

DISSERTATION

FERTILITY IN BEEF CATTLE SYNCHRONIZED WITH PROSTAGLANDIN,
GONADOTROPIN AND PROGESTOGENS

Submitted by

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In partial fulfillment of the requirements

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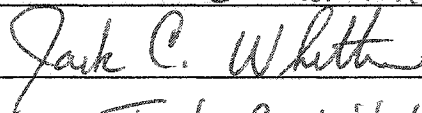
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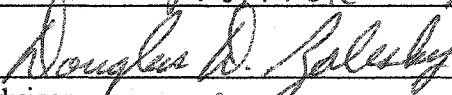
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ABSTRACT OF DISSERTATION

FERTILITY IN BEEF CATTLE SYNCHRONIZED WITH PROSTAGLANDIN, GONADOTROPIN AND PROGESTOGENS

Three objectives were studied to evaluate maximizing fertility by introducing dietary fat supplementation, exogenous hCG injections and synchronization protocols into management programs.

The first objective (Chapter 2) were to determine if supplementing 0.96 kg of whole safflower seeds for an average of 55 d beginning 35 d prior to AI with an hCG injection post AI would increase fertility in beef heifers. Nulliparous crossbred beef heifers from separate breeding seasons in the same year (early breeding heifers [EBH], n=48; late breeding heifers [LBH], n=48) were stratified by BW, BCS and age within breeding season and assigned to treatments in a 2 x 2 factorial arrangement with factors being a control diet (CON) consisting of mixed alfalfa/grass hay and oat grain or a safflower seed diet (SAFF) consisting of the control diet plus safflower seed high in oleic (69.9%) acid with hCG or saline injections given 5 d post AI. Diets were formulated to be isocaloric and isonitrogenous for both groups. Heifers were fed their respective diets beginning 35 d prior to AI and continued 11 to 28 d post AI. All heifers were synchronized with the 7-11 MGA Select Synch protocol. Blood samples were collected from all heifers 5 consecutive days beginning 4 d after AI and analyzed for progesterone concentrations. On day -35, -20 and 0 prior to AI, blood samples were collected and

analyzed for fatty acid composition in all heifers. First-service conception rates were higher ($P < 0.05$) for EBH (79.5%) vs LBH (51.4%) heifers; however, there were no diet x treatment interaction on fertility ($P > 0.10$), therefore data from both breeding seasons were pooled. Conception rates were similar ($P > 0.10$) for heifers in the CON vs SAFF and hCG vs saline groups. Serum progesterone levels were similar ($P > 0.10$) for CON and SAFF heifers; however, progesterone concentrations increased over time ($P < 0.05$) for hCG vs saline injected heifers. Serum stearic acid increased and serum linolenic acid decreased ($P < 0.01$) for both diet groups over time. Serum oleic acid increased ($P < 0.01$) over time for heifers in the SAFF vs CON group. Feeding 0.96 kg of whole safflower seed for an average of 55 d beginning 35 d prior to AI with hCG given 5 d post AI did not improve fertility; however, hCG increased serum progesterone levels.

The second objectives (Chapter 3) were to evaluate optimal timing for timed AI (TAI) using a Co-Synch protocol with or without GnRH at TAI and calf removal. Multiparous Composite and Hereford suckling beef cows ($n=226$) were synchronized for AI from two separate calving seasons in the same year. Early (ECC) and late calving cows (LCC) were randomly assigned to one of four treatment groups for a 2 x 2 factorial arrangement. All cows were injected with 100 μg of GnRH on day 0, followed by 25 mg of $\text{PGF}_{2\alpha}$ (PG) on day 7. All calves were removed at time of PG and returned to nurse at TAI. Half of the cows were inseminated 48 h post PG with (48-TAI-G) or without (48-TAI) GnRH and the other half were inseminated 72 h post PG with (72-TAI-G) or without (72-TAI) GnRH. Pregnancy rates were not affected ($P > 0.10$) by calving

season; therefore data from both ECC and LCC were combined. Body condition and PPI did not influence fertility ($P > 0.10$); however pregnancy rates increased ($P < 0.01$) 20.5% for cows inseminated at 72 vs 48 h, and 15.9% for cows receiving GnRH vs no GnRH ($P < 0.05$) at TAI. Pregnancy rates varied ($P < 0.05$) for cows inseminated to sires A (33.3%) and sires B and C (51.8 and 54.3%). We concluded that fertility levels may have been compromised from one of the three sires used; however, delaying TAI 72 h after PG with GnRH improved timed AI pregnancy rates.

The third objective (Chapter 4) were to determine if a second injection of GnRH at timed AI (TAI) increases the percentage of induced ovulations and improves pregnancy rates in beef heifers synchronized with the CO-Synch plus CIDR protocol. Nulliparous crossbred beef heifers ($n = 375$, BW = 362.7 kg, body condition score, BCS = 5.6) from three locations (Colorado [CO], Wyoming [WY] and South Dakota [SD]) were stratified by BW within BCS and randomly allotted to one of two treatments. All heifers received 100 μg of GnRH with a CIDR insert on day 0, followed by CIDR removal and 25 mg of $\text{PGF}_{2\alpha}$ (PG) on day 7. At 54 hours post PG, heifers in the control (CON) and treatment (TRMT) groups were mass mated and heifers in the TRMT group were given a second injection of GnRH at that time. Blood samples were collected in heifers at d -10 and 0 to determine cyclicity status at CO and WY. Ultrasonography was used to classify follicle diameter at TAI and determine percentage of heifers ovulating 40 h after TAI at both CO and WY locations. Heifers at the SD location were assigned to a time group (1 to 6; 1 = 54 h and 6 = 59 h) based on the hour inseminated beginning 54 h

post PG. Cyclicity rates were higher ($P < 0.01$) for heifers at CO (97.4 %) vs WY (46.4 %). Pregnancy rates were similar ($P > 0.10$) between treatment groups and for cycling and non-cycling heifers at CO and WY; however, pregnancy rates were higher ($P < 0.05$) for heifers in the TRMT (54.2 %) vs CON group (40.4 %) at SD. Body weight did not affect pregnancy rates for either treatment group across all locations ($P > 0.10$); however, pregnancy rates tended to decrease ($P = 0.08$) for heifers with body weights greater than 409.1 kg (39 %) vs heifers with body weights less than 409.1 kg (53.2 %) at SD. The percentage of heifers ovulating were similar ($P > 0.10$) between CO and WY and ovulation rates tended to be higher ($P = 0.10$) for heifers in the TRMT (81.3 and 73.9 %) vs CON (62.5 and 66.7 %) groups at CO and WY. Pregnancy rates were higher ($P < 0.05$) at CO and WY for heifers ovulating by 40 h after TAI (50 and 61.8 %) vs heifers that had not ovulated at that time (16.7 and 16.7 %). Effect of time group, over time, on pregnancy rates were not seen ($P > 0.05$) for SD heifers. We conclude that synchronizing beef heifers with a modified CO-Synch plus CIDR protocol induces ovulation in cycling and non-cycling heifers and produces acceptable pregnancy rates at 54 h TAI. The value of incorporating a second injection of GnRH at timed AI remains questionable.

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At the start of my Ph.D program at Colorado State University, I focused on research that would enable me to gain the knowledge and experience to educate people in the cow/calf industry today. The research I have conducted focuses on improving fertility in beef cattle by utilizing different management strategies for enhancing reproduction. The objectives were developed based on issues of concern in the industry which have allowed me to focus on contributing to improving production efficiency within the seedstock business today.

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CHAPTER 1

HISTORY

Decades ago, the understanding of basic mammalian physiology has enabled scientists and researchers to develop technologies that have improved management practices within some of the primary species used in the food chain today. For instance, artificial insemination (AI) was reported to be the first known use of biotechnology that led to the understanding and management of reproductive tools for genetic selection in farm animals (Foote, 2002), as well as other large species of animals. The idea of collecting semen from a female tract after mating dates back to the Arabs and their quest to improve the genetic diversity of their equine herd. From these undocumented tales, the use of AI was reported in 1784 in dogs (Spallanzani, 1784); 1897 in rabbits and horses (Heape, 1897); 1899 in foxes, poultry and swine (Ivanow, 1907); and 1938 in sheep and goats (Milovanov, 1938). Development and use of AI for dairy cattle in the United States did not begin until the first cooperative dairy AI organization was developed in Denmark

in 1936 by Sørensen. The development of this dairy AI organization in Denmark stimulated some of the first dairy work done with inseminating cows in the US at Cornell University by Brownell in 1936 (Sipher, 1991), and in Minnesota and Wisconsin in the late 1930s. For many years, interest in using artificial insemination in dairy and beef cattle declined because of poor fertility that was seen in cattle synchronized for estrus. It was the use of an exogenous hormone, progesterone, which would in fact regulate the estrous cycle in cattle, but hinder pregnancy rates (Trimberger and Hansel, 1955). The use of biotechnology initiated the developments of newer, more advanced technological applications to reproductive management. These technologies include cryopreservation of sperm for the purpose of introducing new genetics into different herds; harvesting, freezing, culturing and transfer of embryos and cloning to preserve the use of superior genetics in certain populations of species; sexing of sperm to regulate sex of offspring populations and estrous cycle regulation to manipulate and control breeding seasons in sheep and cattle.

The use and growth of AI in beef herds later became popular in the US. Much of the early work on AI primarily focused on dairy herds because of the ease of confinement. Dairy operations then, and still today, are managed in small acreage farms because of operational facilities for dairy products. Beef cattle, because of their extensive range management programs, required additional labor and facilities for incorporation and use of AI. The use of estrous synchronization became an integral part of AI use in beef herds in the US, yet currently less than 6 % of the national beef cow herd is inseminated artificially each year (NAHMS, 1997).

The use of AI alone was the beginning of advancements in reproductive management and production. Combining the use of AI with technologies such as superovulation, synchronization of estrus and manipulation of embryos would lead to the great advancements in reproduction, predicted by Rowson (1971). It soon became a popular practice to combine two or more of these developed technologies to enhance reproductive management and create genetic diversity among beef and dairy herds. With the use of synchronization to regulate estrous cycles, managing small groups of beef cows in close confinements was not as much of an economic problem as in the past. In order to incorporate the use of estrous synchronization and AI as one management practice, the timing of inseminations after a pronounced heat required critical detailed research to fully optimize the potential of inseminated sperm.

