release did not differ between treatments (CO-Synch + CIDR vs CO-Synch). Non-cyclic cows had an induced LH release greater ($P < 0.05$; Fig 3) than cyclic cows after GnRH-1, but concentrations of LH did not differ between cyclic and non-cyclic cows after GnRH-2. A time x cyclic status interaction ($P < 0.05$) associated with GnRH-induced LH release was observed after GnRH-2. Also, overall mean concentrations of LH were greater ($P < 0.01$) after GnRH-2 than after GnRH-1 (7.2 ± 0.71 and 4.3 ± 1.1, respectively).

Distribution of Estrus and Ovulation in Cows Programmed with Select-Synch + CIDR

Timing of insemination relative to ovulation is one of the most important factors affecting the outcome of synchronized, timed breeding in cattle. This becomes even more critical when TAI is combined with an injection of GnRH (GnRH-2) as in the CO-Synch + CIDR protocol. Based upon previously published reports, we employed TAI at 48 h after CIDR removal/PGF injection in the experiments summarized above. However, since TAI pregnancy rates were low and not similar to those reported for *Bos taurus* cattle, a third experiment was performed to determine when GnRH-2 and TAI would be most appropriate

in our animal model.

Procedures. Fifty postpartum, suckled Brahman x Hereford (F-1) females were used. Criteria for inclusion were the same as for Experiments 1 and 2. Cows in the study were primiparous heifers (n = 32), and pluriparous cows (n = 18). Females were placed in pens as in Experiment 1, with 8 cow-calf pairs per pen, and fed according to NRC recommendations (1996). All cows received the same synchronization regimen as described in Experiment 1, but the second GnRH injection (GnRH-2) was not administered. Transrectal ultrasonography was performed the day of CIDR removal, and then every 12 h until ovulation or d 11, whichever occurred first. Estrus detection was performed by visual observation every 3 hours from CIDR removal through d 11. Blood samples were collected on d -21, -11, 0 (CIDR insertion), 7, 8 and 9 following the same procedures described in Experiment 2. Serum was assayed by RIA for progesterone in all samples collected as described in Experiment 2 to retrospectively estimate cyclicity and luteal regression.

Results. Neither ovarian cyclic status (cyclic 60%, non-cyclic 40%) nor parity affected the number of cows exhibiting estrus or ovulating. Mean age (± SEM), BCS, BW, and d postpartum were 5.81 ± 0.5, 5.6 ± 0.1, 565 ± 10.2 kg and 60 ± 1.1 d, respectively. On d 7, a majority of cows (72%) had a visible CL at ultrasound and 97% of those exhibited CL regression after PGF, as evidenced by a reduction in ultrasonographic size and morphology of the CL and a reduction in serum concentrations of progesterone to less than 1 ng/mL. No cows were observed in estrus during the first 48 h after CIDR removal. The majority (75 %) of estrual events was observed between 60 and 82 h after CIDR removal (**Figure 3**). Mean size of the largest follicle at CIDR removal and 48 h after removal were 9.45 ± 0.26 and 11.65 ± 0.26 mm, respectively (**Table 12**). Follicular diameter was greater for cows showing standing estrus than for cows showing only non-standing estrous behavior or no estrous behavior at both CIDR removal ($P < 0.05$) and 48 h after removal ($P < 0.01$). Cows that showed standing estrus had more ($P < 0.01$) ovulations than cows not standing.

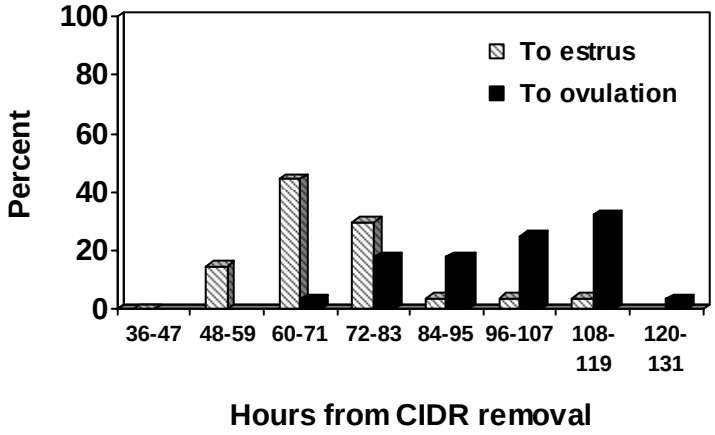

Figure 3. Distribution of estrus (n = 27) and ovulation (n=28) in suckled *Bos indicus x Bos taurus*, F_1 cows treated with Select Synch + CIDR

Table 12. Follicular, Estrual, and ovulatory events in suckled *Bos indicus x Bos taurus,* F_1 cows programmed with Select Synch + CIDR

Variable	All Cows	Estrus		
		Standing	Non-Standing	None
Number of Cows	50	27	14	9
Mean follicle size, mm				
At CIDR removal (range)		10.1 ± 0.4[a] (6.1 - 13.5)	8.8 ± 0.6[b] (6.0 - 12.8)	8.62 ± 0.3[b] (7.2 - 10.2)
48 h after CIDR removal (range)		12.6 ± 0.4[c] (9.6 - 14.7)	10.4 ± 0.4[d] (8.3 - 13.8)	10.83 ± 0.4[d] (9.7 - 13.9)
Ovulating, %	56	93[c]	21[d]	0[d]
Mean ovulatory follicle Size, mm (range)	12.9 ± 0.3 (9.4 - 15.1)	12.9 ± 0.3 (9.4 - 15.1)	13.5 ± 0.5 (12.5- 14.1)	-
Mean interval from CIDR removal to:				
Standing estrus, h (range)		70 ± 2.9 (49 - 108)	-	-
Ovulation, h (range)	99 ± 2.8 (68 - 127)	99 ± 3 (68-127)	104 ± 11 (82 - 117)	-
Mean interval from estrus to ovulation, h (range)		29 ± 2.2 (5 - 55)	-	-

[a,b] Percentage within row with uncommon superscripts letters differ ($P < 0.05$).

[c,d] Percentage within row with uncommon superscripts letters differ ($P < 0.01$).

SUMMARY AND CONCLUSIONS

Ovsynch vs SMB. Synchronization of ovulation in *Bos indicus*-influenced beef cows managed in a subtropical environment using an earlier-generation, progestin-based (SMB) or GnRH/prostaglandin-based (Ovsynch) synchronization protocol resulted in TAI conception rates comparable to each other (Williams et al., 2002) and similar to those reported in *Bos taurus* cattle (Geary and Whittier, 1998). The progestin-based treatment utilizing only the norgestomet implant and a prostaglandin (NP) tended to give lower results than both SMB and Ovsynch in suckled cows, and this difference was observed primarily in cows less than 60 d PP.

In nulliparous heifers confirmed pubertal, the NP protocol yielded TAI conception rates greater than both SMB and Ovsynch (Williams et al., 2002). The lower conception rate

in Ovsynch-treated heifers compared to SMB can only be obviated by inseminating heifers observed in estrus (non-synchronized females) for 9 d during the synchronization period in combination with timed AI of the balance of heifers on d 11. In the current study, and in previous reports, failure of heifers to respond to the first GnRH injection results in a reduced number of heifers with a functional CL. Therefore, these heifers are not synchronized and exhibit estrus before the targeted insemination period.

CO-Synch + CIDR. From these series of experiments summarized above (Saldarriaga
et al., 2005 a, b), we concluded that the CO-Synch + CIDR protocol in which GnRH-2 and TAI are employed at 48 h after CIDR removal/PGF fails to synchronize ovulation and optimize TAI pregnancy rates in *Bos indicus x Bos taurus* females. This appeared to occur in the current experiments primarily because the proportion of cows that exhibited a synchronized follicular wave after GnRH-1 was only 60%. The remaining 40% of females, those without a synchronized follicular wave, introduced marked variability into the system relative to follicular maturity and health, and oocyte fertility at the time of GnRH-2. In addition, the timing of GnRH-2 for inducing ovulation does not appear to be optimal relative to follicular maturity in the 60% of cows that developed what appeared to be a synchronized follicular wave after GnRH-1.

In order to optimize TAI pregnancy rates utilizing CO-Synch + CIDR or similar approaches, it will probably be necessary to delay the time of GnRH-2/TAI past 48 h. Recent reports from the Midwestern U.S. indicate that TAI at 66 h can markedly increase pregnancy rates in *Bos taurus* females (Schafer et al., 2004; Walker et al., 2005). However, given that only 60% of cows in the current studies formed a new follicular wave after GnRH-1, it will also be necessary to elucidate why GnRH-1 does not result in a higher number of ovulations and new follicular wave recruitment. While it is known that the stage of the cycle when the Ovsynch, CO-Synch and CO-Synch + CIDR protocols are initiated can affect the efficiency of synchronization of a new follicular wave, it is not likely that use of pre-synchronization procedures to improve this outcome can be economically-employed in commercial beef cattle enterprises in the southern U.S. Other options include the use of an estrogen to improve synchrony of follicular wave emergence in place of GnRH-1 (Martinez et al., 2002). However, given the fact that there are currently no commercially-available estrogens on the market in the U.S. and their use is not approved by the FDA, employing estrogens in synchronization of beef cattle is problematic at best. Another potential option is the use of hCG in place of GnRH-1 as a means of pharmacologically inducing a greater number of ovulations and thereby improving follicular wave synchrony; however, no data are available on this approach.

Finally, efforts to account for lower pregnancy rates in *Bos indicus* x *Bos taurus* influenced compared to straight *Bos taurus* cattle in relation to synchronization of ovulation and TAI often lead to conjecture about potential differences in overall fertility of straightbred Bos taurus and Bos indicus x Bos taurus crossbreds . Based upon the data presented herein, it should be clear that low pregnancy rates in these systems are accounted for mainly by failure to precisely control follicular growth and ovulation. Cumulative pregnancy rates after 30 and 90 d of breeding in our studies with both older (SMB) and

newer (CO-Synch + CIDR) technologies consistently average greater than 75 and 90%, respectively, confirming that the cattle used for these experiments were highly fertile. Owing to the effects of hybrid vigor, the Brahman x Hereford F_1 (used extensively in our trials) is universally considered to be one of the most fertile of all commercial beef females in subtropical environments. Nonetheless, TAI pregnancy rates of 39% or less using the most advanced technology available are unacceptable and must be improved. In order for CO-Synch + CIDR and other similar, high input technologies to be economically-employed in the southern region of the U.S. and in other sub-tropical and tropical environments, it is likely that TAI pregnancy rates will have to consistently exceed 50%.

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NOTES

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ESTRUS SYNCHRONIZATION METHODS FOR EMBRYO TRANSFER IN *BOS INDICUS* CATTLE

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Introduction

Reproduction is the main limiting factor in production efficiency of beef cattle. The largest loss of the potential calf crop occurs because cows fail to become pregnant due to anestrus and postpartum infertility (Short et al., 1990). Estrus synchronization is a method that has been studied for 40 years to control reproductive efficiency of beef cattle. Its purpose is to manipulate the estrous cycle of a herd to allow for timed artificial insemination and/or superovulation with subsequent embryo transfer into recipient cows or heifers at a predetermined time (Odde, 1990). Estrus synchronization allows for increased production efficiency of a herd by achieving shorter breeding and calving seasons, along with possible control of anestrous cows (Odde, 1990). In turn, the shorter seasons allow for lower labor requirements throughout the year and increase the percentage of the herd that calves early in the calving season. Calves that are born earlier have heavier weaning weights and allow for a longer postpartum period before re-breeding (Burke and Macmillan, 1996). Estrous synchronization also allows for disease control among herds and genetic improvements through the use of AI/ET.

Estrous Synchronization Methods

Estrous synchronization, as the name implies, is the manipulation of the estrous cycle in order to bring a group of females, at random stages of the estrous cycle, into estrus at a precise time. The following section will discuss means to manipulate estrous cycles and synchronization in Bos Indicus influenced cattle.

Progesterone is the dominant ovarian hormone present in the circulation during the estrous cycle and is secreted from the corpus luteum (CL). This period of the estrous cycle is also referred to as the luteal phase and lasts from the time of ovulation until regression or luteolysis of the CL near the end of the cycle. Progestins suppress estrus in cattle and have been used extensively to alter the estrous cycle. Studies during the 1940s revealed that estrus could be delayed and therefore synchronized by administration of exogenous progesterone to cattle or sheep. This led to many studies in which progestins were administered by injection, released by an intravaginal sponge, or fed for a period of up to and exceeding the length of the estrus cycle to synchronize estrus following the cessation of administration. It was determined that an increased duration of progestin

administration resulted in an increased rate of estrus synchronization. However, fertility was compromised following administration of progestins for 14 d or longer and pregnancy rates were unacceptable (Odde, 1990).

One of the first methods used to synchronize estrus in cattle was the long-term feeding of melengestrol acetate (MGA; Zimbelman and Smith, 1966). MGA is a synthetic progestin that suppresses estrus when fed at the rate of 0.5 mg/hd/d. MGA is still utilized extensively today by feedlots to suppress estrus in beef heifers that are being fed for harvest and used for estrous synchronization of heifers with a 14 d feeding program followed by a single injection of PGF 17 d after withdrawl of MGA feeding. It is well established that administration of exogenous progesterone can hasten the attainment of puberty in heifers and cause postpartum anestrous cows to become estrous cycling. The ability of exogenous progestins to induce estrus in anestrous cattle has been attributed to, in part, increased LH secretion both during and after treatment. It has been reported that progestin treatment increased LH secretion in postpartum beef (Garcia-Winder et al., 1986) as well as seasonal dairy cows (Rhodes et al., 2002). In addition, LH secretion following weaning was increased in cows with prior exposure to progestin (Bruel et al., 1993). This induced increase in LH is important because it mimics the proestrus increase in LH leading to the preovulatory LH surge (Day, 2004).

Prostaglandins are lipids consisting of 20-carbon unsaturated hydroxy fatty acids derived from arachidonic acid. Prostaglandin $F_2\alpha$ (PGF) is produced by the uterine endometrium and is responsible for luteolysis, or degradation of the CL, in cattle. The bovine estrous cycle can be divided into two phases, the follicular phase and the luteal phase. The follicular phase is characterized by follicle growth culminating in selection of a dominant follicle and subsequent ovulation. The luteal phase is the longest phase of the cycle (approximately d 6 to d 16 of the estrous cycle). The luteal phase is characterized by the functioning CL secreting progesterone. During the late luteal phase (d 16 – 18 of the cycle) PGF is released from the uterus and binds to the CL causing luteal regression. During the 1970s, it was discovered that PGF was luteolytic in cattle and could be used to synchronize estrus (Lauderdale et al., 1974). It was later determined that PGF had limited utility in synchronizing estrus because it was only effective in cattle that were cycling and had a CL (d 5 to 17 of the cycle). Therefore, prepubertal heifers, anestrous females, females on d 0 to 4 of the estrous cycle, and females in the final days of the estrous cycle subsequent to luteolysis were not responsive. It was later determined that the interval from treatment with PGF to estrus was dependent upon the stage of the follicular wave at treatment (Lucy et al., 1992). Larger, more mature follicles ovulated sooner than their smaller, less mature counterparts.

Another method of estrous synchronization includes the use of gonadotropin releasing hormone (GnRH) or GnRH agonists in combination with and injection of PGF. This method is available to consumers as the Ovsynch program (Pursley et al., 1996). The protocol includes an injection (im) of GnRH (100 ug) and an injection of PGF (25 mg, im) 7 days later. Through day 9 of the protocol, 80% of treated cows and heifers were detected in estrus and fertility rates were as high as 85%. GnRH eliminates the large follicles by ovulation or atresia and induces emergence of a new follicular wave

within 3 to 4 days after treatment during any stage of the estrous cycle (Twagiramungu et al., 1995). By the addition of CIDR with this GnRh program (Co-Synch) the additive effects of progesterone along with the ability to initiate a new follicle wave with the GnRh.

Bos Indicus vs. Bos Taurus Cattle

Brahman cattle are significantly different in several reproductive aspects than European and Continental cattle. Brahman cattle have longer gestations (292 vs. 285 days) shorter and less intense estrus and puberty occurs at an older age. In addition the twinning rate is less in *Bos Indicus* than in *Bos taurus* (Rutledge, 1975). In addition, secretory patterns of hormone production in *Bos indicus* have been shown to be different than *Bos taurus* and may change due to photoperiod or season. However, the single most cited negative factor of the Brahman cow is the sub-standard fertility when compared to the English breeds of beef cattle (Warnick 1956, Reynolds 1963).

There are other differences in reproductive physiology between *Bos Indicus* and *Bos Taurus,* with Brahman cattle having reduced duration of estrus and a shorter period from onset of estrus to the LH surge as well as from the LH surge to ovulation (Randel, 1984). In addition, *Bos Indicus* females have lower preovulatory LH surges than *Bos Taurus females* and their luteal cells are less responsive to LH in vitro especially in the winter (Randel, 1984). *Bos Indicus* also have higher number of follicles and higher serum concentrations of insulin growth factor I. Researchers have recently found differences in timing of ovulation, fertilization or events leading up to cleavage of early embryos in Brahman cattle compared to Holsteins (Krininger et.al. 2003). Brahman and Brahman influenced females are at times more difficult pass catheters through their crooked and large cervixes and their disposition make it more difficult to handle them in pens and corrals.

Donors

Traditionally, bovine superovulation programs have utilized detected estrus followed by FSH treatments beginning between days 9 through 13 of the estrous cycle. These programs require a large amount of time commitment and are inefficient at controlling follicular waves in cattle. The objective of this study was to compare traditional superovulation regimes with those implementing the use of an intravaginal progesterone releasing device (CIDR) with injections of estradiol benzoate (2.5 mg, im) and progesterone (50 mg, im) at CIDR insertion.

Meyer et al. (2000) detected estrus and performed 103 embryo collections (Table 1). Cows that showed estrus days 1-7 of the estrous cycle, days 8-16, and days 17-24 prior to CIDR, P4, EB treatment began FSH injections four days after CIDR insertion. Superovulation was achieved with varied doses of FSH (140-400 mg, im) given bid for 4 d followed by PGF on day 3 (Brahman influenced breeds) or day 4 (Continental and English breeds) of CIDR removal depending on breed, on the third or fourth day of superovulation. Estrus was detected and artificial inseminations performed at onset of

estrus, 12 h and 24 h post-estrus. Embryos were collected 7 to 7.5 d from onset of estrus. Here were no differences in any parameter based on timing of CIDR insertion. From the results the authors concluded that donors could successfully be superovulated without regard to estrous cycle using the CIDR+P4+EB program in a commercial setting. Figures 1 and 2 compare collection results from initiation of FSH at different stages of the estrous cycle. No significant differences were detected, however it appears trends may be present and difference may not be seen due to low experimental numbers.

Table 1. Least Squares Means of superovulatory responses with or without CIDR-P4-EB.

	Total ova		Viable embryos		Degenerate		Unfertilized	
	Brahman	Cont.	Brahman	Cont.	Brahman	Cont.	Brahman	Cont.
Control	11.5^{a}	12.4^{a}	6.5^{a}	5.9^{a}	2.4^{a}	2.5^{a}	2.6^{a}	3.9^{a}
	± 0.86	± 0.86	± 0.57	± 0.58	± 0.31	± 0.31	± 0.50	± 0.50
CIDR	9.7^{b}	9.4^{b}	5.8^{a}	5.1^{a}	1.8^{a}	2.1^{a}	2.0^{a}	2.2^{b}
	± 0.97	± 0.70	± 0.65	± 0.47	± 0.34	± 0.25	± 0.56	± 0.40

$^{a-b}$Data in columns with different superscripts are different ($P < 0.05$, ± SEM, Student's t-test).
Cont = is Continental and English breeds.

Figure 1. Embryo recovery in Bos Taurus-influenced females by stage of cycle at intitiation of the CIDR protocol.

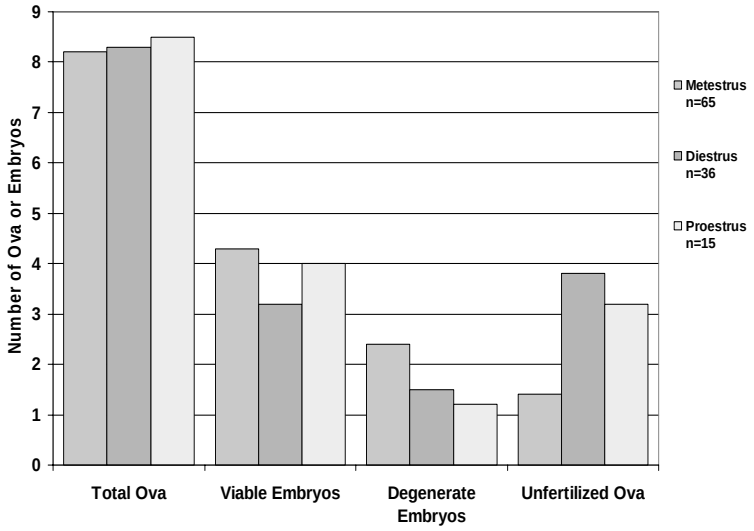
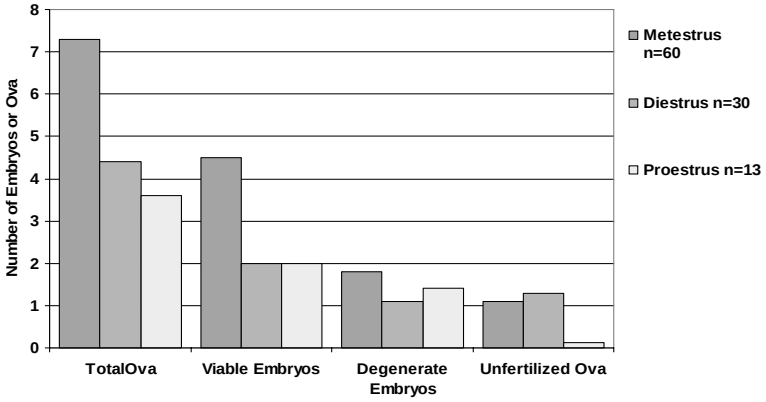


Figure 2. Embryo recovery in Bos Indicus-influenced females by stage of cycle at initiation of the CIDR protocol.



Meyer 2002

Recipients

Although much progress has been made in estrus synchronization in cattle in recent years, it remains to have the most limiting factors in widespread implementation of AI and ET technology. Most of the early protocols were developed by lengthening or shortening the luteal phase with either progesterone or prostaglandin. As researchers began to understand that estrus synchrony entailed ovulation control did programs develop to control the stage of follicular development at the beginning of treatment. Steroid hormone treatments can also be used to alter follicular growth. Both progesterone and estradiol influence the onset of subsequent follicular wave emergence and the combined effects of these steroids have demonstrated a controlled suppression on growth of the dominant follicle and is the most effective and consistent new wave emergence and subsequent ovulation control developed. Synchronization protocols allowing that enable tighter synchrony have been developed and higher overall pregnancy rates achieved (Table 2).

Table 2. Comparing ET service and pregnancy rate of PGF single injection synchronization method to CIDR+P4/E2+PGF+E2.

Synch Method	N	Synchrony (%)	Interval to Estrus (d)	Service (%)	FSCR (%)	FSPR (%)
25 mg PGF	1390	51[a]	3.6	93	63	29[a]
7d CIDR+ P4/E2+PGF+E2	753	94[b]	2.1	83	53	41[b]

[a b] Differing subscripts significant at P<0.05.
Data from Ovagenix's In-Clinic Programs

The following tables contain data from synchronization studies performed at a large Registered Brangus ranch in Central Texas. Information gained from these trials were instrumental in gaining the initial approval issues of the CIDR insert in beef cattel Post-partum Interval (PPI) has a significant effect on first service conception rate and first service pregnancy rate to embryo transfer and final pregnancy rate to natural service (Table 3). Early post-partum females had a lower ($P < 0.01$) first service conception rate and first service pregnancy rate to embryo transfer when compared to medium and late post-partum females. In addition, early post-partum females had a lower ($P < 0.01$) final pregnancy rate to natural service when compared to medium and late post-partum females. PPI did not have an effect ($P = 0.7$) on synchrony rate, interval to onset of estrus from time of CIDR removal, and service rate to embryo transfer.

Table 3. Synchrony rate, interval to estrus, first service conception rate (FSCR) and first service pregnancy rate (FSPR) to embryo transfer after CIDR administration and final pregnancy rate (Final PR) by post-partum interval (PPI) in females.

PPI	n	Synchrony (%)	Interval to Estrus (d)	Service (%)	FSCR (%)	FSPR (%)	Final PR (%)
Early (≤ 45 d)	120	99.2	1.9	79.0	46.9 [b]	31.6 [b]	84.2 [b]
Medium (46 – 75 d)	207	99.5	2.0	89.6	60.3 [a]	53.4 [a]	96.1 [a]
Late (≥ 76 d)	106	99.1	1.9	83.9	57.5 [a]	51.8 [a]	96.2 [a]

Columns with different superscripts are different; $P < 0.01$.
Meyer 2002.

Lactational state had a significant effect on synchrony rate and interval to onset of estrus from time of CIDR removal (Table 4). Non-lactating females had a higher ($P < 0.01$) synchrony rate and a significantly longer ($P < 0.01$) interval to onset of estrus from time of CIDR removal than lactating females. Lactational state had no effect ($P = 0.6$) on service rate, first service conception rate, and first service pregnancy rate to embryo transfer and final pregnancy rate to natural service.

Table 4. Synchrony rate, interval to estrus, service rate, first service conception rate (FSCR) and first service pregnancy rate (FSPR) to embryo transfer after CIDR administration and final pregnancy rate (Final PR) by lactational state of females.

Lactational State	n	Synchrony (%)	Interval to Estrus (d)	Service (%)	FSCR (%)	FSPR (%)	Final PR (%)
Non-lactating	253	95.6 [a]	2.5 [a]	90.1	50.8	43.3	87.7
Lactating	1165	86.3 [b]	2.0 [b]	89.3	55.3	43.4	83.8

Columns with different superscripts are different; $P < 0.01$.
Meyer 2002.

Duration of CIDR treatment (7 or 8-d) had a significant effect on synchrony rate (Table 5). Females that were administered a 7-d CIDR had a lower ($P < 0.01$) synchrony rate than did those females that received an 8-d CIDR. There was no difference ($P = 0.8$) in interval to onset of estrus from time of CIDR removal, service rate, first service conception rate or first service pregnancy rate to embryo transfer and final pregnancy rate to natural service between females that received either a 7 or 8-d CIDR.

Table 5. Synchrony rate, interval to estrus, service rate, first service conception rate (FSCR) and first service pregnancy rate (FSPR) to embryo transfer after CIDR administration and final pregnancy rate (Final PR) by CIDR administration for a duration of either 7 or 8 days.

Days of CIDR Insertion	n	Synchrony (%)	Interval to Estrus (d)	Service (%)	FSCR (%)	FSPR (%)	Final PR (%)
7	712	82.9 [b]	1.8	90.2	54.6	41.9	78.2
8	457	92.2 [a]	1.7	87.9	56.5	46.6	86.7

Columns with different superscripts are different; $P < 0.01$.
Meyer 2002.

Administration of estradiol benzoate (EB) 24 h post-CIDR removal had a significant effect on synchrony rate, interval to onset of estrus from time of CIDR removal, and first service pregnancy rate to embryo transfer (Table 6). Females that received EB 24 h post-CIDR removal had a higher ($P < 0.01$) synchrony rate and first service pregnancy rate to embryo transfer than females that received No EB. Females that received No EB 24 h post-CIDR removal had a longer ($P < 0.01$) interval to onset of estrus from time of CIDR removal when compared to females that received EB. There was no difference ($P = 0.5$) in service rate, first service conception rate to embryo

transfer, and final pregnancy rate to natural service between females that were treated with EB and those that were not.

Table 6. Synchrony rate, interval to estrus, service rate, first service conception rate (FSCR) and first service pregnancy rate (FSPR) to embryo transfer after CIDR administration and final pregnancy rate with estradiol benzoate (EB) or without estradiol benzoate (No EB) administration 24 h post-CIDR removal.

Treatment	N	Synchrony (%)	Interval to Estrus (d)	Service (%)	FSCR (%)	FSPR (%)	Final PR (%)
EB	1041	94.3 [a]	1.5 [b]	88.7	55.2	46.1 [a]	85.3
No EB	377	73.5 [b]	2.5 [a]	91.5	52.8	37.2 [b]	82.2

Columns with different superscripts are different; P < 0.01. Meyer 2002.

Parity had a significant effect on synchrony rate, interval to onset of estrus, service rate to embryo transfer and final pregnancy rate (Table 7). Primiparous females had a higher (P < 0.01) synchrony rate and final pregnancy rate than did multiparous females. Multiparous females had a longer (P < 0.01) interval to onset of estrus from time of CIDR removal than did primiparous females. Multiparous females also had a higher (P < 0.01) service rate than did primiparous females. There was no difference (P = 0.4) between first service conception rate and first service pregnancy rate to embryo transfer between primiparous and multiparous females.

Table 7. Synchrony rate, interval to estrus, service rate, first service conception rate (FSCR) and first service pregnancy rate (FSPR) to embryo transfer after CIDR administration and final pregnancy rate (Final PR) by parity in both lactating and non-lactating females.

Parity	n	Synchrony (%)	Interval to Estrus (d)	Service (%)	FSCR (%)	FSPR (%)	Final PR (%)
Primiparous	250	91.4 [a]	1.8 [b]	83.6 [b]	58.4	44.4	95.2 [a]
Multiparous	1168	87.2 [b]	2.1 [a]	90.7 [a]	53.8	43.1	82.2 [b]

Columns with different superscripts are different; P < 0.01. Meyer 2002.

There was a significant interaction between parity and EB administration on synchrony and service rate (Table 8). Primiparous and multiparous females that were administered EB 24 h post-CIDR removal had a higher (P < 0.01) synchrony rate than primiparous and multiparous females that were not administered EB. Multiparous

females that received No EB had a higher ($P < 0.01$) service rate to embryo transfer than multiparous females that received EB.

Between treatments, primiparous females that were not administered EB had a higher ($P < 0.01$) synchrony rate than multiparous females that received No EB. However, multiparous females that were not administered EB had a higher ($P < 0.01$) service rate than did primiparous females that received No EB. There was no difference ($P = 0.3$) between synchrony rate and service rate to embryo transfer in both primiparous and multiparous females that received EB 24 h post-CIDR removal.

Table 8. Synchrony rate and service rate after CIDR administration by parity of female of female with estradiol benzoate (EB) or without estradiol benzoate (No EB).

Parity	Synchrony (%)				Service (%)			
	EB	(N)	No EB	(N)	EB	(N)	No EB	(N)
Primiparous	93.7 $^{a\,1}$	(191)	86.2 $^{b\,1}$	(87)	85.8 $^{a\,1}$	(191)	78.4 $^{a\,2}$	(87)
Multiparous	94.5 $^{a\,1}$	(923)	70.9 $^{b\,2}$	(412)	89.3 $^{b\,1}$	(923)	94.7 $^{a\,1}$	(412)

Columns with different numerical superscripts are different and rows with different alphabetical superscripts are different; $P < 0.01$. Meyer 2002.

There was a significant interaction between parity and EB administration 24 h post-CIDR removal on first service conception rate and first service pregnancy rate to embryo transfer (Table 9). Primiparous females that received No EB had a higher ($P < 0.01$) first service conception rate and first service pregnancy rate to embryo transfer than did multiparous females that received No EB. There was no difference ($P = 0.6$) in first service conception rate and first service pregnancy rate in both primiparous and multiparous females that were treated with or without EB administration 24 h post-CIDR removal.

Table 9. First service conception rate (FSCR) and first service pregnancy rate (FSPR) to embryo transfer after CIDR administration by parity of female with estradiol benzoate (EB) or without estradiol benzoate (No EB).

Parity	FSCR (%)				FSPR (%)			
	EB	(N)	No EB	(N)	EB	(N)	No EB	(N)
Primiparous	54.9 $^{a\,1}$	(191)	67.2 $^{a\,1}$	(87)	43.7 $^{a\,1}$	(191)	45.9 $^{a\,1}$	(87)
Multiparous	55.2 $^{a\,1}$	(923)	49.8 $^{a\,2}$	(412)	46.6 $^{a\,1}$	(923)	35.4 $^{b\,2}$	(412)

Columns with different numerical superscripts are different and rows with different alphabetical superscripts are different; $P < 0.01$. Meyer 2002.

Estradiol benzoate (EB) administration, (1 mg, im) at CIDR insertion had a significant effect on final pregnancy rate to natural service (Table 10). Females that were treated with EB at CIDR insertion had a higher ($P < 0.05$) final pregnancy rate than did those females that received No EB at CIDR insertion. EB administration at CIDR insertion had no effect ($P = 0.7$) on synchrony rate, interval to estrus from time of CIDR removal, service rate, first service conception rate and first service pregnancy rate to embryo transfer.

Table 10. Synchrony rate, interval to estrus, first service conception rate (FSCR) and first service pregnancy rate (FSPR) to embryo transfer after CIDR administration and final pregnancy rate (Final PR) with estradiol benzoate (EB) or without estradiol benzoate (No EB) at time of CIDR insertion.

Treatment CIDR In	n	Synchrony (%)	Interval to Estrus (d)	Service (%)	FSCR (%)	FSPR (%)	Final PR (%)
EB	85	98.8	2.0	82.8	64.6	54.1	98.8 [a]
No EB	348	99.4	1.9	84.9	55.1	47.5	91.4 [b]

Columns with different superscripts are different; $P < 0.05$. Meyer 2002.

Table 11. Use of CIDR and Estradiol Synchronization in Brahman-Influenced Recipients.

	Total	No. Trans. %	No. Preg. (conception %)
Estrus Observed	860(87%)	765(89%)	443(58%)
Estrus Not Observed	137(13%)	77(56%)	50(65%)
Total	997(100%)	842(85%)	493(58%)

Total preg (493) / Total Treated (997) x 100 = 49.3%
Data from V8 Ranch, Boling, Texas

Table 11 shows data from a single Brahman-influenced client owned recipient herd in South Texas. A total of 997 cows were synchronized using CIDR and 50 mg P4 and 2.5 mg E17β upon insertion and removal of the CIDR along with an im injection of PGF on day 7 or 8 followed by 1 mg E17β 24 h post CIDR removal. Calves were removed and estrus was detected for 2 d following the final estradiol injection.

Estrus was observed in 87% of the recipients synchronized. All recipients were ultrasounded prior to ET on day 7 and only recipients with CL>10mm in diameter received either a fresh of frozen embryo. Overall service rate was 85%. Conception rate was detected by ultrasound at 35-45 days of gestation was 58%. The overall pregnancy

rate was 49.3%. On one replicate or day of work on 123 head of recipients we achieved 72.4% overall pregnancy rate. We have found no other estrus synchronization protocol work better.

Conclusions

The most significant improvement in the last 10 years of embryo transfer technology has been the ability to synchronize estrus and ovulation of cattle. These improvements have enabled AI and ET to be performed utilizing less total labor. The advances in timed embryo transfer and artificial insemination has somewhat eliminated the necessity to monitor estrus without sacrificing overall success rates. More research needs to be performed to better synchronize Bos Indicus influenced cattle. This is very important along the gulf coast as their ability to thrive in this region makes Brahman crossed cattle essential in many embryo transfer programs as recipients.

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NOTES

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NUTRITION AND REPRODUCTION INTERACTIONS

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Introduction

Direct reproductive traits, as we are able to measure them currently, tend to be low in heritability, therefore, the environment in which a beef female is produced is of pivotal importance to assure reproductive success. Large cow size and high milk production translate into increased nutrient requirements for the cow. Increased milk production and cow size increase both energy and crude protein requirements. Animals with higher milk production potential have higher maintenance requirements even when not lactating. Excess milk production and cow size can significantly limit the carrying capacity of any ranch. It is important that animal nutrient requirements match feed resources or reproduction will be compromised.