REGULATION OF THE ESTROUS CYCLE

Estrous Detection. There are many variables that may have an impact when trying to optimize pregnancy after use of AI in any herd. Two of the major contributors to a successful outcome are proper detection of estrus and skillful insemination. Standing heat or detection of estrus refers to an animal (female) standing to be mounted by another animal (Lordosis). Other behavioral characteristics that may occur prior to sexual receptivity includes increased movement of that animal, phonation (vocal expression), attempts to mount other animals and nervousness (Senger, 1999). Physical signs of receptivity are clear mucus discharge from the vulva, roughed up tail head and mud or dirt on the side of the hips of that animal. This period of receptivity is at the end of her

21-day estrous cycle when initiation of ovulation occurs on a large dominant follicle due to endogenous hormones acting on the ovary.

Timing of Insemination. Timing of insemination after a pronounced standing estrus became a major contributing factor to the variation in pregnancy rates within different herds for both dairy and beef cattle when AI was first used in the US. The life span for sperm and ovum in the reproductive tract of cattle vary between the male and female. Ovulation typically occurs 18 to 32 hours after the first time a female stands to be mounted. Trimberger (1944) reported that average standing estrus lasted for 17.77 h in dairy cows and 15.33 h in dairy heifers, with a range of 2.5 to 28 h, and ovulation occurred on average 10.49 h after cessation of estrus for both heifers and cows, with a range of 3 to 18 h. Life of the ovum in the uterine horns, once ovulation occurs, can last approximately 12 hrs. Once semen is either ejaculated into the female tract from a bull or deposited by an inseminator, the life span of sperm is approximately 24 hours inside the female tract. Inside the female tract, sperm will travel to the area where fertilization occurs (oviduct) in about 6 hours. Once you understand the time period in which one has to synchronize the intervention of both the ovum and sperm, increased fertilization rates can be achieved. The time period from which the first standing heat occurs to when the viability of that ovum can compromise fertilization is 18 – 40 h. Once the ovum reaches the area where fertilization occurs in the uterine horns, cells in the ovum begin apoptosis (cell death). Therefore, sperm must be in the ampulla region of the uterine horns hours prior to arrival of the ovum from the ovulatory site of the ovary.

An evaluation was done to determine pregnancy rates upon timing of insemination relative to time after first standing estrus in dairy cattle. Dairy cows were

artificially inseminated at 10 different times following the beginning of standing estrus with pregnancy rates varying (44, 82.5, 84, 75, 62.5, 32, 28, 12, 8 and 0 %) depending upon timing of AI: at the beginning, middle, middle and rebred 24 h later, or end of estrus, or at 6, 12, 18, 24, 36 or 48 h after end of estrus (Trimberger and Davis, 1943). Insemination of semen beyond 24 hrs from standing heat compromised fertilization due to aging of the ovum in the female tract. Insemination prior to standing estrus compromised fertilization due to aging of the sperm in the female tract. Due to physiology and life span of the male and female germ producing cells, timing of insemination needs to occur between standing estrus and 24 hours post standing estrus. Barrett and Casida (1946) reported pregnancy rates of 52.4 % for cows inseminated within a 25 h period beginning 3 h from first estrus and pregnancy rates of 43.6 % in cattle bred later than 25 h. In regards to timing of insemination based upon standing estrus, work was done to determine pregnancy rate differences based upon timing of insemination relative to the time of ovulation. Trimberger (1944) looked at breeding dairy cows and heifers at four different times prior to ovulation compared to four different times after ovulation and reported 69.4 % of cattle bred before ovulation conceived to AI and 33.3 % of cattle bred after ovulation conceived to AI. Trimberger concluded that bovine ovums may remain fertile only for a relatively short time period after ovulation. Because detecting the timing of ovulation becomes more labor intensive and requires experienced technicians, timing inseminations was not commonly used because of the lack of experienced technicians able to palpate ovaries.

A popular method used for timing insemination after detected standing estrus is the AM/PM rule developed by Trimberger (1948). This system was based on

observation, palpation of ovaries and breeding data that reported the highest fertility by using this rule. The AM/PM rule requires cattle that come into standing heat in the AM should be inseminated during the afternoon of the same day and cattle that come into standing heat during the PM should be inseminated during the morning of the next day. Work done in New Zealand (MacMillan and Watson, 1975) concluded that the average interval, in hours, from insemination to ovulation and fertilization is closely related to the stage of the estrous cycle at insemination and that this interval can be a major contributor to fertility differences between sire groups. This interval has also proven to be a major contributor to fertility differences in previous studies showing that maximum conception rates can be achieved when insemination occurs from mid-estrus (period between first standing heat to when an animal is coming out of heat) to the end of standing estrus (Trimberger and Davis, 1943; Trimberger, 1948). Because duration of estrus varies between breeds of cattle and especially within herds of cattle, applying the AM/PM rule treats each individual animal the same even though length of standing estrus differs. Over the years, un-experienced individuals observing detected estrus within a herd have shown that accuracy to detect standing estrus has been a problem in the US (Foote, 1975). One of the main problems is missed heats due to the inability to observe standing estrus for 24 h constant.

ESTROUS SYNCHRONIZATION

Pregnancy rates in dairy cattle, per insemination, have declined over the last 30 years (Lucy, 2001) and a wide array of research topics has been studied to try and correct this reduction in fertility. Estrous synchronization has become one of many tools used in

research to allow producers to reach those improved fertility goals. Today, there are three approaches to estrous synchronization in cattle that manipulate the estrous cycle and mandate control of ovulation for artificial insemination, as well as for natural service purposes. The three approaches to estrous synchronization currently being used are prostaglandin $F_{2\alpha}$ (PG) and its analogues (control of luteolysis), progestogens (suppress estrus) and gonadotropins (ovulation and/or luteinization of dominant follicle).

Prostaglandins were first used in the late 70's to initiate luteolysis (regression) of the corpus luteum (CL; Lauderdale, 1972; Louis et al., 1972; Rowson et al., 1972) during certain stages of the estrous cycle, thus inducing cattle to exhibit standing estrus.

Progestins were used to suppress the estrous cycle, or inhibit estrus from occurring (Zimelman and Smith, 1966; Roussel and Beatty, 1969; DeBois and Bierschwal, 1970; Randel et al., 1972) in order to synchronize a larger number of heifers exhibiting heat at one time. Progestins were also used in feedlot heifers to refrain them from cycling while on feed. Gonadotropins were incorporated into synchronization protocols in the mid 90's (Pursley et al., 1995; Pursley et al., 1998; Geary et al., 1998; Geary and Whittier, 1998) to manipulate the timing of estrus and to allow for fixed-time insemination programs to be used.

Prostaglandins. Luteolysis of the corpus luteum was the basis for developing estrous synchronization protocols in dairy and beef herds across the world. Because prostaglandins induce luteolysis, practical application of these compounds was to synchronize estrus in cattle. With an average 21 day estrous cycle in cattle, the question was, "Can luteolysis occur during any stage of the estrous cycle"? Lauderdale (1972) and Rowson (1972) reported giving an exogenous source of PG was ineffective in

causing luteolysis in cattle during the early stages of their estrous cycle. During the early stages of an estrous cycle (day 1-6; day 0 = ovulation), the developing CL is producing low amounts of progesterone and is non-responsive to prostaglandins acting on that CL. If prostaglandins have no effect on the developing CL, the effects of PG during the later stages of a cycle (day 17-21) are very minimal because of low progesterone production from a regressing CL. During late stages, the CL is regressing and the animal will naturally fall into standing estrus within 1 to 6 days based upon the follicular wave pattern and length of a cycle in an animal. Research demonstrated that beef heifers synchronized with a single injection of PG can increase 5 d pregnancy rates compared to a control when AI was performed after standing heat (Lauderdale et al., 1980). Depending upon stage of the estrous cycle, PG administration during the luteal phase can have varying effects on the number of heifers exhibiting a standing estrus and the time interval between PG injections to standing estrus (King et al., 1982). King's results concluded that heifers given PG between days 10 to 15 have a greater estrous response rate with delayed estrus versus heifers given PG between days 5 to 9. King did notice that the interval to estrus was shorter for heifers treated with PG than for cows. Williams et al. (1999) reported similar results indicating that estrous response was higher in beef heifers given PG on day 14 (91.7 %) of the estrous cycle vs day 6 (54.2 %) and 10 (63.6 %). Williams concluded that sensitivity of the corpus luteum may be dependent upon stage of the estrous cycle when PG is administered and size of a dominant follicle, but circulating estradiol levels or level of gonadotropic administration does not affect this response. This response to PG was reported to be similar for dairy cattle (Macmillan, 1983; Macmillan and Henderson, 1984), as well as dairy heifers (Johnson, 1978; Refsal

and Seguin, 1980; Stevenson et al., 1984). Conception rates were reported to also improve in cattle injected with PG during the late luteal phase of the estrous cycle versus the early phase of their cycle (Watts and Fuquay, 1985). A separate study reported no differences in conception rates on stage of the estrous cycle when PG was given (Stevenson et al., 1984). Administration of PG will not have an effect during the early and late stages of the estrous cycle, although a big area of concern with the single-shot PG system is timing the injection to target the luteal phase of an estrous cycle. Another method of using PG is to detect estrus for 4 days and breed off of heat. At day 5, inject PG to those that have not been detected in estrus and continue observation for standing heats and breed for another 5 days. Moody (1979) and Lauderdale (1980) reported improved pregnancy rates on cows and heifers using this system compared to controls using the single PG injection system. Labor costs and time are valuable considerations when developing a protocol to synchronize cattle. Work done by Lauderdale (1979) used a two injection PG protocol, where prostaglandin was given 10 to 12 days apart (first injection given at a random stage of the estrous cycle) to reduce the amount of labor and time needed for estrous detection. A comparison of a 5 day estrous response and pregnancy rates were greater for cattle treated with a two injection PG protocol versus cattle treated as controls. Based on stages of events that occur during the estrous cycle, cattle should be at a stage of their cycle that would allow them to respond to the second injection of PG in order to induce luteolysis. Cattle in the early (1-6) and late (16-21) stages of their estrous cycle will not respond to the first injection of PG. Those cattle in the early stages of their cycle will complete that cycle, while the second injection of PG, 10-12 days later, should target between d 12-18 and will respond or naturally come into

heat within 7 days of the second PG injection. Cattle in the late stages of their estrous cycle will exhibit a natural heat, close to first PG, due to a decrease in progesterone from the regressing CL and will be at day 7-12 of their cycle, which will cause a response to the second injection of PG. Cattle in the luteal phase of their estrous cycle at the time of first PG injection will go through luteolysis and exhibit estrus within 7 days. Those cattle will be at day 7-10 of their estrous cycle and should respond to the second injection of PG. Approximately 70 % of cycling cattle should exhibit standing estrus after the first injection of PG if all cattle are distributed equally across a 21 day estrous cycle (Odde, 1990). Over the years, research has shown that estrous response and pregnancy rates are not always equal for every herd. There can be some variation in the length of an estrous cycle (17-24 days) and whether an animal is cycling or not within or between herds.

Cyclicity status in heifers (puberty) and cows (postpartum recovery) has become an important factor in synchronizing the majority of animals to exhibit estrus. In non-cycling females (peripubertal or postpartum anestrus), there are no dominant follicles (> 10 mm in diameter) or functional CLs present on the ovary, with the exception of developing small antral follicles. The lack of a functional CL prevents cattle from initiating and sustaining a normal 21-day estrous cycle. In order to induce early cyclicity in non-cycling cattle, a dominant follicle must develop to produce estradiol that will allow for the eventual release of an LH surge and induce first pubertal ovulation (Gonzalez-Padilla et al., 1975) or first postpartum ovulation (Perry et al., 1991). Past estrous synchronization systems that have incorporated either a single or double injection of PG were not developed for synchronizing estrus and inducing ovulation in peripubertal heifers and postpartum non-cycling suckled cattle (Stevenson et al., 1997). Stevenson

reported that the percentage of non-cycling cows conceiving to AI was dramatically reduced for cows receiving a double injection of PG (26.5 %; 14 days apart) versus a PG + GnRH + norgestomet implant system (62.2 %). Similarly, Fike et al. (1999) revealed a dramatic decrease in pregnancy rates from non-cycling cows when treated with a two injection PG system (16 %; 10 days apart) versus an 18 day MGA plus PG system (64.7 %). Successful synchronization systems must employ protocols that induce ovulation in non-cycling heifers and suckling cows. Research has indicated that estrous synchronization rates can be improved by increasing LH secretion during and after a progestin-based program (Higgins et al., 1986), thus inducing estrus in peripubertal and postpartum anestrus cows (Anderson et al., 1996).

Currently there are four prostaglandin products available that are approved by the FDA for synchronization of estrus in cattle: Prostaglandin $F_{2\alpha}$ or Lutalyse (Pharmacia & Upjohn Company, Kalamazoo, MI), cloprostenol or Estrumate (Bayer Corporation, Shawnee, KS), dinoprost tromethamine or Prostamate (Pro Labs) and dinoprost or In-Synch (Pro Labs, St. Joseph, MO). The label approved dosages for these four products are: $PGF_{2\alpha}$, 25 mg; cloprostenol, 500 μ g; dinoprost tromethamine, 25 mg; and dinoprost, 25 mg. These products have been effective in initiating luteolysis of the corpus luteum, but there is little information on direct comparisons of the four prostaglandin products on luteolysis.

Progestogens. The use of progesterone came about in the late 1940s and 50s to delay or suppress estrus in cattle. While results from giving a progesterone source were unclear, the principle behind the role that exogenous progestins played on the reproductive system was encouraging. It was suggested that progesterone can control the

estrous cycle by inhibiting the gonadotropin-hypophyseal axis (mainly LH release) from acting upon the ovary. Research began looking at giving progesterone to dairy heifers as a daily injection to synchronize estrus in cattle. Results indicated that the interval from the end of progesterone treatment to beginning of standing estrus decreased as the dosage level decreased (Christian and Casida, 1948; Ulberg et al., 1951). Use of an injectable progesterone source to synchronize cattle, by controlling heats, in the late 1950's was a promising challenge that would soon diminish. Soon the use of a feed additive as a progesterone source acquired popularity with researchers. Melengestrol Acetate (MGA; Pharmacia & Upjohn Company, Kalamazoo, MI) is a progesterone analogue that suppresses estrus and ovulation in nearly all animals when administered orally at 0.5 mg/hd/d (Zimbelman and Smith, 1966; Roussel and Betty, 1969; DeBois and Bierschwal, 1970; Randel et al., 1972). Along with the use of MGA, studies were done using a different source of progesterone to see if the effects were the same as MGA or could be improved. Previous work administered either 6-methyl-17 acetoxy-progesterone (MAP; Hansel et al., 1961), 6-chloro-6-dehydro-17-acetoxy progesterone (CAP; Hansel et al., 1966) or dihydroxyprogesterone acetophenide (DHPA; Wiltbank et al., 1967) and reported reduced fertility after long-term feeding of these progesterone agents, yet supplementing MAP produced higher first service pregnancy rates in two different experiments (65.4 and 48.7 %) in beef cows vs supplementing CAP (35.6 and 34.8 %; Hansel et al., 1966). The use of supplementing MGA alone has been shown to be 300 to 900 times more potent than MAP (Zimbelman and Smith, 1966).

The use of MGA soon became popular for synchronization of estrus in cattle. Zimbelman and Smith (1966) reported supplementing a progestin for 15 to 18 days at

levels of 0.25 to 8.0 mg/hd/d inhibited estrus and ovulation in all but one heifer from the group that received 0.5 mg of MGA. Zimbelman's group also indicated that the average length, in days, of estrous synchrony occurring for heifers receiving a low dose (0.2 mg) of melengestrol acetate was 2.7 and for heifers receiving a high dose (2.0 mg) of melengestrol acetate was 6.3, thus concluding that the level of MGA fed was correlated to the interval from MGA withdrawal to standing estrus. The amount of progesterone supplemented does not seem to affect ovulation rates in cattle, yet the number of animals exhibiting estrus, in a particular time period, may vary depending upon level of progesterone fed. Hansel et al. (1961) speculated that estrous rate may have been compromised due to the presence of too much or too little progesterone left in circulation after supplemental treatment and those high levels of fed progesterone may still suppress these manifestations of exhibiting estrus after supplementation has ended. The use of supplementing MGA as a feed source was effective in inhibiting standing estrus, but the mechanism for delaying estrus was unknown at the time. Imwalle et al. (2002) concluded that MGA blocks estrus and ovulation by inhibiting the preovulatory LH surge.

Several studies have reported that the percentage of females receiving MGA and exhibiting estrus within a 6-day period was equivalent to the percentage of females receiving a control and exhibiting estrus within a 20-day period. Many of these studies did notice a significant decrease (14 %) in first-service conception rates within 10 days after MGA withdrawal for females receiving MGA, versus females that did not. Guthrie et al. (1970) and Lamond et al. (1971) reported that this decrease in fertility for females receiving MGA may be due to altered follicular growth patterns and increasing atretic follicle numbers. Long feeding intervals of 14 – 18 d with MGA resulted in reduced

fertility from AI after first synchronized estrus (Zimbelman and Smith, 1966; De Bois and Bierschwal, 1970; Lamond et al., 1971). Incorporating PG at MGA removal successfully synchronized estrus in a majority of cows and induced some non-cycling cows to cycle, however fertility was compromised due to supplemental progestin (Beal et al., 1988).

The reduced fertility, commonly referred to as infertile heats, following a progesterone treatment was found to be associated with increased concentrations of estrogen (Taft et al., 1999) from the present dominant follicle during and after a progestin based treatment (Patterson et al., 1989), thus leading to persistence of a dominant follicle that can lead to compromised oocyte quality and ultimately reducing fertility (Taft et al., 1999). To avoid this reduced fertility, an experiment was conducted to induce regression of the persistent dominant follicle present during long feeding intervals of MGA and increase first-service conception rates in heifers and cows following MGA removal. Anderson and Day (1994) fed MGA for 14 days and either administered 200 mg of progesterone or no hormone on day 12 of MGA feeding and found that synchronized conception rates were increased for both heifers and cows with non-luteal structures that were injected with progesterone (50 % and 66.7 %) vs heifers and cows given no progesterone injections (16.7 % and 25 %). Anderson's group concluded that administering acute progesterone during the later part of MGA supplementation will induce regression of a persistent follicle, stimulate growth of a new follicular wave and increase fertility. McDowell et al. (1998) also concluded that administering progesterone towards the end of MGA feeding reduced the incidence of persistent dominant follicles and effectively synchronized estrus with normal fertility. Fike et al. (1999) looked at

comparing MGA supplementation for 18 d with both progesterone and estradiol or no hormones given exogenously 7 d prior to MGA removal to induce regression of any persistent dominant follicle present and improve first service conception rates. Fike indicated that conception rates to AI from the MGA plus hormone group were higher overall and for anestrual females (62.7 % and 45.8 %) vs females just receiving MGA alone (50 % and 33.9 %).

New research was conducted to avoid using infertile heats for insemination after MGA removal by incorporating prostaglandin injections to resynchronize the estrous cycle. The combination of MGA and exogenous PG for synchronizing estrus was heavily studied to see if reduced variability in the time interval from PG injection to standing estrus could be accomplished (Stevenson et al., 1984; Watts and Fuquay, 1985). In order to achieve optimal results from the use of both supplemental MGA and PG, it was suggested that prostaglandin injection should be administered 17-19 days after the last day of MGA feeding. Work done by Brown et al. (1988) incorporated PG 16-17 d after MGA removal and compared estrous response and conception rates to a 9-d norgestomet implant plus both norgestomet and estradiol injections at implant insertion in yearling beef heifers. Brown reported similar estrous response for MGA+PG vs norgestomet (83.4 % vs 90.2 %); yet higher synchronized conception rates for MGA+PG were reported vs norgestomet (68.6 % vs 40.6 %). Brown speculated that the increased fertility for MGA+PG heifers was due to timing of PG by targeting the late luteal phase of the estrous cycle. Patterson and Corah (1992) fed MGA for 14 d with PG administered 17 d after MGA removal and looked at the effects of synchronizing or inducing estrus in yearling beef heifers. Patterson's results indicated that 79 % of the

heifers exhibited estrus in a 6 day breeding season vs 32 % for heifers that received a grain carrier with no MGA or PG, yet first service conception rates were not different. The MGA plus PG system would place most animals in the late luteal phase of the estrous cycle at the time of PG injection.

These results show a promising future for improving synchronized responses to a fed progestin with PG administered during the late luteal phase after MGA removal. Mauck et al. (1988) looked at trying to improve conception rates by reducing the amount of days MGA was fed with PG given at MGA removal, but conception rates were not improved when compared to studies using a 14 d MGA feeding period with PG given 16-18 d later (Brown et al., 1988; Patterson and Corah, 1992; Patterson et al., 1995). By administering PG during the late luteal phase of the estrous cycle, 17 d after the 14 day MGA feeding period, estrous synchrony and conception rates in suckled beef cows improved by 16 and 21 % vs cows that received no MGA (Patterson et al., 1995). Patterson reported estrous responses within 6 d after PG injection to be higher (76.5 %) for cows receiving MGA vs cows not receiving MGA (60 %). The reported increases in estrous synchrony and first-service conception rates using an MGA+PG system is due in part to the number of animals developing a functional CL prior to PG injection. This response illustrates the influence progestins have on fertility in cattle.