Body Condition Score

Body condition score (BCS) is correlated with several reproductive events such as postpartum interval, services per conception, calving interval, milk production, weaning weight, calving difficulty, and calf survival; greatly affecting net income on a cow/calf operation (Table 1; Kunkle et al., 1994). The most important factor influencing pregnancy rate in beef cattle is body energy reserves at calving (Wettemann et al., 2003). Body condition at calving is the single most important factor determining when beef heifers and cows will resume cycling after calving. Body condition score at calving also influences response to postpartum nutrient intake. Spitzer et al., (1995) fed primiparous cows differing in body condition (BCS 6 vs. 4; 1 = emaciated, 9 = obese) to gain either 1.87 or .97 lb/d. The percentage of BCS 6 cows in estrus during the first 20 days postpartum increased from 40 to 85% when fed to the higher rate of gain, the cows in BCS 4 only increased estrous response from 33 to 50% during the first 20 d postpartum when fed to gain at the higher rate. Cattle should have an optimum body condition score of 5 to 6 at calving through breeding to assure optimal reproductive performance. Body condition score is generally a reflection of nutritional management; however, disease and parasitism can contribute to lower body condition scores even if apparent nutrient requirements are met.

Specific Nutrients and Reproduction

Feeding a balanced diet to beef females in the last trimester of pregnancy through the breeding season is of critical importance. Nutritional demands increase greatly in late gestation and even more in early lactation. Reproduction has low priority among partitioning of nutrients and consequently, cows in thin body condition often don't rebreed. Plane of nutrition the last 50-60 days before calving has a profound effect on

postpartum interval (Table 2, Randel, 1990). The importance of pre- and postpartum protein and energy level on reproductive performance has been consistently demonstrated (Table 2). Positive energy balance postpartum is essential for prompt rebreeding of heifers calving in thin condition (Table 3; Lalman et al., 1997).

Table 1. Relationship of body condition score (BCS) to beef cow performance and income

BCS	Pregnancy rate, %	Calving interval, d	Calf ADG, lb	Calf WW, lb	Calf Price, $/100 lb	$/cow Exposed[a]
3	43	414	1.60	374	96	154
4	61	381	1.75	460	86	241
5	86	364	1.85	514	81	358
6	93	364	1.85	514	81	387

[a] Income per calf x pregnancy rate.

Table 2. Effect of pre- or postpartum dietary energy or protein on pregnancy rates in cows and heifers

Nutrient and time	Adequate	Inadequate	
	Pregnant, %		Difference, %
Energy level pre-calving[a]	73	60	13
Energy level post-calving[b]	92	66	26
Protein level pre-calving[c]	80	55	25
Protein level post-calving[d]	90	69	21

[abcd] Combined data from 2, 4, 9 and 10 studies, respectively.

Table 3. Influence of postpartum diet on weight change, body condition score (BCS) change and postpartum interval (PPI)

	Diet			
Item	Low	Maintenance	Maint./ High	High
Post-calving weight, lb	835	822	826	821
BCS at calving	4.27	4.26	4.18	4.10
PPI, d	134	120	115	114
PPI wt. change, lb	12	40	70	77
PPI BCS change	-.32	.37	1.24	1.50

Bearden and Fuquay (1992) summarized the effects of inadequate and excessive nutrients on reproductive efficiency (Table 4).

Table 4. Influence of inadequate and excessive dietary nutrient intake on reproduction in beef cattle

Nutrient Consumption	Reproductive Consequence
Excessive energy intake	Low conception, abortion, dystocia, retained placenta, reduced libido
Inadequate energy intake	Delayed puberty, suppressed estrus and ovulation, suppressed libido and spermatozoa production
Excessive protein intake	Low conception rate
Inadequate protein intake	Suppressed estrus, low conception, fetal reabsorption, premature parturition, weak offspring
Vitamin A deficiency	Impaired spermatogenesis, anestrus, low conception, abortion, weak offspring, retained placenta
Phosphorus deficiency	Anestrus, irregular estrus
Selenium deficiency	Retained placenta
Copper deficiency	Depressed reproduction, impaired immune system, impaired ovarian function
Zinc deficiency	Reduced spermatogenesis

Protein and Energy

Inadequate daily energy intake is a primary cause of reduced cattle performance on forage diets. In many instances with warm-season perennial forages (and possibly with cool-season perennial forages at advanced stages of maturity), there is an inadequate supply of crude protein, which will limit energy intake (Mathis, 2000; Paterson et al., 1991). An example of the relationship between crude protein content of forages and forage intake is presented in Figure 1. Dry matter intake declined rapidly as forage crude protein fell below 7%, a result attributed to a deficiency of nitrogen (protein) in the rumen, which decreased microbial activity. If forage contains less than approximately 7% crude protein, feeding a protein supplement generally improves the energy and protein status of cattle by improving forage intake and digestibility. For example (Figure 1), with a crude protein content of 5%, forage intake was about 1.6% of body weight, while at 7% crude protein, forage intake was 44% higher and consumption was 2.3% of body weight.

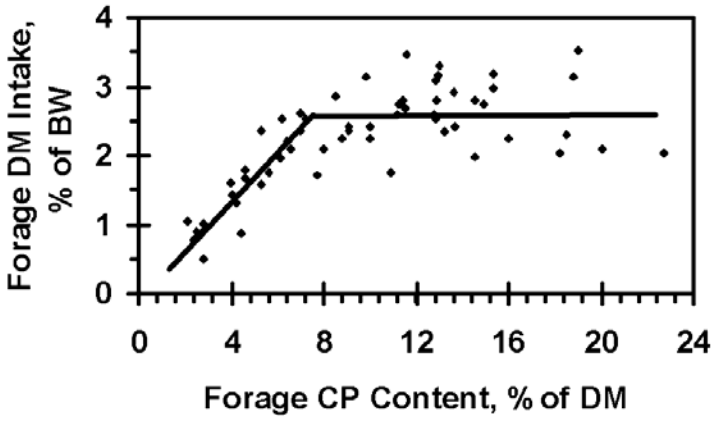

Figure 1. Effect of forage crude protein (CP) on dry matter (DM) intake

Improved forage intake increases total dietary energy intake, and is the reason correcting a protein deficiency is usually the first step in formulating a supplementation program for animals grazing poor quality forage. As suggested, when the crude protein content of forages drops below about 7%, forage intake declines. However, intake of other forages may decline when forage crude protein drops below 10%. Part of the variation can be attributed to differences in nutrient requirements of the cattle, with the remainder of the variation attributed to inherent differences among forages that present different proportions of nutrients to rumen microbes. Response of intake to a single nutrient such as crude protein should not be expected to be similar among all forages (Mathis, 2000).

Livestock producers are often concerned excessive dietary nutrients during the last trimester of pregnancy may negatively influence calf birth weights and dystocia. Selk (2000) summarized the effects of providing either adequate or inadequate amounts of

dietary energy and protein on calving difficulty, reproductive performance, and calf growth. These summaries are presented in Tables 5 and 6.

Reducing energy pre-partum had virtually no effect on dystocia rates, even though birth weights were altered in some experiments. Of the nine trials summarized, seven indicated increased energy intakes during the last trimester of gestation did not increase calving difficulty.

Table 5. Summary of studies on supplemental prepartum energy intake on calving difficulty, subsequent reproductive performance and calf growth

Researcher	Supplementation[a]	Summary of Effects
Christenson et al., 1967	HE vs. LE for 140 d prepartum	HE increased birth wt., dystocia, milk & estrus activity
Dunn et al., 1969	ME vs. LE for 120 d prepartum	ME increased birth wt. and dystocia
Bellows et al., 1972	HE vs. LE for 82 d prepartum	HE increased birth wt. but had no effect on dystocia or weaning wt.
Laster & Gregory, 1973	HE vs. ME vs. LE for 90 d prepartum	HE increased birth wt. but had no effect on dystocia
Laster, 1974	HE vs. ME vs. LE for 90 d prepartum	HE increased birth wt. but had no effect on dystocia
Corah et al., 1975	ME vs. LE for 100 d prepartum	ME increased birth wt., estrus activity, calf vigor and weaning wt. but had no effect on dystocia
Bellows and Short, 1978	HE vs. LE for 90 d prepartum	HE increased birth wt., estrus activity, pregnancy rate and decreased post partum interval but had no effect on dystocia
Anderson et al., 1981	HE vs. LE for 90 d prepartum	HE had no effect on birth wt., milk or weaning wt.
Houghton et al., 1986	ME vs. LE for 100 d prepartum	ME increased birth wt. and weaning wt. but had no effect on dystocia

[a]HE = high energy (over 100% NRC or National Research Council's recommended dietary need); ME = moderate energy (approximately 100% NRC); LE = low energy (under 100% NRC)

In addition, producers are often concerned with levels of crude protein and possible effects on calf birth weight. Selk (2000) summarized studies conducted to specifically measure effects of varying protein intake to the prepartum beef female on calving difficulty (Table 6). Reducing dietary crude protein prepartum does not decrease calving difficulty and may compromise calf health and cow reproductive performance.

Table 6. Summary of studies on feeding supplemental protein during gestation on calving difficulty, subsequent reproductive performance and calf growth

Researcher	Supplementation[a]	Summary of Effects
Wallace & Raleigh, 1967	HP[a] vs. LP for 104 - 137 d prepartum	HP increased cow wt., birth wt. and conception rate but decreased dystocia
Bond & Wiltbank, 1970	HP vs. MP throughout gestation	HP had no effect on birth wt. or calf survivability
Bellows et al., 1978	HP vs. LP for 82 d prepartum	HP increased cow wt., cow ADG, birth wt., dystocia, weaning wt. and decreased conception rate
Anthony et al., 1982	HP vs. LP for 67 d prepartum	HP had no effect on birth wt., dystocia or postpartum interval
Bolze et al.,1985	HP vs. MP vs. LP for 112 d prepartum	HP had no effect on birth wt., dystocia, weaning wt., milk or conception rate but decreased the postpartum interval

[a]HP = high protein (over 100% NRC); MP = moderate protein (approximately 100% NRC); LP = low protein (under 100% NRC)

Excess Protein and Energy

Caution should be used with feeding excessive amounts of nutrients before or after calving. Not only is it costly, but animals with excess body condition (BCS >7) have lower reproductive performance and more calving difficulty than animals in moderate body condition (BCS 5-6). Excessive protein and energy can both have negative effects on reproduction. Overfeeding protein during the breeding season and early gestation, particularly if the rumen receives an inadequate supply of energy may be associated with decreased fertility (Elrod and Butler, 1993). This decrease in fertility may result from decreased uterine pH during the luteal phase of the estrous cycle in cattle fed high levels of degradable protein. The combination of high levels of degradable protein and low

energy concentrations in early-season grasses may contribute to lower fertility rates in females placed on such pastures near the time of breeding. Negative effects of excess rumen degradable intake protein on reproduction are well documented in dairy literature (Ferguson, 2001).

Effects of supplementing feedstuffs high in undegradable intake protein (UIP) on reproduction are inconclusive and appear to be dependent on energy density of the diet (Hawkins et al., 2000). Recent research (Kane et al., 2004) demonstrated negative effects on reproductive hormones when high (.71 lb/d) levels of UIP were supplemented but not at low (.25 lb/d) or moderate (.48 lb/d) levels. Heifers fed additional UIP (.55 lb/d) during development reached puberty at a later age and heavier weight and had fewer serviced in the first 21 d of the breeding season. Fall pregnancy rate was not affected (Lalman et al., 1993). Further research is needed to elucidate potential mechanisms UIP may stimulate or inhibit reproductive processes and under what conditions.

Distillers grains are a co-product from the ethanol industry being utilized in beef cattle diets and are also high (65% of CP content) in UIP. Two research projects were conducted to determine the effects of feeding dried distillers grains to beef heifers during post weaning development and to 2-yr-old cows during the postpartum period (Funston, unpublished data). In both experiments distillers grains were included in a total mixed diet and fed at approximately 2.76 lb DM (3 lb as fed; approximately .55 lb/d UIP). Diets were formulated to be similar in crude protein and total digestible nutrients. Heifers (n = 100) were fed diets with either distillers grains or whole soybeans (3 lb as fed) from late October through early June when they were artificially inseminated after being synchronized with melengestrol acetate (MGA)/$PGF_{2\alpha}$. There were no differences in cycling activity (98%) before MGA feeding, synchronization rate (86%), AI conception rate (69%) or AI pregnancy rate (59%).

The second experiment utilized 54, 2-yr-old cows, which were assigned to treatment by calving date and fed diets with either distillers grains or wet corn gluten feed as a protein source beginning approximately one week after the last calf was born for a period of 60 d. At 67 d postpartum (based on average calving date), cows were given an injection of GnRH and a CIDR inserted; 7 d later the CIDR was removed and $PGF_{2\alpha}$ injected. Cows were then heat detected and AI'd 12 h later for 96 h, at which time all cows not detected in estrus were inseminated and injected with GnRH. Cow-calf pairs were trucked approximately 225 miles shortly after the last AI and ultrasounded for pregnancy 47 d later. Pregnancy rate (65%) to AI did not differ between treatments.

Shike et al. (2004, and personal communication) also did not observe a negative effect on reproduction when distillers grains were fed to postpartum Simmental cows. One-hundred cows were blocked by age and calving date and fed postpartum diets containing either 13 lb corn gluten feed and 10 lb alfalfa or 12.26 lb dried distillers grains and 10 lb alfalfa (DM basis) until the beginning of the breeding season (approximately 74 d). Pregnancy rate to AI (60 vs. 60.5% for corn gluten and distillers, respectively) and after a 45 d bull breeding (97.1 vs. 90.7 for corn gluten feed and distillers, respectively; *P* = 0.13) period did not differ. Cows fed corn gluten feed lost more weight, had greater

milk production, and greater calf average daily gain during the postpartum period. Milk urea nitrogen levels were above levels reported to negatively influence reproduction in other studies (Butler, 1998). Differences may be due to energy balance and lactation potential.

Minerals

Minerals are important for all physiological processes in the beef animal including reproduction. Therefore, the question is not whether minerals are important for reproduction, but rather, when do minerals have to be supplemented in the basal diet.

Salt (NaCl) is the most important mineral in terms of need for the beef animal. Sodium and chloride normally do not appear in feedstuffs in adequate amounts to meet animal requirements and should be provided free choice at all times.

Calcium is generally adequate in forage-based diets but is often included in commercially available mineral supplements because many phosphorus sources also contain calcium. There has been much debate and research conducted on the effects of phosphorus supplementation on reproductive function. Phosphorus and crude protein content generally parallel each other in pasture or rangeland. Mature forages are generally deficient in phosphorus and impaired reproductive function has been associated with phosphorus deficient diets (Dunn and Moss, 1992; Lemenager et al., 1991). Diets should be evaluated for phosphorus content and supplemented accordingly. Caution should be used to not overfeed phosphorus -- it is costly, of potential environmental concern, and does not positively influence reproduction in beef (Dunn and Moss, 1992) or dairy (Lopez et al., 2004) cattle.

Other macro minerals include magnesium, potassium, chlorine, and sulfur. Need for supplementation, as with the previously mentioned minerals, is dependent on content in the basal diet and water. Both deficiencies and excesses can contribute to suboptimal reproductive function.

The micro or trace minerals include copper, cobalt, iodine, iron, manganese, and zinc. Inadequate consumption of certain trace elements combined with antagonistic effects of other elements can reduce reproductive efficiency (Greene et al., 1998).

Vitamins

Most of the vitamins (C, D, E, and B complex) are either synthesized by rumen microorganisms, synthesized by the body (vitamin C) or are available in common feeds and are not of concern under normal conditions. Vitamin A deficiency, however, does occur naturally in cattle grazing dry winter range or consuming low quality crop residues and forages (Lemenager, et al., 1991). The role of vitamin A in reproduction and embryo development has been reviewed by Clagett-Dame and Deluca (2002). Supplementation before and after calving can increase conception rates (Hess, 2000).

Water

Water is more essential to life than any other single nutrient. Feed intake is directly related to water intake. Water may also contribute significant macro and micronutrients that may benefit or impair production and reproduction. The contribution of these nutrients from water sources must be considered to accurately design a supplementation program.

Strategies to Enhance Reproduction

Ionophores

Bovatec® and Rumensin® have been shown to influence reproductive performance during the postpartum period. Cows and heifers fed an ionophore exhibit a shorter postpartum interval provided adequate energy is supplied in the diet (Table 7; Randel, 1990). This effect appears to be more evident in less intensely managed herds that generally have a moderate (60-85 d) or longer postpartum interval. Scientists have also demonstrated heifers fed an ionophore reach puberty at an earlier age and a lighter weight (Patterson et al., 1992).

Table 7. Effect of ionophore feeding on postpartum interval (PPI) in beef cows and heifers

Study	Ionophore (PPI, d)	Control (PPI, d)	Difference (d)
1	30	42	-12
2	59	69	-10
3	67	72	-5
4	65	86	-21
5	92	138	-46

Fat Supplementation

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 (Coppock and Wilks, 1991) sometimes experienced with cereal grains (Bowman and Sanson, 1996) in high roughage diets.

Supplemental lipids may also have direct positive effects on reproduction in beef cattle independent of the energy contribution. Lipid supplementation has been shown to positively affect reproductive function at several important tissues including the hypothalamus, anterior pituitary, ovary, and uterus. The target tissue and reproductive response appears to be dependent upon the types of fatty acids contained in the fat source.

Fat supplementation is a common practice in dairy cattle production, primarily to increase the energy density of the diet. Associated positive and negative effects on reproduction have been reported (Grummer and Carroll, 1991; Staples et al., 1998).

Research with supplemental fat has been conducted on cows that have had one or more calves, and 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, 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.

The effects of fat supplementation on reproduction in beef heifers and cows has recently been reviewed (Funston, 2004). Following is a summary from that review.

Fat Supplementation to Replacement Heifers. Studies are limited on the use of fat supplements in replacement heifer diets. In general, heifers in the studies cited were on a positive plane of nutrition and developed to optimum weight and age at breeding. There may have been a positive response to fat supplementation had heifers been nutritionally challenged. It appears from the studies cited here, there is limited benefit of fat supplementation in well-developed replacement females and is probably only warranted when supplements are priced comparable to other protein and energy sources.

Fat Supplementation Prepartum. Results from feeding supplemental fat prepartum are inconclusive. However, response to supplementation appears to be dependent on postpartum diet. Beef animals apparently have the ability to store certain fatty acids, supported by studies in which fat supplementation was discontinued at calving but resulted in a positive effect on reproduction. Postpartum diets containing significant levels of fatty acids may mask any beneficial effect of fat supplementation. There appears to be no benefit and in some cases, a negative effect of feeding supplemental fat postpartum, particularly when supplemental fat was also fed prepartum. Fat supplementation has been reported to both suppress and increase $PGF_{2\alpha}$ synthesis. In situations in which dietary fat is fed at high levels for extended periods of time, $PGF_{2\alpha}$ synthesis may be increased and compromise early embryo survival. Hess (2003) summarized research on supplementing fat during late gestation and concluded that feeding fat to beef cows for approximately 60 d before calving may result in a 6.4% improvement in pregnancy rate in the upcoming breeding season.

Fat Supplementation Postpartum. Supplementing fat postpartum appears to be of limited benefit from studies reported here. The majority of the studies reported approximately 5% fat in the diet supplemented with fat. It is not known if more or less fat would have elicited a different response (either positive or negative). If supplementing fat can either increase or decrease $PGF_{2\alpha}$ production, it seems reasonable the amount of fat supplemented might affect which response is elicited. Recent research (Hess, 2003) demonstrated a decrease in first service conception rates (50 vs. 29%) when young beef

cows were fed high linoleate safflower seeds (5% DMI) postpartum. The same laboratory has also reported (Grant et al., 2002) an increase in $PGF_{2\alpha}$ metabolite (PGFM) when high linoleate safflower seeds are fed postpartum and a decrease in several hormones important for normal reproductive function (Scholljegerdes et al., 2003 and 2004).

Feeding Considerations. The amount of supplemental fat needed to elicit a positive or, in some cases, a negative effect on reproductive function is largely unknown and titration studies are needed in all situations in which supplemental fat has been fed. Dose response studies indicate the amount of added plant oil necessary to maximize positive ovarian effects is not less than 4% (Stanko et al., 1997; Thomas et al., 1997). Staples et al. (1998) indicated 3% added dietary fat (DM basis) has often positively influenced the reproductive status of the dairy cow. Lower levels of added dietary fat (2%) have also been shown to elicit a positive reproductive response (Bellows et al., 2001) and in studies with fishmeal less than 1% added fat (Burns et al., 2002) produced a positive reproductive response, indicating both the amount and types of fatty acids are important. Feeding of large quantities of fat (> 5% of total DMI) has not been recommended due to potential negative effects on fiber digestibility and reduction in DMI (Coppock and Wilks, 1991). The duration and time (pre or postpartum) of supplement feeding needed to elicit a positive response is not precisely known, many of the studies have supplemented fat at least 30 d. 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 nutrients. 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 adequate pasture or range resources may not provide any benefit beyond energy contribution to the diet.

The majority of fat supplementation in beef cattle diets has been in the form of oilseeds added to a total mixed diet or fed as a supplement. A challenge has been making a supplement high in fat that can be pelleted or blocked and fed on the ground. Levels above 8% fat have resulted in pellets and blocks that are soft and of poor quality (Bellows, personal communication). Whole soybeans, sunflower, and cottonseeds have been fed without processing; it appears 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).

Additional Compounds in Oilseeds. Gossypol levels may be a concern when high levels of whole cottonseed are fed. However, levels of gossypol present in typically fed quantities of whole cottonseed for protein or fat supplementation provide only a fraction of the amount of gossypol fed in studies in which gossypol toxicity has been reported (Williams and Stanko, 1999). Other factors such as phytoestrogens may be present in some oilseeds (legumes in particular) and have been shown to negatively affect reproduction in cattle (Adams, 1995). The precise effect of these factors and possibly others on reproductive function has not been fully elucidated and is probably dependent

on level of inclusion, basal diet, and stage of physiological maturity of the female being supplemented.

In a recent study (Funston, unpublished data), beef heifers (n = 106; approximately 10 mo of age; 660 lb) were fed 3 lb/d (4% added fat) whole soybeans or wet corn gluten feed as a protein source in a total mixed diet approximately 110 d before AI. There was no difference in cycling activity (98%) before heifers were synchronized with MGA/ $PGF_{2\alpha.}$ Fewer (81 vs 96% for soybean and control, respectively) heifers fed soybeans were detected in estrus through 120 h after $PGF_{2\alpha}$. Estrous response (time after $PGF_{2\alpha}$) was also delayed (3.2 vs 2.9 d for soybean and control, respectively) in the heifers fed soybeans. Neither AI conception rates (81 vs 72% for soybean and control, respectively) nor AI pregnancy rates (65 and 69% for soybean and control, respectively) were affected by treatment. Overall pregnancy rates (90 and 94% for soybean and control, respectively) were also not different after the breeding season. The reason for the delayed estrous response and delayed time of estrus is not known. However, analysis of the extracted soybeans indicated the presence of three different phytoestrogens, which may have affected estrous response, and time of estrus.

In a subsequent heifer development study utilizing whole soybeans (3 lb/d), discussed previously, distillers grains were used as the protein source in the control diet. Heifers (n = 100) were approximately 500 lb and 7 months of age when placed on experimental diets. There were no differences in cycling activity (98%) before MGA feeding, synchronization rate (86%), time of estrus (2.9 d) after $PGF_{2\alpha,}$ AI conception rate (69%) or AI pregnancy rate (59%). It is not understood why there was not a difference in estrous response or delay in time of estrus in this experiment. Only 16% of heifers were cycling when feeding of experimental diets initiated compared to 81% the previous year. Soybeans were also fed longer (230 d) than the previous year (110 d). Differences in physiological maturity and duration of feeding may have contributed to the inconsistencies between years.

An additional study was conducted to determine if time of feeding whole soybeans before AI had an effect on estrous response or pregnancy rates. Heifers (n=100) were synchronized with MGA/PGF and fed 3 lb/d whole soybeans for approximately 120 or 210 d before PGF injection. Heifers weighed approximately 570 and 730 lb at initiation of soybean feeding. There was no difference in synchronization rate (77%), time of estrus (78 h) after PGF, AI conception rate (57%), AI pregnancy rate (44%) or final pregnancy rate (90%). Serum samples will be analyzed to determine cyclic activity before each treatment was initiated.

Howlett et al. (2003) also fed whole soybeans, whole cottonseed, or pelleted soybean hulls for 112 d in a total mixed diet to replacement heifers. Soybeans and cottonseeds contributed approximately 2% added fat to the diet. Heifers were synchronized with $MGA/PGF_{2\alpha}$ and experimental diets were discontinued approximately one week before the first MGA feeding. Treatment did not affect the proportion of heifers pubertal before beginning MGA feeding. First service conception rates were also not affected by treatment. However, there was a 20% increase ($P = 0.27$) in first service conception rates

in the soybean fed group (57%) compared to controls (37%). In this study 96 heifers were split into three treatments and a control group. Neither estrous response nor time of estrus was reported.

Five hundred-sixty Angus x Simmental cows were utilized to evaluate the effects of supplemental fat on performance, lactation, and reproduction (Shike et al., 2004). Cows were fed one of four dietary supplements: whole raw soybeans, flaxseed, tallow, and corn-soybean meal (control). Flaxseed and tallow were added to the control supplement to provide similar fat levels as supplied by whole soybeans. Supplements (4 lb/d) were fed for 105 d after calving and ended at breeding. Cows grazed endophyte infected tall fescue and red and white clover pastures. There were no differences in cow or calf ADG or milk production. Soybean supplemented cows had greater milk fat and milk urea nitrogen than flaxseed supplemented cows. There were no differences in AI conception rates. However, conception rates to bulls were lower in cows fed soybeans (65%) compared to flaxseed (79%) or tallow (76%). Overall pregnancy rates were lower in cows fed soybeans (83%), compared to cows fed flaxseed (91%) or tallow (89%). It was stated the flaxseed, tallow, and control supplements were isonitrogenous but apparently not the soybean supplement. It is not clear why there would be a reduction in bull, but not AI, pregnancy rates. Apparently protein levels were higher in the soybean supplement as demonstrated by higher milk urea nitrogen levels. Overall dietary protein may have been in excess throughout the supplementation period, depending on forage quality. Artificial insemination pregnancy rates were also apparently quite low. Cessation of supplement feeding may have actually benefited reproduction. This also appears to be a high supplementation rate of soybeans. Compounding this apparent problem may have been endophyte from tall fescue and phytoestrogens from clover (Adams, 1995).

Summary of Fat Supplementation. Currently, research is inconclusive on exactly how to supplement fat to improve reproductive performance beyond the energy contribution. 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. 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, 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.

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 is greatest. Postpartum fat supplementation appears to be of limited benefit and adding a fat source high in linoleic acid postpartum may actually have a negative effect on reproduction.

Summary

Nutrition has a profound effect on reproductive potential in all living species. Body condition is a useful indicator of nutritional status and when used in conjunction with body weight change can provide a useful method to assess reproductive potential. Energy and protein are the nutrients required in the greatest amounts and should be first priority in developing nutritional programs to optimize reproduction. Minerals and vitamins must be balanced in the diet to optimize reproductive performance. Consider water quantity and quality when balancing diets. Caution should be taken not to overfeed nutrients or reproductive processes may be adversely affected. There does not appear to be any magic feed ingredient that will compensate for a diet greatly deficient in any of the nutrients or poor body condition score.

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DEVELOPEMENT AND MANAGEMENT OF HEIFERS FOR CONTROLLED BREEDING PROGRAMS

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Introduction

Cow-calf producers require a consistent source of replacement females in order to maintain the size of the breeding herd at the desired level. Replacement females may be produced internally or acquired from external sources. This discussion will focus upon the critical control points that range from the selection criteria used in mating decisions to produce potential replacement heifers to the factors that influence successful rebreeding of the first-calf heifer. Heifers must attain puberty and conceive before 15 months of age to meet the goal of calving at 2 years of age. In order to successfully enter the cow herd, replacement females must be developed, conceive early in the breeding season, produce a live calf and conceive as a first-calf heifer. Heifer age and body weight (BW) significantly influence the onset of puberty. General guidelines have been proposed for target BW at specific stages of development relative to expected mature BW.

Genetic Factors

Mating decisions for production of potential replacement heifers should consider breed type, expected progeny difference for selected performance traits and age of dam. In a study of nine different sire breeds, breed group of sire significantly affected age and weight of heifers at puberty (Thallman et al., 1999). However, pregnancy rate of heifers was not affected by breed group of sire. Wolfe et al. (1990) reported that selection for growth traits did not have a detrimental effect on age at puberty in Hereford heifers.

Although reproductive traits are generally considered to have low heritability, recent studies indicate that selection for certain traits may allow identification of females with higher genetic potential for fertility. Doyle et al. (2000) conducted a study to determine whether heifer pregnancy (HP) and subsequent rebreeding (SR) were heritable in an experimental population of Angus cattle. The genetic relationships among HP, SR and stayability (probability of a female having at least 5 calves with the first calf born when the heifer is 2 years old) were determined in the same population. The authors concluded that HP (average heritability = .21) and stayability (average heritability = .15) were heritable and should respond favorably to selection. SR did not appear heritable in the same Angus cattle population. However, Buddenberg et al. (1989) and Snelling et al. (1996) concluded that SR was heritable in the Hereford and Angus populations in their studies. Doyle et al. (2000) suggested that the nonlinear relationships among HP and

stayability indicate that selection for improved female fertility would be most effective by having predictions on both traits.

Martinez et al. (2005) reported moderate estimates of heritability in Hereford cattle for stayability to calving and to weaning. The authors concluded that it is possible to select for sires whose daughters have an increased probability to remain longer in the herd. Utilization of an EPD for stayability can enhance reproductive lifespan of females through sire selection. Future development of an EPD for heifer pregnancy probability could allow selection of sires with daughters that have increased genetic potential to conceive at first breeding.

Preweaning Management

Preweaning growth is significantly influenced by calf age, level of maternal milk production and genetic potential for growth. Evans et al. (1999) reported a 10 percentage point advantage in the probability of pregnancy for each 20-day increment earlier that the heifer is born during the calving season. Heifers born to dams from 2 to 4 years of age had a 6 to 14% lower probability to become and remain pregnant to 120 days than heifers born to mature dams (5 to 9 years of age; Doyle et al., 2000). Creep feeding of suckling calves is one option for enhancing postnatal growth but does increase cost of heifer development. Although use of a growth-promoting implant after weaning can reduce fertility of replacement females, the use of an appropriate implant in suckling calves may be beneficial in certain management systems. A single implant at 2 months of age increased early weight gain and decreased subsequent calving difficulty scores without affecting reproduction or calf production of 2-year-old cows (Hancock et al., 1994).

An effective vaccination program is essential for calves to realize their genetic potential for gain and to minimize the potential for adverse effects on reproductive performance. Programs to control internal and external parasites should be designed for the particular production environment. For example, liver flukes (Fasciola hepatica) can decrease performance and impair reproduction in some regions including the Southern United States. Experimental infection of 4-month-old heifers with F. hepatica delayed the onset of puberty by 39 days compared with non-infected controls (Lopez-Diaz et al., 1998). Paczowski et al. (2004) infected Angus-sired heifers with F. hepatica at 4 months of age in south-central Texas. Age and weight at puberty did not differ significantly between infected and control heifers under the conditions of this study.

Weaning Management

Innovative weaning strategies provide potential to minimize stress and reductions in weight gain that are frequently associated with conventional methods of weaning. Providing fenceline contact for cows and calves at weaning increases calf average daily gain compared with the traditional method of weaning by separation (Price et al., 2003). A two-stage method of weaning cattle further reduced distress of calves (Haley et al., 2005). Calves were prevented from nursing their dams by placement of a plastic

antisucking device (noseflap) for 3 to 14 days (Stage 1) before calf separation (Stage 2). The authors recommended an optimum duration of Stage 1 is 4 to 5 days.

Postweaning Management

Postweaning growth rate, age at puberty, and pregnancy rate affect both the cost of developing replacement heifers and subsequent productivity of those replacements. Funston and Duetscher (2004) compared the effects of developing British X Continental heifers to either 53 or 58% of mature BW at breeding on reproduction and calf production. Costs of developing heifers to 53% of mature BW were lower than costs of developing heifers to 58% of mature BW while not adversely affecting reproduction through the fourth pregnancy or calf production through the third gestation.

The effects of three heifer development strategies based upon timed nutrient limitation (High, Medium or Low-High) on primiparous heifer performance were reported by Freetly et al. (2001). The authors concluded that pattern of growth may not affect the ability of a heifer to conceive or calf growth potential if heifers achieve a minimal BW before mating.

Ionophores can improve average gain by .1 to .2 lb/day, inhibit coccidiosis and enhance the onset of puberty in growing heifers by approximately 2 weeks. Supplements containing ionophores have been shown to decrease the age and weight at puberty of beef heifers (Moseley et al., 1977). Anthelmintics can reduce the gastrointestinal parasite load and increase weight gain. Purvis and Whittier (1996) reported that age and weight at puberty were reduced by administration of an ionophore or an anthelmintic. However, the effects of the ionophore and the anthelmintic on age and weight at puberty were not additive under the conditions of their study. Modified live vaccines to booster protection against respiratory diseases can be administered at least 30 days before the start of the breeding season.

Initial studies failed to demonstrate a beneficial effect of exposure to bulls on age at puberty in heifers. Subsequent reports indicate that heifer growth rate may interact with the biostimulatory influence of bulls on age at puberty in beef heifers. Roberson et al. (1991) assigned heifers (approximately 8.5 months of age) to either bull exposure (175 days) or isolated from bulls (NE). Heifers were fed to gain at either a moderate (1.3 lb/day) or high (1.8 lb/day) growth rate. Heifers exposed to bulls attained puberty at younger ages than NE heifers, and the effect on puberty was greater for high than for moderate growth rate heifers.

Breeding

The period from weaning to puberty is critical in the management of replacement heifers. However, body weight and condition score at the beginning of the breeding season appear to be more important than rate of gain. Grings et al. (1999) reported that age at puberty was delayed by 28 days in heifers produced by 2-year-old cows compared to heifers produced by 5-year-old cows. Although ADG from weaning to breeding was

similar between heifers born to either 2- or 5-year-old dams, the heifers from 2-year-old dams were 73 lb lighter at weaning.

The 5-point reproductive tract scoring (RTS) system was developed by Anderson et al. (1991) to estimate pubertal status of heifers. Lafever and Odde (1986) reported higher estrous and conception rates to artificial insemination after synchronization for heifers determined to be pubertal (RTS of 4 or 5) than for prepubertal heifers (RTS of 1, 2 or 3). Rosenkrans and Hardin (2003) conducted a study which validated the repeatability and accuracy of the RTS system to evaluate pubertal status of heifers prior to the onset of the breeding season. Rathmann (2005) quantified the relationship between RTS of heifers and 2-year reproductive performance in 90-day natural mating breeding systems under range conditions. Heifers with a RTS of 1 were lighter at weaning and younger at the start of breeding than heifers of RTS from 2 to 5. Pregnancy rate as a yearling and as a first calf heifer was only 62.5% for heifers with RTS of 1 or 2 compared with 91.2% for heifers with RTS of 3, 4 or 5 ($P<0.01$).

First-calf Heifer Management

Heifers should achieve a body condition score of 6 at first calving in order to optimize rebreeding efficiency and enhance the probability of conception early in the breeding season. Restriction of the suckling stimulus can accelerate the initiation of ovarian cyclicity after parturition. Methods of suckling restriction include early weaning, once- or twice-daily suckling and short-term calf removal (typically 48 to 72 hours). These management techniques are more labor intensive and require appropriate feeding and health strategies to ensure adequate growth rate of the calf.