Any percentage of prepubertal heifers and acyclic cows at the beginning of a breeding season can become a major limitation to the success of a synchronization protocol utilizing PGF_{2α} alone. Work was done to determine the effects of supplementing subnormal levels of progesterone using a PRID (progesterone releasing intravaginal device) device on pulsatile release of LH from the time period of PRID

removal to the LH surge and progesterone secretions during the estrous cycle. Roberson et al. (1989) indicated that supplementing half a PRID for 12 d resulted in lower mean concentrations of progesterone during the subsequent estrous cycle and higher pulse frequency of LH versus cows that received 2 PRIDs during a 12 d period. The onset of preovulatory LH surges also occurred 17.1 h earlier in cows receiving a half a PRID vs cows receiving 2 PRIDs, thus concluding that lack of normal luteal function may be due to altered ovarian processes in response to changes in the secretion of gonadotropins (Roberson et al., 1989). It was discovered that administering 2 PRIDs can release the same amount of progesterone that is produced endogenously from a cow during the midluteal phase of her estrous cycle (Kojima et al., 1992). Kojima's work was in concurrence with Roberson et al. (1989) where supplementing subnormal levels of progesterone (SMB) produced greater frequencies of LH pulses, stimulating early preovulatory LH surges and lower concentrations of progesterone during the subsequent estrous cycle than cows treated with 2 PRIDs or cows treated with no hormones, resulting in normal estrous cycles.

Custer et al. (1994) reported higher incidences of persistent non-ovulatory follicles from follicles that were dominant at the beginning of a 7 d MGA treatment vs cows that were receiving a 7 d PRID. MGA supplementation secretes a lower dose of progesterone when fed versus the insertion of PRIDs or a Controlled Intravaginal Drug Release-Bovine (CIDR-B) device, thus resulting in increased incidence of persistent dominant follicles when using a lower dose progesterone source (Savio et al., 1993; Custer et al., 1994). The addition of a CIDR-B device has been marketed in over thirty countries including the United States. The CIDR-B consists of a "T" shaped nylon

backbone that is coated with a silicone layer containing 10 % progesterone by weight. The CIDRs that are marketed in other countries contain 1.9 g of progesterone, whereas CIDRs marketed in the US contain 1.38 g of progesterone. These CIDRs are inserted into the vagina with a lubricated applicator gun following disinfection of the vulva and surrounding area. Removing the CIDRs involves pulling a flexible nylon tail that can easily be done from a chute or loading alley. Work done by Savio et al. (1993) reported giving an exogenous source of subnormal levels of progesterone (CIDR-B; 1.2 g) for 9 d stimulated growth of persistent dominant follicles vs cows receiving a high dose of progesterone (CIDR-B; 1.9 g). Savio concluded that a dominant follicle from the first wave in cows receiving a low dose CIDR failed to ovulate at CIDR insertion, thus resulting in a persistent dominant follicle that was larger in diameter at ovulation and resulted in poor pregnancy rates to AI (37.1 %) vs cows receiving a high dose CIDR that ovulated a new synchronized dominant follicle at ovulation (64.8 %).

Once eliminating persistent dominant follicles was accomplished using new CIDR inserts, studies were done to determine the efficacy of an intravaginal insert and PG on estrous response and first-service conception rates. Postpartum beef cows were synchronized with a 7 d CIDR insert and PG given on day 6 with results indicating an improved estrus response with higher first service pregnancy rates to AI (59 % and 36 %) vs cows treated with PG alone (33 % and 22 %) or just a control (15 % and 7 %; Lucy et al., 2001). Lucy also implicated that the 3 d estrous response was higher for beef and dairy heifers that received a CIDR+PG (64.7 % and 84 %) vs heifers that received PG alone (26.6 % and 57 %) and conception rates were also higher in beef heifers that received a CIDR+PG (60.1 %) vs PG alone (52.2 %), yet no differences were reported for

treatment effects on conception rates in dairy heifers. Lucy concluded that using a synchronization protocol consisting of a CIDR insert with PG injection 1 day prior to CIDR removal will result in improved synchronization rates in beef cows and heifers, but only improved estrous synchrony in dairy heifers.

Estrous cycles in beef and dairy cattle are controlled by endocrine changes and physiological events that occur within the animal's body and environment. These changes and events may determine that animal's fate for conceiving to AI or natural service. Progesterone levels remain low following parturition, keeping animals from exhibiting normal estrous cycles so that recovery of the female's reproductive tract may occur (Rawlings et al., 1980; Wetteman, 1980). This transient increase in progesterone initiates the start of a short-lived corpus luteum that is sometimes necessary for initiation of the first estrous cycle (Odde et al., 1980). Only 70 % of first calf heifers show a transient increase in progesterone levels prior to first estrus and the remaining 30 % exhibited estrus without an increase in progesterone prior to first heat (Werth et al., 1996). Werth also reported pregnancy rates to be higher for heifers showing a transient increase in progesterone prior to estrus and AI (76 %) vs heifers that did not (41 %), concluding that the preceding transient increases in progesterone just prior to first postpartum estrus may be critical for enhancing first service conception rates in beef cattle.

Gonadotropins. Gonadotropins' role on follicle manipulation depends upon the stage of follicular development during the estrous cycle. Due to the physiological role gonadotropins have, new protocols were researched and developed for synchronization of estrus in cattle. The traditional progestin and prostaglandin systems were successful in

synchronizing estrus, yet length of these systems were long and the consistency of fertility was not meeting producer demands. Incorporating gonadotropins may greatly reduce the length of time it takes to synchronize estrus and inseminate groups of cattle in producer operations. By incorporating drugs to induce ovulation with products that suppress estrus and induce luteolysis, precise controlled synchrony of estrus should be accomplished.

With an initial treatment of GnRH to synchronize selection of new follicles, growth and dominance of these follicles will lead into subsequent ovulation after PG induced luteolysis. This system can reduce the time needed for estrous detection by synchronizing estrus in 70 to 80 % of cyclic animals within a 4 day interval and not affect fertility (Twagiramungu et al., 1995). The short interval to standing estrus is primarily due to control of the LH surge that is responsible for ovulation. Sirois and Fortune (1990) confirmed that synchrony of the LH surge depends upon stage and growth of a population of large follicles in response to PG induced luteolysis. It has been speculated that high levels of progesterone, due to high exogenous progesterone sources, may have an indirect relationship in decreasing LH pulse frequency via negative feedback affects on LH release (Sirois and Fortune, 1990). If LH release is reduced due to these high levels of exogenous progesterone, androgen synthesis and 17β -estradiol concentrations in the ovary may be reduced, preventing actual presence of visual standing heats and ovulation. Some reports have shown that artificial maintenance of subnormal progesterone levels reduces the interval from removal of progesterone to the LH surge vs supplying above normal levels of progesterone (Roberson et al., 1989; Sirois and Fortune, 1990), thus reducing the interval from luteolysis to standing estrus. It is

believed that supplying subnormal levels of progesterone would mimic the end of the luteal phase of a normal estrous cycle, thus leading to an increase in LH pulse frequency, as seen in previous studies. If subnormal levels of progesterone at time of luteolysis is critical for quick release of LH, then it was hypothesized that the ovarian hypothalamo-pituitary axis is in a complete "ready state" to initiate an increase in LH release for the ovulatory LH surge just after withdrawal of progesterone or the beginning of luteolysis (Sirois and Fortune, 1990). If sufficient support for LH release is absent, possibly due to normal or above normal levels of progesterone in a normal estrous cycle, initiation of new follicular waves due to regression or atresia of nonovulatory dominant follicles would prevent development to the ovulatory stage in an estrous cycle. When a gonadotropin is administered during the luteal phase of an estrous cycle, progesterone concentrations are high, thus preventing an LH surge, initiating dominant follicle regression and onset of new follicular waves. When gonadotropins are administered during the follicular phase of an estrous cycle and progesterone levels are low, depending upon presence or absence of a large dominant follicle, ovulation can occur 24 to 32 h after GnRH administration (Pursley et al., 1995).

In normal estrous cycles, the dominant follicle of the first follicular wave normally undergoes atresia and initiation of a new follicular wave; however Lavoit and Fortune (1990) reported that a large dominant follicle from the first follicular wave can have the potential to ovulate during the growth stage if luteolysis is induced. By knowing that modifications can be accomplished in the endocrine environment, precise timing of exogenous hormones administration to mimic certain stages of the estrous cycle may lead to precise control of ovulation for artificial insemination. This modification to the

endocrine environment specifically involves administering gonadotropins to mimic GnRH release from the hypothalamus (Conn and Crowley, 1990). Administration of GnRH or GnRH agonist/analogues acts to increase peripheral circulation of LH and FSH within 2 to 4 h (Chenault et al., 1990; Rettmer et al., 1992) of administration. The action of exogenous gonadotropins on the anterior pituitary is due to the lack of specific receptors for GnRH on the bovine ovary (Brown and Reeves, 1983; Ireland et al., 1990). Small follicles (1 to 5 mm) do not acquire LH receptors until the growing phase, so GnRH induced LH release will not compromise follicular development through growth of an incompetent oocyte (Spicer and Echterkamp, 1986). Though GnRH acts to stimulate luteinization or induce ovulation, work done by Prescott et al. (1992) indicated that the fate of a large dominant follicle committed to atresia will not reverse its fate by being rescued due to administration of exogenous gonadotropins. The response and quick release of FSH causes stimulation of new follicular waves of medium size (6 to 10 mm) follicles that emerges within 2 – 3 days after GnRH treatment (Twagiramungu et al., 1994a). This stimulation of growing follicles was also reported to be affected by a natural delayed increase in FSH that occurred 1 to 2 days before regression or ovulation of a present dominant follicle (Ko et al., 1991; Adams et al., 1992), due to an FSH inhibitor, inhibin (Beard et al., 1990). Twagiramungu et al. (1994a) reported that the stimulation of growing follicles occurs regardless of the phase of an estrous cycle. Although there is an increase in developing medium class follicles, rate of atresia for developing follicles increases as well and is likely due to lack of gonadotropin support (Jablonka-Shariff et al., 1994) from an estradiol negative feedback effect that is usually responsible for continuing the growth and development of follicles. The rate of atresia

for medium class follicles is typically seen 6 days after administration of GnRH and could be a stimulatory response to the selection of a dominant follicle for ovulation if induced luteolysis occurs. Dramatic improvements were seen by decreasing the variability in the estrous interval without compromising fertility using a GnRH agonist 6 day prior to a PGF_{2α} agonist injection (Twagiramungu, 1994). Decreasing the variability from luteolysis to estrus relies on the fate of a newly selected dominant follicle after administration of GnRH. The fate of a dominant follicle depends on complete or incomplete luteolysis. If luteolysis is complete, estrus and subsequent ovulation occur, along with a decrease in estradiol concentrations. If luteolysis is incomplete, the present dominant follicle becomes persistent (non-ovulatory), estrus will not occur and estradiol concentrations are either maintained or increased (Twagiramungu et al., 1994a).

Persistent dominant follicles have become an issue in early developments of synchronization protocols that have resulted in fertility problems (Sanchez et al., 1993; Savio et al., 1993; Wehrman et al., 1993; Schmitt et al., 1994b). These persistent follicles occur when below luteal concentrations of circulating progesterone occur for a prolonged period of time, resulting in decreased frequency of LH pulses and inhibiting the induction of an ovulatory LH surge (Roberson et al., 1989; Savio et al., 1993; Stock and Fortune, 1993; Sanchez et al., 1995).

By regressing a dominant follicle present on the ovary and stimulating development and growth of a new dominant follicle with administration of exogenous gonadotropins, the occurrence of a spontaneous heat is reduced up to 6 - 7 days (Thatcher et al., 1989). The reduction of spontaneous heats post gonadotropin administration is due to level of estradiol during time of gonadotropin administration. During the growing and

active phase of a developing dominant follicle in early luteal and late follicular phases of the estrous cycle, serum concentrations of estradiol increase (Guilbault et al., 1993; Fortune et al., 1988). As dominant follicles undergo regression or ovulation, subsequent estradiol levels decrease. Hughes et al. (1987) reported peripheral circulations of estradiol decrease dramatically after demise of a dominant follicle, which coincides with work done by Twagiramungu et al. (1994b) reporting decreases in estradiol concentrations, were associated with ovulation of a large dominant follicle induced by GnRH. During early development of a follicular wave or just after ovulation of a present dominant follicle, peripheral concentrations of estradiol are low and spontaneous heats (recurring estrus) are inhibited.

Controversy exists over the influence of GnRH on peripheral progesterone concentrations in circulation and its effects on fertility. Progesterone concentrations were reported to increase (Webb et al., 1977; Garverick et al., 1980; Macmillan et al., 1985; Stevenson et al., 1993; Thompson et al., 1999), decrease (Ford and Stormshak, 1978; Lucy and Stevenson, 1986; Rodger and Stormshak, 1986; Ryan et al., 1994) or remain the same (Prescott et al., 1992; Rettmer et al., 1992) after treatment with GnRH or a GnRH agonist in both dairy and beef cattle. Progesterone concentrations were reported to increase for 30 days when GnRH was given at insemination (Mee et al., 1993); however effects on fertility were not seen. Due to this variation in peripheral concentrations of plasma progesterone, it is safe to say that the variability GnRH has on progesterone levels may be due to stage of the estrous cycle at time of administration.

Later work revealed changes in the number of luteal cells and volume of luteal tissue after administration of GnRH, GnRH analogues or GnRH-like products.

Niswender et al. (1985) discovered increases in the number of large luteal cells (LLC) and decreases in the number of small luteal cells (SLC) in ewes after administration with either LH or hCG and Twagiramungu et al. (1995a) reported an increase in corpus luteum volume and the number of LLCs that were present in cattle at time of busserelin administration. The number of LLCs in 10 d old CLs increased when cystorelin was administered at standing estrus (Mee et al., 1993). Because LH concentrations rapidly increase in peripheral circulation after administration of gonadotropins, it may be that increases in LH stimulate LLC populations.

In two separate studies, use of estradiol valerate (EV) with a subsequent injection and implant of norgestomet induced puberty in prepubertal heifers (79 and 89 %) compared to prepubertal heifers not synchronized (6 and 25 %; Gonzalez-Padilla et al., 1975). With the incorporation of gonadotropins into synchronization systems, inducement of cyclicity in some anestrous cattle is possible. Postpartum anestrous cattle maintain continuation of follicular wave-like patterns of small and medium size follicles (Savio et al., 1990) on their ovaries. Larger follicles can be induced to ovulate through stimulation of increased frequencies of LH pulses due to exogenous administration of gonadotropins in dairy and beef cattle (Kesler et al., 1978; Irvin et al., 1981). After ovulation of a dominant follicle, estradiol concentrations decrease, formation of a new CL occurs and progesterone concentrations increase in peripheral circulation (Twagiramungu et al., 1995a). Early studies reported induced ovulations in both cycling and non-cycling suckled beef cows after administration of GnRH (Troxel and Kesler, 1984; Troxel et al., 1993). Normal fertility, following a detected standing estrus, was observed in acyclic cows when EV was given with a norgestomet implant (Gonzalez-

Padilla et al., 1975) and PG was administered 6 days after an injection of GnRH (Twagiramungu et al., 1992b). Normal fertility was also reported following ultrasonic observations of ovulating or regressing dominant follicles in 75 % of anestrus cows following first injection of GnRH (Thompson et al., 1999). With 75 % of those anestrus cows ovulating or regressing a dominant follicle, 62.5 % had a CL at PG injection 7 days after GnRH administration. Anestrus cows may not ovulate a present dominant follicle after GnRH administration due to follicle size or maturity at time of GnRH. Presence or absence of LH receptors on granulosa cells at time of GnRH administration determines its ovulatory success (Ginther et al., 1996). Prado et al. (2002) reported that the length of time an animal is anovulatory has no effect on follicular growth when treated with GnRH. Other studies have also induced cyclicity in non-cycling cows by treatment of anestrus cattle with GnRH or GnRH agonist (Gonzalez-Padilla et al., 1975; Twagiramungu et al., 1995; Geary et al., 1998; Thompson et al., 1999). As we can see, administration of GnRH or GnRH agonist can induce a normal estrus in anestrus cows, yet not all non-cycling cows will be affected by these gonadotrophs.

Lemaster et al. (2001) synchronized cycling and non-cycling beef cows of *Bos Indicus* breeding with GnRH 7 days prior to PG injection and reported a 20.8 % pregnancy rate. The authors speculated that the high rate of anestrus cows in this study (64 %) at first GnRH injection could have caused the low estrous response resulting in poor pregnancy rates. Even though GnRH can induce estrous cycles, only a small percentage of these animals will be affected if a larger number of cattle are non-cyclic. Cattle of *Bos Taurus* breeds were also shown to have poor estrous response and pregnancy rates when the percentage of cattle cycling at the start of breeding is less than

50 % (Stevenson et al., 2000), yet non-cycling cattle synchronized with GnRH+PG versus a 2-shot PG system still had higher detected estrous rates (41.3 % vs 29.4 %). Stevenson et al. (1997) reported pregnancy rates of 62.2 % in non-cycling females that utilized a norgestomet implant, 2 injections of PG and GnRH vs a two injection PG protocol alone (26.5 %). When a 7 day norgestomet implant was incorporated into a GnRH+PG protocol, estrous response in non-cycling cows increased (59.1 %) vs GnRH+PG (41.3%) or PG (29.4%) protocol alone (Stevenson et al., 2000), thus resulting in a greater proportion of cows, both cycling and non-cycling, detected in estrus. Stevenson et al. (2000) also reported increased conception rates in non-cycling cows that received GnRH vs cows only receiving two injections of PG.

Typically, anestrus cattle will undergo a short estrous cycle after a natural or first induced ovulation from GnRH administration, but addition of a norgestomet implant has shown to reduce the incidence of short cycles (Stevenson et al., 2000). This occurrence of a short cycle is basically the luteal phase of an estrous cycle being shortened by premature secretion of $\text{PGF}_{2\alpha}$ from the uterus (Inskeep, 1995).

The use of GnRH or GnRH agonist with PG administered 6 to 7 days later has been developed into a widely used synchronization protocol. Both Twagiramungu et al. (1992a) and Geary et al. (1998) reported that a 5 day breeding season synchronized with GnRH+PG, with an 83.3 and 87.5 % estrous rate for both studies, would be acceptable in most producer breeding programs when taking into account factors that may prevent estrous response such as anestrus, suckling, nutrition and environment. Both studies also reported a 76 % estrous response rate within 72 h of PG treatment. These findings coincide with Geary et al. (2000) who reported that the average interval to standing estrus

after GnRH+PG is typically 70 h. The GnRH+PG protocol (Select Synch; DeJarnette, 1999; Geary et al., 2000) was developed from an existing protocol (Ovsynch; developed for dairy cattle; Pursley et al.; 1995) to synchronize estrus in beef cattle. When comparing pregnancy rates for the Select Synch protocol versus a 2 injection PG system in non-cycling cows, rates were higher for GnRH+PG (27 %) versus the 2 injection PG system (12 %; Thompson et al., 1999). Estrous response has been known to vary (38 and 94 %) in anestrous cows synchronized with the Select Synch protocol (Stevenson et al., 2000; Geary et al., 2000). By comparing these two systems in cycling postpartum cows, pregnancy rate differences (Forbes et al., 1997) and conception rate differences (Stevenson et al., 2000) were not seen.

Based on numerous studies using the Select Synch protocol, research has shown that the variation in response to the Select Synch protocol over the years raises a concern with producer breeding programs and their accepted goals. Ovarian and estrous response to GnRH and PG is dependent upon the stage of the estrous cycle when this system is initiated (Geary et al., 2000). The biggest variation seen is the interval from PG injection to detected standing estrus (Geary et al., 2001), along with the number of cows anestrous at initiation of treatment, nutritional status, weather or environment conditions and follicular wave patterns. This presents a problem when research today is working on incorporating fixed-time insemination systems into breeding programs.

When combining both gonadotropins and progestins with prostaglandins, estrous response was high between 48 and 72 h after PG injection for pubertal heifers that received MGA+Select Synch (76 %) and heifers receiving MGA+PG (63 %; Wood et al., 2001). Wood reported that 100 % of GnRH treated heifers either ovulated or had a

luteinized dominant follicle following GnRH administration and in response, initiated a new follicular wave. If the estrous interval for most postpartum suckling beef cows is between 48 and 72 h, then timing insemination later than 48 h might improve fertility.

Reducing the feeding period of MGA was further evaluated in an effort to minimize the amount of time it took for the duration of an MGA+PG synchronization system (33 days long). MGA was fed for 7 days with PG given at MGA removal and a second PG given 11 days after MGA removal (Kojima et al., 2000). Kojima reported 100 % of heifers exhibited a standing estrus by 54 h and 50 % of cows exhibited estrus by 64 h. The addition of GnRH given 4 d after MGA removal and 7 d prior to the second injection of PG (7-11 Synch; Kojima et al., 2000) was studied to try and achieve ovulation or luteinization of all dominant follicles in order to improve estrous response and synchronization rates. Synchrony of estrus occurred from 42 to 66 h and conception rates were higher for cows synchronized with the 7-11 Synch (91 % and 66 %) vs cows synchronized with the Select Synch protocol alone (69 % and 40 %; Kojima et al., 2000). With a tighter estrous response and improved pregnancy rates from a 7-11 Synch, fixed-time insemination may be considered as an alternative method for synchronizing cattle.