The presence of bulls hastens the onset of ovarian cyclicity (Custer et al., 1990) and improves reproductive performance (Fernadez et al., 1993) in first-calf, suckled beef cows. This management practice typically requires that the bulls are modified to prevent the presence of spermatozoa in the ejaculate and (or) to prevent their ability to achieve copulation. A recent study by Berardinelli and Joshi (2005) reported an alternative approach to achieve the desired response without joining bulls with the cows. Beginning on day 35 after calving, primiparous cows were restricted to suckling twice-daily and assigned to one of four treatments. Cows were exposed to a bull (BE), exposed to excretory products of a bull (EPB), not exposed to a bull (NE) or exposed to excretory products of cows (EPC). Cows in the EPB and EPC groups were placed in enclosures from 1830 to 0800 daily, and the enclosure was either occupied by bulls (EPB) or left empty (EPC) from 0800 to 1830 daily. Mean interval to resumption of luteal function reduced for BE and EPB groups. In addition, the exposure to exretory products of cows hastened resumption of luteal function compared to NE cows by 60 daysof treatment.

Conclusions

Successful development and management of replacement heifers requires formulation of a plan that spans the continuum from the mating decisions involved with production of the heifer calf through timely rebreeding of the young cow. Technologies

that encompass genetics, nutrition, health, reproduction and animal behavior need to be effectively integrated in order to accomplish the objective of producing replacement females that will calve early as a two-year-old and continue calve early in subsequent years.

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PHYSIOLOGY AND MANAGEMENT OF THE POSTPARTUM SUCKLED COW FOR CONTROLLED BREEDING PROGRAMS

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Introduction

After an approximate 30-d period of uterine repair and involution, the resumption of normal postpartum ovarian cycles is regulated mainly by the rate of recovery of the hypothalamic-pituitary axis. Mechanisms controlling the re-initiation of patterns of LH secretion that are needed to support follicular development and ovulation include physiological recovery of the pituitary from effects of high circulating concentrations of estradiol produced by the placenta, nutritional status (body condition), suckling, season of calving, and genetics. The purpose of this review is to 1) summarize our understanding of mechanisms controlling the length of the postpartum anovulatory period in the suckled beef cow; and 2) consider management approaches that can exploit our understanding of postpartum physiology, nutrition and management to enhance reproductive performance.

Physiology of Postpartum Reproduction

Gestational Effects on the Hypothalamic-Pituitary Axis

Pituitary stores of LH are very low at parturition in cattle, owing to the effects of high circulating concentrations of placental-derived estradiol that are observed during late gestation (Nett, 1987). High circulating concentrations of estradiol inhibit the synthesis of the β subunit, and to some degree, the α subunit of the LH molecule in gonadotrophs. Storage and release of follicle-stimulating hormone (FSH) does not change appreciably during the postpartum period. Following parturition, the rapid decline in circulating estrogens allows a rapid re-accumulation of anterior pituitary LH, which requires 2 to 3 wk to complete. During this period of recovery, circulating concentrations of LH and frequency of LH pulses are usually low. This occurs initially because of a lack of releasable LH in all cows, regardless of whether they are suckled, nonsuckled, or milked (Silveira et al., 1993; Griffith and Williams, 1996)). That synthesis and accumulation of pituitary LH requires only a low level of GnRH stimulation accounts for the ability of the pituitary to accumulate LH during this period. After the second or third week, the pulsatile release of LH increases in weaned beef cows and milked dairy cows, resulting in the resumption of ovarian follicular development and ovulation (Carruthers et al., 1980; Williams, 1990). However, in suckled cows, the suppressive effects of suckling on hypothalamic GnRH secretion continue to prevent an increase in pulsatile LH release. Eventually, the suckled cow escapes from the effects of suckling, or is weaned, and the frequency and amplitude of GnRH pulses increases

dramatically, the frequency of LH pulses increases, and ovarian cycles resume (Williams and Griffith, 1995; Gazal et al., 1998). Although the ability of the hypothalamus to stimulate a preovulatory LH surge through estradiol positive feedback is blunted or absent immediately after calving, the normal feedback response returns within about 2 wk postcalving.

Effects of Suckling and the Maternal-Offspring Bond

For over a half century it was assumed that chronic sensory stimulation of the teat (suckling) was the primary cause of lactational anovulation in numerous species, including cattle. However, our laboratory and others have shown that somatosensory pathways within the teat and udder are unnecessary for suckling to suppress LH secretion. Neither chronic milking nor the physical presence of the calf in the absence of suckling have measurable effects on the pulsatile pattern of LH release, and neither denervation of the udder (Williams et al., 1993) nor mastectomy (Viker et al., 1993) shortens the postpartum anovulatory interval if calves remain with their dams. Additional work has clearly shown that the maternal-offspring bond is a requisite feature of postpartum Anovulation (Williams and Griffith, 1995; Griffith and Williams, 1996). Beef females forced to suckle an alien calf for up to 6 d undergo the same neuroendocrine changes that occur with weaning: a rapid increase in the frequency of LH pulses, development of a preovulatory follicle, ovulation, and the resumption of ovarian cyclicity. Formation of a selective maternal bond by the cow plus the physical interaction of the calf in the inguinal region (bunting, oral manipulation of the flank, or suckling) appear to be responsible for neural changes that create the anovulatory state (Viker et al., 1989; Williams et al., 1993; Silveira et al., 1993; Williams and Griffith, 1995). These include an increase in hypothalamic sensitivity to estradiol negative feedback and an increase in opioid tone that causes a suppression of GnRH and LH secretion for variable periods (Acosta et al., 1983). However, the time of day during which calves suckle (eg., night vs day) has no effect on length of the postpartum interval to first ovulation or conception (Gazal et al., 1999).

Genetics and Season of Calving

The resumption of the appropriate pattern of LH secretion to promote ovarian cyclicity can be affected by at least two other factors: genotype of the cow and season of calving. Purebred *Bos indicus* cattle tend to be affected more strongly by both the negative effects of suckling and undernutrition than most purebred *Bos taurus* females. Crossbreeding, either within or between species, results in greatly improved reproductive performance, including a reduction in length of the postpartum interval (Gregory, 1969). Size of the cow and lactation potential represent genotypically-driven features that also impact length of postpartum anovulation. Both of these factors increase nutritional requirements, which in turn affect reproductive performance if nutrients are limiting. In addition, the season of calving can affect the length of the postpartum anovulatory interval by 15 to 20 days or more. Although not usually considered to be seasonal breeders, cattle are affected by photoperiod to some degree. Cows bred to calve during the late summer or early fall will invariably have shorter postpartum anovulatory intervals than cows bred to calve in winter or early spring (Hansen and Hauser, 1984). However, because the calving season is often managed to coincide with maximum forage quality and quantity, herds are

more frequently managed to calve in the spring.

Nutritional Status and Body Condition

Undernutrition, particularly a deficit in dietary energy intake, is probably the most prevalent natural and man-made cause of delayed rebreeding in cattle (Randel, et al., 1990; Short et al., 1990; Williams, 1990). Moreover, the effects of undernutrition have their greatest effects when they occur during late gestation. Cows that calve in thin body condition have greatly extended intervals to first postpartum estrus and ovulation. This occurs because of a slowing of the pituitary repletion of LH after calving and heightened effects of suckling on hypothalamic GnRH secretion. As a result, LH secretion is low and the development of ovulatory follicles is delayed for periods often exceeding 100 days or more. Many experiments have been conducted showing the effects of cow body condition and postpartum nutrition on reproductive performance. Although some of the effects of low body condition at calving can be remedied by increasing feed intake after calving, this is generally not an economically-feasible approach. Therefore, the best approach is to realiment cows during the dry period after calves are weaned and before the next calving. It is during this period that the most economical gain can be achieved and during which the cow's nutrient requirements are lowest.

Dietary Fat Supplementation

For many years, we examined the potential of dietary fat supplementation to enhance reproductive performance in beef cows. The original studies on this topic were conducted in North Dakota and addressed the effects of dietary fat supplementation on circulating concentrations of progesterone in dairy heifers (Talavera et al., 1985). In that study, and in others conducted subsequent to it, fat supplementation enhanced circulating concentrations of progesterone and enhanced the lifespan of induced CL in early postpartum beef cows (Williams, 1989; Ryan et al., 1995).

Metabolic and ovarian effects. The initial studies in beef cattle were conducted with the objective of determining whether certain metabolic changes could be created to improve reproductive performance in cows in marginal to thin body condition at calving, independent of BW/BCS gain (Williams, 1989; Wehrman et al., 1991; Ryan et al., 1994; 1995). Our overriding goal was to create metabolic changes that would allow range beef cows in moderately thin condition to perform more efficiently than would be expected without such changes. It was assumed that most of these effects would occur directly at the ovarian level, without effects on the hypothalamic-pituitary axis, and for the most part, this has been confirmed. Results indicated that increasing dietary fat consumption increased the number of follicles in the medium-sized classification by 1.5- to 5-fold within 3 to 7 wk and these changes occurred coincident with changes in serum insulin, GH and intraovarian insulin-like growth factor (IGF-1) (Wehrman et al., 1991; Ryan et al., 1992; Thomas et al., 1997). All studies employed an experimental design in which treatment and control diets were isocaloric and isonitrogenous. Using this approach, it was shown unequivocally that the effects of fat supplementation did not depend upon increased dietary energy or weight gain of cattle (Wehrman et al., 1991; Thomas et al., 1997). The greatest increase in medium

follicle populations occurred in response to plant oil consumption, which as discussed below, is likely a direct result of the effects of high levels of linoleic acid in the rumen. Sources of plant oil have included whole cottonseed, soybean oil and rice bran. Unfortunately, we were not able to increase the number of ovulatory follicles in superovulation regimens using this dietary strategy (Thomas and Williams, 1996). Maximum follicular growth responses to plant oil supplementation have occurred when plant oils were fed at 4 to 6% of diet dry matter, with lesser increases noted with lower levels of added fat. Animal tallow, calcium salts of saturated fatty acids or fish oil have been shown to have less robust effects on follicular growth than plant-derived oils. Moreover, postpartum beef cows calving in very thin body condition (BCS of 3; 1-9 scale) were unable to develop medium or large follicles at a rate equal to those with a body condition score of 4 or greater after 3 wk of fat consumption (Ryan et al., 1994). Longer feeding intervals in cows in very thin condition (BCS 3) have not been examined. **Table 1 s**ummarizes the effects of dietary fat supplementation on follicular physiology and growth as observed in our lab and in others.

Table 1. Summary of effects of dietary fat supplementation in cattle on ovarian follicular growth and steroidogenic potential of follicle cells in vitro (From Williams and Stanko, 2000 with permission)

Source	Characteristics Affected
Wehrman et al., 1991; Ryan et al., 1992; Hightshoe et al., 1991; Lucy et al., 1991; Thomas and Williams, 1996; Thomas et al., 1997; Lammoglia et al., 1996; Stanko et al., 1997; De Fries., et al., 1998	Increased number of medium-sized follicles (polyunsaturated fat > saturated and highly polyunsaturated fat effects)
Wehrman et al., 1991; Ryan et al., 1992	Increased granulosa cell progesterone production in vitro; increased follicular fluid progesterone
Ryan et al., 1992; Thomas and Williams, 1996	No effect on superovulation rate
De Fries et al., 1998	Increased number of large follicles; increased size of largest follicle

Effects on postpartum ovarian cyclicity. In early studies conducted at the Animal Reproduction Laboratory, Beeville, supplementation of postpartum, lactating beef cows with whole cottonseed beginning 30 d before the start of the breeding season increased the number of cows cycling at the start of the breeding season by up to 18% (Wehrman et al., 1991). This response was most evident when environmental conditions resulted in a loss of body condition during the postpartum period, in spite of supplementation (**Table 2**). Work at other locations has confirmed that fat supplementation reduces the postpartum anovulatory interval and may enhance rebreeding performance (**Table 3**). However, several of the latter trials were conducted with saturated or bypass fat. Therefore, we have speculated that performance would be further enhanced if polyunsaturated plant oils had been used, since ovarian responses to saturated fats appear less robust than to polyunsaturated fats.

Table 2. Effects of feeding high fat supplements to suckled, postpartum beef cows for 1 mo prior to the start of breeding on incidence of luteal activity at the start of the breeding season (From Wehrman et al., 1991 with permission)

Group[a]	Year	No. Cows	Luteal activity, %
High fat	1	61	72.0
Control	1	59	57.6
High fat	2	31	42.0
Control	2	32	18.8
High fat	Both	92	61.9[b]
Control	Both	91	43.9[c]

[a]High Fat and Control supplements were isocaloric and isonitrogenous
[b,c]Means with differing superscripts differ ($P < .05$)

Table 3. Summary of reports summarizing the positive effects of fat supplementation on postpartum reproductive performance.

Reference	Class of Cattle	Type of Fat	Response
Wehrman et al., 1991	Postpartum	Polyunsaturated	Earlier Cyclicity
Hightshoe et al., 1991	Postpartum	Saturated/Polyunsat.	Earlier Cyclicity
De Fries et al, 1998	Postpartum	Polyunsaturated	Earlier Pregnancy
Espinoza et al., 1995	Postpartum	Saturated/Polyunsat.	Earlier Cyclicity
Whitney et al., 2000	Heifers	Polyunsaturated	Earlier Pregnancy

Effects on first-service conception and cumulative pregnancy rates. Recently, in a review by Funston et al. (2005), it was implied that first-service conception rates could be substantially reduced in heifers by feeding high linoleic acid supplements (ie, soybeans, whole cottonseed; Howlett et al., 2003) or could reduce overall pregnancy rates in pasture-bred cattle (Shike et al., 2004). In one of these studies, the supplement added fat at only 2% of DM, which would be expected to have marginal effects on any variable. Examination of the Howlett paper suggested a misinterpretation, as no deficits in first-service conception rate were reported. Several other studies that were reviewed showed no effect of dietary fatty acid supplementation on these variables. In my opinion and experience, high fat supplements fed within the 4-5% of DM limit present no potential for adversely affecting reproduction. This includes the feeding of whole cottonseed which contains some gossypol. This subject has been exhaustively evaluated and discounted as a concern in the vast majority of beef cattle supplementation regimes (Gray et al., 1993; Jones et al., 1991).

Table 4 summarizes a trial at Beeville involving 199, Brahman x Hereford, F_1 females (87 pluriparous cows; 53 primiparous and 59 nulliparous heifers) fed either a high fat (3.5 lb whole cottonseed) or an isocaloric/isonitrogenous, corn/cottonseed meal-based control supplement beginning 30 d before the start of the breeding season and continuing for 30 d into the breeding season. Heifers were 14-15 months of age and averaged 725 lb (329.5 kg). All cattle had a BCS of at least 5 (5.2 $\pm$ 0.4). Females were stratified by age, parity, date of calving and BCS and allocated randomly to a 2 x 2 factorial arrangement of treatments: 1) Control-Normal Fat, 2) Control, High Fat 3) SMB, Normal Fat and 4) SMB,

High Fat. Synchronized females (SMB-treated) received the standard 9-day SMB regimen, and calves were removed from all cows for 48 h at the time of SMB implant removal. All SMB females were inseminated by TAI at 48-54 h after implant removal (SMB) and females in all groups were placed with fertile bulls 5 d after TAI in the SMB groups. Results indicated no beneficial or detrimental effects of fat supplementation on TAI conception rates or 45-day cumulative pregnancy rates. However, there was a tendency for fewer synchronized cows to be pregnant on d 45 than non-synchronized.

Table 4. Effects of SMB synchronization and high fat supplementation on TAI conception and cumulative 45-day pregnancy rates in Brahman x Hereford, F_1 females

Group	Age	No.	TAI conception, %	45-Day Pregnancy, %
SMB-Normal Fat	Pluriparous	22	54.5	82.0
	Primiparous	13	46.1	78.6
	Nulliparous	15	47.0	100
	Total	**50**	**50.0**	**86.3**
SMB-High Fat	Pluriparous	22	50.0	81.8
	Primiparous	14	42.8	92.8
	Nulliparous	15	53.3	80
	Total	**51**	**49.0**	**84.3**
Control-Normal Fat	Pluriparous	22	N/A	100
	Primiparous	13	N/A	100
	Nulliparous	14	N/A	92.8
	Total	**49**	**N/A**	**97.9**
Control-High Fat	Pluriparous	21	N/A	95.2
	Primiparous	13	N/A	92.3
	Nulliparous	15	N/A	92.8
	Total	**49**	**N/A**	**93.8**

Fat supplementation and onset of puberty. Several studies have examined the effects of high fat diets on age at puberty. Because sexual maturation is a brain-mediated event, we have no basis to expect and have not observed any effect of fat supplementation on age at puberty in *Bos indicus x Bos taurus* heifers (Garcia et al., 2003). A review of published studies in heifers by Funston et al. (2004) generally confirms this view, although one or two studies have shown small effects on reducing age at puberty.

Effects on uterine prostaglandin release. In addition to the studies summarized above that have examined the effects of dietary fat intake on lipoprotein cholesterol metabolism, insulin, growth hormone, and IGF-1 secretion, and ovarian follicular growth, other laboratories have focused on the role of fat supplementation and fatty acid metabolism on prostaglandin synthesis by the uterus. The primary basis of this work relates to the desire to modulate uterine prostaglandin synthesis during early pregnancy so as to avoid premature

luteal regression. This information is reviewed in more detail elsewhere (Staples et al., 1998; Thatcher and Staples, 2000). However, suffice it to say in the context of this overview that no definitive studies have been published to demonstrate that supplemental fats high in linoleic acid or in the n-3 fatty acids found in fish oil can consistently improve or diminish reproductive performance of beef or dairy cattle.

Management of Postpartum Reproduction

Selection for Fertility

Heritability of reproductive traits has traditionally been considered low, making genetic progress for reproductive efficiency slow. However, much of this lack of robustness is caused by environmental x genotypic interactions which make it difficult to accurately assess genetic worth. As already stated, crossbreeding has a large positive effect on reproductive efficiency. The use of physiological or genetic markers for reproduction has begun to be examined for their value in identifying superior individuals early in their life. One approach used at the Animal Reproduction Laboratory in Beeville was to examine responsiveness of the pituitary to GnRH early after calving (days 5-8 postpartum) and in heifers during pubertal development (Fajersson et al., 1999). We found that great variability exists in pituitary responsiveness to GnRH, forming essentially a normal distribution. In this herd, which has been selected for fertility, cows with high responses to GnRH did not have postpartum anovulatory intervals different from low-responding cows. However, cows exhibiting an early LH peak after a pharmacological challenge with GnRH had a longer postpartum interval than those with a late peak. The same measures in heifers did not predict age at puberty. Nevertheless, further work is needed in these areas, as the heritability for pituitary responsiveness to the gonadotropins has been shown to be near 0.45 in sheep. It is assumed that, in the future, it will be more likely that genotypic markers will be used for the selection of superior traits rather than physiological markers. Unfortunately, reproduction is a complex trait controlled by many genes. Therefore, identifying and selecting for increased frequency of a single gene may result in changes in products of that gene without improving overall reproductive performance.

Body Condition and Postpartum Reproduction

Body condition scoring (BCS) is an important element in management of beef cattle. On a 1 to 9 scale (1 = emaciated; 9 = obese), it is desirable to maintain cows in at least a BCS of 5 (good condition). However, cattle are managed throughout the world in environments that often result in BCS falling below this recommended level, and economics may not allow its prevention by supplemental feeding. Therefore, if BCS is allowed to vary with changes in environment and forage availability, attempts should be made through management to achieve a BCS as high as possible before calving. A low BCS at calving has greater negative effects than losses in BCS after calving or after conception (Short et al., 1990; Randel et al., 1990). If cows calve in excellent to moderate (BCS 5-6) condition, they can often rebreed early enough to withstand nutritional challenges during lactation. Therefore, they should be managed to recover body condition during the dry period and before the next parturition. Alternatively, positive effects on reproductive performance can

be realized if cows calving in less than optimum BCS are fed to gain body weight and condition after calving. However, **this is not a very economical approach** as significant amounts of supplemental nutrients will be used for milk production as opposed to reproduction. Therefore, it is best to calve cows in good body condition and then use strategic supplementation with protein to enhance intake and digestion of low to medium-quality forages for maintaining body condition.

Practical Supplementation Strategies Using Fat: Claims and Controversy

Fat supplementation and postpartum reproductive performance. 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 kg of calf produced is dependent upon an array of interactive management practices and environmental conditions. No studies have been conducted demonstrating that long-term use of fat supplementation during the postpartum, rebreeding period will contribute to markedly improved pregnancy rates or enhanced economic outcome. The majority of field studies that have examined the effects of fat supplementation on reproductive performance have suffered from the use of small numbers of animals, feeding strategies (e.g., prepartum; Bellows et al., 2001) that failed to appropriately exploit the physiological basis for fat supplementation established in earlier studies, or used cattle whose reproductive performance would not be expected to be compromised. Therefore, although a host of important physiological responses to fat supplementation have been documented, optimal strategies that provide predictable and consistent enhancements to reproductive performance have not been developed.

Feeding ruminant animals excessive quantities of fat (> 5% of total dry matter intake) can result in a marked negative effect on fiber digestibility and on dry matter intake. This occurs because of selection against microorganisms with cellulolytic capability. The level of fat that can be fed is also dependent upon the form of the feedstuff from which it is derived, and 5% of total dry matter may not be the maximum tolerable amount under all conditions. Fat contained in whole oilseeds can be fed at much higher levels than free oils mixed throughout the diet, as the oil is released into the rumen more slowly. Due to the lack of reactive double bonds, saturated fatty acids, such as those that predominate in animal tallow, pass through the rumen undegraded and are considered bypass fats. Some of the effects that these bypass fats have on the metabolism and physiology of the animal are potentially quite different from those created by polyunsaturated fatty acids metabolized in the rumen, although their caloric values are similar.

Sources of fat. The majority of the early work studying fat supplementation effects on reproduction employed either whole oilseeds, soybean oil, or Megalac®, which contains calcium salts of palm oil. Depending upon oil content, oilseeds were fed at a rate of 15 to 30% of the diet on a dry matter basis, and supplied 4 to 6% added fat. Oilseeds, particularly cottonseed, provide a unique blend of energy, protein, fiber, and fat and make an excellent supplemental feed when fed at 0.9 to 2.2 kg per head daily. An issue that has been raised regarding the use of whole cottonseed is that of gossypol toxicity. Beef cows consuming up to 20 g daily of dietary free gossypol for up to 2 months, via diets containing direct solvent-

extracted cottonseed meal (high gossypol) and whole cottonseed, exhibited no effects on reproductive endocrine function, estrous cycles, or pregnancy rates (Gray et al., 1993). Although high levels of gossypol do produce increased red blood cell fragility, this effect does not appear to create a clinically-significant pathology in beef cows under normal management conditions. Moreover, the levels of gossypol present in typically fed quantities of whole cottonseed for protein or fat supplementation (as described above) provided only a fraction of the amount of gossypol fed in the studies summarized above. In mature female cattle, the only reports of gossypol toxicity have been in the dairy industry involving diets containing up to 45% direct solvent cottonseed meal for 14 weeks. The reader is referred to a complete treatise on the subject of gossypol-containing feeds and gossypol toxicity in beef cattle (Jones et al, 1991).

Oilseeds are not universally available or economically practical under all conditions in which beef cattle supplementation is employed. Therefore, other alternatives are needed. One of these alternatives is molasses-based liquid supplements containing soybean oil soapstocks. Technology to maintain fat in a homogenous suspension for long periods continues to be the major challenge, and optimization of blends containing urea, sugars, fat and other constituents to promote consistent intake will be required. Recently, dry fat supplements containing 18 to 20% plant oil have been marketed for grazing beef (CONCEPT; Purina Mills, St. Louis, MO) and dairy cattle (High Fat Product; ADM, Decatur, IL) to exploit the benefits of fat supplementation on reproductive performance. Animal tallow has been used in supplements designed to enhance reproductive performance; however, there are marked palatability problems associated with high feedstuff concentrations of tallow. Therefore, it appears that plant-derived oils, when recommended for use at levels shown to maximize ovarian physiological responses, will continue to be the source of choice. Alternative commercial supplements or other by-products containing up to 20% plant oils are needed. Yellow grease, a by-product of the restaurant trade (20 to 25% linoleic acid), can be used as one of those alternatives.

Suckling Management

An increased understanding of how suckling mediates its negative effects on postpartum reproduction has aided our attempts to develop management protocols to reduce those effects. The following is a list of procedures that have been utilized to obviate the effects of suckling.

1. Temporary Calf Removal. This practice has been used since the early 1970's, particularly in association with estrous synchronization protocols. For example, removal of calves for 48 hours beginning at the time of removal of a progestin implant (SYNCRO-MATE-B; CRESTAR) or after GnRH treatment (OvSynch) will improve synchrony and timed-AI conception rates. However, we do not recommend that 48-hour calf removal be used alone to stimulate ovulation in anovulatory cows. In our experience, 48-hour calf removal is inadequate to achieve ovulation in more than 30% of anovulatory cows. This occurs because many cows that are responding to calf removal will again be suppressed by suckling if the calf is returned at 48 h (Williams et al., 1995). Moreover, this first ovulation is often not accompanied by

estrus. As it is not prudent to leave calves off of cows for more than 48 h due to health considerations, we recommend 48-h calf removal only when it can be combined with synchronization treatments that tend to induce ovulation in anovulatory cows.

2. Early Weaning. This technique is used in the U.S. when it is more economical to feed the calf than it is to feed the lactating cow. It is usually reserved for severe drought conditions and can allow managers to rebreed their cows without the high nutrient requirements associated with lactation.

3. Once-Daily Suckling. This is also a tool that is beneficial, particularly with first-calf heifers, when environmental conditions are challenging. First-calf, grazing heifers have been shown to return to estrus at a dramatically earlier rate than heifers suckled *ad libitum* (Randel, 1981).

4. Alien Suckling. As reviewed above, we now know that the maternal bond between a cow and her suckling calf is an important element in suckling-mediated anovulation. However, if cows are forced to suckle an alien for up to 6 d, cows will be "physiologically weaned" and ovarian cycles will resume. In the U.S., there are few if any management systems in which this tactic is practical. However, in countries in which cattle are managed for dual purposes (eg, milk and beef production), the use of alien suckling could prove beneficial and practical. Using this system, small groups of cows are usually intensively managed on a daily basis for both milking and suckling by the calf. Therefore, it should be possible to temporarily replace the cow's own calf with an alien for approximately 1 wk under controlled suckling conditions. This will result in the induction of ovulation in anovulatory cows, continue to allow milking of the cow, and provide adequate milk for the alien during the 6-day period. However, we have observed that suckled, Brahman-influenced cows tend to resist milk let-down when suckled by an alien; therefore, these calves often obtain milk only from the cisternae. As a result, total milk production is likely to decline during the 6-d alien suckling period.

5. Alien Cohabitation. This is a modification of the system described above and has been implemented successfully in estrous synchronization protocols. Since alien suckling does not have negative effects on LH secretion, we hypothesized that cohabitating alien or unrelated calves with cows during synchronization could substitute for 48-h calf removal and perhaps benefit the husbandry of calves weaned from their own dams. In those experiments, approximately 30% of Brahman x Hereford, F_1 females allowed some degree of suckling by an alien calf when housed in pens together with alien calves. Total suckling time by these calves over the 48-h period averaged 14.7 to 24 min, and the proportion of calves attempting suckling ranged from 24 to 44%. Alien suckling did not reduce calf weight losses compared to weaned calves. However, timed AI conception rates in cows treated with SMB were equal for cows subjected to 48-h weaning and alien cohabitation, but greater than cows allowed to suckle their own calves *ad libitum*.

Summary and Conclusions

An extended and variable period of anovulation occurs in suckled beef cows after parturition. This phenomenon exerts both biological and economic limitations on the efficiency of beef production world-wide. Intensive research efforts over the last 40 years have identified factors that regulate the length of the postpartum anovulatory interval, including post-gestational recovery of the hypothalamic-pituitary axis, nutrition, suckling, season of calving, and genotype. Moreover, a detailed understanding of many of the physiological, cellular, and molecular mechanisms underlying these effects has evolved and has, in some cases, yielded enlightened approaches to cattle management. Increased consumption of dietary fat influences ovarian follicular growth, steroid hormone production, growth factor synthesis or accumulation in follicular fluid, luteal activity, and postpartum anovulatory intervals in cattle. However, methods to consistently improve rebreeding performance have not been demonstrated. Major challenges remain in our efforts to link increased scientific understanding with management strategies and biotechnologies that are economically relevant.

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NOTES

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INSEMINATION RELATED FACTORS AFFECTING FERTILIZATION IN ESTRUS-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 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. Recent 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 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 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 the minimum number of motile sperm required for maximum fertility (threshold) differed among bulls and bulls also differed in the maximum fertility at any

dosage (Figure 1). They also observed low fertility bulls required more sperm be inseminated than high fertility bulls in order to reach their respective maximum fertility. They postulated the

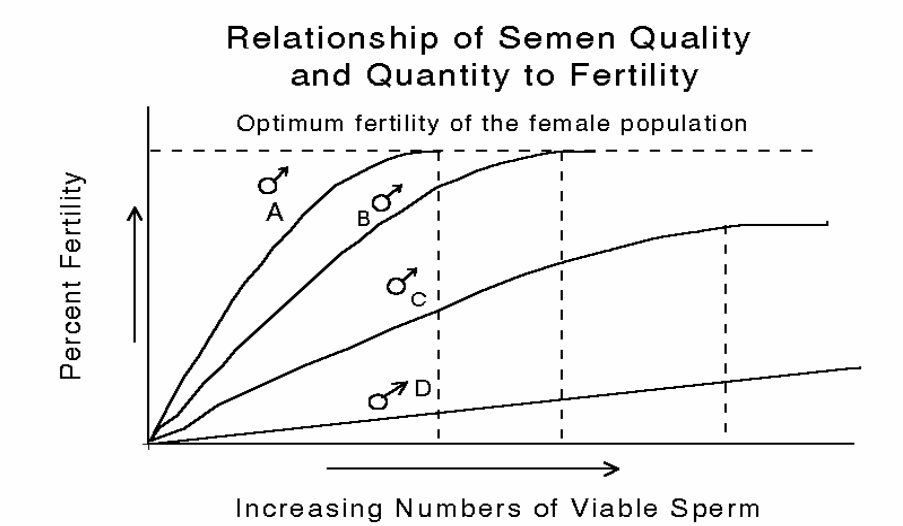

Figure 1. Relationship between pregnancy rate and the number of spermatozoa inseminated. The semen of different bulls varies in the maximum non-return rate and in the rate at which the maximum fertility is achieved with increasing sperm dosage (modified from Sullivan and Elliott (1968).

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 to be true in a later study (Saacke et al., 1998) where sperm with classically misshapen heads did appear as accessory sperm following artificial insemination. From AI data in the Netherlands, den Daas et al. (1992) found 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 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 would 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 competent sperm may be delivered to the cow. Seminal deficiencies that are **uncompensable** would be those that result in subfertility to AI or natural service 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 incompetent sperm can preempt 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. Bulls providing semen with unacceptable

levels of abnormal sperm appear to be the main source of uncompensable traits and should not be offered for semen preservation and use in AI.

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 ovulated 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 interest is the association of accessory sperm number per egg/embryo to the fertilization status and embryo quality. This is best described by the median number (50 percentile of cows) 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 embryo quality tends to be positively related to median accessory sperm number. Good to excellent embryos have more accessory sperm than do degenerate or fair to poor embryos. This rather small difference has been interpreted to suggest 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. There is evidence this competition favors a more competent sperm (i.e., there is sperm selection at the zona pellucida of the egg, Howard et. al., 1993) as well as at other locations in the female tract (previously reviewed, Saacke et al., 2000). 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 804 embryos recovered from the 927 ova/embryos represented in Figure 2 and Table 1. It is apparent from Figure 3 that nearly 10 sperm per embryo were necessary to reach the maximum embryo quality index, after which increasing accessory sperm numbers had no influence on embryo quality. Regardless of embryo quality, the mode in accessory sperm number remained 0 suggesting that nature intended few sperm to approach the egg. On the otherhand, this exercise 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., approach 10 sperm/egg) necessary to maximize both fertilization rate and embryo quality for a general population of bulls. It should also be clear that the large variation in accessory sperm within and across fertilization/embryo status categories (Table 1) would preclude any use of accessory sperm numbers in predicting male fertility. However, increasing accessory sperm numbers could indicate

directions to be taken in adopting reproductive practices and strategies favoring improved pregnancy rates. Improving accessory sperm number would not be expected to bring inseminates or bulls harboring uncompensable traits into a normal reproductive range, thus, use of such bulls should continue to be discouraged.

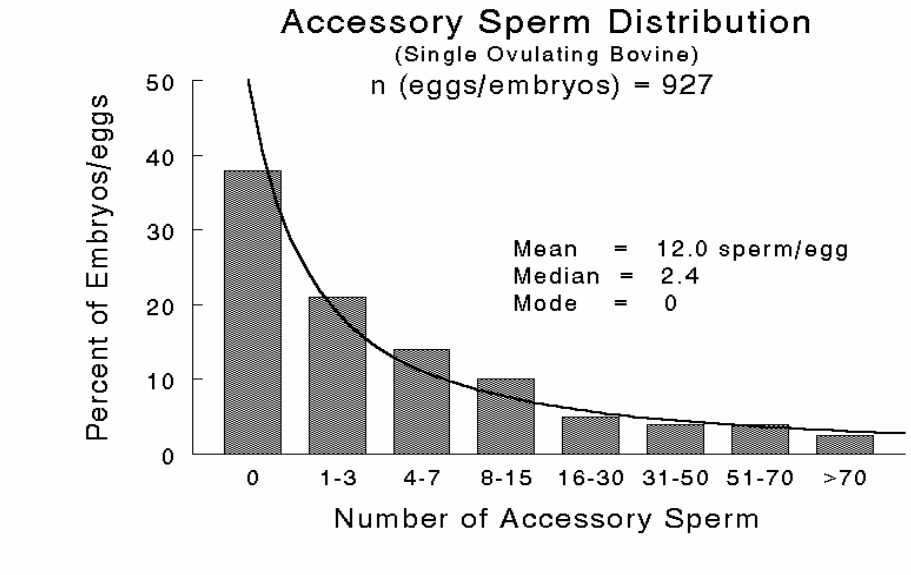

Figure 2. Frequency distribution of accessory sperm per embryo or ovum in artificially inseminated single-ovulating cows. Quality and quantity of semen used varied, but was within acceptable standards for commercial artificial insemination. Similar distributions have been reported for individual experiments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

Table 1. Relationship of accessory sperm per embryo/ovum to fertilization status and embryo quality (n=927)

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

Embryo quality based upon Lindner and Wright, 1983 as modified for degenerate embryos by DeJarnette, et al., 1992

It is important one understands how embryo quality affects pregnancy rate. The best data on this point is Lindner and Wright (1983), who developed the embryo scoring system we used in the data presented above. They showed embryos classified as excellent to good produce twice as many pregnancies upon transfer to recipients as those 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 nearly 10 sperm per ovum/embryo to optimize embryo quality (Figure 3), it is clear 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, i.e., choice of bull and time of insemination relative to ovulation.

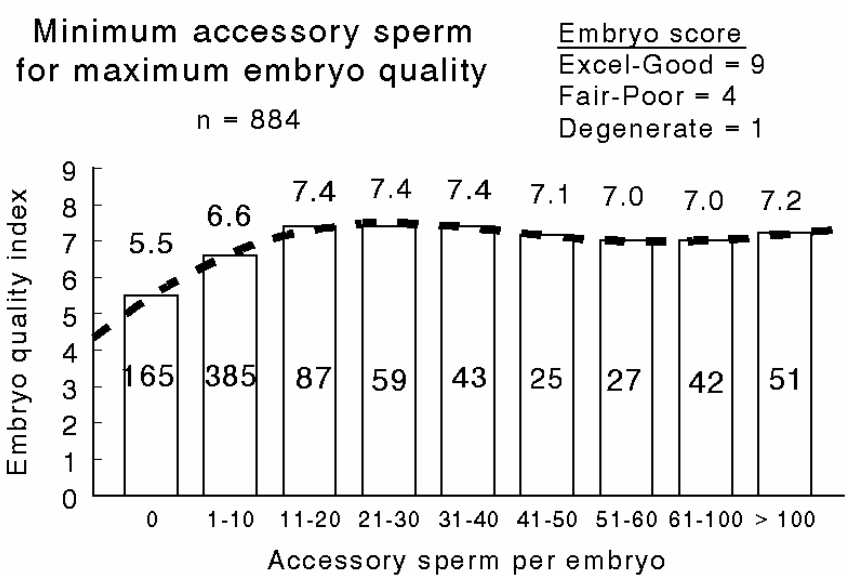

Figure 3. Histogram showing the numbers of accessory sperm required to maximize embryo quality index for 6 day-old embryos (morulae) derived from artificial insemination of single-ovulating cows. Embryo grading was according to Lindner and Wright (1983) as modified by DeJarnette et al., (1992). Embryo 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.