Another alternative for supplementing progesterone was inserting a PRID for 12 d with an injection of progesterone and/or estradiol valerate at PRID insertion. Sprott et al. (1984) reported endogenous progesterone concentrations, one day after PRID removal, to be higher for cows receiving a PRID insert alone or PRID + progesterone vs PRID + estradiol valerate or PRID + progesterone + estradiol valerate. Sprott also concluded that estrous response was higher for cows receiving a PRID + progesterone and/or EV (88 % and 77 %) vs PRID alone (38 %) or PRID + progesterone (36 %), within 96 h after PRID

removal. Sprott also noted that 31 % fewer cows, treated with a PRID on days 5 through 8 of their estrous cycle, exhibited standing estrus within 96 h after PRID removal. It was suggested that estrous synchronization might be effective if EV is used in conjunction with a 12 d PRID insertion and estrous response may be affected based on day of estrous cycle PRIDs are inserted (Sprott et al., 1984).

Ryan et al. (1995) compared synchronization treatments in dairy cattle with either estradiol benzoate (EB) or buserelin on the day of CIDR insertion vs no hormones given, PG, PG+EB, or a control on estrous response rate and first-service pregnancy rates. Estrous response was higher and the interval from CIDR removal to AI was shorter for cows synchronized with CIDR + buserelin for 8 d (88.5 % and 1.6 d) vs CIDR (85 % and 1.7 d), CIDR+EB (75.9 % and 2.3 d), PG+EB (35.1 % and 5.2 d), PG (32 % and 5.5 d) and control (26.5 % and 10.4 d). It was concluded that a high estrous response with a shorter interval to estrus and high pregnancy rates could be achieved if an 8-d progestin is combined with a gonadotropin-releasing hormone at the onset of progestin treatment (Ryan et al., 1995).

Schmitt et al. (1996) reported that administering a GnRH agonist two days after a 9 day norgestomet implant insertion induced ovulation in 100 % of the dominant follicles present at the beginning of implant insertion in Holstein cows. This led to looking at synchronizing heifers with a 9 d CIDR insert with or without GnRH injection 7 d prior to CIDR removal (PG given on day of CIDR insertion and removal) and its effects on fertility. The percentage of heifers detected in estrus within 4 d was 96.8 and 94.3 %, and pregnancy rates were 60.6 and 43.4 % for both CIDR+GnRH and the CIDR insert alone (Schmitt et al., 1996). It was possible that heifers receiving only a CIDR insert

ovulated a persistent dominant follicle after CIDR removal, thus causing reduced fertility. Schmitt concluded that occurrence of a persistent dominant follicle ovulating and reducing the chance for conception can be reduced if incorporation of GnRH or GnRH agonist occurs at CIDR insertion.

Another study looked at the effects of synchronizing suckled beef cows with either a 7 d CIDR insert plus EB given 24 to 30 h after CIDR removal, CIDR alone, a sham device for 7 d (no progesterone) plus EB or sham device alone to see if the progesterone insert can induce estrus in anestrous cattle and if EB would enhance reproductive responses after insert removal. Fike et al. (1997) reported synchronizing cows with a progesterone device resulted in replacing the function of a CL in anestrous cattle and administration of EB enhanced the incidence of estrus.

Determining the optimal dose of EB to enhance reproductive responses in beef heifers and cows were evaluated when synchronized with a 7 d CIDR insert and PG given one day prior to CIDR removal. Lammoglia et al. (1998) reported administering 0.38 mg of EB to heifers and 1 mg of EB to cows 24 to 30 h post CIDR removal increased estrous response (86 % and 100 %) vs administering 0, 0.2 or 0.75 mg of EB in heifers and 0, 0.25 or 0.5 mg of EB in cows. Peak LH was also highest for heifers receiving 0.38 mg of EB and cows receiving either 0.5 or 1 mg of EB. Pregnancy rates were higher for cows that received 1 mg of EB after CIDR removal. Bogacz et al. (1999) reported ovulations to be higher for cattle that received either 1 or 2 mg of EB/500 kg of body weight vs cattle that received either 0 or 0.5 mg of EB/500 kg of body weight just prior to a 7 day CIDR insert. Dose response may play a role in the level of activity needed for improved fertility.

Other protocols have been looked at to determine the effects of incorporating two injections of EB with progesterone insert to enhance fertility in dairy cattle. Comparisons of two dose levels (1 mg and 2 mg) of EB given at the beginning of a 7 d CIDR insert with or without a second injection of EB 2 d after CIDR removal, resulted in no difference in estrus or conception rates; however cows in estrus 2 d after CIDR removal tended to be less fertile than cows in estrus 3 d post CIDR removal (Day et al., 2000). Day also reported that the 4 d estrous response was high (90 %) for cows just receiving either 1 or 2 mg of EB at CIDR insertion.

Richardson et al. (2002) looked at comparing a modified version of the Select Synch protocol (GnRH given 6 d prior to PG) to a protocol that consisted of a CIDR insert for 7 d with PG given 1 d prior to CIDR removal with or without GnRH at CIDR insertion and its effects on fertility in beef and dairy heifers. Estrous response and conception rates were reported to be higher for both the CIDR+PG (87.1 and 58.6%) and CIDR+GnRH+PG (84.1 and 58.2%) vs the modified Select Synch (77.7 and 53%) protocol for both beef and dairy heifers combined, although estrous response for all three systems combined was higher for beef heifers (87.6 %) vs dairy heifers (79.4 %; Richardson et al., 2002). Richardson commented that the current study was conducted with previously used CIDR inserts that contained either 1.38 or 1.9 g of progesterone initially.

Cavalieri et al. (2003) looked at synchronizing non-lactating Holstein cows with a CIDR insert for 7 d and PG injection at CIDR removal followed by 1 mg of EB 24 h after CIDR removal with either progesterone, EB, progesterone + EB or no hormones given at CIDR insertion to look at ovulation rate and peripheral concentrations of gonadotropins

and steroid hormones. Only 64.7 % of the cows that exhibited standing estrus had ovulated within 96 h of CIDR removal. While estrous response was high, Cavalieri concluded that cows synchronized with EB+CIDR insert produced LH concentrations with a more synchronous emergence of new follicular waves and shorter intervals from the time of new wave emergence to ovulation vs cows synchronized with a CIDR insert alone. Cavalieri et al. (2003) also reported combining EB and progesterone at initiation of a CIDR implant resulted in no significant improvements on follicular dynamics.

Early Heats. A problem that has persisted with protocols that give GnRH prior to PG injection without any additional progestins (Select Synch, Co-Synch, Ovsynch) is the incidence of early heats. Kojima et al. (2000) noticed that 18 % of cows synchronized with the Select Synch protocol exhibited estrus 30 h prior to and 18 h after the initial PG injection. These animals likely do not respond to the initial GnRH injection and are exhibiting a natural heat prior to or immediately after PG administration. Downing et al. (1998) concluded that a percentage of cows treated with the Select Synch protocol are likely to be in the later stages of their estrous cycle (d 15 to 17) at time of GnRH injection. These early heats often require longer intervals for heat detection, if breeding off of detected estrus, with a decreased estrous response after PG injection (Pursley et al., 1995). These early heats also present a problem when considering fixed-time insemination. Geary et al. (2000) reported that 66 % of suckled beef cows responded to the initial injection of GnRH and that 9 % of the remaining cows were in their later stages of the estrous cycle (d 15 to 17) and exhibited natural heats within 24 h prior to PG injection. Although there is an incidence of early heats prior to PG, fertility from

inseminating off of these early heats has shown to be normal (T. W. Geary, unpublished data).

Fixed-Time Insemination. In order to control some of the inconsistencies of heat detection and the variability of interval between onset and observation of detected estrus, synchronizing cattle for insemination at a pre-determined time was developed. Protocols were designed to initiate a timed artificial insemination program so that cows could be inseminated without estrous detection. Synchronizing a pronounced standing heat entails bringing a large percentage of a group of females into estrus at a predetermined time. There are many benefits, from a producer's standpoint, that led to an increase in the use of timed AI. The ability to shorten the calving season so 50 % of the herd would calve within a 10 day period would decrease labor during the calving season. One of the concerns producers had with calving cows during a short time period was the chance of calving in bad weather. As with most herds, the variation in gestation length is consistent and only 20 % of those synchronized for estrus would be expected to calve on their due dates (Odde et al., 1987).

Successful fixed-time AI protocols require precise control of an animal's estrous cycle and timing of estrus in the majority of cattle being synchronized. Synchronizing follicular growth and luteal regression may aid in coordinating a pronounced time where ovulation is tighter and standing estrus is more accurate. The concepts behind coordination of synchrony came from numerous studies done with gonadotropins and its ability to reprogram follicular waves. It has been reported that 56 to 76 % of GnRH administered cows exhibit standing estrus within 24 to 72 h after PG-induced luteolysis (Twagiramungu et al., 1992a,b). With this response in estrous synchrony, new

developments for fixed-time insemination were developed. Initial studies done to look into timing of insemination after a GnRH+PG protocol (PG given 6 days later) with fixed-time insemination at 48 and 60 h post PG injection resulted in marginal pregnancy rates (38.4 vs 53.8 %; Zeroual, 1994).

Twagiramungu et al. (1995b) countered Zeroual's protocol by comparing fixed-time insemination 54 h after PG administration with or without a second injection of GnRH vs breeding off of heats in both beef cows and heifers. Overall pregnancy rates for all treatments were higher in heifers (62.7 %) vs cows (48.4) and the interval from PG to standing estrus was shorter for heifers (47.3 h) vs cows (56.2 h) suggesting that timing of AI at 54 h was closer, in heifers, to the peak estrous response (in hours) vs peak estrous response in cows (Twagiramungu et al., 1995b). Twagiramungu also concluded that pregnancy rates were higher for both heifers and cows combined that were bred off of standing heats (63.4 %) vs both timed AI groups (GnRH = 57.1 % and no GnRH = 48.7 %), however timed AI pregnancy rates were high for heifers and cows receiving a second injection of GnRH at breeding. Timed AI at 54 h seemed to potentially be the right time for AI with GnRH given at insemination in heifers because ovulation of the selected dominant follicle typically occurs between 24 to 32 h after the second injection of GnRH (Pursley et al., 1994). Twagiramungu et al. (1995b) concluded that by incorporating a second dose of GnRH to a modified Select Synch protocol eliminated the need for heat detection before and after PG induced luteolysis without negatively affecting fertility.

A study was done to compare AI after detected estrus or timed-insemination on pregnancy rates in beef cattle and whether inseminating in the uterine body or uterine

horns would make a difference in fertility. Grieger et al. (1998) reported no differences by inseminating in the uterine body (70 %) vs the uterine horns (73 %) in cows bred off of standing estrus with a Select Synch protocol. Grieger did report that pregnancy rates to TAI with a Co-Synch protocol were higher for cows inseminated in the uterine body (53 %) vs cows inseminated in the uterine horns (42 %) suggesting that placing semen in the uterine body is more beneficial when inseminating either by detection of estrus or by timed-AI.

As we are seeing some improvements on pregnancy rates by incorporating fixed-time insemination utilizing gonadotropins and prostaglandins and taking into account the economics behind heat detecting versus timed AI, future work is needed to look at determining the exact timing of AI. Controversies over timed-insemination and past results have boggled researchers and producers for the last decade as to whether the benefits are substantial. Timing ovulation of an animal has become difficult; however recent research to induce or synchronize ovulation in heifers and suckled cows for timed-insemination have resulted in acceptable pregnancy rates when bred off of TAI vs detected estrus. Pregnancy rates were similar for females timed-inseminated 72-74 hrs after two injections of PG with GnRH given 18-20 hours prior to TAI (58.3 %) vs females inseminated following detected estrus after two injections of PG alone (63.1 %; Stevenson et al., 1997). These females were synchronized with two injections of PG, a norgestomet implant with GnRH given 7 days prior to the second injection of PG and the norgestomet implant removed one day after the second injection of PG. Stevenson concluded that fertility after timed AI was equal to AI after detected estrus in both heifers and suckled beef cows. Stevenson et al. (2000) reported pregnancy rates to a fixed time

AI 64 h following a Select Synch protocol with a 7-day norgestomet implant (57.1%) were comparable to AI off of detected estrus (68.8 %). Based on these reports, pregnancy rates were reduced for cows bred by appointment without the addition of a norgestomet implant vs cows synchronized with a norgestomet implant and GnRH.

Two protocols have been developed for use of fixed-time AI without incorporation of a progestin: the Ovsynch (Pursley et al., 1995) and Co-Synch protocols (Geary and Whittier, 1998) used for both dairy and beef cattle. The Ovsynch protocol consists of an injection of GnRH on day 0, PGF_{2α} on day 7, a second injection of GnRH on day 9 with timed AI occurring on day 10. The Co-Synch protocol consists of an injection of GnRH on day 0, PGF_{2α} on day 7 and a second injection of GnRH on day 9 with timed insemination. With these two protocols, many studies have been done to compare the effects of pregnancy rates in beef and dairy cattle. Geary and Whittier (1998) reported pregnancy rates to be higher for cows synchronized with the Ovsynch protocol (57 %) vs cows synchronized with the Co-Synch protocol (49 %), but cattle being handled a fourth time with the Ovsynch protocol will depend on goals that a producer sets prior to breeding. Because pregnancy rates were improved for cows inseminated 72 h post PG injection vs cows inseminated 48 h and the average interval to standing estrus in cows after PG was found to be 70 h (Geary et al., 2000), delaying AI in a fixed-time insemination protocol to 72 h may result in improved fertility. Geary et al. (1998) also looked at pregnancy rate differences when comparing the traditional Syncro-Mate B protocol with timed-insemination beginning at 48 h and the Ovsynch protocol with 48 h calf removal. Geary concluded that pregnancy rates for cows treated with the Ovsynch protocol were higher (54 %) vs cows synchronized with the Syncro-Mate B

protocol (42 %) suggesting that delaying timed-insemination after 48 h, even without a progesterone source, may improve pregnancy rates. Geary also indicated that pregnancy rates were slightly higher for anestrous cows synchronized with the Ovsynch protocol vs the Syncro-Mate B protocol.

Another study looked at timing of insemination relative to timing of ovulation using the Ovsynch synchronization protocol in lactating dairy cows. With ovulation occurring 24 to 32 h after administration of GnRH (Pursley et al., 1994), pregnancy rates were compared when fixed-time insemination occurred at 0, 8, 16, 24 or 32 h after the second GnRH injection. Pursley et al. (1998) reported pregnancy rates to be higher for cows inseminated at 0 (37 %), 8 (41 %), 16 (45 %) and 24 h (41 %) vs cows inseminated at 32 h (32 %), and pregnancy loss was lowest for cows inseminated at 0 h and highest for cows inseminated at 32 h. They concluded that the variability in timing of AI after administration of a second GnRH injection tends to be more flexible as long as AI does not occur after ovulation.

Moreira et al. (2000) looked at comparing different times during the estrous cycle when the Ovsynch protocol was introduced on follicular dynamics. Dairy heifers were administered with a second injection of GnRH 36 h after PG with TAI 16 h later. Ovulation rates varied in heifers synchronized with the first injection of GnRH on d 2 (0 %), 5 (100 %), 10 (25 %), 15 (60 %) and 18 (100 %) of their estrous cycle. Moreira concluded that injection of GnRH on day 15 of the estrous cycle caused heifers to ovulate prior to insemination, thus compromising fertility.

Stevenson looked at comparing 54 h TAI with a Co-Synch protocol and GnRH administered at TAI vs the Select Synch protocol and reported lower pregnancy rates to

AI (33.1 vs 69.6 %). Another study compared pregnancy rates in cows synchronized with the Select Synch protocol with insemination occurring 72 h after PG with (33 %) or without (27 %) a second injection of GnRH vs cows inseminated after a detected estrus (60 %; Geary et al., 2001). With peak estrous response ranging from 30-72 hours after PG (Geary et al., 2000), those animals inseminated after detected estrus between 48 to 72 h would possibly have an equal chance of becoming pregnant to TAI if inseminated at 72 h. There seems to be no difference in the effects on fertility with a second injection of GnRH at TAI.

A protocol consisting of an initial injection of GnRH with a 7 day norgestomet implant and PG given at implant removal was used to determine the effects of inducing luteal structures in non-cycling suckled beef cows and its effects on pregnancy rates to a fixed-time AI protocol. Thompson et al. (1999) reported 75 % of non-cycling cows had induced luteal structures subsequent to the initial injection of GnRH. Thompson also reported pregnancy rates from TAI 16 h after GnRH was given 48 h post PG injection were greater for cows synchronized with GnRH + norgestomet + PG (71 %) vs the Select Synch (31 %) or norgestomet + PG (15 %) protocols. By incorporating a norgestomet implant with GnRH upfront, induced ovulation or follicular turnover will stimulate new follicular growth, followed by increases in estradiol 17β and progesterone, thus resulting in higher pregnancy rates (Thompson et al., 1999).

A follow up study was done to determine if incorporating MGA into a modified Co-Synch protocol would result in improved fertility with fixed-time AI. Perry et al. (2002) indicated that pregnancy rates may be improved from a fixed-time AI protocol if MGA is incorporated into a Co-Synch protocol with TAI at 72 h (61 %) vs a Co-Synch

protocol alone (47 %). By delaying TAI until 72 h post PG injection, Perry synchronized ovulation to optimize pregnancy rates based on when the peak window of standing estrus would hit, thus allowing for more cows that exhibited standing estrus to fall within 24 h of mass mating.

Stevenson et al. (2002) looked at replacing the second GnRH injection, in an Ovsynch or Ovsynch + CIDR protocol, with estradiol cypionate (ECP) to determine effects on LH surge, ovulation and timing of estrus. Stevenson reported higher estrous rates for ECP cows than GnRH cows but ovulation rates were lower for ECP cows (59 %) vs cows given GnRH (83 %). When a CIDR insert was incorporated into the Ovsynch protocol, the incidence of LH surges was less for cows receiving ECP (42 %) vs cows receiving GnRH (89 %; Stevenson et al.; 2002). Stevenson concluded that ECP did in fact increase the number of cows in estrus; however the incidence of LH surges and ovulation rates were less for ECP cows than cows receiving GnRH.

When comparing GnRH protocols, it is sometimes difficult to determine if inseminating off of heats or breeding at a predetermined time is of more value to a producer. Three synchronization protocols were compared to determine the efficacy of a GnRH+PG system for synchronizing estrus and (or) ovulation. Lemaster et al. (2001) reported pregnancy rates to be greater for lactating crossbred beef cows synchronized with a Co-Synch (31 %) and Hybrid-Synch protocol (35.5 %) vs the Select Synch protocol (20.8 %). The Hybrid-Synch protocol allows producers to breed off of standing estrus for 72 h after administration of PG and mass mate the remaining cows at 72 h with a second injection of GnRH on those remaining cows. Lemaster reported that the Co-Synch and Hybrid-Synch protocols resulted in greater pregnancy rates over the Select

Synch protocol and incorporating a timed-insemination protocol into your breeding system may be beneficial. Lemaster concluded that low pregnancy rates for all protocols used were likely due to poor estrous response. Even though estrous response was poor, the interval to estrus after PG was highest from 48 to 72 h post PG injection.

Hixon et al. (2001) compared pregnancy rates in beef cows using either a two-injection PG protocol, 7-11 Synch protocol or 7-11 Synch where all cows received a second injection of GnRH 48 h after second PG injection with TAI. Hixon concluded that first service conception rates were 58.4, 61.8 and 72.7 % for cows synchronized with the two-shot PG, 7-11 Synch and 7-11 Synch with TAI protocol, respectively. With these results, breeding at a pre-determined time can yield acceptable pregnancy rates that are comparable to pregnancy rates achieved with breeding off of detection of estrus, therefore resulting in an alternative method for synchronizing cattle that may benefit producers.

Introduction of the CIDR-B device into fixed-time AI systems was evaluated to determine its ability to control ovulation in postpartum suckled beef cows compared to a traditional Co-Synch protocol. Lamb et al. (2001) reported higher pregnancy rates for cows synchronized with the Co-Synch protocol plus a 7 d CIDR insert between initial GnRH and PG injections (58 %) vs cows synchronized with the CO-Synch (48 %) protocol alone. The Co-Synch protocol yielded acceptable pregnancy rates for fixed-time AI, yet pregnancy rates for non-cycling cows were higher for the CIDR + Co-Synch protocol (59 %) vs cows synchronized with the Co-Synch protocol (39 %), indicating that incorporation of a CIDR insert can induce more non-cycling cows to become cyclic vs just a single injection of GnRH. Lamb also reported that pregnancy rates were higher for

cows inseminated 72 h after PG injection at one of the four locations (62.2 %) vs the other three locations where AI was performed at the second injection of GnRH (60.4, 47.5, 43.8 %). By delaying TAI and GnRH injection post 48 h, improved pregnancy rates in beef cattle may be achieved as seen using the Ovsynch protocol (Geary et al., 2001) vs inseminating at the traditional 48 h time period. Lamb et al. (2001) also reported increased pregnancy rates for cows whose ovulatory follicle was > 12 mm vs ovulatory follicles < 12 mm.