The Effect of Bulls and Time of Insemination on Sperm Access to the Egg and Embryo Quality

Even 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 four 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 three 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 this bull would be less vulnerable to inseminator error in semen placement and handling than would other bulls.

Table 2. Accessory sperm differences per embryo/egg among bulls used at the same insemination dosage

Bull	n	Median	Mean ± SD
A	25	40	53 ± 61
B	37	8	15 ± 23
C	16	13	36 ± 65
D	20	2	11 ± 16

Nadir et al., 1993

Such a bull would be considered to have little to no compensable deficiencies and easily meet threshold numbers of sperm to the cow by AI. 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 could be expected to depend more heavily on dilution rates, inseminator competence and timing of insemination. Based on a median of two sperm per egg, bull D might be more marginal in optimizing fertilization rate and embryo quality under current use in AI. The seminal differences we are addressing 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 understand and can only determine by fertility data from the artificial insemination of adequate numbers of cattle.

With respect to differences among bulls important to embryo quality, i.e., the competence of a bull's fertilizing sperm or the uncompensable deficiency in his semen; our best judge of this is the occurrence of abnormal sperm in the semen. Abnormal sperm in the semen reflect the health of the spermatogenic process in the testes of the bull 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 is 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 bull's 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. Of interest is that abnormal sperm in semen (the best indicator of uncompensable deficiencies), rarely get to the egg in vivo (Saacke et al., 1998). However, these abnormal sperm represent a deficiency in DNA quality that extends to the normal appearing sperm in the same samples (Acevedo et al. 2001) rendering them incompetent in sustaining the embryo after fertilization. Posing a particular problem in uncompensable semen deficiencies among beef breeds in particular, are fat bulls and a percentage of those coming off "hot rations" from test stations, where testicular thermoregulation has been impaired by inguinal fat (Kastelic et al. 1996).

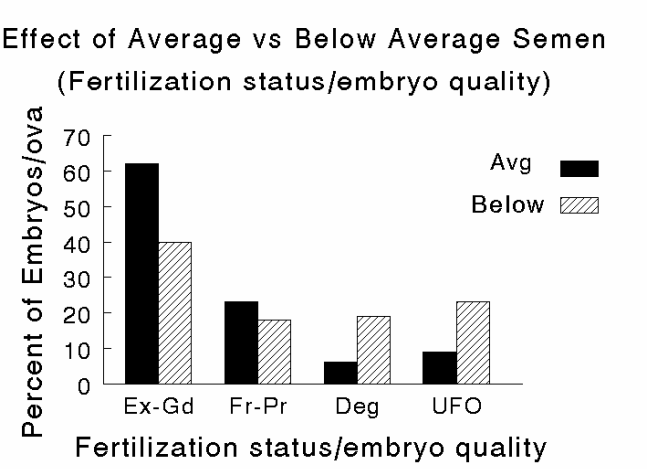

Figure 4. Effect of average and below average semen (based upon content of abnormal sperm) on fertilization status/embryo quality in single ovulating cattle. Both, fertility and embryo quality were influenced by the semen as noted in the shift in distribution across categories (n = 21 and 22 for the 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). 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 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. 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 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. In the current study, this would indicate that a rather large portion of eggs would be aging awaiting sperm arrival. This probably attributes most of the degenerate embryos to late insemination rather than a male-related uncompensable trait. On the other hand, the high embryo quality associated with early insemination suggests 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.

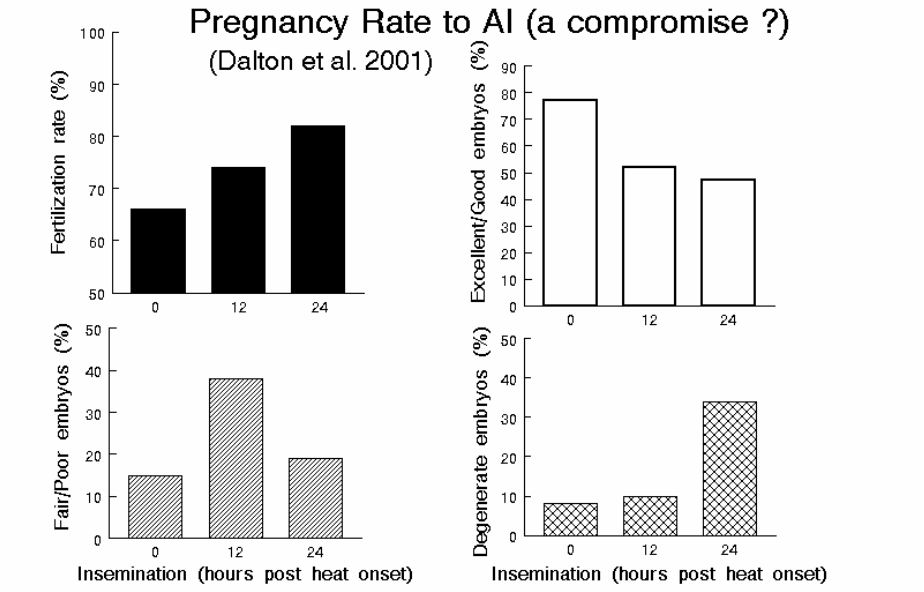

Figure 5. Effect of time of artificial insemination following onset of standing heat (Heat Watch System®) on fertilization status and embryo quality judged 6 days following artificial insemination (n = 117). (Dalton et al. 2001)

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

(breeding time post onset of estrus based on HeatWatch System®)

Treatment	n	Mean ± SD	Median	% Fert
0 hour	39	9 ± 23	1	66
12 hour	39	21 ± 46	2	74
24 hour	39	33 ± 53	4	82

- Ovulation 27. 6 ± 5.4 hours
- 25 x 10^6 sperm/dose

(Dalton et al., 2001)

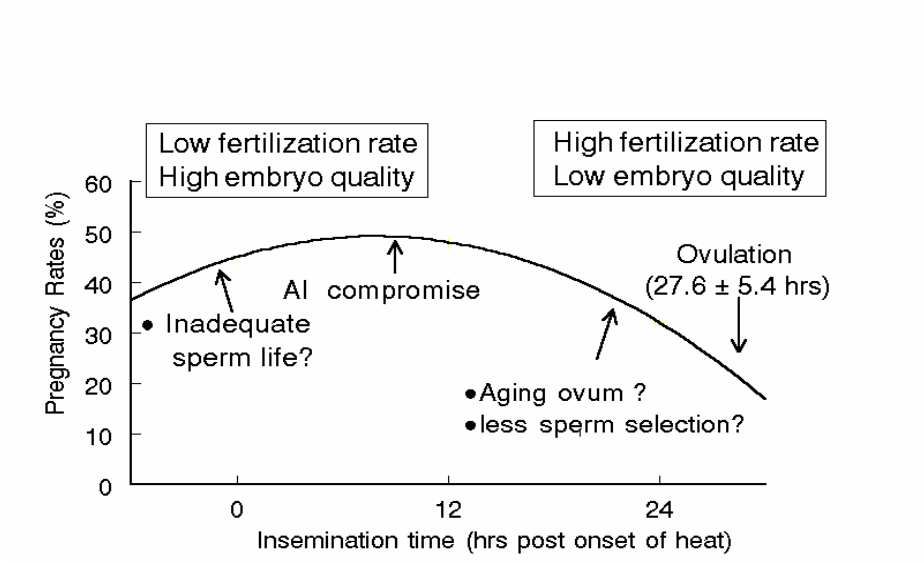

Figure 6. Calculated pregnancy rate from data presented in Figure 5 and based upon the ability of embryos classified excellent to degenerate to constitute a pregnancy (according to Lindner and Wright, 1983). AI as a compromise is based upon early inseminations being inadequate due to high levels of unfertilized 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).

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 in a group of treated animals. 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 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 ovulation and tightening the variation in time of ovulation.

Finally, I would end this discussion by again recognizing the magnitude of bull differences that can greatly influence results to a synchronization program. Differences we have seen among bulls in response to time of insemination for one of our studies are 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, 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 know the expected time and variation in ovulation in order to choose an insemination time maximizing results to most bulls. Lastly, subscribing to a reputable semen source is the best protection against the use of poor quality semen.

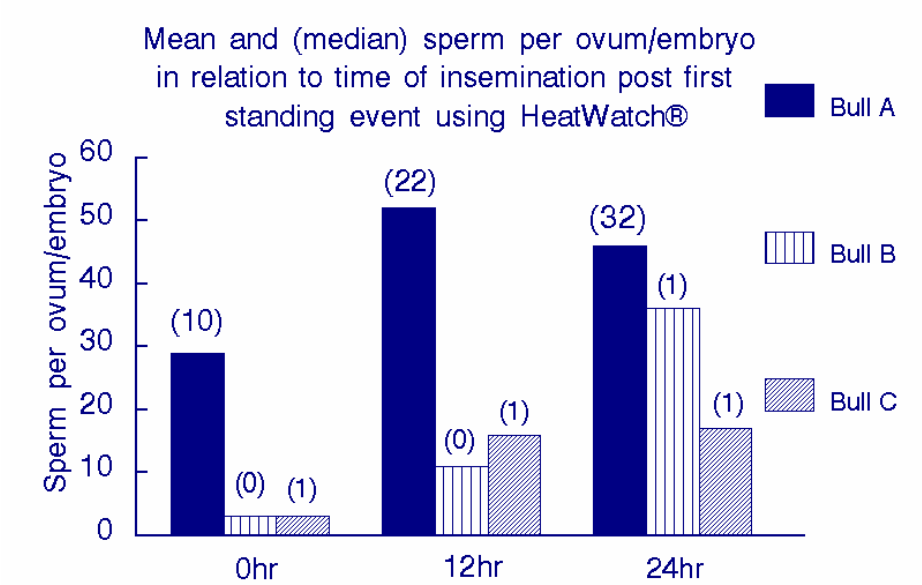

Figure 7. Variation among bulls in sperm access to the egg relative to time of insemination post heat onset. Mean sperm per egg/embryo is shown by the bars and the median number in brackets. Bull A has adequate numbers of sperm accessing the egg at all breeding times while bulls B and C require insemination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

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NOTES

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FACTORS THAT INFLUENCE FERTILITY IN NATURAL AND SYNCHRONIZED BREEDING PROGRAMS

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Introduction

Reproductive failure is a major source of economic loss in the beef industry. The majority of this loss occurs because cows do not become pregnant during a defined breeding season. Therefore, the goal of any breeding program is to maximize the number of females that become pregnant. This means that fertility plays a major role in the success of any breeding program. This review will focus on the factors that affect, pregnancy rates over specific days of the breeding season, in both natural service and synchronized breeding programs. Since pregnancy rates are a product of both estrous detection rates and conception rates, comparisons will be made between synchronized and non-synchronized cows bred by natural service or by artificial insemination.

Artificial insemination provides a method to inseminate a large number of females to a single sire that has been selected/proven to be an industry leader for economically relevant traits. Thus, genetic change in a herd can occur quickly through the use of artificial insemination. With natural service, herd bulls are also selected for economically relevant traits but are limited on the number of cows/heifers they can service during the breeding season. During the breeding season, a herd bull's job is to detect cows/heifers in standing estrus and breed them at the appropriate time. For successful artificial insemination of cattle to occur, the producer (herd manager) must take the place of the herd bull in detecting the cows/heifers that are ready to be inseminated.

Synchronizing estrus is an effective way to minimize the time and labor required to detect standing estrus in cattle that are going to be artificially inseminated. However, estrous synchronization can also benefit overall herd management. Cows that respond and conceive to a synchronized estrus have the following advantages: 1) exhibit standing estrus at a predicted time, 2) conceive earlier in the breeding season, 3) calve earlier in the calving season, and 4) wean calves that are older and heavier at weaning. In addition, some estrous synchronization protocols (progestin-based protocols) can induce a proportion of anestrous cows to begin estrous cycles. This will decrease the anestrous postpartum interval and allow for more chances for cows to conceive during a defined breeding season. A study conducted at Colorado State University indicated cows that conceived to a synchronized estrus calved on average 13 days earlier and weaned calves 41 pounds heavier than cows that were not synchronized (Schafer et al., 1990).

Estrous Synchronization simply implies the estrous cycles of a group of heifers/cows are manipulated to cause them to exhibit standing estrus around the same time. However, the question is often asked, "Do estrous synchronization protocols increase or decrease fertility?" To answer this question fertility must be compared between non-synchronized and synchronized females bred by natural service or artificial insemination.

Fertility of Synchronized and Non-synchronized Females
Natural Service

Nonsynchronized females: When cows are bred by natural service, the time required to detect estrus is not a concern, since the bull will be detecting the cows that exhibit standing estrus, but the serving capacity of the bull becomes a critical management consideration. Recommendations for the bull to female ratio in nonsynchronized cows ranges from 1:10 to 1:60. No differences were detected between a bull to female ratio of 1:25 and 1:60 for estrous detection or pregnancy rates in the first 21 days of the breeding season provided the bulls were highly fertile and had large scrotal circumferences (Rupp et al., 1977).

Synchronized females: When cows are synchronized and bred by natural service, management considerations should be made for the serving capacity of the bull. Healy et al., (1993) reported a tendency ($P < 0.10$) for pregnancy rates over a 28-day synchronized breeding season to be reduced when a bull to female ratio of 1:50 (77%) was used compared to a bull to female ratio of 1:16 (84%); however, no difference was detected between a bull to female ratio of 1:16 and 1:25 (84% and 83%, respectively). In the following studies, a bull to female ratio of up to 1:25 was used.

A single injection of prostaglandin $F_{2\alpha}$ (**PG**) on day 4 of the breeding season (bulls introduced on day 1) resulted in more cycling cows becoming pregnant during days 5 to 9 of the breeding season compared to cycling cows not injected with PG (55.7 vs. 25.0%, respectively; Whittier et al., 1991). In addition, pregnancy rates were similar ($P > 0.10$) for cows in which estrus was synchronized with a single injection of PG and exposed to a bull for 80 hours (19%) compared to non-synchronized cows exposed to a bull for 21 days (33%; Landivar et al., 1985). When cows were synchronized with a single injection of PG on day 4 of the breeding season, there were no differences in pregnancy rates over the first 25 days of the breeding season (1 cycle) between synchronized and non-synchronized cows (Whittier et al., 1991). Therefore, the greatest benefit of estrous synchronization (PG) with natural service is the ability to get more cows pregnant during the first 5 to 7 days of the breeding season (**Table 1**). Cows that exhibit estrus early in the breeding season will also have additional chances to conceive during a defined breeding season. The average estrous cycle is 21 days (range 18 to 23 days), allowing one chance every 21 days for a cow to conceive. During a 65-day breeding season, cows that cycle naturally have only three chances to conceive, but cows that are synchronized and show estrus the first few days of the breeding season have up to four chances to conceive.

Table 1. Comparison between synchronized and non-synchronized pregnancy rates when bred by natural service in cows and heifers

Study	Cows/ Heifers	Period of Time	Synchronization Method	Pregnancy Rate Anestrual	Pregnancy Rate Estrual Unknown
(Whittier et al., 1991)	Cows	4 days	1 shot PG	13.6%	55.7%[a]
			Not synchronized	22.7%	25.0%[b]
(Plugge et al., 1989)	Heifers	7 days	MGA + PG	62%[a]	
			Syncro-Mate B	67%[a]	
			Not synchronized	23%[b]	
(Landivar et al., 1985)	Cows	80 hours	1 shot PG	19%	
		21 days	Not synchronized	33%	
(Whittier et al., 1991)	Cows	25 days	1 shot PG	59.1%	86.1%
			Not synchronized	59.1%	76.3%

Pregnancy rates within a study and estrous cycling status having different superscripts are different [ab]$P < 0.01$

Some estrous synchronization protocols that utilize progesterone (CIDR), norgestomet (Syncro-Mate B), or GnRH can initiate estrous cycles resulting in a shorter anestrous postpartum period or earlier onset of puberty (Yavas and Walton, 2000a; Lucy et al., 2001; Perry et al., 2004a). In a small study, peripubertal heifers treated with melengestrol acetate (**MGA**, an orally active progestin) for 10 days resulted and a similar number of MGA treated heifers and control heifers attaining puberty by day 7 after MGA withdrawal, but by day 10 following MGA treatment, 50% more of the treated heifers attained puberty compared to the control animals (Imwalle et al., 1998). Heifers synchronized following progestin exposure [norgestomet (Syncro-Mate B) or MGA] resulted in more ($P < 0.01$) heifers becoming pregnant (67% and 62%) during the first 7 days of the breeding season compared to non-synchronized heifers (23%, Plugge et al., 1989), but when a single injection of PG was administered to a group of anestrous cows, no difference was detected between synchronized and non-synchronized cows (13.6% and 22.7%, respectively, Whittier et al., 1991). Therefore, estrous synchronization protocols capable of inducing puberty and shortening the anestrous postpartum period can result in an even greater percentage of cows having a chance to become pregnant during the first few days of the breeding season.

Artificial Insemination

Artificial insemination (**AI**) with semen collected from genetically superior sires is the most efficient and economical method for the genetic improvement of economically important traits in the beef industry. Estrous synchronization makes AI more feasible due to the reduction in time and labor required for estrous detection. Therefore, it is also necessary to compare fertility between synchronized and non-synchronized females bred by AI (**Tables 2 and 3**). When AI is combined with estrous synchronization, the limitation on serving capacity of a single bull is removed, and a large number of females can be bred to a single sire during the first few days of the breeding season. This can result in a more uniform calf crop that is older and heavier at weaning.

Table 2. Comparison between synchronized and non-synchronized pregnancy rates when bred by artificial insemination during the synchronized period

Study	Cows/ Heifers	Period of Time	Synchronization Method	Pregnancy Rate Anestrual	Pregnancy Rate Estrual
				Unknown	
(Lucy et al., 2001)	Cows	3 days	1 shot PG	11%[b]	34%[c]
			Progesterone + PG	26%[a]	46%[b]
			Not synchronized	4%[c]	11%[a]
(Lucy et al., 2001)	Heifers	3 days	1 shot PG	6%[b]	19%[b]
			Progesterone + PG	28%[a]	49%[a]
			Not synchronized	6%[b]	9%[c]
(Landivar et al., 1985)	Cows	80 hours	1 shot PG	19%	
		21 days	Not synchronized	30%	
(Heersche et al., 1979)	Heifers	5 days	Norgestomet + PG	60%	
		21 days	Not synchronized	61%	
(Beal et al., 1988)	Cows/ Heifers	7 days	MGA-PG		40%[a]
			Not synchronized		24%[b]
(Beal, 1983)	Cows	5 days	2 shots PG	28%[ab]	
			Progesterone + PG	49%[a]	
			Not synchronized	10%[c]	
(Miksch et al., 1978)	Heifers	5 days	Syncro-Mate B	36%[b]	
			Not synchronized	17%[c]	
(Miksch et al., 1978)	Heifers	5 days	Syncro-Mate B	39%	
			Not synchronized	28%	
(Miksch et al., 1978)	Cows	5 days	Syncro-Mate B	48%[a]	64%[a]
			Not synchronized	8%[b]	20%[b]
(King et al., 1988)	Cows	5 days	Syncro-Mate B	50%[a]	
			Not synchronized	16%[b]	

Pregnancy rates within a study and estrous cycling status having different superscripts are different [ab; ac]$P<0.01$ [bc]$P < 0.05$

Table 3. Comparison between synchronized and non-synchronized pregnancy rates when bred by artificial insemination during the first cycle of the breeding season

Study	Cows/ Heifers	Period of Time	Synchronization Method	Pregnancy Rate	
				Anestrual	Estrual
(Lucy et al., 2001)	Cows	31 days	1 shot PG	47%	65%[a]
			Progesterone + PG	46%	71%[a]
			Not synchronized	42%	58%[c]
(Lucy et al., 2001)	Heifers	31 days	1 shot PG	25%[b]	56%[c]
			Progesterone + PG	50%[a]	69%[a]
			Not synchronized	31%[b]	64%[c]
(Beal et al., 1988)	Cows/ Heifers	30 days	MGA-PG		72%
			Not synchronized		69%
(Beal, 1983)	Cows	24 days	2 shots PG	52%	
			Progesterone	53%	
			Not synchronized	56%	
(Miksch et al., 1978)	Heifers	27 days	Syncro-Mate B	64%	
			Not synchronized	62%	
(Miksch et al., 1978)	Heifers	27 days	Syncro-Mate B	74%	
			Not synchronized	67%	
(Miksch et al., 1978)	Cows	21 days	Syncro-Mate B	67%	79%
			Not synchronized	45%	76%
(King et al., 1988)	Cows	21 days	Syncro-Mate B	67%[a]	
			Not synchronized	56%[c]	
(King et al., 1988)	Cows	25 days	Syncro-Mate B	75%[a]	
			Not synchronized	61%[b]	

Pregnancy rates within a study and estrous cycling status having different superscripts are different [ab]$P < 0.01$; [ac]$P < 0.05$

Cows synchronized with a single injection of PG and artificially inseminated for an 80 hour period had similar ($P > 0.10$) pregnancy rates (19%) compared to cows artificially inseminated for a 21-day period (30%; Landivar et al., 1985). However, when fertility is compared over the synchronized period, a single injection of PG 2 days before the start of the AI breeding season resulted in more ($P < 0.01$) cows pregnant during the first 3 days of the breeding season (22%) compared to non-synchronized females (7%, Lucy et al., 2001). Furthermore, cows synchronized with two injections of PG 11 days apart also resulted in more ($P < 0.01$) cows pregnant (28%) during the first 5 days of the breeding season compared to non-synchronized cows (10%, Beal, 1983).

When estrous synchronization protocols are used that will initiate estrous cycles [progesterone (CIDR), norgestomet (Syncro-mate-B), and GnRH protocols], an even greater benefit can be realized. Cows treated with a CIDR for 7 days before the start of

the breeding season and an injection of PG at time of CIDR removal resulted in 26% of anestrous and 46% of estrous cycling cows becoming pregnant during the first 3 days of the breeding season compared to only 4% of anestrous and 11% of estrous cycling control cows (Lucy et al., 2001). Cows synchronized with Syncro-Mate B (**SMB**) resulted in more cycling and anestrous cows pregnant ($P < 0.01$; 64% and 48%, respectively) during the first 5 days of the breeding season compared to cycling and anestrous non-synchronized cows (20% and 8% respectively, Miksch et al., 1978). Furthermore, when heifers were synchronized with SMB, a greater ($P < 0.05$) percentage became pregnant (36%) during the first 5 days of the breeding season compared to non-synchronized heifers (17%, Miksch et al., 1978). Estrous synchronization protocols that utilize GnRH are also able to initiate estrous cycles in anestrous cows. When a GnRH-based protocol (Ovsynch; 100 μg GnRH, i.m. on d -9; 25 mg PG, i.m. on d -2; 100 μg GnRH, i.m. on d 0 and timed AI on day 1) was compared to SMB with timed-AI, similar pregnancy rates were obtained ($P > 0.10$) by both protocols among anestrous cows (43% and 49% respectively, Geary et al., 1998). Therefore, estrous synchronization protocols capable of inducing puberty and shortening the anestrous postpartum period can result in anestrous cows having a chance to become pregnant during the first few days of the breeding season and more opportunities to conceive during the breeding season.

Initiation of Estrous Cycles

The anestrous postpartum interval is a major contributing factor to cows failing to become pregnant and calving on a yearly interval (Short et al., 1990; Yavas and Walton, 2000b). However, treatment with some progestins can induce ovulation in anestrous postpartum cows (Yavas and Walton, 2000a; Lucy et al., 2001; Perry et al., 2004a), thereby shortening the anestrous postpartum interval. Consequently, many estrous synchronization protocols include progestin exposure. However, all progestins are not equally effective at inducing the initiation of estrous cycles in anestrous postpartum cows. Evidence for this difference is based on differences in the ability of progesterone (CIDR) and MGA to induce ovulation in anestrous cows (**Figure 1**). Fewer anestrous cows treated with MGA (0.5 mg MGA$\bullet cow^{-1}\bullet d^{-1}$ for 7 days) ovulated compared to progesterone-treated [1.9 g of progesterone contained in a controlled internal drug releasing device (**CIDR**) for 6 days] cows (33% and 91%, respectively, Perry et al., 2004a), and fewer anestrous cows that spontaneously initiated estrous cycles (23%) or MGA-treated anestrous cows (46%) exhibited normal length luteal phases compared to progesterone-treated cows (100% and 100%, Smith et al., 1987; Perry et al., 2004a). However, by day 22 after treatment withdrawal there was no difference ($P > 0.05$) between the percentage of CIDR treated cows that had ovulated (91%) and the percentage of MGA-treated cows that had ovulated (61%, Figure 1, Perry et al., 2004a). These data indicate that following a CIDR protocol (progesterone exposure) a large percentage of cows should exhibit estrus, and following a MGA protocol (14 day of MGA and an injection of PG on day 33) an equally large percentage of cows should exhibit estrus. For example, when heifers were synchronized by progestin exposure (MGA or norgestomet), more heifers became pregnant ($P < 0.01$, MGA 62% and SMB 67%) during the first 7 days of the breeding season compared to non-synchronized heifers (23%), but there was no difference between MGA and norgestomet in the percentage of heifers pregnant during the first 7 days of the breeding season (Plugge et al., 1989). Furthermore, when a group of cycling cows and heifers were synchronized

with a 7-day MGA protocol (MGA-PG), pregnancy rates after 7 days (40%) of artificial insemination were greater in synchronized animals compared to non-synchronized animals (24%, Beal et al., 1988).

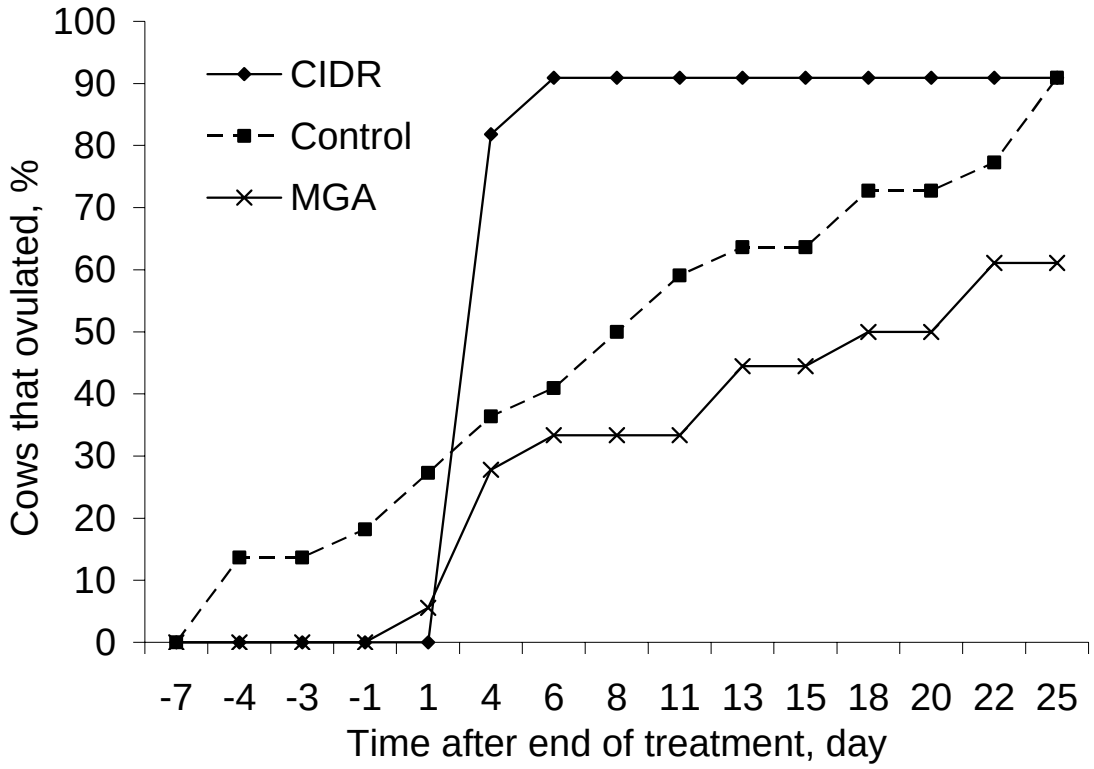

Figure 1. Effect of treatment on the cumulative percent of animals that had ovulated (ovulation is shown as having occurred 4 days before the first day circulating concentrations of progesterone were > 1 ng/mL) by day of treatment (day 0 = last day of feeding melengestrol acetate [MGA], and day of controlled internal drug-releasing device [CIDR] removal). Control animals received no treatment. Treatment $P < 0.01$; Day $P < 0.01$; Treatment x Day $P < 0.01$. (Perry et al., 2004a)

Estrous Detection

When pregnancy rates from 13,942 first service artificial inseminations were compared to 6,310 first services by natural service, no difference ($P > 0.10$) was detected between artificial insemination and natural service (Williamson et al., 1978). Furthermore, no differences were detected between synchronized pregnancy rates when cows were bred by AI or natural service (Plugge et al., 1989). However, for successful artificial insemination of cattle to occur, the producer (herd manager) must take the place of the herd bull in detecting the cows/heifers ready to be inseminated. Detecting standing estrus (also referred to as heat detection or detecting standing heat) is simply looking for the changes in animal behavior associated with a cow/heifer standing to be mounted by a bull or another cow/heifer. Detecting animals in standing estrus is the goal of good estrous detection and plays a vital role in the success of any artificial insemination program. However, when estrus was detected in 500 Angus cows with Heat Watch estrus-detection aids (24 hour a day estrus detection), the length of estrus averaged

around 10 hours (ranged from 0.5 hours to 24 hours), and 26% of cows exhibited estrus for less than 7 hours and had fewer than 1.5 mounts per hour (Rorie et al., 2002).

In a study conducted at Colorado State University, animals were administered an estrous synchronization protocol, then monitored for standing estrus 24 hours a day or twice a day for 30 minutes. By day 5 after estrous synchronization, 95% of animals monitored 24 hours a day were detected in standing estrous, while only 56% of animals observed twice a day for 30 minutes were detected in standing estrus (Downing et al., 1998). With a 95% estrous detection rate and a 70% conception rate (95% X 70% = 67%), 67% of the animals will be pregnant; whereas, only a 39% (55% X 70% = 39%) pregnancy rate will occur with a 55% estrus detection rate (**Table 4**). Therefore, a successful artificial insemination program requires good estrous detection.

Table 4. Effect of estrous detection rate on increasing pregnancy rate

Estrous Detection Rate	**55%**	**60%**	**65%**	**70%**	**75%**	**80%**	**85%**	**90%**	**95%**
Conception Rate	70%	70%	70%	70%	70%	70%	70%	70%	70%
Pregnancy Rate	**39%**	**42%**	**46%**	**49%**	**53%**	**56%**	**60%**	**63%**	**67%**

To maximize detection of standing estrus, it is extremely important to visually monitor cattle as much as possible. Observations should occur as early and as late as possible as well as during the middle of the day. Continuous observation of over 500 animals exhibiting natural estrus in 3 separate studies indicated 55.9% of cows initiated standing estrus from 6 p.m. to 6 a.m. (**Table 5**). Furthermore, when cows were observed for standing estrus every 6 hours (6 a.m., noon, 6 p.m., and midnight), estrous detection increased by 10% with the addition of a mid-day observation and by 19% when observed four times daily (every 6 hours) compared to detecting standing estrus at 6 a.m. and 6 p.m. alone (Hall et al., 1959). Therefore, detection of standing estrus can be one of the most time-consuming chores related to artificial insemination. However, the success of any artificial insemination program requires detecting the animals that are ready to be bred (standing estrus) and inseminating them at the correct time. Failing to detect estrus and mis-detection of estrus can result in significant economic losses (Heersche and Nebel, 1994). Several estrous detection aids have been developed to assist with this time consuming chore. These estrus-detection aids can effectively determine which cows are or have been in standing estrus, therefore relieving some of the time required to visually observe cattle for standing estrus. However, increased visual observation, in addition to the use of estrous-detection aids, could improve fertility by detecting the most possible number of animals ready to be inseminated and indicating the most appropriate time for insemination.

Table 5. Time of day when cows exhibit standing estrus

Time of day	Cows exhibiting standing estrus
6 a.m. to 12 noon	26.0 %
12 noon to 6 p.m.	18.1 %
6 p.m. to midnight	26.9 %
Midnight to 6 a.m.	29.0 %

Data adapted from (Hurnik and King, 1987; Xu et al., 1998, G.A. Perry unpublished data).

Fixed-Time Insemination

To expand the use of artificial insemination and increase the adoption rate of other emerging reproductive technologies, precise methods of controlling ovulation must be developed. Therefore, numerous studies have been conducted to induce ovulation in cattle at a specific time, thereby eliminating the time and labor required to detect estrus. Methods of inseminating cattle at a fixed-time with consistently high pregnancy rates may be a reality in the near future. Stevenson et al. (2000) reported higher pregnancy rates ($P < 0.05$) for cattle artificially inseminated following detection of standing estrus (44%; Select Synch - GnRH on day -9, PG on day -2 and detect estrus) compared to cattle bred by timed AI (33%; CO-Synch – Select Synch with timed insemination and a second injection of GnRH on day 0). However, Lemaster et al., (2001) reported higher ($P < 0.05$) pregnancy rates for timed AI following the CO-Synch protocol (31%) compared to AI following estrus detection with the Select Synch protocol (21%).

Currently, most fixed-time insemination protocols (ovulation synchronization protocols) utilize GnRH to ovulate a dominant follicle around the time of insemination. The Ovsynch (Pursley et al., 1998) and CO-Synch (Geary and Whittier, 1998) protocols include the same hormonal treatments to synchronize ovulation [on day -9, GnRH is administered, on day -2, PG is administered, and 48 hours later (day 0) GnRH is administered to induce ovulation around the time of insemination]. The MGA-select timed-AI protocol (MGA is fed for 14 days, on day 26 GnRH is administered, on day 33 PG is administered, and 72 hours later GnRH is administered to induce ovulation around the time of insemination, Perry et al., 2002b) also utilizes GnRH to induce ovulation around the time of insemination. The use of GnRH at the time of insemination resulted in a wide range of follicle sizes being induced to ovulate (Perry et al., 2005), and although dominant bovine follicles (≥ 10 mm) have the ability to ovulate in response to a GnRH-induced gonadotropin surge, a larger dose of LH was required to induce ovulation of a 10 mm follicle compared to larger follicles (Sartori et al., 2001).

A decrease in pregnancy rates occurred when small follicles were induced to ovulate following fixed-time AI in both heifers and cows (CIDR Protocol – Lamb et al., 2001 T.W. Geary unpublished data; CO-Synch protocol – Perry et al., 2004b; Perry et al., 2005; **Figure 2**). In addition, when the length of proestrus was varied to induce ovulation of small (< 12 mm) or large (≥ 12 mm) follicles, pregnancy rates were decreased in animals induced to ovulate small follicles compared to animals induced to ovulate large follicles (Mussard et al., 2003). The ovulatory follicle may affect fertility through the preparation of the oocyte for embryonic development, preparation of follicular cells for

luteinization, and/or preparation of the uterine environment for the establishment and maintenance of pregnancy. However, when embryos of similar quality were transferred into cows induced to ovulate small (< 12 mm) or large (> 12 mm) follicles, cows induced to ovulate small follicles had significantly lower pregnancy rates compared to cows induced to ovulate large follicles (Mussard et al., 2003). The preceding study indicates the uterine environment is likely a major factor in decreased fertility following induced ovulation of small dominant follicles.

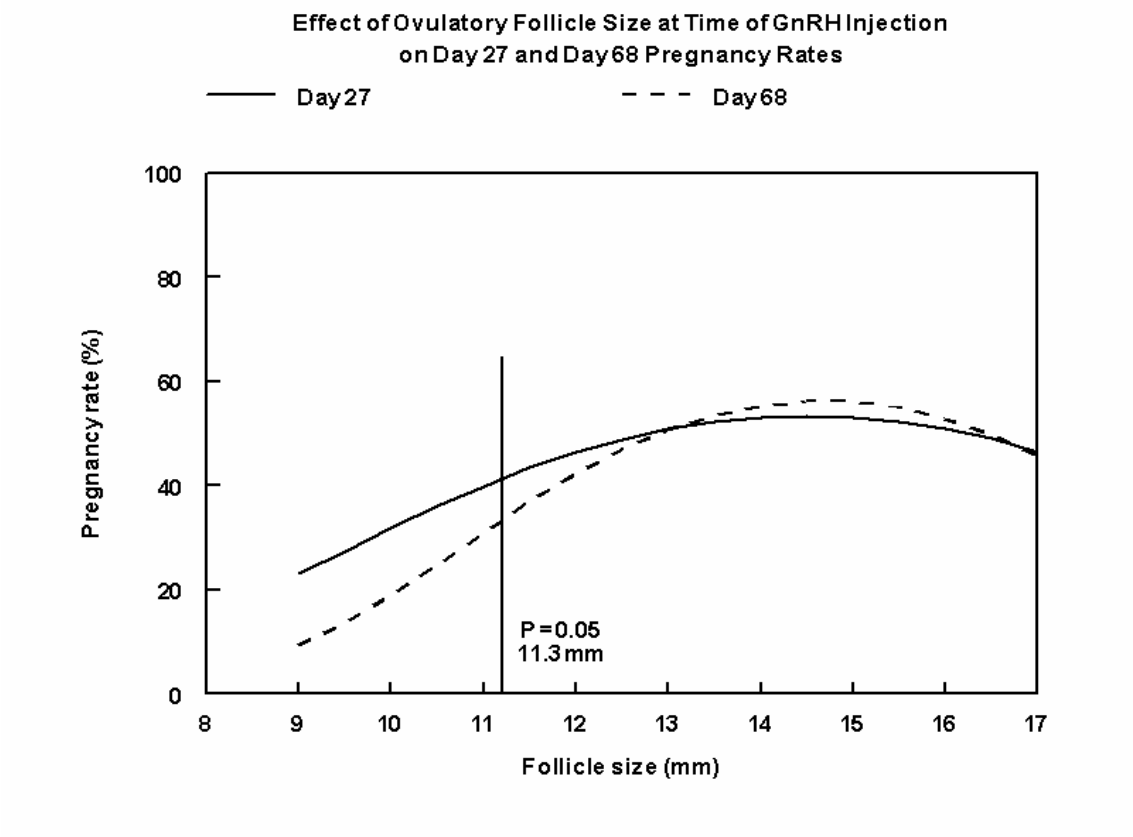

Figure 2. Regression analysis of the effect of ovulatory follicle size at time of GnRH injection/insemination on pregnancy rates 27 and 68 days after insemination. Follicle sizes at which pregnancy rates were decreased ($P < 0.05$) below the maximal pregnancy rates are indicated with vertical lines. (Perry et al., 2005)

Luteal secretion of progesterone is required for the survival of the embryo/fetus (McDonald et al., 1952), and has been associated with fertility in cattle by stimulating both uterine secretions (Geisert et al., 1992) and embryonic growth and development (Garrett et al., 1988; Mann et al., 1996). Uterine secretions including nutrients, growth factors, immunosuppressive agents, enzymes, ions, and steroids contribute to early conceptus growth/survival (Geisert et al., 1992; Gray et al., 2001). Cows with normal developing embryos had higher concentrations of progesterone on days 3 and 6 after insemination compared to cows with degenerating embryos (Maurer and Echternkemp, 1982). Following timed-AI protocols, serum concentrations of progesterone were affected ($P < 0.04$) by the size of the dominant follicle induced to ovulate (**Figure 3**). More specifically, the rise of progesterone following GnRH-induced ovulation was

decreased ($P < 0.01$) in cows that ovulated ≤ 12 mm follicles compared to cows that ovulated larger follicles. Furthermore, cows induced to ovulate ≤ 12 mm follicles had decreased ($P < 0.05$) pregnancy rates compared to cows induced to ovulate larger follicles (29% vs. 71%, respectively, Perry et al., 2002a).

Variation does exist in the proportion of animals induced to ovulate small follicles by different fixed-time insemination protocols. Following the CO-Synch protocol, 30% of cows and 52% of heifers (G.A. Perry unpublished data) were induced to ovulate follicles < 11.5 mm in diameter. However, when fixed-timed AI was performed in cows with or without a CIDR from day -9 to -2 [on day -9, GnRH was administered, on day -2, PG was administered, and 48 hours later (day 0) GnRH was administered and animals were inseminated], the percentage of cows that ovulated follicles < 11.5 mm was 7% for CIDR-treated cows and 15% for cows not receiving a CIDR (T.W. Geary unpublished data). Therefore, different timed-insemination protocols are more effective at reducing the percentage of small follicles induced to ovulate. However, regardless of synchronization protocol, reduced fertility does appear to occur whenever small follicles are induced to ovulate.

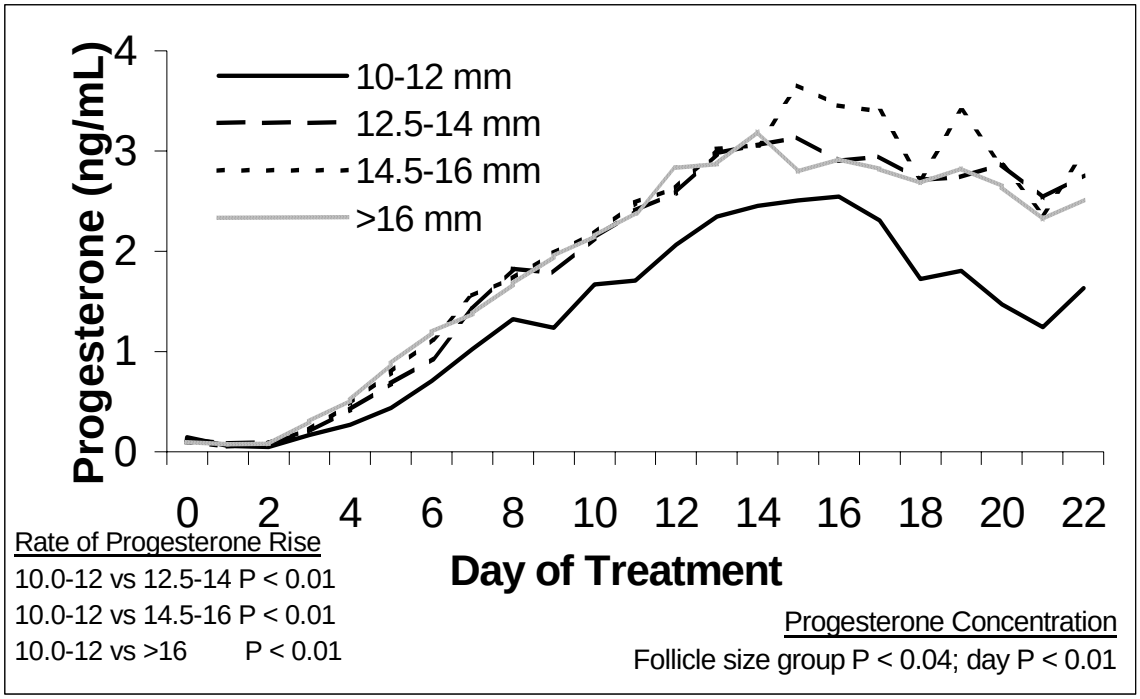

Figure 3. Effect of ovulatory follicle size, across both anestrous and cycling cows, on mean serum concentrations of progesterone from day 0 (second GnRH injection) through day 22, and rate of progesterone increase from day 0 to peak progesterone concentration. (Perry et al., 2005)

Implications

Synchronizing estrus in cows and heifers is an effective way to maximize the use of time and labor required to detect standing estrus in cattle. In addition, by using estrous synchronization more cows can conceive and become pregnant early in the breeding

season with no decrease in fertility. Some estrous synchronization protocols can even induce estrous cycles and shorten the anestrous postpartum period allowing cows to conceive earlier in the breeding season. However, when estrous synchronization is used together with artificial insemination, one of the largest factors that influences fertility is efficiency and accuracy of estrous detection. With fixed-timed insemination protocols, fertility can be reduced in a proportion of animals (cows induced to ovulated follicles < 11.5 mm). However, if the appropriate amount of time and effort cannot be spent detecting estrus, fixed timed-insemination protocols may result in overall greater pregnancy rates. In conclusion, when fertility is defined as the percentage of cows that conceive in the first few days of the breeding season, synchronized cows will have increased fertility compared to non-synchronized cows. When fertility is defined as the percentage of cows that conceive during the first cycle (first 21 to 25 days) of the breeding season, estrous synchronized females will have similar or better fertility than non-synchronized females depending on the percent of animals that are anestrous or prepubertal and the synchronization protocol used. Therefore, estrous synchronization can be a tremendous management tool to get more cows pregnant early in the breeding season.

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NOTES

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COSTS AND COMPARISONS OF ESTRUS SYNCHRONIZATION SYSTEMS

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Introduction

To incorporate desired genetics into cattle breeding programs, producers have an increasing number of options available for synchronization of estrus or ovulation and artificial insemination (AI). Low-cost production continues to be essential for survival in the beef industry. Understanding the costs of producing pregnancies via various methods and their associated value is very important. For some, the need to do more than turn a bull out with the cows is sufficient analysis for them to not consider AI. Others will take a broader view of the issue and may find AI is a tool that can improve profitability.

This paper examines the costs associated with producing pregnancies via natural service and various estrous synchronization systems. Some parts of the process are relatively easy to assign costs and make comparisons, whereas for others, assigning economic values is much more difficult. As always, to make the most informed decisions, each producer must know costs of production for their own operation.

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 1999 to 2004 was $2320. Annual bull costs were adapted from the 2003 Kansas Cow-Calf Enterprise Budget cost estimates and annual bull costs were separated using the method of Kasari et al. (1996). Additional assumptions included: the use of each bull for four breeding seasons; 10% death loss; 7% interest rate; and a 94% pregnancy rate. Annual feed costs for cow herds vary by as much as $200 per cow and this same variability is expected in feed costs for bulls. Increasing annual feed costs by $100, increased cost per pregnancy by $7.34 for a low bull to cow ratio (15 cows/yr) and $2.21 for heavy bull use (50 cows/yr), given a $2,300 purchase price.

Producers who use breeding pastures with carrying capacities less than the serving capacity of the bull, will increase cost per pregnancy. Conversely, cost per pregnancy will be reduced if highly fertile bulls are identified and exposed to more females compared to more conservative recommendations.

Cost of Synchronization of Estrus Plus AI

The partial budget in Table 2 gives an overview of cost differences between an AI program and natural service. Compared to natural service, increased costs of an AI program

Table 1. Annual bull costs ($) based on purchase price and associated cost per pregnancy.

Purchase price	1,500.00	1,700.00	2,000.00	2,300.00	2,500.00	3,000.00
Salvage value	896.00	896.00	896.00	896.00	896.00	896.00
Summer pasture	174.00	174.00	174.00	174.00	174.00	174.00
Crop residue	8.50	8.50	8.50	8.50	8.50	8.50
Hay	102.98	102.98	102.98	102.98	102.98	102.98
Protein, mineral	25.00	25.00	25.00	25.00	25.00	25.00
Labor	50.00	50.00	50.00	50.00	50.00	50.00
Vet	40.00	40.00	40.00	40.00	40.00	40.00
Repairs	31.00	31.00	31.00	31.00	31.00	31.00
Misc.	7.00	7.00	7.00	7.00	7.00	7.00
Interest	15.35	15.35	15.35	15.35	15.35	15.35
Total variable	453.83	453.83	453.83	453.83	453.83	453.83
Deprec. on equipment	12.39	12.39	12.39	12.39	12.39	12.39
Deprec. on bull	150.85	200.85	275.85	350.85	400.85	525.85
Interest on bull	83.88	90.88	101.38	111.88	118.88	136.38
Death loss	15.00	17.00	20.00	23.00	25.00	30.00
Total fixed	262.12	321.12	409.62	498.12	557.12	704.62
Total cost/yr	715.95	774.95	863.45	951.95	1,010.95	1,158.45
Purchase price	1,500.00	1,700.00	2,000.00	2,300.00	2,500.00	3,000.00
Cows Exposed Per Year			Cost per pregnancy			
15	50.78	54.96	61.24	67.51	71.70	82.16
20	38.08	41.22	45.93	50.64	53.77	61.62
25	30.47	32.98	36.74	40.51	43.02	49.30
30	25.39	27.48	30.62	33.76	35.85	41.08
35	21.76	23.55	26.24	28.93	30.73	35.21
40	19.04	20.61	22.96	25.32	26.89	30.81
50	15.23	16.49	18.37	20.25	21.51	24.65

included synchronization products, labor for synchronization of estrus and AI, time for planning, and perhaps improvements in facilities. Decreased returns include income from the sale of cull bulls because fewer bulls will be needed. 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 less calving assistance 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 also will increase returns from a more uniform calf crop and by producing offspring with 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., improved genetics, more concentrated calving season) are much more difficult to value, 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 synchronization of estrus plus AI

Budget Effect	Source	Budget Effect	Source
Increased returns	Heavier calves (earlier average birth date) Improved genetics (calves and replacement females) Uniformity of calf crop (fewer sires could be used, total breeding season could be shorter)	Decreased returns	Fewer cull bulls to sell
Decreased costs	Fewer bulls to purchase and maintain Less labor for more concentrated calving season More predictable calving ease	Increased costs	Planning and management for synchronization of estrus and AI Synchronization products and supplies Labor Improved facilities?

An example of the potential value of improved genetics is in Table 3. Carcass characteristics and boxed beef values from Angus sires with 10 or more carcass data records are illustrated. The carcass value was $206 per head greater for sires grouped in the top 10% than the bottom 10% for carcass value. It is clear a few more dollars could be invested in breeding costs to produce a product worth $206 more at harvest. Because the industry has been selling commodity cattle based on average values for so long, it is difficult for many producers to market calves so they are 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 is one of four keys for high returns on assets for cow/calf enterprises in the Northern Great Plains (Dunn, 2000).

Table 3. Average carcass characteristics and boxed beef values for Angus sires with 10 or more carcass data records*

Trait	Top 10%	Bottom 10%	Difference
No. of progeny	2728	1751	
No. of sires	109	110	
% Prime	7.7	0.7	+7.0
% CAB	47.4	.7	+46.7
% Choice & above	93.7	48.1	+45.6
% Select	6.1	35.0	-28.9
% Standard	0.2	16.9	-16.7
% YG 1&2	60.0	38.2	+21.8
% YG 4&5	1.4	18.2	-16.8
Carcass price/cwt	$110.19	$94.15	$16.04
Carcass value	$822.27	$616.36	**$205.91**

*Source: Angus Beef Bulletin, January 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. If the cost per pregnancy is higher for a particular method of breeding, what are the chances those costs can be recouped achieving higher marketing returns on the superior genetics? This example illustrates the complex decision-making framework that exists in the cow/calf industry today.

Genetic improvement made from the use of AI sires represents a gain sometime in the future from dollars spent today. The amount of actual profit will be influenced by semen price and quality (EPD values and accuracies) of the selected AI sires. Selecting the most appropriate AI sire(s) to use in a particular setting is difficult and requires defined production goals. Baker et al., 2004, have developed a spread sheet to estimate the value of AI sires for various production goals. The program is available at http://www.farm-mgmt.wsu.edu/beef.html.

Whole Herd Cost of Pregnancy

To evaluate breeding costs under different breeding systems, estimates of the hours of labor required for various synchronization systems were obtained from a survey of beef producers using AI in Nebraska (Loseke, 1989). From that survey, regression equations were estimated for total labor hours required for various AI programs.

Nonsynchronized program:
$TM = 19 + .036(CD)$ $R^2 = .83$

Lutalyse synchronization program:
$TM = 2.65\ (CD)^{.5}$ $R^2 = .60$

SyncroMate-B synchronization program:
$TM = 2.53\ (CD)^{.5}$ $R^2 = .87$

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 labor equation for the SyncroMate-B system was used for all the estrous synchronization systems in this report. Breeding systems were evaluated for various herd sizes. 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 to AI 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. Pregnancy rate for the total breeding season was 94%. The number of bulls required for clean-up was based on the assumption 50% would conceive to AI and that one bull was used per 30 nonpregnant females. This approach to the number of clean-up bulls reflects that a decision on number of bulls needed must be made before the AI pregnancy rate is known. Variable and fixed costs for AI are shown in Table 4. Synchronization product costs represent the average of 30 sources for both GnRH and $PGF_{2\alpha}$ products available on internet sites in 2004. The annual interest rate charged for cash costs was 7%. The labor rate used was $10.77 per hour (Fogleman et al). Annual bull costs ($2,300 purchase price) were $952 per bull as illustrated in the Table 1. Budget items from the partial budget in Table 2 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

Item	Cost per unit ($)
Semen	14.00 – straw
Prostaglandin $F_{2\alpha}$	2.54 – dose
GnRH	3.21 – dose
CIDR	9.02 - dose
Supplies	0.50 – insemination
Fixed costs[a]	175.00

[a]Semen tank, carrying case, pipette gun, thaw box, and liquid nitrogen

Cost per pregnant female as calculated in this model reflects costs for both AI and natural service pregnancies. 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 where only cows observed in estrus are inseminated. 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. To understand the impact of number of bulls used for cleanup on the cost of the system, the number of clean-up bulls was varied in Table 5. An additional bull for natural service adds from $8.73 per pregnant female for herds of 100 head and only $2.91 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. For smaller herds especially, the number of bulls used has a significant effect on costs of the breeding system. Successfully identifying bulls that can reliably service more than the 30 cows used in this example would be extremely valuable.

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

Calving herd size	AI pregnancy rate	No. of bulls for natural service	Breeding cost per pregnant female	% of total cost attributed to:			
				Bulls	Semen	Labor	Treatments
100 hd	75%	1	$42.75	20%	37%	19%	14%
	75%	2	$51.48	34%	31%	16%	12%
	50%	2	$45.59	38%	23%	18%	13%
	40%	2	$43.23	40%	20%	19%	14%
	40%	3	$51.96	50%	16%	16%	12%
300 hd	57%	5	$39.77	37%	30%	12%	15%
	57%	6	$42.68	41%	28%	11%	14%
	50%	6	$41.03	43%	26%	11%	15%
	40%	6	$38.67	45%	22%	12%	16%
	40%	7	$41.58	49%	20%	11%	15%

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 lb heavier at weaning, thus increasing the return at weaning by $25, if the additional weight is worth $1.25/lb. For this model, calves sired by AI sires 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 costs per exposed female were 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.

Breeding system costs and the standardized cost per cwt for various breeding systems assuming equivalent AI pregnancy rates (50%) are in Table 9. Breeding system costs per pregnant female were least for natural service followed by MGA + PGF, MGA-Select and Select Synch; CO-Synch + CIDR was most expensive. On a standardized production scale, 500-lb equivalent weaned-calf breeding cost per cwt, several systems have costs less than natural service. These include MGA + PGF, MGA Select, and Select Synch for all herd sizes and include 7-11 Synch 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 cwt between MGA + PGF and natural service was $2.08/cwt and $1.65/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 must be $2.08/cwt greater for a natural service breeding system than one using MGA + PGF to generate equal returns with all else being equal. The CO-Synch+CIDR system standardized cost per hundred was $2.52 and $3.36 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 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 AI system such as CO-Synch to Select Synch where cows are inseminated after an observed estrus, the standardized costs per cwt are less with the Select Synch system, and the difference is greatest for the largest herd size. Therefore, although in most cases estrus-AI may produce more pregnancies with less cost, timed AI may allow a producer to use AI who would not have considered AI if heat detection was necessary. This situation may occur because of herd size, a pasture too large for efficient heat detection, or if labor was unavailable. This type of producer may have a 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 6. For the herd size of 30, there are several situations where the breeding costs per cwt are less for CO-Synch than Select Synch. These include at low semen costs and medium to high labor costs, at medium semen cost and medium to high labor costs at a 60% AI pregnancy rate and at the highest semen and labor costs at an AI pregnancy rate of 60%. For a herd size of 100 (data not shown), the only situation where the cost of Co-Synch is less than Select Synch is for the low semen cost and high labor cost and 60% AI pregnancy rate (difference of $0.14). The differences in cost per cwt between CO-Synch and Select Synch are greatest when semen costs are high. For a herd size of 300, 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 $0.62/cwt less than CO-Synch and this increases to $1.44/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 $0.29 and increases to $1.78/cwt at high semen costs.

Table 6. 500 lb equivalent weaned calf breeding costs per cwt for a herd size of 30 at various labor and semen costs

		Semen cost ($)								
		4/unit			14/unit			24/unit		
		Labor Cost ($/hour)								
System	Preg. Rate (%)	*5.77*	*10.77*	*15.77*	*5.77*	*10.77*	*15.77*	*5.77*	*10.77*	*15.77*
CO-Synch	40	10.44	11.34	12.24	13.05	13.95	14.85	15.66	16.56	17.46
CO-Synch	50	9.92	10.82	11.73	12.53	13.43	14.34	15.14	16.04	16.95
CO-Synch	60	9.41	10.31	11.21	12.02	12.92	13.82	14.63	15.53	16.43
Select Synch	40	9.86	11.42	12.99	11.35	12.91	14.48	12.84	14.40	15.97
Select Synch	50	9.51	11.08	12.64	11.37	12.94	14.50	13.24	14.80	16.37
Select Synch	60	9.16	10.73	12.29	11.40	12.97	14.53	13.64	15.20	16.77

Pregnancy rates to AI will vary based on a variety of factors and the effect of changing pregnancy rate on the standardized cost per cwt was calculated within each system (Table 10). Notice for a herd size of 30 using CO-Synch, the cost per pregnant 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 similar. The benefit of more AI pregnancies is reflected in the standardized production scale.

Table 10 allows a comparison of systems at different AI pregnancy rate outcomes. Comparing Select Synch to Select Synch + CIDR, the CIDR allows for two 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 cwt 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 cwt for MGA + PGF at a 40% pregnancy rate are less than a 50% pregnancy rate with MGA + Select. CO-Synch and MGA-Select +TAI have very similar costs and returns, because there is little added cost with the MGA-Select +TAI in this model. This is based on the assumption 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 (CIDR + PGF6) or at CIDR removal (CIDR + PGF7) indicates the CIDR + PGF7 system reduces cost from $0.90 to $0.28 per pregnant 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 well managed small herds can be as profitable as large herds.

Another Look at Labor Costs

To verify the estimates of labor required for AI in the model developed by Loseke, (1989), a survey of producers using AI was conducted at the 2003 Range Beef Cow Symposium, Mitchell, NE. Comparisons of the labor cost (all at $10.77/hour) between the Loseke model and that estimated from the Range Beef Cow Symposium (RBC) survey are show in Table 7. Cost per pregnancy determined by the survey estimate is lower for herd sizes of 30 but slightly higher for herd sizes of 100 and 300. Given the variability of conditions these estimates are intended to represent, the "average" values are fairly close especially when compared on a breeding cost per hundred basis.

Limited survey information was collected on costs associated with hiring outside labor to do all AI and heat detection. Technicians surveyed represent the states of Kansas, Nebraska, Colorado and Missouri. Technicians were asked to estimate the costs associated with an operation 30 miles from their base location, "good facilities" and no semen sales involved. Scheduling of smaller herds, especially with no semen sales involved may be a problem during peak season. For days when extra help was required beyond the technician, most had people they called on for help in the price range of $100 to $150 (with horse) per day. Costs were higher for herd sizes of 100 and 300 when priced in this fashion. Technicians were also asked to estimate a cost per head to inseminate in a strict timed AI setting. The insemination cost per head was $7 to $10, $5 to $10 and $3.5 to $6.5 for herd sizes of 30, 100 and 300, respectively. Higher prices are not unheard of in the author's own experience, particularly when facility quality is lower.

Table 7. Comparisons of labor costs from three different sources* for a Select Synch breeding system resulting in a 50% AI pregnancy rate.

	Total Labor cost ($)			Cost per pregnancy ($)			500 lb equivalent weaned calf breeding cost ($) per hundred		
Herd Size	Loseke Model	RBC Survey	AI Tech Survey	Loseke Model	RBC Survey	AI Tech Survey	Loseke Model	RBC Survey	AI Tech Survey
30	484	369	463	67.66	64.19	67.03	12.94	12.14	12.80
100	880	965	1243	45.59	46.36	48.91	7.88	8.06	8.64
300	1525	2186	2997	41.03	43.05	45.53	6.83	7.30	7.87

*Loseke, 1989, RBC Survey – Date collected at 2003 Range Beef Cow Symposium, AI Tech survey – responses from a limited number of commercial AI technicians

Pregnancy Rates to AI

The costs and returns based on various AI pregnancy rates and estrous synchronization systems have been shown. The question then becomes, what pregnancy rate can be expected from various systems in my herd? Age, body condition, and days postpartum will all impact the proportion of cows cycling at the onset of the breeding season and thus the pregnancy rate to AI. AI-pregnancy rates will vary widely for the same synchronization system. Table 8 depicts ranges in pregnancy rates that might be expected during a 5-day AI period or a single timed AI (CO-Synch). The value under the "typical" column is a conservative estimate that might be used for planning in **well-managed herds** with optimal conditions. For the synchronization systems

recommended by the Beef Cattle Reproduction Leadership Team, a list of the references and reported pregnancy rates that have been used to create Table 8 can be found in Johnson, 2005.

Table 8. Pregnancy rates (%) to a 5-day AI period or a single timed insemination*

	Heifers		Cows	
System	Range	Typical	Range	Typical
MGA + PGF	40-70	60	40-60	55
MGA Select	40-65	60	55-70	60
MGA Select - TAI*			45-65	55
Select Synch	40-65	50	35-65	45
CO-Synch*	-		30-55	45
CO-Synch+CIDR*	30-80	55	30-75	55
CIDR + PGF7	40-80	50	35-60	50
7-11 Synch	30-55		35-65	
2 × PGF	30-65	50	20-45	35

Exercise caution when evaluating field reports of pregnancy rates from various systems. In some cases, only part of the herd (mature or early calving cows) was studied. This may be a wise and practical way to implement an AI program, but the results will likely be better than when the entire herd is synchronized. The method of determining AI pregnancies also may be misleading. To ensure clear distinction between AI and natural service pregnancies, a common research practice is to wait at least 10 days after AI before turning out bulls for clean-up in order to make an accurate early pregnancy diagnosis (30 to 40 days after first AI). Even with this 10 day break in breeding, known AI-sired calves have been born on the same day as natural service sired calves.

It is clear reliable estrous synchronization systems exist that produce AI pregnancy rates of 50% or more with a single timed AI. Producers who refine their management in preparation for the breeding season, 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.

Other Factors to Consider in Protocol Selection

Although costs of a breeding system are important, other factors should be considered when selecting a synchronization protocol. For example, the duration 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 proper treatment will be given on the correct day or that the facilities are adequate to allow detection of estrus and sorting of breeding females and their calves.

The proportion of heifers or cows expected to be cycling at the start of treatments will be a major factor in synchronization success and thus is also important in protocol selection. Systems that incorporate MGA, GnRH or CIDRs and/or calf removal should be considered for groups of animals where anestrus may be a problem. In long-term MGA systems, the MGA is delivered before some of the anestrous animals are ready to respond to the induction. Both GnRH and CIDRs can provide a progestin exposure just prior to

the start of the breeding season. However, on a given day, not all animals will have a follicle that is responsive to GnRH. Insertion of CIDRs ensures every animal receives the progestin exposure. Uniform consumption of MGA can be a limitation in some production settings and is necessary to obtain the desired effect. While the CIDR works well on anestrous animals the added treatment cost may be hard to justify for cycling animals. Selective administration of CIDRs to animals that are late calving, thin or young may be a good compromise.

Other considerations in protocol selection include length of treatment protocol as it relates to other management issues (i.e., movement to summer pastures), ability and facilities to detect heat, ability and ease of treatment administration (i.e., can MGA be fed?), and prior experience with AI or synchronization. Further discussion of the various synchronization systems and associated strengths and weaknesses can be found in other papers in this proceedings.

Conclusions

Cost per pregnancy is often used to evaluate various breeding systems but it fails to account for added value from AI sired calves. When even a small value is associated with an AI-sired calf, several synchronization systems have standardized production costs lower than natural service. While timed AI systems are in demand by producers, if labor is available and heat detection is feasible, cost analyses indicate AI after estrus rather than timed AI should produce greater returns. Some timed AI systems have standardized costs similar to natural service at a 50% pregnancy rate and lower costs at 60% depending on herd size. Given all the demands on the CEO's of today's cow-calf herds, hiring highly skilled, specialized people to apply estrous synchronization systems and AI makes 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.

A variety of synchronization systems are available to fit a range of production settings and requirements for implementation. Producers that can identify and market high value genetics will profit most from this technology.

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Figure 1. Diagram of systems for synchronization of estrus included in cost analysis

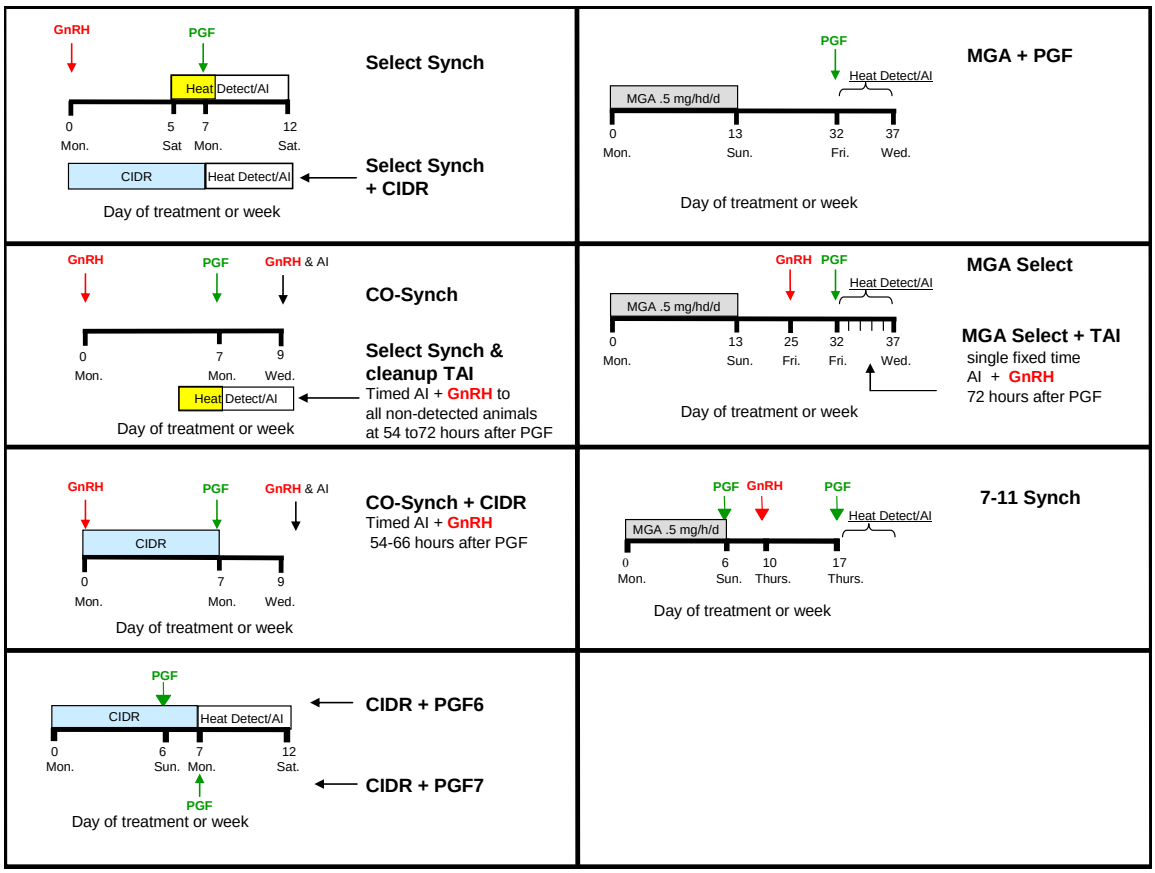

Table 9. Breeding system costs and 500 lb equivalent weaned calf breeding cost per cwt.

System*	Days worked	Preg. rate (%)	Total labor hours			No.of bulls			Cost ($) per pregnancy			500 lb equivalent weaned calf breeding cost ($) per cwt					
			Herd size														
			30	100	300	30	100	300	30	100	300	30	Diff[a]	100	Diff[a]	300	Diff[a]
Natural Service						2	4	12	58	35	35	**13.27**	-	**8.01**	-	**8.01**	-
MGA/PGF	6	50	37	67	116	1	2	6	62	41	37	11.61	1.65	6.83	1.17	5.93	2.08
MGA Select	7	50	40	72	125	1	2	6	66	45	41	12.67	0.60	7.79	0.22	6.84	1.17
Select Synch	9	50	45	82	142	1	2	6	68	46	41	12.94	0.33	7.88	0.13	6.83	1.17
7-11 Synch	8	50	42	77	133	1	2	6	70	48	44	13.47	(0.21)	8.50	(0.49)	7.50	0.51
CO-Synch	3	50	26	47	82	1	2	6	70	51	47	13.43	(0.17)	9.02	(1.01)	8.30	(0.29)
MGA-Select + TAI	3	50	26	47	82	1	2	6	70	51	48	13.56	(0.30)	9.15	(1.14)	8.43	(0.43)
CIDR+PGF d7	7	50	40	72	125	1	2	6	73	51	47	14.06	(0.79)	9.18	(1.17)	8.22	(0.22)
Select Synch & cTAI**	7	50	40	72	125	1	2	6	73	52	47	14.13	(0.86)	9.24	(1.24)	8.12	(0.12)
CIDR+PGFd6	8	50	42	77	133	1	2	6	73	52	47	14.26	(1.00)	9.29	(1.28)	8.29	(0.28)
Select Synch +CIDR	7	50	40	72	125	1	2	6	76	55	51	14.90	(1.63)	10.01	(2.01)	9.06	(1.06)
CO-Synch + CIDR	3	50	26	47	82	1	2	5	80	61	55	15.79	(2.52)	11.37	(3.36)	9.99	(1.98)

*Descriptions of these systems are shown in Figure 1.

**Assumes 40% of cows bred based on observed estrus (no GnRH at AI), cTAI=cleanup fixed-time AI

[a]Diff=difference between natural service and breeding system, $/cwt

Table 10. Breeding system costs ($) and 500 lb equivalent weaned calf breeding cost ($) per cwt at various AI pregnancy rates.

System*	Days worked	Preg. rate (%)	No. of bulls			Cost ($) per pregnancy			500 lb equivalent weaned calf breeding cost ($) per hundred					
			Herd size											
			30	100	300	30	100	300	30	Diff[a]	100	Diff[a]	300	Diff[a]
Natural Service			2	4	12	58	35	35	13.27	-	8.01	-	8.01	-
CO-Synch	3	40	1	2	6	70	51	47	13.95	(0.68)	9.53	(1.52)	8.82	(0.81)
	3	50	1	2	6	70	51	47	13.43	(0.17)	9.02	(1.01)	8.30	(0.29)
	3	60	1	2	6	70	51	47	12.92	0.35	8.50	(0.49)	7.79	0.22
MGA-Select + TAI	3	40	1	2	6	70	51	48	14.08	(0.81)	9.66	(1.65)	8.95	(0.94)
	3	50	1	2	6	70	51	48	13.56	(0.30)	9.15	(1.14)	8.43	(0.43)
	3	60	1	2	6	70	51	48	13.05	0.22	8.63	(0.62)	7.92	0.09
CO-Synch+ CIDR	3	40	1	2	5	80	61	55	16.30	(3.03)	11.88	(3.88)	10.50	(2.50)
	3	50	1	2	5	80	61	55	15.79	(2.52)	11.37	(3.36)	9.99	(1.98)
	3	60	1	2	5	80	61	55	15.27	(2.01)	10.85	(2.85)	9.47	(1.47)
MGA/PGF	6	40	1	2	6	60	39	35	11.59	1.68	6.81	1.20	5.90	2.10
	6	50	1	2	6	62	41	37	11.61	1.65	6.83	1.17	5.93	2.08
	6	60	1	2	6	64	43	39	11.64	1.63	6.86	1.15	5.96	2.05
MGA Select	7	40	1	2	6	64	43	39	12.65	0.62	7.76	0.24	6.81	1.19
	7	50	1	2	6	66	45	41	12.67	0.60	7.79	0.22	6.84	1.17
	7	60	1	2	6	69	48	43	12.70	0.57	7.82	0.19	6.86	1.14
CIDR+PGF7	7	40	1	2	6	70	49	45	14.03	(0.76)	9.15	(1.14)	8.20	(0.19)
	7	50	1	2	6	73	51	47	14.06	(0.79)	9.18	(1.17)	8.22	(0.22)
	7	60	1	2	6	75	54	49	14.08	(0.82)	9.20	(1.20)	8.25	(0.24)
Select Synch+CIDR	7	40	1	2	6	74	53	48	14.87	(1.60)	9.99	(1.98)	9.04	(1.03)
	7	50	1	2	6	76	55	51	14.90	(1.63)	10.01	(2.01)	9.06	(1.06)
	7	60	1	2	6	79	57	53	14.92	(1.65)	10.04	(2.03)	9.09	(1.08)
Select Synch & cleanup TAI	7	40	1	2	6	73	52	47	14.64	(1.37)	9.76	(1.75)	8.64	(0.63)
	7	50	1	2	6	73	52	47	14.13	(0.86)	9.24	(1.24)	8.12	(0.12)
	7	60	1	2	6	73	52	47	13.61	(0.34)	8.73	(0.72)	7.61	0.40
7-11 Synch	8	40	1	2	6	68	46	42	13.45	(0.18)	8.47	(0.47)	7.47	0.53
	8	50	1	2	6	70	48	44	13.47	(0.21)	8.50	(0.49)	7.50	0.51
	8	60	1	2	6	72	51	46	13.50	(0.23)	8.53	(0.52)	7.53	0.48
CIDR+PGF6	8	40	1	2	6	71	49	45	14.24	(0.97)	9.26	(1.26)	8.26	(0.26)
	8	50	1	2	6	73	52	47	14.26	(1.00)	9.29	(1.28)	8.29	(0.28)
	8	60	1	2	6	76	54	50	14.29	(1.02)	9.32	(1.31)	8.32	(0.31)
Select Synch	9	40	1	2	6	65	43	39	12.91	0.35	7.85	0.15	6.81	1.20
	9	50	1	2	6	68	46	41	12.94	0.33	7.88	0.13	6.83	1.17
	9	60	1	2	6	70	48	43	12.97	0.30	7.90	0.10	6.86	1.15

[a]Diff=difference between natural service and breeding system, $/cwt *-See Figure 1 for descriptions of system

NOTES

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MANAGEMENT OF INFECTIOUS REPRODUCTIVE DISEASES IN BEEF CATTLE HERDS

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Reproductive diseases are the greatest disease threats to the production and profitability of beef cattle herds. Infection by reproductive tract pathogens results in a wide array of losses including *embryonic deaths, abortions, stillbirths and weak calves*. Abortions are the visible tip of the iceberg in reproductive tract infections. Embryonic deaths appear clinically as repeat breeders and low pregnancy rates. Devastating losses occur when a reproductive tract pathogen is introduced into a naïve herd, often reducing pregnancy rates to 40 or 50 percent. Thereafter, they can cause losses in a cyclic pattern: great losses one year followed by several years of minimal loss, then major losses again. *Low reproductive performance robs a beef herd of profitability.*

There are 4 parts to a successful program to control infectious reproductive diseases in beef herds:

1. *Maintain a high level of general resistance to infectious disease.*
    - Proper nutrition*:* including minerals (especially those needed for a strong immune system - copper, selenium and zinc).
    - Minimize stress*:* avoid crowding – don't mix first calvers and adults.
    - Control internal and external parasites.

2. *Keep infectious agents out of the herd*
    - Purchase animals from well-managed, reputable herds.
    - Test purchase animals for carrier state - Prior to purchase!
    - Quarantine purchased animals on arrival.
        - 60 days – no nose to nose contact.
        - Administer vaccines, treat for parasites and give LA-200 to eliminate *Leptospira* hardjo-bovis carrier state.

3. *Minimize spread of infectious agents within the herd.*
    - Identify and cull carrier animals.
    - Isolate sick animals – bury dead animals.
    - Don't use same equipment for feed and manure handling.
    - Reduce wildlife reservoirs of neosporosis.

4. *Maintain a high level of specific resistance to infectious disease.*
   - Proper vaccination program: especially for bovine viral diarrhea (BVD) and *Leptospira borgpetersenii* serovar hardjo-bovis.

All 4 parts of the program are necessary for its success!!

This article gives general principles on control of infectious reproductive diseases in beef herds. There are many details to the *design* and *implementation* of a successful reproductive disease control program. It must be stressed at the onset that **a specific herd's reproductive disease control program should be based on the herd's unique management practices and knowledge of the diseases that are a significant threat to the herd.**

There are 7 reproductive pathogens that are of main concern because they each are capable of inflicting major disease losses in a beef cattle herd. They are *Brucella abortus, Leptospira* hardjo-bovis, *Campylobacter fetus*, infectious bovine rhinotracheitis (IBR) virus, bovine viral diarrhea (BVD) virus, *Tritrichomonas foetus* and *Neospora caninum*. A discussion of practical control measures for of each of these diseases follows.

Brucellosis

The Disease - We've come a long, long way in eradication of this bacterial reproductive disease since the late 1960's when a negative herd was rare in most Texas counties! Even though this disease is almost eradicated, we *must continue our control programs* well into the future years.

Control Program - Control of brucellosis in beef herds will continue to be based on **calfhood vaccination and biosecurity**. Continue calfhood vaccination and only purchase brucella-vaccinated females, preferably from Brucella Certified herds or females who have been tested for brucellosis before purchase. Bulls should be required to pass a brucella test prior to purchase.

Leptospirosis

The Disease - There are many different leptospira organisms that can potentially infect cattle. Five of the most common ones are included in the 5-way leptospirosis vaccine which contains *Leptospira Pomona, L. hardjo, L. canicola, L. icterohemorrhagica* and *L. grippotyphosa*. Over the past 30 years, hardjo-bovis infection has emerged as the most common cause of reproductive losses in North American cattle caused by leptospiral organisms and there's lots of it in Texas! Transmission of infection is very efficient: chronic carrier cows harbor the bacteria in their kidneys and shed massive numbers of organisms in urine. A recent survey of Texas beef herds found cows shedding hardjo-bovis in 6 of 12 herds (50% prevalence).

Leptospira hardjo-bovis has become widespread in our cattle population, inflicting significant reproductive losses. The reason for its unchecked rampage is that while our 5-way leptospira vaccines have provided moderate protection against most leptospira serovars, they give *minimal protection against hardjo-bovis.* They do not

contain that organism! They contain *L. interrogans* serovar hardjo (hardjoprajitno) which is present in Europe, not North America, and gives little cross protection against our form of hardjo, (hardjo-bovis). Fortunately, a highly effective vaccine against hardjo-bovis (*Spirovac*® - Pfizer Animal Health) has recently become available in the United States. At this time, it is the only vaccine available in the U.S. that has been proven to be effective against hardjo-bovis.

Control Program – This disease is widespread in Texas beef cattle herds and *a control program is highly recommended.* Prevention of losses due to hardjo-bovis infection in beef herds is based on a combination of **biosecurity, antibiotic treatment to eliminate the carrier state and vaccination**. Sampling and testing for the hardjo-bovis carrier state is time-consuming and expensive. To keep from introducing the infection into a herd, purchased animals should be kept separate from the herd for the standard quarantine period of 60 days and handled as if they were hardjo-bovis carriers instead of testing for carrier status. Upon arrival, they should be placed in isolation from the herd (no nose to nose contact), treated with LA-200 which clears the carrier state and given a primary vaccination with Spirovac. Four weeks later they should receive a booster vaccination with Spirovac. They can be introduced to the herd at the end of their quarantine period.

Control of hardjo-bovis within the herd is accomplished by *antibiotic treatment to eliminate the carrier state and vaccination.* The program is designed to insure that carrier animals are not present in the herd and that cattle in the herd have a protective degree of immunity against hardjo-bovis. The older calves are, the more likely they are to be renal carriers. Also, the more contact that calves have with adults, the more likely they are to be carriers of hardjo-bovis. Thus, at a young age, calves must be treated with LA-200 to eliminate the possible carrier state and given their primer vaccination. In 4 to 6 weeks they should receive a booster vaccination.

In beef herds, a practical control program could be to administer LA-200 and a primer vaccination to calves at weaning and then give a vaccine booster 4 to 6 weeks later. Thereafter, they and adults should receive an annual booster vaccination.

The first year of a herd control program, in a beef herd all yearlings and adults could be treated with antibiotics to eliminate the carrier state and given their primer vaccination. Four to 6 weeks later, the entire herd should receive their booster vaccination. Thereafter, calves should be started with antibiotic treatment and primer vaccinations at a young age and adults should receive annual boosters. An alternative approach would be to limit antibiotic treatments to yearlings and calves at weaning and rely solely on vaccination in adults.

To protect the herd against the other leptospiral organisms, the 5-way leptospira vaccine must continue to be administered to the herd unless the just introduced Spirovac vaccine that contains the other leptospiral organisms plus campylobacter is used. It should be given to heifers at first working and then boostered at weaning and 1 month prior to breeding.

Campylobacteriosis (Vibriosis)

The Disease - Vibriosis is a bacterial venereal disease of cattle characterized by embryonic deaths which appear clinically as repeat breeders and low pregnancy rates. Abortions occur occasionally, usually between the 4th and 7th months of gestation. Bulls become infected from breeding an infected cow and then pass the infection to a naïve cow during breeding. Young bulls (under 3 to 4 years of age) tend to have transient infections of hours to days while older bulls (4 to 5 years and older) become life-long asymptomatic carriers. Cows are capable of mounting an immune response and clearing themselves of the organism. The resistance is temporary, however, and re-infection is possible 3 or 4 months later. In herds with long breeding seasons (6 months or more), this phenomenon can result in a pregnancy pattern characterized a cluster of pregnant cows the first month or so of the breeding season followed by 2 or 3 months of a few scattered pregnancies and then another cluster of pregnancies the last 2 months of the breeding season.

Control Program - Vibriosis is widespread in the cattle population of Texas. It can be **controlled very effectively by vaccination**. *All herds must vaccinate their cattle against Campylobacter fetus!* An **oil-based vibriosis vaccine** results in the longest lasting immune response and a single dose is effective with no advantage to using 2 injections initially. Unfortunately, oil-adjuvanted vaccines cause swellings at the vaccination site due to formation of granulomas and fibrosis. Replacement heifers should be vaccinated 1 month prior to the start of their breeding season. Cows should be given an annual booster, preferably 1 month prior to breeding, however, annual boosters given at pregnancy examinations have been found to provide adequate protection. Bulls should receive two-5ml doses of oil-based vaccine (21/2 times the cow dosage) at 4-week intervals beginning 8 weeks before the start of the breeding season. This has been shown to not only prevent infection in bulls, but to clear infections from carrier bulls.

IBR and BVD Viruses

The Diseases – These 2 viruses are discussed together because infection of cattle with either of them results in early embryonic deaths, abortions, stillbirths and weak calves. In addition, BVD virus infection can result in birth defects, especially cerebellar hypoplasia. Calf crops are reduced due to lower pregnancy rates, abortions and higher calf mortality rates. Also, weaning weights are reduced due to infection of calves with these viruses during the nursing period.

Herds become infected with these viruses by purchasing chronically infected animals who spread the viruses throughout the herd. Calves are born persistently infected (PI) with BVD virus when their non-immune dams become infected with BVD virus at 42 to 125 days of gestation. PI animals shed massive amounts of BVD virus into the environment. About 50% of the PI calves die by 1 year of age, however, the rest survive longer. Some become pregnant replacement heifers and infect new herds when sold to a naïve ranch. Introduction of a PI animal into a naïve herd results in serious losses from BVD virus infection.

Control Program – Prevention of infection of beef herds with these viruses is based on **biosecurity and vaccination.** Biosecurity involves not buying an animal

persistently infected with BVD virus! The best test for PI status is immunohistochemistry on skin biopsies collected with pig ear-notchers. All *herd additions should be tested for PI status* for BVD virus prior to purchase. Purchased heifers must ***remain in quarantine until they have calved*** *and* ***their calf has been proven non-PI*** by a negative immunohistochemistry skin test.

Modified-live (MLV) or killed IBR/BVD virus vaccines can be used in vaccination programs. The current recommendations of veterinary virologists on the most effective use of vaccines in prevention of IBR and BVD virus infections is to *use MLV vaccines as much as possible* in a herd vaccination program. MLV vaccines provide more complete protection against infection of the fetus than killed vaccines. Careful though: **non-immune pregnant cattle that are vaccinated with MLV IBR/BVD vaccines will abort their fetuses from vaccine virus.**

An approach that safely utilizes MLV vaccines is to administer them to replacement heifers at weaning and then give a booster of MLV vaccine to the heifers 1 month prior to the onset of breeding. Thereafter, as adults they should receive MLV vaccine 3 to 4 weeks prior to breeding when they are not pregnant. There are some recent developments in the available MLV vaccines and their use that allows the more practical time of booster administration to be pregnancy examinations. This should only be practiced under close veterinary supervision with close adherence to vaccine insert recommendations.

Trichomoniasis

The Disease - Trichomoniasis is a protozoan disease that like vibriosis is a venereal disease of cattle characterized by embryonic deaths which appear clinically as repeat breeders and low pregnancy rates. Abortions also occur beginning in early pregnancy and continuing right up to the time of calving. Trichomoniasis is probably one of the most economically devastating disease of cattle, second only to foot and mouth disease!

Control Program **–** Trichomoniasis is common in Texas beef cattle herds, but it is not as widespread as vibriosis and expensive vaccines are only partly effective. Most herds should use **biosecurity as the main control measure for this disease**. Don't buy trichomoniasis into the herd! All purchased **non-virgin bulls** must be cultured or tested by PCR for *T. fetus.* In addition, don't expose cows to bulls from other herds:

1) Don't borrow or lease bulls.
2) Don't graze common lands with other herds.
3) Keep your fences in good repair to keep the neighbor's cattle out.

Another defense against establishment of trichomoniasis into a herd is to *keep the bull battery as young as possible*. Younger bulls (less than 5 years) have shallower epithelial crypts in the mucosa of the prepuce than older bulls. The *T. foetus* organisms require deep epithelial crypts to establish chronic infection.

Vaccination of cows and bulls against *T. foetus* in a beef herd is **recommended under certain circumstances:**

1) High-risk herds (eg. neighbor's herd is infected, communal grazing).
2) In suspected trichomoniasis herds.
3) As part of the control program in trichomonas-infected herds.

Neosporosis

The Disease – Abortions at any stage of gestation, but most commonly between 5 and 6 months is the main damage caused by infection of cattle with the protozoan *Neospora caninum. Stillbirths* also occur. This disease is *widespread in Texas beef herds* and is probably *one of the most common causes of abortion* in our beef cattle. A survey of calves in 2000 found that 59% of the herds that sent calves to the Texas Ranch to Rail Program were infected with *N. caninum*.

Neosporosis is a "new disease" of cattle, first reported in 1989 as the cause of an outbreak of abortions on a New Mexico dairy. Researchers later uncovered genetic evidence that indicates that this organism has been present on earth as long as cattle! The reason we did not recognize this disease until years after we had the technology to do so is that the organisms are mainly present in the brain of aborted fetuses and until recently, veterinarians conducting autopsies of aborted fetuses usually did not remove the brain!

This protozoan organism has a life cycle that involves dogs (coyotes, dingoes?) and foxes as definitive hosts and cattle as intermediate hosts. In Australia, the ranchers call this "wild dog disease". Dogs infected with *N. caninum* have been shown to shed *N. caninum* oocysts in their feces. Cows can become infected by ingesting the oocysts. Once a cow becomes infected, she is a *life-long carrier* of the disease. She also will almost always (85 to 95%) pass the infection to her calf in-utero. Most calves infected in-utero are normal healthy carrier calves who have a much *greater chance of aborting their first and second pregnancies than non-infected calves*. Thus, there are two possible ways that cattle can become infected with this protozoan:

1) Ingestion of oocysts in feed or water contaminated by feces of dogs or foxes that have neosporosis. Ingestion of placentas or fluids of aborted fetuses. (*Post-natal* or *horizontal* transmission)

2) In-utero infection of a fetus whose dam is a life-long carrier. (*Congenital* or *vertical* transmission)

In infected beef herds, *both* of these routes of transmission probably are ongoing to some extent. As has been proven in dairies, however, in-utero transmission is probably by far the most common means of transmission.

Replacement heifers or cows that are carriers of *N. caninum* are more likely to abort their fetus than those who are not infected. In an investigation of abortions in a Texas purebred herd, the author found that cows who were carriers of *N. caninum* were 10 times as likely to lose their calf as cows who were not carriers.

Control Program – Control programs at this early stage of our understanding of such a new disease are not well-proven. Much research is being performed on this

disease and successful control strategies will evolve as new information becomes available. Various combinations of biosecurity, testing and culling of carrier cows and vaccination are being tested. Research by Dr. Barling of our College of Veterinary Medicine has identified some of the management factors that are associated with a higher likelihood of a beef herd in Texas having neosporosis. These include a spring calving season or split calving season, a higher stocking density, use of round bale hay feeders and allowing wildlife access to the weaning supplement. A very interesting finding of his study was that beef herds in Texas who had a cattle dog were less likely to have neosporosis. Our cattle dogs must be having success at keeping wild canids away from cattle feed sources! Only one vaccine, (*NeoGuard*™ - Intervet Inc) is commercially available against *N. caninum*. Unfortunately, we do not know if it is effective. There have been no published independent studies on its efficacy. Carrier cows can be accurately identified by detection of antibodies in their serum. Use of the vaccine in a herd may make a test and cull program impossible because vaccinated cattle may remain seropositive for long periods of time. The role of vaccination in control of neosporosis will not be known until properly conducted field trials are performed on its ability to protect against abortion due to *N. caninum.*

Biosecurity appears to be the **soundest approach to control** of neosporosis with our current knowledge of the disease:

1) Test for antibodies in the serum of female potential herd additions. Only purchase seronegative females.
2) Lower the number of neospora carrier cows in the herd (they create more neospora positive females and they are more likely to abort than a neospora negative cow). Two approaches:
    a. Test the entire herd of breeding females and do not keep the offspring of infected cows as replacement heifers. It's highly likely they are infected! (This is the most economical control program for a commercial beef cow/calf herd)

    Or

    b. Test the entire herd of breeding females, cull all positive cows and replace them with cows that have been tested negative. (This option is probably the best one for a purebred herd using embryo transfers into recipient cows because the value of the fetus is too high to risk losing. ***There is no need to cull donor cows, their embryos are safe to use***.)
3) Protect feed and water sources from fecal contamination of wild canids.
4) Promptly dispose of aborted fetuses and their placentas.

The author has found testing and culling cows to be very effective in controlling abortions due to *N. caninum* in a purebred beef herd in central Texas for 4 years after initiation of the program. Simulation models have recently concluded, however, that the most economically effective approach to control of neosporosis in a beef herd is to test all females and keep infected cows in the herd, but not use their daughters as replacements. *It is very important to accompany a test and cull program with an effort to reduce the number of potential wildlife carriers on the ranch.* When a vaccine is available that is proven to be effective, vaccination will become an important part of a neospora control program.

Conclusions

Reproductive tract pathogens pose a great threat to the production and profitability of beef cow/calf operations. **They usually enter a herd through purchase of a chronically infected carrier** heifer, cow or bull and cause the most damage the first year they are introduced into a naïve herd. Replacement heifers and first-calf heifers are very susceptible to infectious diseases and experience the greatest loses from reproductive tract infections. Thus, it's wise to make special efforts to implement an effective reproductive diseases control program in a herd's young breeding stock. **Control programs for infectious reproductive diseases generally utilize a combination of biosecurity and vaccination, and should be closely supervised by the herd's veterinarian.**

NOTES

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BREEDING SOUNDNESS EVALUATION OF BULLS

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Introduction

Fertility of breeding bulls is one of the most important economic considerations to the commercial cattle producer. Other factors such as genetics and nutrition of the breeding stock become secondary in economic importance when you consider that the use of a healthy, well managed, **infertile** bull could result in no calves and the loss of 100% of the marketable calf crop. In a herd of 550 bulls, approximately 33% failed a breeding soundness exam performed according to the Society for Theriogenology standards.

Prior to June 1993 the Texas Department of Criminal Justice(TDCJ) beef cattle operation consisted of 13 farms on 13 separate Prison Units and consisted of approximately 6,000 cows and 550 breeding bulls. In June 1993 the format changed. One centrally located farm became designated as the unit to house and care for the breeding bulls, when they were not out with the cows. All the bovine females on that unit were dispersed to other farms. This programmatic change allowed improved attention to bull health and improved record keeping.

The recorded data included the age, breed, date, body-condition score, results of the breeding soundness exam of the individual bulls and results of fecal exam on selected bulls. The objective was to determine if age, breed, season, body-condition score, intestinal helminths, coccidia, liver flukes, the interaction of parasites and body condition, and the interaction of breed and season were associated with failed breeding-soundness examinations. Individual defects in the morphology exam were recorded as: 1. Head defects 2. Midpiece defects 3. Spermatogenic tail defects and 4 handling and storage defects.

Methods

Age

Registered beef breeding bulls are purchased from breeders. The date of birth and breeder's identification brand are recorded. As the replacement bulls are purchased, a

unique identification number is assigned to each bull that tracks him through the computer and, in addition to the brand, is used to record the result of the various evaluations done on a periodic basis. The bulls in the bull herd ranged from 2 years to 8 years.

Breed

The following breeds were represented in the bull herd: Angus, Brangus, Brahman, Hereford and Simmental.

Season

The cow herd is divided into a fall, winter and spring herd. The fall herd is exposed to the bulls during December and January. The winter herd is exposed to the bulls during February and March. The spring herd is exposed to the bulls during May and June. Breeding soundness exams(BSE) are completed on the bulls within 30 days prior to each breeding season. Results are recorded using the criteria approved by the Society of Theriogenology. Thirteen different units comprise the beef enterprise. The number of breeding females and the breeding season utilized is determined by the land acreage available on the different units. Bulls are assigned to the units based on the number and breed type of the females on each unit.

Body condition score

Body condition scores are recorded as part of the BSE and are also recorded after each breeding season.

Fecal evaluation

Fecal samples were collected as the bulls came through the working chute for BSE evaluation. If a bull did not pass the BSE his fecal sample was blocked with the next two bulls through the chute that passed the BSE

Intestinal helminths. Parasite eggs were identified and recorded by fecal flotation

Coccidia. Were identified in the same fecal flotation as used for intestinal helminths

Flukes. Were identified using the "Wisconsin fluke finder" to show fluke eggs and identify the species.

Herd Health

The bulls were treated for flukes and intestinal helminths in the fall while BSE evaluations were completed. They were vaccinated for leptospirosis, and campylbacteriosis and 8-way clostridia at the time of this working. In the spring the bulls were treated for intestinal helminths and vaccinated for leptospirosis, cammpylobacter, anaplasma and 8 way clostridia.

Analysis

For initial analysis, season was divided into winter, spring and summer, breed was coded as a 5-level variable (Angus, Brahman, Brangus, Herefords and Simmental) and age treated as classification variable with a separate class for each birth year. Variables for intestinal helminths, coccidia and liver flukes were coded as positive, if any oocytes or helminth eggs were found to be present in the fecal examination. Initial analyses were performed using chi-square analysis for association and also chi-square for trend for the variable age.

For multi variate analysis, breed was recorded as Hereford or other, season was recorded as summer or other, and age was coded as a continuous variable. Variables significant at $p<0.05$ in the initial analysis were then modeled using path analysis.

Results

Results were obtained from 381 bulls during the months January to July in 1994.

Figure 1. Age of bull and passing proportions for breeding-soundness examinations.

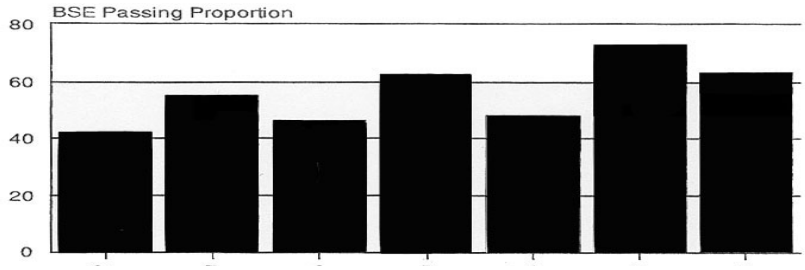


Figure 2. Breed of bull and passing proportions for breeding-soundness examinations.

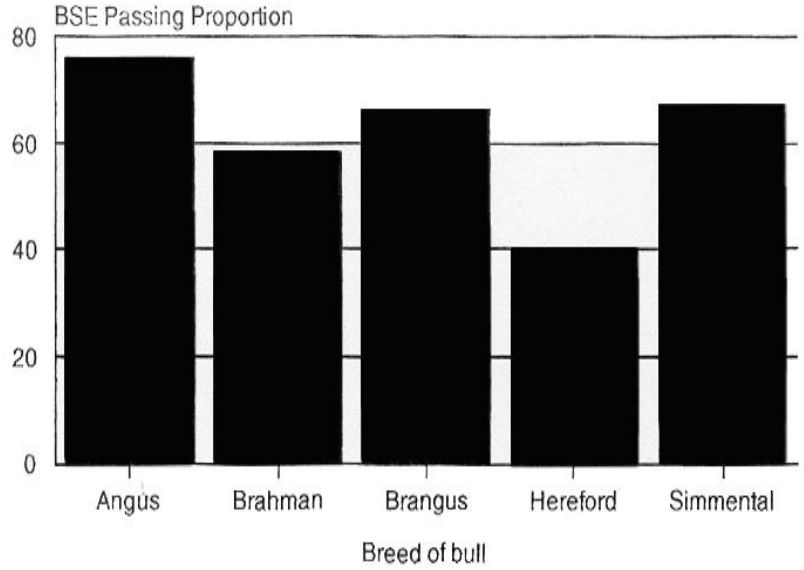
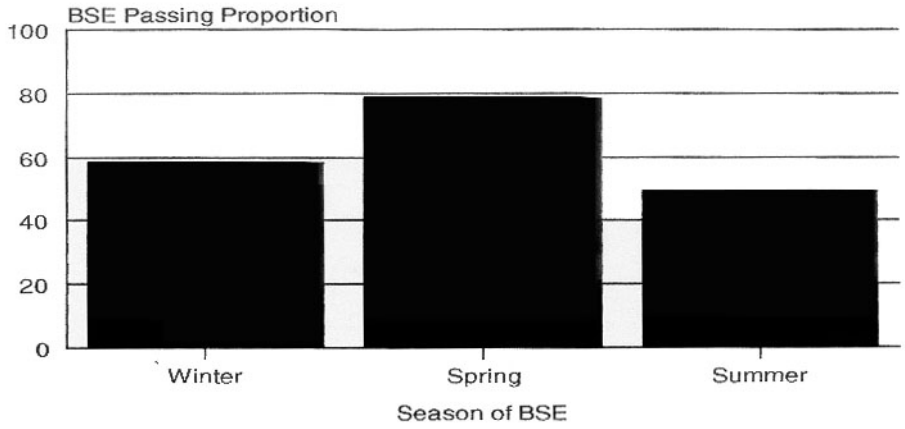
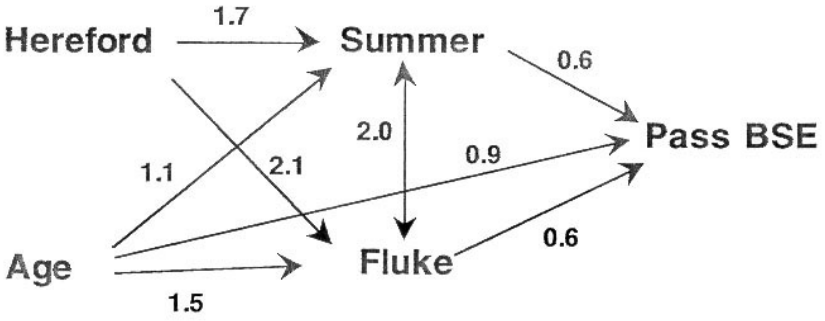


Figure 3. Season of breeding-soundness examination and passing proportions.

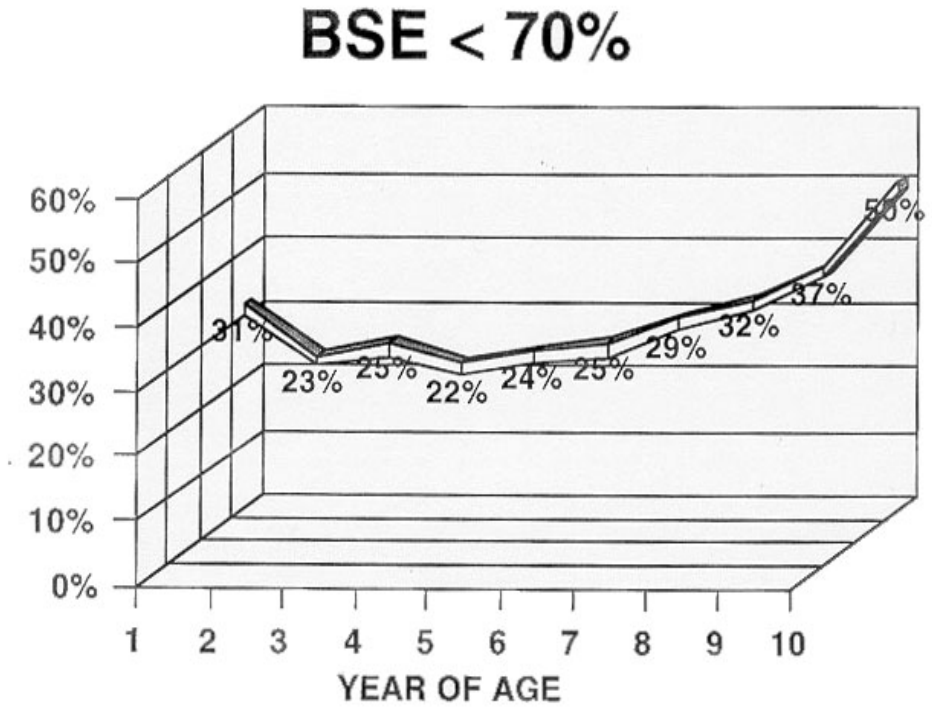


Body-condition score, intestinal helminths and coccidia were not significantly ($p > 0.1$) associated with results of the breeding-soundness examinations. Bulls positive for fluke infestation were more likely to have a failed breeding-soundness examination (odds ratio = 1.29; 95% confidence interval 1.05 to 1.56). This association was independent of body condition score.

Figure 4. Results of Path Analysis. All odds ratios were significant at $p < 0.1$



	<2 year	2 year	3 year	4 year	5 year	6 year	7 year	8 year	9 year	10 year
1998		17	22	9	6	12	100	25	100	50
1997	0	12	21	13	11	33	35	0	17	0
1996	100	17	14	10	62	24	33	40	14	0
1995	39	15	14	37	37	17	32	35	22	0
1994	24	14	37	25	25	32	34	41	67	0
1993	24	65	43	39	67	33	38	50	0	0
	187	140	151	133	141	151	272	191	220	
Average	**31%**	**23%**	**25%**	**22%**	**24%**	**25%**	**29%**	**32%**	**37%**	**50%**



Total number bulls evaluated =3,638

Six year summation of Bulls scoring under 70% normal morphology

Seventy percent normal morphology score is necessary for a bull to be designated as a "satisfactory potential breeder". The graph is based on this morphology score. Bulls that failed to pass a physical exam or a semen motility score are not included in the data, The data records that 31% of all the bulls evaluated at less than 2 years of age failed to pass the morphology exam. This should not be used as an excuse not to test these yearling bulls. On the contrary, early maturity is an inherited trait and beef herds trying to upgrade the fertility of the cow herd would be well served to evaluate these young bulls and use the morphology score as a selection tool for future herd sires. This is not to say that young bulls that fail an BSE exam at an early will not pass at a later date when they have had more time to mature.

The failure rate from 2 years of age to 6 years of age is relatively constant at about 25% of each age group. This answers the question of the necessity of testing all bull prior to each breeding season. The breeding-soundness exam does not predict libido and, therefore, it is necessary to observe bulls soon after they are put with cycling females to assess their actual performance.

There is some evidence that a correlation exists between a BSE score and the number of calves sired. Two bulls that we evaluated never produced a semen sample in spite of numerous attempts to collect them. Another one in the group never had a morphology score over 46% normal sperm. The administration maintained that these 3 bulls could be called "non-breeders" until they were exposed to fertile females. The 3 bulls were put into single sire traps, each with 5 normal, cycling heifers for 45 days. The two aspermatic bulls did not achieve any pregnancies during this time and the bull with less than 46% normal morphology only achieved one pregnancy. In a similar small study from South Africa, there were similar correlations to pregnancy rates achieved by 3 bulls; one with acceptable semen morphology achieving over 80% pregnancies, one with intermediate semen morphology achieving 50% pregnancies and one with low semen morphology achieving almost no pregnancies. Even though these are very small studies, they do indicate that if libido is acceptable, then morphology scores are important predictors of pregnancy rates. Beyond age 7 the failure rates begin to increase significantly.

Texas Department of Criminal Justice Beef Cattle Program:

Prior to 1993 the beef cattle program was conducted on 16 different farms, and consisted of a total of 6,000 cows and 550 bulls. In June 1993 all the bulls were moved to a central farm unit and all the cows on that unit were redistributed to other units. A detailed health record was instituted, as well as a detailed BSE record for each bull. That is when the data already presented was initiated. Over the last 12 years the cow herd(including replacement heifers) has grown to about 16,000 females. The bull herd has been reduced to 300 bulls. The cow herd is divided into spring calving herd, fall calving herd, and a winter calving herd. The replacement females are bred one month ahead of the corresponding cow herd. Until very recently a 60 day breeding season has been utilized. The pregnancy percent has averaged approximately 88

Discussion

Age

The path model suggests that as a bull grows older, his chances of passing the morphology part of the BSE decrease. Analysis of the larger set of data accumulated over the whole five-year period would suggest that a yearling bull has a greater risk of failing the BSE than bulls over 2 years of age. This is probably due in large part by the fact that young bulls of various breeds mature at different rates. The *Bos indicus* bulls mature at a later age than do the *Bos tarus* and are the best example of this interaction between age and sexual maturity. There seems to be no significant increase in the BSE failure rate between the year of age and the sixth year of age. Beginning the seventh year of age the BSE failure rate begins to increase at a significant rate.

Breed

The number of different breeds failing a BSE was not different significantly with the exception of the Herefords. This probably does not represent the total population of the Hereford breed, but is rather an interaction of our particular set of Hereford bulls

.

Body Condition Score

The ideal body condition score (BCS) for breeding bull is thought to be between BCS 5 and BCS 6 on a scale of 1 to 9 (1 equates to very thin and 9 to very fat). Bulls that lost condition in the 60 days prior to evaluation exhibited a severe negative impact on the BSE. This is evidenced by young bull's BSE scores coming off "gain trials" where they were BCS 8 or 9 at the end of the trial and were then allowed to lose BCS rapidly. Older bulls allowed to gain BCS up to 8 or 9 react the same as yearling bulls when allowed to lose BCS rapidly. The problem is that the fat in the neck of the scrotum insulates the testis and causes the temperature pf the testis to be elevated. Thus, you may be presented with bulls with a BCS of 5 and have them to fail the BSE if they were BSC 8 or 9 in the previous 60 days prior to examination. Bulls that consistently maintained BCS of 4 ("hard keepers") showed no detrimental effects on fertility caused by the BCS 4.

Intestinal Helminths and/or Coccidia

The data suggest that the presence of intestinal parasites does not have a detrimental impact on BSE scores unless overt signs of parasitism are present.

Flukes

The presence of liver flukes has a definite impact on fertility, but this effect seems to have additional factors which are necessary to create the most apparent impact on

fertility. This effect may be an interaction with the number of adult flukes in the liver and the length of time they have been present. The present techniques of fecal egg counts do not correlate to the number of adult flukes present in the liver. Controlled research will be necessary to actually delineate the interaction necessary to have a negative impact on BSE scores.

Interaction with BCS and Parasites

An older bull with liver flukes is over twice as likely to fail the BSE as a younger bull with no liver flukes. As long as the bull is not losing BCS due to internal parasites (other than flukes), the presence of internal parasites has no adverse effect on fertility.

Interaction with Breed and Season

The data shows that the Hereford bulls experienced a tremendous negative impact with the presence of flukes, particularly in the summer months. This may not be representative of the Hereford breed as a whole, but may be the result of our particular population of Hereford bulls. It may also be a function of different levels of exposure on different farm units. The data have not been examined to evaluate this supposition.

Conclusions

The failure rate of the morphology exam has decreased at the present time to approximately 12%. The pressure applied to the bulls is at least partially responsible for this decline in failure rates. Additional changes are the deworming schedule, which has been changed to the use of a flukacide preparation at least twice a year, if not more often. Increased attention is being paid to the body score of individual bulls, in order to have them in optimum body condition prior to each breeding season. The bulls are managed as groups , but also as individuals.

Bulls that fail the Breeding Soundness Exam, and those that are inconsistent in their record of evaluation are culled. This makes room for the bulls that are consistent performers. The different breeding seasons make it possible to expose the bulls to increased numbers of females that might not otherwise be possible.

All the emphasis can not be placed on the bulls. The cow herd must also be in acceptable body condition, in order for them to become pregnant. Adverse weather conditions can place a strain on pregnancy rates. When heat and humidity are too high the cows may cycle, but will not sustain a pregnancy. Cold, wet, windy conditions can also have detrimental effects on pregnancy rates.

NOTES

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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

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first exposed for breeding depend on management systems, forage quality and 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).

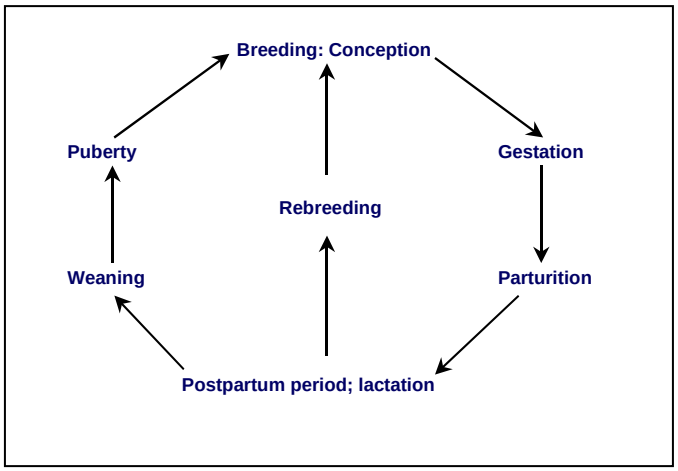

Figure1. Reproduction cycle of the beef female (Bellows, 1985).

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 1. Selected management procedures used on replacement beef heifers[a]

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

[a]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 2. Optimum growth rate for breeding herd replacement heifers[a]

	Frame size				
	1	3	5	7	9
Optimum weight at first estrus, lb	572	669	761	858	955
Mature weight, lb	880	1027	1173	1320	1467

[a]From 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[a]

RTS	Uterine horns	Ovarian length (mm)	Ovarian height (mm)	Ovarian width (mm)	Ovarian structures
1	Immature, < 20 mm diameter, no tone	15	10	8	No palpable follicles
2	20-25 mm diameter, no tone	18	12	10	8 mm follicles
3	20-25 mm diameter, slight tone	22	15	10	8-10 mm follicles
4	30 mm diameter, good tone	30	16	12	10 mm follicles, CL possible
5	> 30 mm diameter	> 32	20	15	CL present

[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).

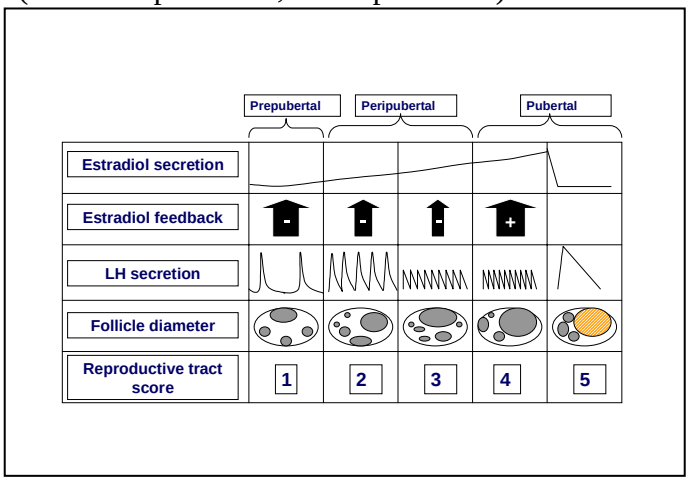

Figure 2. Endocrine and ovarian changes associated with puberty onset in the heifer and associated reproductive tract score (adapted from Day and Anderson, 1998 and Anderson et al., 1991).

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[a]

	Neonatal age at treatment[c]			
Response[b]	Birth	Day 21	Day 45	Control
Uterocervical weight[d] (g)	113.7[e]	123.5[e]	101.3[e]	173.9[f]
Myometrial area (mm^2)	123.7[h]	141.8[h]	111.3[h]	162.8[I]
Endometrial area (mm^2)	29.9[j]	32.4[j]	37.7[j]	45.4[k]
Gland density (hits/mm^2)	172.2[e]	380.3[f]	328.2[f]	486.9[g]
Uterine luminal protein content (mg/flush)	2.8[e]	2.9[e]	2.3[e]	4.9[f]

[a]Adapted from Bartol et al., (1995).

[b]Data were collected from cyclic adult heifers on d 12 of an induced estrous cycle.

[c]Treated heifers received a single Synovex-C® 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.

[d,e,f,g,h,i,j]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.

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 estrous 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 (Table 5).

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 estrous response during the synchronized period is up to 80 percent (Table 5). Estrous 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, estrous response improved.

Table 5. Prebreeding weights, measurements, and subsequent estrous response after synchronization of estrus with MGA-PG[a]

RTS	n	Weight (lb)	Pelvic height (cm)	Pelvic width (cm)	Pelvic area (cm^2)	Estrous response (%)
1	61	594[b]	13.9[b]	10.9[b]	152[b]	54[b]
2	278	620[c]	14.1[b]	11.2[b]	158[b]	66[c]
3	1103	697[d]	14.5[c]	11.4[c]	166[c]	76[d]
4	494	733[e]	14.7[d]	11.7[d]	172[d]	83[e]
5	728	755[e]	14.7[d]	11.7[d]	172[d]	86[e]

[a]Adapted from Patterson and Bullock, 1995. Weights and measurements were taken within 2 wk prior to the first day of MGA. Estrous response is the percentage of heifers that exhibited estrus and were inseminated within 144 h after PG.
[b,c,d,e]Means within a column with different superscripts differ ($P < .05$).

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 lead 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.

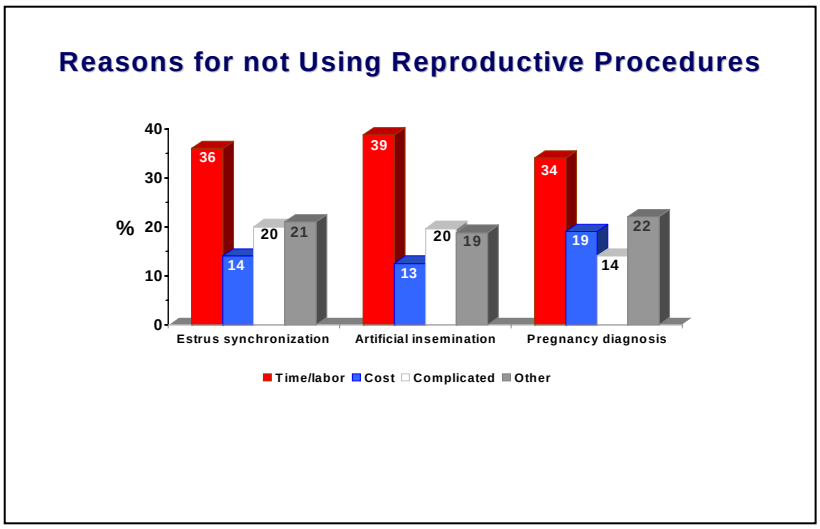

Figure 3. Reasons for not using reproductive procedures (adapted from NAHMS, 1998).

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

	Import and domestic beef semen sales (units sold)		
Country	1993	1998	% change
Brazil[a]	1,874,996	3,256,259	+74
United States[b]	1,117,798	906,923	-19

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

	Number of head			
	1-49	50-99	100-499	≥ 500
Percent of U.S. beef operations by herd size	79.8	11.9	7.7	0.6
Percent of U.S. beef cow inventory by herd size	30.8	19.5	35.7	14

[a]Percentages 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 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, semen 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.

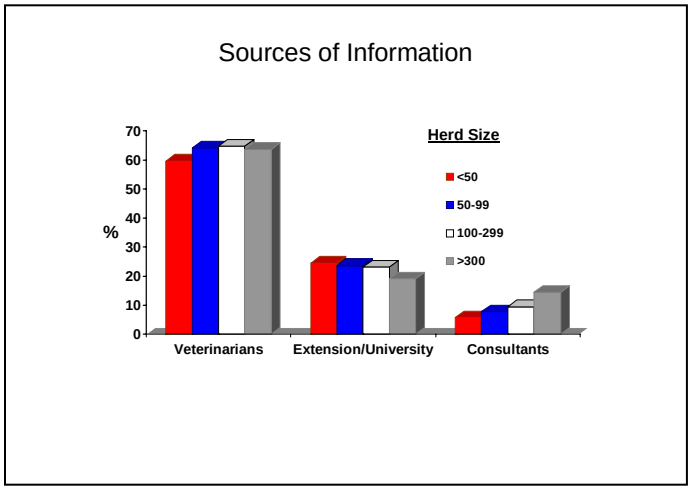

Figure 4. Sources of information (adapted from NAHMS, 1997c).

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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NOTES

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Bovine Fetal Sexing Using Ultrasound

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Introduction

The economics of food animal production is the driving force behind advanced reproductive technologies in the cattle industry. For the past 15 years, the use of ultrasound has proven to be a valuable tool for cattle breeders to assess carcass characteristics and to provide valuable reproductive information beyond the scope of rectal palpation. There are many reproductive scenarios that ultrasound addresses; early pregnancy diagnosis as early as day 21 (Pierson et al., 1984), normal vs. cystic ovarian disease (Pierson, 1984), cycling vs. anestrous females, multiple pregnancies (Stroud, 1991), early embryonic death (Fissore et al., 1986), live vs. dead fetus, response to superovulation (Guibault et al., 1991), oocyte aspiration for IVF (Callensen et al., 1987), endometritis (Fissore, 1988), and fetal sexing. Knowledge of the sex of a fetus, male or female, by days 60 – 90 of gestation provides extremely valuable management information for breeders.

Fetal sexing by ultrasound was first reported in the early 1990's (Curran et al., 1991). Since then, tens of thousands of first trimester pregnancies have been diagnosed by skilled ultrasonographers. The accuracy of the procedure is determined primarily by the skill and experience level of the technician, quality of the ultrasound unit, and the ambient conditions during the examinations, but experienced personnel should be at least 95% accurate in diagnosing fetal sex. Since 1993 the author has performed more than 12,000 fetal sexing procedures with less than five reported missed diagnoses.

Physics of Ultrasound

The physics of real-time ultrasonography have been described in elaborate detail by previous investigators (Pierson et al., 1988), but for the purpose of this article, a brief overview should suffice. A transducer, or probe, has an array of crystals that, when electrically stimulated, produce high-frequency sound waves in a linear, convex linear, or sector (pie-shaped) direction. For bovine reproductive applications, a linear-array transducer is used transrectally in order to facilitate proximity (one to three inches) to the target object. A highly resolved and focused image is thus produced. A linear transducer transmits ultrahigh frequency (inaudible) sound waves along a three- to four-inch axis. The width of the ultrasound waves is approximately one millimeter; therefore, any image projected on the monitor would be comparable to viewing the same structure at necropsy that is opened by a knife in either cross, longitudinal, or oblique sections.

The transmitted sound waves travel through body tissue in a direction determined by the angle of the transducer until they reach a dense tissue reflector. Some of the sound waves are absorbed (fluid) and some are reflected (various tissues and bone) and return to

receiving crystals in the transducer. The force of the returned waves compresses and expands the crystals which, in turn, produce a voltage that is amplified and converted into lifelike images on a high-resolution monitor.

Tissues have different densities that reflect sound at various amplitudes (strengths). For example, the echo produced from amniotic fluid would be weak or anechoic (black on the monitor), whereas the echo from fetal bone, a dense tissue, would be strong or highly echogenic (almost white on the monitor). Significant reproductive tissues of the bovine uterus and ovary (follicular and luteal tissue) as well as various fetal organs have different densities and therefore reflect sound at various amplitudes. These densities are depicted as various shades of gray on the monitor. Most modern, linear ultrasound units produce at least 128 shades of gray that result in high-resolution images of clinically important tissues. The gray-scale image is refreshed with current data at the rate of 30 frames-per-second thus creating a *real-time* or moving image. Figuratively, a real-time ultrasonogram is similar to a moving x-ray.

Applications of fetal sexing

The management applications of fetal sexing by ultrasound are numerous. Prior knowledge of the sex of a fetus can influence the sale value of bred heifers or cows especially in the purebred industry. Also, grouping bred heifers by sex of fetus can be advantageous for calving since the incidence of dystocias are significantly higher with male calves than female. In the dairy industry, sexing the fetus of a marginally efficient older cow can determine whether or not she should be culled if she carries a bull or heifer calf inside. In the case of twins, ultrasound fetal sexing can distinguish between same sex twins and freemartins. Additionally, embryo transfer recipients can be sexed to determine if an adequate number of a desired sex has been achieved from a particular flush or group of transfers. For example if a breeder has sold a flush with a guarantee of two heifer calves, and only one recipient is diagnosed as having a female fetus, the donor should be flushed again in an attempt to satisfy the terms of the sale. There are numerous other scenarios where knowing the sex of the fetus can be advantageous to the owner or buyer of a particular female.

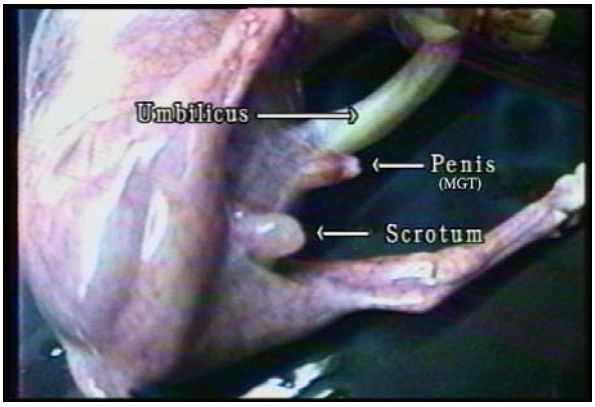


Figure 1

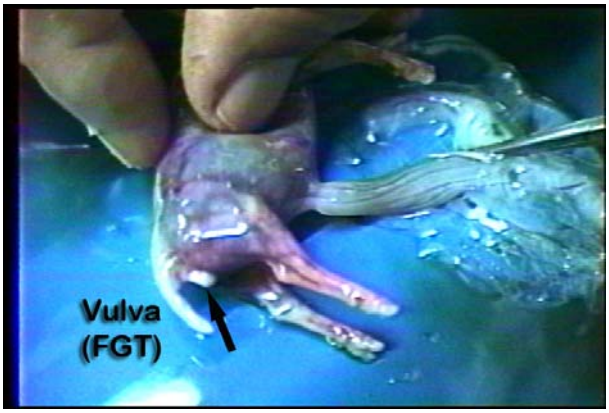


Figure 2

Fetal anatomy

At approximately day 60 of gestation, male and female genital tubercles can be visualized on a high-resolution ultrasound monitor. The fetal sex organs are composed of dense, highly echogenic tissue similar to skeletal structures and therefore are depicted as bright or white structures on the monitor. Male and female genital tubercles appear bilobed on the monitor; each lobe is in the shape of an oval, which aids in differentiation from surrounding structures[6]. The male genital tubercle is found just caudal to the umbilicus (Figure 1), whereas the female genital tubercle is located under the tail (Figure 2).

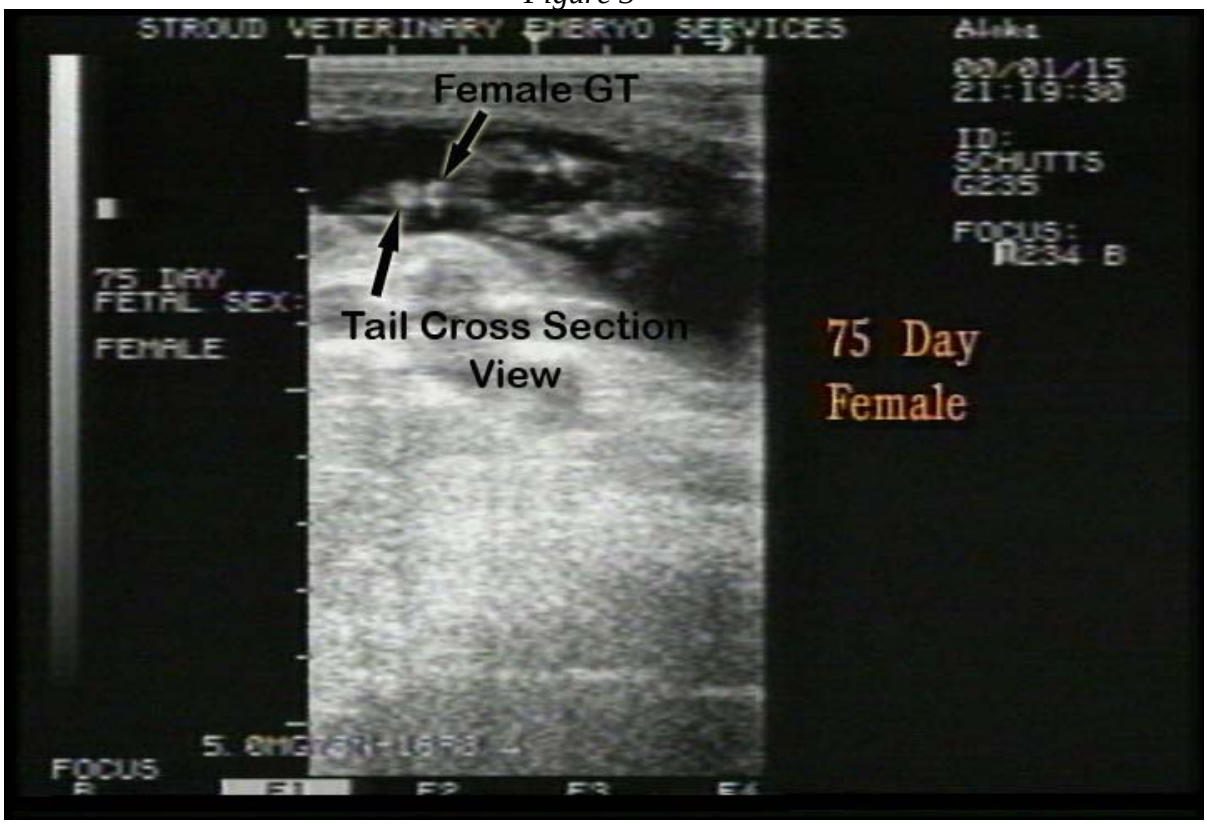
Fetal sex examination

A systematic approach should be taken by the ultrasonographer when performing fetal sexing. There are three very important anatomic references on a fetal sonogram that are critical in achieving proper orientation of the fetus: (1) the head, (2) the beating heart, and (3) the umbilicus (Stroud, 1994). These structures are relatively easy to recognize on an ultrasound monitor. It is sometimes difficult to differentiate the front legs from the rear legs; therefore, these structures have been excluded from the list of anatomic references. Once the fetus has been located on the monitor, the three anatomic references should be systematically examined to ensure cranial-to- caudal orientation.

The following three views can be used to observe a fetus during an ultrasonographic examination: a lateral view (seldom seen), a frontal view (routinely seen and easiest for orientation), and a cross-sectional view (the most often presented). Angled or oblique variations of these views are often presented during routine ultrasound exams, but, for teaching purposes, all three views are discussed in principle.

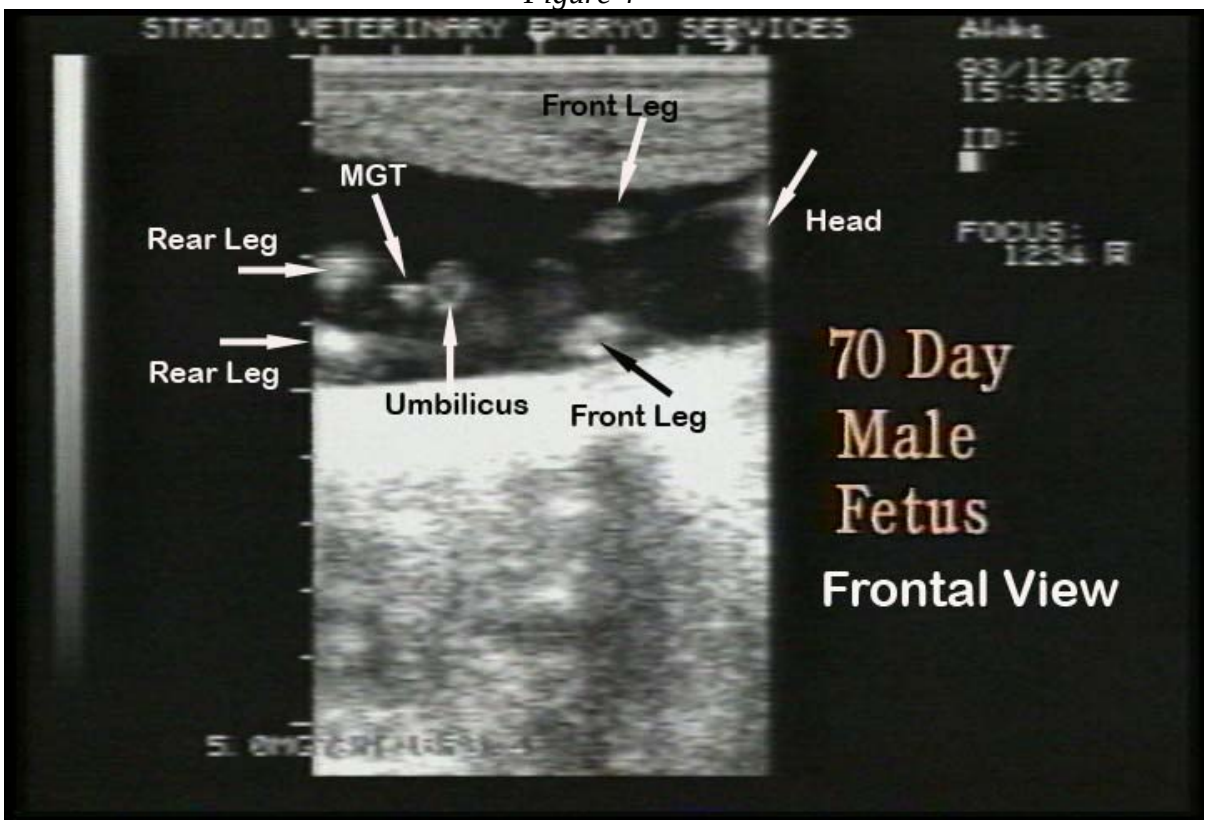
During a cross-sectional examination of the fetus, the transducer is placed over the cranium and moved distally through the thorax to review the beating heart; no heartbeat indicates a dead fetus. The transducer is moved further distally to where the umbilicus attaches to the abdomen. At this time, the transducer should be moved slowly back and forth to diagnose the presence or absence of a male genital tubercle. In males, the genital tubercle is immediately caudal to the umbilicus, appears very bright or highly echogenic on the monitor, and is usually bilobed.

Figure 3



If a male genital tubercle is detected, the examination is complete. If a male genital tubercle is not observed, the transducer must be moved distally to the perineal area to detect the presence of a female genital tubercle. The perineal area is the most difficult region of the fetus to focus; therefore, patience is required. The ultrasonographer should move the transducer slowly and must establish the difference between a cross-sectional view of the tail and the female genital tubercle (Figure 3). The female genital tubercle is generally bilobed, whereas the tail is a monolobed structure. Frequently, the tail and female genital tubercle are seen simultaneously and the ultrasonographer should definitively distinguish one structure from the other.

Figure 4



When the fetus is in a frontal position, the head, thorax, abdomen, and inguinal area can be viewed. The transducer should be manipulated so that the umbilical attachment to the abdomen comes into focus. In males, immediately caudal to the umbilicus is the hyperechogenic male genital tubercle (Figure 4). The frontal view is excellent for diagnosing gender because the perineal area can also be viewed; however, some finesse by the technician is required. The female genital tubercle is sometimes superimposed over the tail. If the transducer is titled either to the left or right, creating a slightly oblique angle, the two structures can be effectively separated optically.

Lateral-view orientation is presented occasionally. From the author's experience, the female genital tubercle is somewhat difficult to visualize using this position. The male genital tubercle at 60 to 100 days and often the entire sheath/prepuce/penis complex of a 90-day pregnancy examination is easily seen on a lateral-view ultrasonogram.

Common mistakes

Before a definitive diagnosis of fetal sex is made, it is imperative that the respective male or female genital tubercle is seen clearly and distinctly by the ultrasonographer. Diagnosing male or female based on the absence of either genital tubercle is ill advised. For example, it is usually faster to diagnose a male simply due to the fact that the penis happens to be located near the attachment of the umbilicus to the abdomen. Since the umbilicus has such an optical presence in an ultrasound exam, it's easy to find, and traceable to the abdomen where the male genital tubercle resides.

However, during some examinations of male fetuses, when the transducer is placed at certain angles, ultrasound waves can become scattered or reflected creating an unresolved and undiagnosable image of the male genital tubercle. So, just because a male genital tubercle is not observed at first glance doesn't mean that the fetus is a female. If a male genital tubercle is not observed, the ultrasonographer must move to the rear of the fetus and see a female genital tubercle before making a final decision.

Conversely, a female fetus can be misdiagnosed as male when the tail is tucked between the hindlegs.[7] The tip of the tail can actually approach the area close to where the umbilicus attaches to the abdomen and create a hyperechogenic structure similar to a male tubercle on a cross-sectional view. Ultrasonographers must be patient and decisive in order to avoid misdiagnosis. With experience, making an accurate diagnosis should not be a problem.

Figure 5

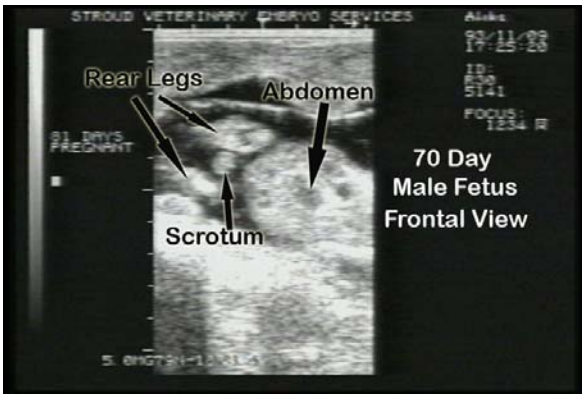


Figure 6

At approximately 75 to 90 days of gestation, fetal sexing is enhanced by secondary reproductive anatomic structures. In males, the scrotum has developed and can easily be seen on a frontal view between the rear legs (Figure 5). In females, the teats are very distinct in the frontal (Figure 6) and cranial-caudal views. Ultrasonographers must be careful when scanning 90 to 120 day male fetuses because some will display rudimentary teats. Also, inexperienced ultrasonographers sometimes see hyperechogenic bits of tissue that can be misconstrued as teats on a female or a scrotum on a male. So, diagnosing sex based on the presence or absence of secondary reproductive structures is not advised. However, once an ultrasound technician becomes confident with ultrasound anatomy, the scrotum and teats are helpful adjuncts to the genital tubercles when diagnosing sex.

Learning curve

Ultrasonographers must (1) have a thorough understanding of ultrasonographic fetal anatomy and (2) develop the skills necessary to produce fetal images that are positioned and focused well enough to accurately diagnose sex. As soon as these criteria are met, ultrasonographers will become proficient in determining fetal sex. A considerable amount of practice is needed in order to achieve a professional level of

expertise in making a consistent and accurate diagnosis. Reaching that level can be quite frustrating, but, with patience, it can be done in a reasonable time frame. The author recommends a two phase learning curve.

Phase one involves learning to accurately read images of both male and female fetuses at various stages between 60 and 90 days and at different angles, i.e., frontal, cross sectional, and obliques. Studying quality still images captured from a sonogram is a good way to begin. Structures such as the umbilicus, head, heart, and fetal sex buds should become recognizable on still images before moving to real time ultrasound exams. Once stills have become mastered, the student should have the confidence to move to videotaped real time exams. Studying edited videotapes with labeled structures transitioning into unedited real time exams can save dozens of hours of frustration for upstart ultrasonographers.

Phase two is simple in principle, but very difficult for most students – producing a quality image with arm in cow. Without having conquered phase one, phase two can be daunting. Assuming phase one has been completed, producing quality images will likely take at least 200 or more exams. The first 50 or so often frustrates many aspiring veterinarians to the point of quitting. Patience and stubbornness are required. The author recommends beginning with five or so exams at a time then progressing to more as confidence grows. Combining both phases culminates in a practitioner being able to accurately diagnose sex.

Selecting an ultrasound unit

A dozen or more companies are currently marketing veterinary ultrasound units in the United States. Major considerations in making a selection are resolution quality, price, serviceability, portability, availability of new as well as loaner units, and the willingness on the part of the salesperson to educate the buyer before and after a sale. The cost of veterinary ultrasound units ranges between $3000 and $20,000, depending on the resolving capabilities, number of transducers, and other technical features.

For most clinical bovine reproductive applications a 5-MHz linear array transducer to be the most versatile and effective. That unit performs adequately on early pregnancy examinations; fetal sexing; pathologic ovaries; and, in general, most all reproductive uses. A 7.5-MHz linear transducer may be more practical if the ultrasonographer intends to do research on follicular dynamics. For transvaginal oocyte recoveries for in vitro embryo production a convex linear transducer gives the technician much more flexibility in gaining access to the hard-to-reach follicles as compared with a linear transducer.

If at all possible, a buyer should sample any potential ultrasound unit and ask for a list of buyers to get feedback before purchase. Most major veterinary conventions have representatives on the trade floor that are more than happy to show their product; however, live cows are recommended as the test host. If portability is a major concern, the buyer should definitely consider the size, weight, stability, and the intended usage for the unit. For example, if fetal sexing is to be done heavily in an ambulatory practice,

resolution and portability are major concerns and the unit should be tested under those conditions before purchase.

Intangibles

Some intangible benefits arise from using ultrasonography in practice. Ultrasonographers inevitably become more proficient in rectal palpation. The difference between a luteal cyst and a normal fluid-filled follicle is easily discernible by real-time ultrasonography but is very subtle by rectal palpation. After having viewed several hundred of each via an ultrasonographic examination, diagnosis by palpation becomes easier. The same holds true for early pregnancy testing. The art of palpation takes literally thousands of cows and years of practice to become proficient. With the help of real-time ultrasonography, an individual inexperienced in rectal palpation could learn skills much more quickly while simultaneously providing a more accurate diagnosis to clients.

Conclusion

Fetal sexing by ultrasound has seen limited use over the last decade due to the steep learning curve necessary to become proficient. However, video training along with well organized short courses with wet labs over the last few years are turning out some well qualified ultrasonographers. Once clients have had bred females accurately sexed they soon demand the service routinely. Having the knowledge of sex before birth is very valuable information. When combining fetal sexing with the other benefits of ultrasound, breeders of valuable purebred livestock begin to rely on the technology.

The bottom line is that ultrasonography in a bovine practice can be profitable to both veterinarians and their clients. Veterinarians must understand that the learning curve is time consuming and sometimes frustrating. The initial investment in a high-quality ultrasound unit also warrants considerable deliberation—ultrasound units are expensive. An extremely busy practitioner may not have the time to learn how to use the unit, which would make its purchase ill-advised; however, if bovine veterinarians want to improve their image, enhance their diagnostic skills, and become leaders in a relatively new discipline of clinical veterinary medicine, ultrasonography may be the tool to achieve these goals.

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NOTES

Proceedings, Applied Reproductive Strategies in Beef Cattle
November 12 and 13, 2005, Texas A&M University, College Station

FROM WISHFUL THINKING TO REALITY: "SEX SELECTION OF SPERMATOZOA"

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Genetic Resources International
Navasota, Texas

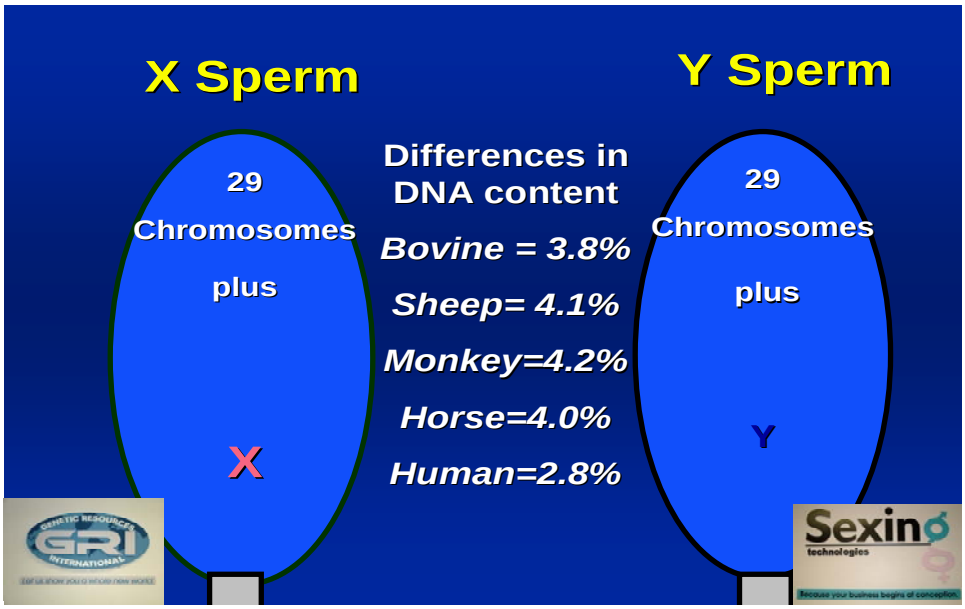
Historical perspective

For thousands of years, mankind has pursued the desire to have reliable, repeatable techniques for sex selection of sperm. In ancient Greece, the philosopher DEMOCRITUS OF ABADERA (460-370 BC) proposed that females originated in the left testicle and males in the right testicle. HIPPOCRATES (460-377BC) proposed the first description of sperm and the theory that strong sperm lead to the development of males and weak sperm to the development of females. In 1677, VAN LEEUWEHOEK used a microscope to describe sperm and new theories of sex determination arouse. It was not until the 1980's and 1990's that the development of sophisticated instruments allowed for an in depth study of the sperm and the scientific proof that sperm carry either the X chromosome for females or the Y chromosome for males. In the same period, the flowcytometer allowed for the separation of sperm based on the X- and Y- chromosome. Finally, in the new century commercialization of sexed semen became a reality.

Mechanisms of sexual differentiation.

The mechanisms of sexual differentiation vary according to species. In some reptiles, temperature depending enzymes regulate the sex of offspring (Dorizzi et al. 1996). While in other reptiles sex is determined chromosomically depending on the combination of the sex chromosomes (ZW vs ZZ) as is seen in lizards and turtles (Coriat et al. 1994). In mammals and avian species, sex determination depends on chromosomal information only. Mammalian sperm carry either the X or the Y chromosome (Jacobs and Strong 1959), and when combined with the X chromosome from the oocyte create an embryo that is ether male or female. Mourizzi in 1979 showed a difference in the size of the X and Y chromosome and Garner et al in 1983 showed on a quantitative basis the difference in DNA content between X- and Y- chromosome bearing sperm.

The simplest, most economical, and most efficient way to influence sex ratios in offspring is to determine the sex before fertilization and therefore to separate the populations of X and Y chromosome bearing sperm. Thousands of attempts and reports have been published regarding different methods to determine the sex of sperm and techniques to separate X- and Y- chromosome bearing sperm. Among the reported techniques were velocity, density, electric surface charge, and immunologically relevant structures. None of those techniques have proven repeatable and accurate. The only technology that has proven repeatable and accurate is the flow-cytometrical separation of X- and Y- chromosome bearing sperm based on the difference of DNA content.



Techniques to identify sex-related characteristics of spermatozoa.

The path to the development of today's current sperm sexing technology

A chronological series of events lead to the development of today's proven, repeatable technology to separate sperm based on the size of the X- and Y- chromosome bearing sperm by flowcytometry.

a. Difference in DNA content of X- and Y- chromosome bearing sperm

Moruzzi 1979

Showed that the X chromosome carries more DNA than the Y chromosome and autosomal cells have identical DNA content.

b. Flowcytometry and sperm sorting

Sprenger et al 1971 and Gledhill et al 1976

First experiments of flowcytometric analysis of sperm with no success.

Pinkel et al 1982

Modified the injection tubes in a flowcytometer to allow for better orientation in front of a laser beam.

Garner et al 1983

First report of detecting DNA content differences in sperm with a flowcytometer.

Bull 3.8% ; Boar 3.7%
Ram 4.1% ; Rabbit 3.9%

Johnson and Pinkel 1986

Added a second detector to a flocytometer and developed a beveled tip for the injection tube.

Johnson et al 1987 and Johnson et al 1987b.

Start using Hoescht 33342 as the stain of choice to selectively bind to the chromosomes and be able to measure DNA differences with a flocytometer. Process very slow 55 sperm/second.

Johnson and Clarke 1988

Show sperm decondensation and pronuclear formation of sex selected sperm by flowcytometry. Do sperm injection in Hamster oocytes.

Morrell et al 1988

Reports the births of the first animals with sorted spermatozoa.

Johnson et al 1989

Report the birth of offspring and gender selection accuracy of 94% and 81% for animals inseminated with X- and Y- chromosome bearing sperm.

Cran et al, 1993

Reports the first use of sex sorted sperm in in-vitro fertilization

Seidel et al 1997

Reports the use of low dose insemination with sex sorted semen

Rens et al 1998

Creates an improved orientation nozzle, high speed flowcytometers are introduced and speed goes up to 30,000 evens per second with a 90% plus degree of accuracy.

Schank et al 1999

Reports the freezing of sex sorted semen with pregnancy results.

COMMERCIALIZATION

XY, Inc 1996

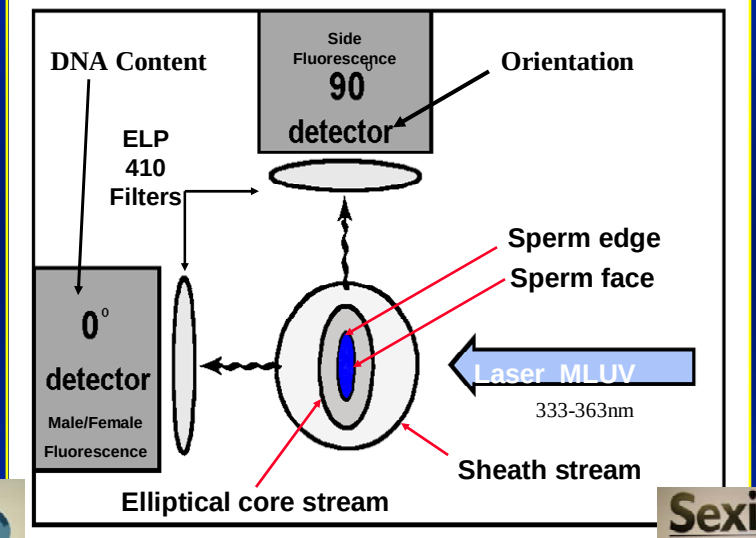
Leads the development of new sperm preparation procedures, handling, medias, and freezing that lead to the use of sex sorted semen in AI, IVF and ET with a high degree of success.

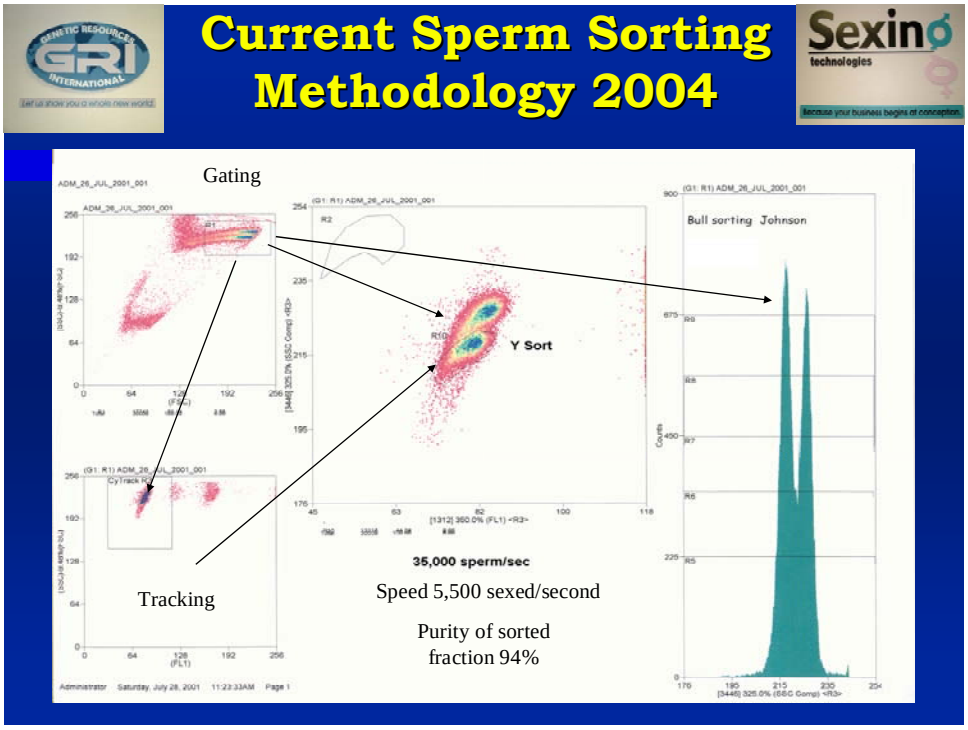
Introduces licensing of its patented technology to the market and leads to the first commercial applications of sex sorted semen:

1. Cogent in England
2. Goyaike in Argentina
3. Sexing Technologies in the US and Brazil

MoFlo® SX

Fluorescence detectors





RESULTS

PREGNANCY RATES

In Artificial Insemination

BULL	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***Total***
DAIRY									
A	***38%***	***30%***	***41%***	***43%***	***42%***	***42%***			***39%***
B	***46%***	***15%***	***58%***	***78%***	***25%^***	***50%***			***50%***
C	***51%***	***51%***	***54%***	***53%***	***12%***	***31%***			***42%***
D	***51%***	***53%***	***56%***	***48%***	***62%***	***47%***	***55%***		***53.5%***
E	***39%***	***62%***	***27%***	***57%***	***52%***	***55%***	***51%***	***48%***	***54%***
F	***10%***	***20%***	***17%***	***32%***	***25%***	***33%***			***24%***
G	***64%***	***62%***	***71%***	***58%***	***49%***	***71%***	***66%***	***55%***	***61.5%***
Total									***52.5%***

All artificial Inseminations were performed with straws of 2 million sperm in virgin dairy heifers. The data se contains more than 10,300 AI's

Sexed Semen In Embryo Transfer

Embryos (1245) were produced with female sexed semen from Brahman and Nelore females during the spring- summer of 2005. Different concentrations of semen and times of artificial inseminations were tested to determine the best protocol. The Heat Watch system was used for heat detection. Embryo average per donor rangeed from 3.2

embryos to 7.5 embryos depending on treatment. Embryos (1032) were transferred fresh for an average pregnancy rate of 68%. Ultrasound fetal sexing on a random group of recipients provided an average of 91% female fetuses.

Sexed Semen in In Vitro Fertilization

During the Spring and Summer of 2005, sexed semen was used in in vitro fertilization trials in Brazil and the United States. Sexed semen samples from 23 different bulls were used. Breeds represented included Brahman, Nelore, jersey, Holstein, Brown Swiss, Gyr, Guzerat.

4324 oocytes were matured
2248 oocytes divide to two cells
1513 embryos were produced

Development to Blastocyst/transferable embryos was 35.2%. Pregnancy rate of the embryos was 48.3% and 93.1 % of the embryos were female.

Economic Analysis

Advancements in sexing procedures have allowed the commercialization of sexed semen at commercially viable prices. Straws of sexed semen in dairy bulls sell for between $35.00 and $60.00 per straw, depending on the bull. For beef sires, sexed semen prices range from $30.00 to $450.00 per straw, depending on the bull.

Dr. Bob Everett of Cornell University has developed an economic analysis spread sheet calculation for the value of sexed semen. The producer can input all of his costs, pregnancy rates, value of their calves, etc and determine if sexed semen is economically viable for his or her operation. We will provide for those interested a copy of that calculation at the time of the conference.

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REPRODUCTIVE TECHNOLOGIES: TECHNOLOGY STACKING

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Introduction

There have been several reproductive technologies developed over the past several years. Although these technologies have great potential to improve productivity of beef and dairy operations, they have only been minimally used. The reproductive technologies that may offer great value include estrus synchronization, embryo transfer, *in vitro* fertilization (IVF), sex-sorted semen, and embryo splitting. Synchronization of estrus protocols have been developed that consistently synchronize fertile ovulations with high fertility–fertility equal to breeding at estrus–and can be as valuable in synchronizing recipient cows as it is for AI cows. These procedures are often viewed as individual technologies. I have a more comprehensive viewpoint and propose combined utilization of the technologies. Within this succinct report, I will provide approaches to increase the reproductive potential of cattle.

Twinning

There are three basic approaches to twinning: transfer of two embryos, embryo splitting, and transfer of one embryo after an AI. With embryo splitting, the embryo is split using an embryo splitter and the demi-embryos are transferred into the cow. Since these are identical twins there is no concern with freemartinism. The following are data (Table 1) published by Dahlen et al. (2002). In that study, there was an increase of 84 pregnancies (calves) per 100 cows.

Table 1. Pregnancy rate and pregnancies with demi-embryos.

Item	One Whole Embryo	Twin Demi-Embryos
Pregnancy Rate (# pregnant / # cows treated)	15/37 (40%)	30/37 (81%)
Twinning (d 27)	0/15 (0%)	16/30 (53%)
Pregnancies (# fetal offspring / # cows treated)	15/37 (40%)	46/37 (124%)
Number of Calves / 100 Treated	40	124

There obviously will be cost associated with using this technology; however, the question is "Does the value of the additional calves justify the cost?"

Similarly, if one transfers two embryos per cow, calf production can be increased. However, one can also transfer an embryo into a cow seven days after an AI. A potential problem with these two concepts is freemartinism (assuming that one wishes to use the females for reproductive purposes). However, with the development of sex-sorted semen one can create embryos using

sex-sorted semen and about 90% of the embryos will be of the desired sex. This greatly reduces the incidence of freemartinism. If one artificially inseminates with sex-sorted semen and follows with an embryo created with sex-sorted semen, then freemartinism will again be greatly reduced. This concept is by no means new; however, when it was first developed, sex-sorted semen was not on the horizon. Because of the high cost of sex-sorted semen, use for AI may be of questionable value. However, when used with embryo transfer, again one must assess if the value of the additional calves justifies the cost. Both Anderson et al. (1979) and Holy et al. (1981) demonstrated the efficacy of these procedures (summarized in Tables 2 and 3).

Table 2. Pregnancy rate and pregnancies in cows transferred one or two embryos (Anderson et al., 1979).

Item	**ET Two Embryos**	**ET One Embryo + AI**
Pregnancy Rate	15/21 (71%)	12/17 (71%)
Aborted	1/15 (7%)	1/12 (8%)
Produced Twins	10/14 (71%)	9/11 (82%)
Number of Calves / 100 Treated	114	117

Table 3. Twinning rate of cows transferred one embryo after AI (Holy et al., 1981).

Item	**Single**	**Twin**
Frequency	35/64 (55%)	29/64 (45%)
Single from AI	13/35 (37%)	—
Single from ET	22/35 (63%)	—

The pregnancy rate in the Holy et al., study was 62%. Clearly, both procedures increased the production of calves. Combined, 114 to 124 calves were produced using these three procedures. The number of animals used in these studies was limited; however, these data demonstrate the feasibility of these procedures.

Technology Stacking

I conducted another study wherein we used multiple reproductive technologies: estrus synchronization, sex-sorted semen, *in vitro* fertilization, and embryo transfer. In this study 486 beef cows were used. These cows were synchronized using CO-Synch with or without the CIDR. The day of the second injection of GnRH was considered day 0. On day 7 cows received either one or two grade 1 balastocysts. The embryos were created *in vitro*. Oocytes were collected from slaughterhouse ovaries and fertilized *in vitro* with frozen-thawed semen that had been sex-sorted to produce female offspring. The presence of corpora lutea were determined *per rectum* without grading quality. If a corpus luteum was present, transfers were done. Luteal tissue was verified via ultrasonography and transfers were done in every cow with luteal tissue. Single embryos were placed in the uterine horn ipsilateral to the ovary with the corpus luteum. In cows that received two embryos, they were transferred bilaterally (one in each uterine horn). Pregnancy was diagnosed 40-55 days post-transfer via ultrasonography.

Luteal tissue was detected in 463 cows (95%) at the time of transfer (Table 4). In thirteen

cows, ultrasound revealed the presence of luteal tissue. Although, embryos were transferred into these cows, only 1 (8%) became pregnant. No luteal tissue was detected in 10 cows and transfers were not done in these cows. Based on this information, the corpora lutea should be detected only *per rectum*. However, clearly the CO-Synch estrus synchronization protocol (with or without the inclusion of the CIDR) was an effective protocol to prepare cows for embryo transfer. We have previously observed this using frozen-thawed embryos (Table 5).

Table 4. Pregnancy rate of cows based on classification of luteal tissue at transfer.

Item	**Number**	**Pregnancy Rate**
Cows:	486	—
with no luteal tissue	10	no transfers
detected only via ultrasound	13[b]	1/13 (8%)
detected *via per rectum* examination	463 (95%)[a]	200/463 (43%)

[a]Ninety-five percent (95%) of the cows received one or two embryos.
[b]These 13 cows are not included in Table 5.

Table 5. Pregnancy rates in synchronized cows transferred embryos.

Item	**Transferred**	**Pregnancies/Treated**
CO-Synch (expt. 1)	94%	50%
CO-Synch + CIDR (expt. 1)	94%	59%
CO-Synch (expt. 2)	92%	62%
CO-Synch + CIDR (expt. 2)	92%	65%

Pregnancy rates (# pregnant / # transferred) were not affected (P>.10) by inclusion of the CIDR; however, pregnancy rates was higher (P<.01) in cows receiving two embryos (51%) vs. one embryo (38%).

Table 6. Pregnancy rate and calving rate of cows synchronized with CO-Synch with and without the CIDR receiving one or two embryos at transfer.

Item	**CO-Synch**	**CO-Synch + CIDR**	**CO-Synch +/- CIDR**
Pregnancy Rate (# pregnant/# transferred):			
single transfer	60/145 (41%)	49/140 (35%)	109/285 (38%)[a]
twin transfer	43/ 88 (49%)	48/ 90 (53%)	91/178 (51%)[b]
Calving Rate (# calves/# recipients):			
single transfer	45/144 (31/100)	39/136 (29/100)	84/280 (30/100)[c]
twin transfer	38/ 85 (45/100)	41/ 87 (47/100)	79/172 (46/100)[d]

[a,b]Values with different superscripts differ [P<.01] (CIDR, P=.80; single/twin X CIDR interaction P=.27).
[c,d]Values with different superscripts differ [P<.01] (CIDR, P=.88; single/twin X CIDR interaction P=.62).

Sex of calf was assessed at calving and 90% (158/176) of the calves were heifers. Twenty-two percent of the cows with twin transfers calved with twins. There tended (P=.06) to

be a higher pregnancy loss (# calving / # diagnosed pregnant) in cows with twin transfers than in cows with single transfers. Inclusion of CIDR in the synchronization did not affect (P>.10) pregnancy loss. Calving rate (# calves born / # recipients) was not affected by inclusion of the CIDR; however, calving rate was higher (P<.01) in cows receiving twins (46/100) vs. one embryo (30/100). Eight of the cows were diagnosed with hydramnios/hydrallantois and was not affected by CIDR inclusion nor number of embryos transferred. Calving difficulty was no greater than in previous years even though the Holstein calves were larger than beef calves and even though a large number of cows gave birth to twins. No excessive large calves were observed.

In summary, synchronization of ovulation with the CO-Synch protocol, with or without the CIDR, effectively prepared cows for transfer and twinning, regardless of transferring unilaterally or bilaterally, improved pregnancy rates. Furthermore, these data demonstrate the utility of the combined use of estrous synchronization, sex-sorted semen, in vitro fertilization, and embryo transfer technologies.

Conclusions

One novelty not mentioned is that the oocytes and semen were all of Holstein genetics. The idea here was to develop a source of income for the research unit. Holstein heifers are of high value. This is only one way to generate profit and I encourage you to explore other possibilities that can be obtained by stacking the technology. Combing these technologies give producers options for increasing calf crop and profit as well. As the technologies mature, utilization will increase and cost will decrease. Consider the possibilities.

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CLONING BOVINE EMBRYOS: CURRENT STATUS AND FUTURE APPLICATIONS

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Introduction

Cloning animals entails transferring the nucleus of a cell obtained from the individual to be cloned into an unfertilized ovum that has had its metaphase chromosomes removed. If successful, the transferred nucleus is re-programmed so to direct development of a new embryo that is genetically identical to the animal from which the cell was obtained. This embryo can then be transferred into a surrogate mother for gestation to term and birth of a clone.

Although the history of animal cloning dates back to the early 1900's (Spemann et al., 1938, McLaren, 2000), the first cloned mammals (sheep) were not reported until 1986 by Steen Willadsen, (Willadsen, 1986). In brief, Dr. Willadsen dissected a pre-implantation embryo into individual cells, and then utilized electrical pulses to fuse individual embryonic blastomeres with unfertilized ova in which he had removed the metaphase chromosomes. The resulting "cloned embryos" were then transferred into recipient females and developed into lambs which were genetically identical. This landmark accomplishment was the key event in history that spawned interest in the utilization of cloning to produce large numbers of genetically identical livestock. At that time, splitting embryos to produce identical twins was already becoming a popular method for increasing embryo production and thus the number of calves that could be derived from valuable embryo donor cows. However, splitting embryos was limited in its ability to produce only twins as pregnancy rates dropped dramatically when trying to split a single embryo more than one time. Cloning on the other hand offered the promise of producing literally thousands of genetically identical calves. This could be accomplished, "in theory", by producing cloned embryos, then allowing them to divide several times in culture prior to using them for a second round of cloning to produce additional cloned embryos, and simply continuing to repeat the process, termed "multiple generation cloning". With this approach, thousands of genetically identical embryos could be produced that when transferred into recipient females would result in thousands of genetically identical calves. The idea spawned visions of large herds of cloned bulls, cloned feedlot steers and cloned dairy cows. However, 20 years later, this idea has still not become reality. What happened? What went wrong? Is there still the possibility (what's the probability) that this will someday occur? It's a long sorted story that I

certainly do not have time here to discuss in detail. I will however try to hit the highlights that bring us to the current status of cloning livestock, in particular, cattle.

Shortly after Dr. Willadsen's successful demonstration of producing cloned sheep he was recruited and hired by Granada Genetics, an entity of Granada Corporation based in Houston Texas. At that time Granada Genetics represented the largest commercial bovine embryo transfer company in the world and was interested in expanding their research program to include cloning and genetic engineering, then transferring these technologies to the commercial market. The challenge for Dr Willadsen was to adapt techniques he had utilized in sheep to produce cloned cattle. Success came quickly. In fact, by the time Willadsen's report involving cloned sheep was made public in *Nature*, pregnancies derived from cloned cattle embryos had already been obtained and the first cloned calves were born in 1986. Successful cloning of sheep and then cattle resulted in two other major companies investing time and resources to establish commercial cloning operations, Alta Genetics, Calgary Canada, and American Breeders Services, DeForrest Wisconsin. Over the next several years, all three of these companies had active cloning programs involving both research in addition to commercial applications.

Unfortunately, even though hundreds (if not thousands) of cloned cattle were produced during this time period and entered the market either as breeding stock, milk cows or for slaughter, the economics of producing cloned cattle using embryos as nuclei donors failed to support an ongoing viable business model, and by the early 1990s commercial activities involving the production of cloned cattle were at best, minimal. Multiple factors contributed to this scenario including the high cost of producing cloned offspring, low pregnancy rates resulting in low efficiency, enormous variability in the outcome (sometimes it worked and sometimes it didn't), and the infamous observation/documentation that a high proportion of the calves produced by nuclear transfer exhibited Large Offspring Syndrome (LOS) resulting in management problems as pertained to dystocia and death of cloned calves.

Although cloning bovine embryos never resulted in large-scale commercial application, interest in nuclear transfer and cloning livestock continued. New research focused on increasing the efficiency of the process and trying to understand problems associated with low pregnancy rates and LOS (Wilson et al., 1993). In addition, numerous studies were initiated to explore the utility of alternative cell types for use as nuclei donors. The most significant outcome of this research was the demonstration that cultured cell lines derived from either embryos or fetuses could be utilized to produce live cloned offspring (Campbell et al, 1996). While the efficiency of using cultured cells as donors for nuclear transfer was no more efficient than when using embryonic blastomeres (in fact it was most times much more inefficient), there were several advantages. First, cell culture allowed for the production of millions of cells with identical genotypes, using standard tissue culture techniques. As a result, millions of cells were readily available for use as nuclei donors thus bypassing the need for multigeneration cloning to produce large numbers of clones. More relevant, and the true driving force behind this work was the fact that cultured cell lines could be genetically engineered prior to utilization for cloning, resulting in a new more efficient approach for

producing genetically engineered livestock (Schnieke et al., 1997) . In fact, it was this approach, with the goal of producing genetically engineered sheep that ultimately led to the utilization of somatic cells derived from adult animals to produce cloned livestock. In 1997, Wilmut et al reported the birth of a cloned sheep (Dolly), produced by nuclear transfer and using nuclei obtained from cultured mammary epithelial cells.

The birth of Dolly set into motion a new wave of interest in cloning. It also caused an enormous uproar in the bioethics community with concerns over adapting this technology for human cloning, a controversy that remains today. What made the birth of Dolly such an amazing feat was the fact that her birth went against more than 50 years of scientific dogma that suggested cloning mammals from differentiated cells collected from an adult mammal was biologically impossible. Obviously, this was not the case. It was simply a matter of trying new approaches and transferring enough embryos i.e. large enough numbers to finally obtain one that would develop to term. Since Dolly's birth, less than 10 years ago, an explosion in research efforts targeted at cloning mammals has occurred. Cloned animals resulting from somatic cell nuclear transplantation have now been reported in more than a dozen different species including all the major livestock species, cattle, goats, sheep, pigs, and horses (Wilmut et al., 1997; Cibelli et al., 1998; Hill et al., 2000; Keefer et al., 2000; Polejaeva et al., 2000). Work involving other species is currently ongoing, and information gathered to date suggests a wide variety of different animal species can be cloned by nuclear transplantation. These animals have all been cloned using cells obtained from adult animals. The most common cell used is normally a fibroblast, obtained by simply taking a sample of skin and applying tissue culture techniques to obtain millions of cells that are suitable for cloning. An obvious advantage of cloning from live animals is the ability to select individuals in which the phenotype is already known. Previous efforts involving the utilization of embryos and even fetal cells as nuclei donors could be used to produce genetically identical offspring, however besides the sex, there was no way to predict how this particular animal might develop as pertained to production characteristics. Cloning from adult cells now allowed one to select animals representing the very best of the best, and clone these e.g. the Grand Champion Bull or the milk cow that produced 45,000 # of milk per year. As such, title of this paper is really somewhat of a misnomer as the current status of cloning does not involve "embryo cloning" at all, rather cloning animals with superior phenotypes, using cells obtained from a small biopsy of skin.

Current Status

It is probably safe to say that world-wide there are more laboratory groups working on cattle cloning than in all other livestock species combined. It is also probably the case that more different cattle genotypes are represented by cloned offspring than genotypes of all other species combined. The ability of a number of different laboratory groups to successfully clone cattle is a result not only of numerous research programs focused on nuclear transfer in cattle, but the enormous base of knowledge that has been developed over the last 20 years involving the application of assisted reproductive techniques in cattle. Successful and repeatable procedures for *in vitro* oocyte maturation, *in vitro* fertilization, and *in vitro* embryo culture are now well established in cattle. Each

of these represent a key step in the cloning process and in some cases are not as well established in other species. This is in part due to the ability to access large numbers of oocytes from abattoirs for use in research and at a relatively low cost. In terms of cloning a specific cow or bull, the ability to access large numbers of oocytes at a relatively low cost also provides the opportunity to carry out numerous attempts at cloning a specific animal. Therefore, even if the overall efficiency is low, chances are given enough trials and enough embryos transferred, a clone of most any cow or bull could be produced.

The efficiency of cloning cattle by nuclear transplantation is extremely variable. Due to the limited number of controlled experiments, it is difficult to determine the source of this variability and analyze potential interactions between different variables which include not only genotype, but the type of nuclei donor cell utilized and of course the laboratory group performing the work, just to name a few. The percentage of nuclear transfer embryos developing to the compact morula or blastocyst stage ranges from less than 5% to greater than 65%. Live births per embryo transferred are also extremely variable (0% - 83%). Of the calves born alive, a significant percentage die within one week of birth due to various health problems. Again, this varies, ranging from 0% to 100% of the calves failing to survive past one week of age (Kato et al., 1998: Hill et al., 2000). Looking at the overall averages when reviewing the scientific literature, the efficiency of producing cloned embryos that develop to the blastocyst stage in culture is similar to those produced by in vitro fertilization i.e. it is not uncommon for 45-50% development to the blastocyst stage. The major losses occur once the embryo is transferred into a recipient female and more specifically beyond 40-45 days of gestations. Initial pregnancy rates can average 40% -50%, again competitive with normal embryo transfer. However pregnancy loss can be extremely high after 50 days of gestation resulting in a calving rate of only 5-10%. Having said this, more recent data suggests these numbers are improving and as a result, large scale application of cloning may in fact be closer to reality than we think.

The Future

The high cost to produce small numbers of clones of individual donor cell lines will likely create self-imposed limits to commercial cloning in the purebred cattle industry. However, that's not the real enigma that besieges leaders in the industry and handcuffs them from using this potentially potent tool. By the time a bull or cow is determined to be worth cloning, i.e., has proven itself with sufficient numbers of superior offspring, it is most likely over five years of age. The breeder must ask himself if the money he spends today would be better spent on reproducing "old genetics", i.e., a clone, or on the next "super calf" with next generation EPDs/TPIs. The time lapse between the gestation of the clone, its birth, subsequent puberty, reproducing, and having its first calf crop on the ground, is normally about three years, and creates some serious head scratching considering that no genetic improvement is gained by cloning. Perhaps only a few proven herd sires and donor females from any breed will qualify as cloning prospects with the real potential to produce profits. None-the-less, these unique animals do indeed exist and several commercial companies currently offer cloning services to replicate the

genotype of these individuals, for a fee. Cloning these superior individuals is certainly worth the cost and effort should unintentional death of the animal occur.

Ironically, the commercial sector of both the beef and dairy industries are likely the best candidates for cloning. Animals with exceptionally valuable phenotypes such as high producing dairy females or beef bulls that produce steers with "perfect" carcasses will be the candidates for genetic replication. However, the expense of cloning will have to be neutralized by high volume production of hundreds or even thousands of copies of each cell donor. In other words, as the volume of clones produced increases the cost will come down, on a per clone basis. Commercial companies are already offering volume discounts. As the cost for producing a cloned calf decreases when compared to other modes of reproduction, cloning large numbers of animals to increase herd size or provide replacement animals starts to make good business sense.

It's important to note that clones of valuable beef bulls won't be eaten, but their offspring will. Uniformity and predictability are two very important economic traits in the beef industry. Some, but certainly not all, of the criteria for selecting a beef bull to clone would be that he produces calves with low birth weight, high weaning and yearling weights, is disease resistant, an efficient feed converter, and has desirable carcass traits. It is rare that a single sire would pass on all or most of those traits to a high percentage of his offspring. A single animal exhibiting all these economically valuable traits in his genotype would be considered a genetic freak. Indeed he would be just that, and exactly the animal that should be cloned for commercial purposes. Imagine the uniformity of putting 5000 such male clones on 125,000 or so related females. What commercial buyer or packer wouldn't want that set of calves? Retained ownership suddenly takes on a different meaning.

Cloning in the dairy industry has even greater ramifications. It's not news that US dairies are getting bigger by the decade. However, expansion is expensive and, in some cases, superior genetics are difficult to acquire. Throw in a lack of biosecurity issues on purchased animals involving diseases such as Bovine Viral Diarrhea (BVD), Neospora, Bovine Leukemia Virus (BLV), Johne's, and Leptospirosis and all of a sudden the task to grow seems very burdensome. Cloning on a massive scale seems a healthy solution. Reproducing or cloning several cell lines of ultra high producing females can generate in one gestation cycle hundreds or thousands of cows representing superior genetics and phenotypes that most dairy breeders spend their lifetime trying to raise just one. It's no wonder considering the fact that most economically valuable traits like volume milk production, milk protein, fat values, good udder, sound feet and legs, highly fertile, plus longevity are all multigene traits. The odds of raising such a cow are mathematically very unlikely – she's literally the one-in-a-million. Cloning her on a high volume basis, at least theoretically, makes sense. Use a biosecure recipient herd to nurture them and the dairyman now has control of his future. The real value of the clones wouldn't be in their own production records, but more likely production of their offspring for generations to come. The biggest problem the dairyman would have is choosing a bull to breed to. Finding one that wouldn't pull them down genetically could be a challenge.

A key word when considering cloning for commercial application is “theoretical”. The history of cloning suggests that there are problems to overcome before cloning will become commercially feasible. Low conception rates of transferred embryos, early embryonic death, abortions, stillbirths, and perinatal deaths have all been reported. However, those problems are being defined and corrected. Current commercial cloning projects look promising with conception rates on nearly 600 NT embryo transfers at 40 days of gestation equal to or greater than *in vivo* produced control embryos from superovulated donors (59% vs. 54% respectively). By day 100 to 120 the cloned pregnancy rates are holding at 35%, but calving rates won’t be available until after this manuscript goes to print (Brad Stroud, unpublished data).

For large commercial dairies looking to substantially expand their numbers cloning is certainly a consideration when compared economically to traditional embryo transfer (ET). For example, assume that a dairy has plans to expand by 1,000 females. A standard starting price for purchasing a frozen dairy breed embryo produced by traditional ET is about $300. The genetic value of that embryo would be at or below average for ET donor females in the dairy industry. Based on industry standards, a 50% calving rate could be expected from purchased frozen dairy embryos. Additionally, half of those calves would be unwanted males. Consequently, the dairyman would have to purchase 4,000 frozen embryos to produce 1,000 replacement females. At $300/embryo the initial investment in genetic material is $1,200,000 or $1200 per heifer calf.

Alternatively, the expansion project could be done utilizing nuclear transfer (cloning) technology. Assuming the dairyman would want to clone his best producing female there would be no initial investment in genetics. However, he could choose to buy a high producing female outside his herd, biopsy and freeze her tissue, then sell her to recover most, if not all, his initial investment. So, the economic model now shifts to the cost of cloning. Obviously, 100% of the offspring will be female, so that factor is removed. Assuming cloned embryos cost $250 each on a volume basis (ViaGen Inc., Austin, Texas), and the calving rates of transferred cloned embryos are 25%, the cost to produce 1,000 heifer calves would be [$250 x 4 x 1000 = $1,000,000. That’s $1,000 per heifer calf produced by cloning as compared to $1200 per heifer calf produced by traditional ET.

The cost of transferring the embryos would be the same. Both scenarios, cloning and traditional ET, would require 4000 transfers to achieve 1000 heifers, so economically that’s a washout. It could be argued that recipient wastage due to embryonic mortality and abortions is greater in the clone group since half of the pregnancies diagnosed at 40 days will be lost. However, data from ViaGen Inc. (unpublished) showed that eight of nine aborted recipients bred back to a bull within 90 days of the original 40 day pregnancy check. Although aborted clone recipients are an economic loss, so too are the recipients giving rise to unwanted bulls in the traditional ET group. At least the aborted clone recipients could be synchronized again for another clone whereas the recipients carrying unwanted male calves to term have wasted an entire gestational period.

When comparing all the economic factors listed above cloning stands pretty close to traditional embryo transfer. However, the model above considers a $300 embryo, which is the price for average quality genetics in the dairy industry. The dairy farmer who chooses to clone one of the most genetically elite females in the industry has an overwhelming advantage for a relatively similar investment. Who wouldn't choose to expand by producing 1000 copies of a lifelong healthy cow with good feet and legs that has had six or more lactations of close to 40,000 lbs plus high fat and protein as compared to 1000 heifers from mediocre donor females? The key of course to making this all work is directly tied to calving rate i.e. the efficiency of producing healthy calves by cloning.

Summary

In the early 1900s, Hans Spemann described what he called "The Fantastical Experiment". In essence, his vision involved the utilization of nuclear transplantation to someday be able to clone animals. Nearly a hundred years later, that vision has become reality. However, the large scale application of cloning cattle (or any livestock species for that matter) has yet to occur. To date, only those animals representing the elite of their breed have been selected for cloning. The efficiency of cloning cattle remains low, and the cost of producing a cloned calf high. However, as research continues, the efficiency of cloning cattle will increase. As seen in the examples provided here, just a slight increase in the efficiency could dramatically increase the utility and benefit of cloning and in turn the demand for cloned cattle. Some modern day visionaries obviously think we are already there.

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