An evaluation was done to compare two progestins (CIDR-B and MGA) and three gonadotropins given at initiation of protocol treatment and at breeding to look at differences in pregnancy rates in beef heifers. Martinez et al. (2002) looked at synchronizing heifers with a 7 d CIDR insert or 6 d feeding period of MGA with GnRH, porcine LH or EB given at the beginning of treatment and PG given on day 7. After PG, additional EB was given 24 h after PG injection and timed AI 28 h after the second EB, and either GnRH or porcine LH was given 48 h after PG injection with a concurrent insemination following. Estrous response and pregnancy rates for heifers detected in estrus vs ones not detected in estrus were higher for those synchronized with the CIDR insert (68.9 and 62.6 %) vs feeding MGA (45.5 and 51.9 %; Martinez et al., 2002), respectively. Pregnancy rates for heifers receiving a CIDR insert and GnRH were higher (65 %) than heifers administered EB (61.5 %) or porcine LH (55.9 %), suggesting that GnRH has similar effects on pregnancy rates as EB, though estrous responses to EB were higher (91.7 %) than GnRH (35.6 %) when fed MGA for 6 days (Martinez et al., 2002).

Final work done by Stevenson et al. (2003) compared three different sources of progesterone with the incorporation of a Co-Synch protocol or the Co-Synch protocol

alone in three different experiments on TAI pregnancy rates in beef cows. Results from experiment 1 indicated that overall pregnancy rates and pregnancy rates in anestrous cows were higher for cows treated with the CIDR + Co-Synch protocol (66.3 and 60 %) vs cows synchronized with the Co-Synch (60.9 and 42.9 %) protocol. Experiment 2 indicated that overall pregnancy rates and pregnancy rates in anestrous cows were higher with the CIDR + Co-Synch protocol (54.6 and 51.3 %) vs cows synchronized with the MGA + Co-Synch (46 and 39.1 %) protocol. Results from experiment 3 indicated that cows synchronized with the CIDR + Co-Synch protocol and TAI at 48 h (44.4 %) vs 60 h (48.1 %) after CIDR removal resulted in no difference in pregnancy rates. There seemed to be increased pregnancy rate differences when GnRH was administered at both breeding intervals (50.3 %) vs no GnRH (42.3 %). With multiple comparisons done on these protocols, pregnancy rates were higher for cows synchronized with a CIDR + Co-Synch protocol vs the MGA + Co-Synch or just Co-Synch protocol alone (Stevenson et al., 2003). Based on the results of delaying TAI to 60 h after CIDR removal, pregnancy rates were not improved vs breeding at 48 h after CIDR removal, although giving a second injection of GnRH at time of AI improved fertility vs no injections.

HUMAN CHORIONIC GONADOTROPIN

It has been reported in early and recent studies that systemic progesterone is important for maintenance of pregnancy and embryo development (Estergreen et al., 1968), but new research has questioned the role of systemic progesterone concentrations on embryo survivability. Whether abnormal progesterone concentrations after insemination influence (Hansel et al., 1978; Butler et al., 1996) or have no effect

(Echternkamp and Maurer, 1983) on pregnancy rates is still unknown; however it has been shown that giving exogenous human chorionic gonadotropin (hCG) increases progesterone synthesis (Eduvie and Seguin, 1982). Increased concentrations of progesterone seen from hCG administration is reported to be caused by gonadotropin induced ovulation of a dominant follicle with formation of a secondary corpus luteum or luteinized follicular cells. This induced ovulation has been seen when hCG is given on d 4 (Breuel et al., 1989; Price and Webb, 1989), 5 (Schmitt et al., 1996a,b), 6 (Fricke et al., 1993), 7 (Breuel et al., 1989), 10 (Breuel et al., 1989) or d 14 to 16 (Price and Webb, 1989). Studies involving hCG timing after insemination in order to enhance fertility by increasing progesterone levels in circulation has reported mixed results. Price and Webb (1989) reported luteinized structures formed from luteal phase large follicles after treatment with hCG in early metestrus. It has been reported that GnRH and hCG will stimulate growth of secondary CLs from induced ovulating dominant follicles present on the ovary, but Schmitt et al. (1996a,b) indicated that hCG given 5 d after estrus induces secondary CLs that are heavier in weight and secrete more progesterone in-vitro (Schmitt et al., 1993) for 8 days vs cows administered GnRH. By administering hCG on d 5 of the estrous cycle, when a selected follicle of the first wave is beginning its dominant stage, it was reported that heifers originally displaying two follicular wave patterns would switch to ovulating a dominant follicle from a third follicular wave (Diaz et al., 1998). Diaz concluded that increased concentrations of progesterone, induced by exogenous hCG during the luteal phase of the estrous cycle, may stimulate follicular turnover that would induce a switch to having three vs two waves of follicular growth.

Pregnancy rates have been reported to improve (Brown et al., 1973) or not change (Echternkamp and Maurer, 1983) when hCG was administered after insemination. Breuel et al. (1989) looked at giving an exogenous source of hCG to increase both progesterone concentrations and fertility. By giving hCG on d 4 of the estrous cycle prior to breeding and 4 days after breeding with insemination taking place at the end of a 21 d cycle, progesterone concentrations increased over a 16 d period prior to breeding with improved pregnancy rates of 37 % (Breuel et al., 1989). It was suggested that this increase in the concentration of progesterone during the luteal phase of the estrous cycle may have positive effects on fertility in cattle (Robinson et al., 1989). These effects of hCG on fertility in both heifers and cows were evaluated when given 5 days after insemination. Schmitt et al. (1996a) reported no differences in pregnancy rates for heifers receiving a control (62.9 %) or hCG (64.8 %). Another study looked at improving pregnancy rates in dairy heifers by administering hCG at insemination in a Co-Synch protocol to prevent additional handling of cattle; however results found a slight decrease in fertility when hCG was replaced with GnRH at insemination (Schmitt et al., 1996c). Salverson et al. (1999) reported similar results when GnRH was replaced twice with hCG in a Co-Synch protocol in beef cattle with a 14 % decrease in fertility. Salverson also found an increasing number of cows exhibiting short luteal cycles when treated with hCG vs cows treated with GnRH.

NUTRITION

There are many factors to consider when managing cattle for reproduction to ultimately produce a product on an annual basis. One of the main factors producers need

to consider is the effects of nutrition on reproduction. The main nutrients required for basic maintenance in cattle are water, energy, protein, minerals and vitamins. The most important nutrient required to live is water. Intake of water in cattle will vary depending upon ambient temperatures. If intake or outside temperature increases, water consumption tends to increase linearly (Mass, 1987). Another important nutrient in most species is energy. Energy requirements in cattle are met by carbohydrates, proteins, fats, any combination or all the above from most diets. Energy is one of the more important nutrients involved in beef cattle reproduction, however when energy is low, typically other nutrients are inadequate as well (Mass, 1987). When considering the source and type of nutrients needed, quality and quantity become more important than cost of any feed source (Mass, 1987). When cattle are in their critical phases of production (heifer growth, late gestation, parturition and lactation) and maintenance requirements are high, the importance of forage quality becomes critical. The National Research Council (NRC) publishes guidelines for the quality and quantity of a particular feed source needed during specific times of production in cattle based on the animal's maintenance requirements. Heifer growth is of particular importance because they must weigh approximately 65 % of their full potential mature weight prior to first breeding (Mass. 1987). This target weight ensures that most heifers will reach puberty by the beginning of the breeding season.

Another major nutrient in the diet of cattle is protein, or crude protein (CP). Based on the assumption that CP contains approximately 16 % nitrogen, it is a calculated value ($CP = 6.25 \times \% \text{ nitrogen}$) that fluxuates linearly with the requirements of energy depending upon stage of production (Mass, 1987). Maintenance of beef cattle usually

requires 8 – 10 % CP in the diet, but as maintenance requirements increase during late gestation, parturition, lactation and heifer growth, CP requirements increase. Protein deficiency can result in reduced dry matter intake, thus resulting in calf morbidity, reduced milk production and infertility (Mass, 1987).

Body Condition Scoring on Reproduction. A valuable tool (body condition score, BCS) was developed to evaluate the nutrient energy status of beef cows for management practices (Bellows et al., 1982; Spitzer, 1986) and uses a numbering system which classifies cattle based on their condition of fat or fat deposits (1 = emaciated and 9 = obese; Whitman, 1975). Based on research, there is enough of an approximated difference in body weight between each numerical score (approximately 35 to 40 kg) to use these numerical scoring values on beef cattle (Mass, 1987). The use of BCS is well adapted for classifying cattle, based on condition prior to calving and prior to breeding, into different feeding groups to allow producers to manage all animals. Adequate condition of an animal prior to parturition is critical to ensure normal fetal growth and development, survivability of the calf, postpartum rebreeding and a concentrated calving season (Mass, 1987). Cattle that calve with a low BCS typically have problems rebreeding during the normal breeding season, which causes those cows to calve later in the calving season. Longer postpartum intervals with decreased pregnancy rates and longer calving intervals all lead to increased cost of production to the producer vs cows that calve with adequate or above adequate body condition. Changing the diet prior to puberty or just after calving from low to high-energy diets can increase fat deposition (Yelich et al., 1995; Perry et al., 1991a). By scoring cattle during their third trimester of

gestation, a producer can adjust the feeding program to target calving cows with a BCS of 5 to 7.

Wiltbank et al. (1962,1963) carefully studied and established early work with nutritional affects on postpartum anestrus in cattle such as quality and quantity of feed intake, stored nutrient reserves in the body and competition for those stored reserves from other physiological functions in the body. There is a physiological order of importance for nutrient targeting in the body of cattle (nutrient partitioning): 1) basal metabolism, 2) activity, 3) growth, 4) basic energy reserves, 5) pregnancy, 6) lactation, 7) additional energy reserves, 8) estrous cycle and initiation of pregnancy and 9) excess reserves (Short et al., 1990). Nutrient partitioning prioritizes reproduction later in the order of importance, so the importance of maintaining nutrient requirements is easily underestimated during critical times of the year. A large issue with fertility after parturition is the condition of that animal before and after calving. Pre-calving conditions in cattle have more of an impact on the postpartum interval than post-calving conditions and there seems to be a non-linear relationship between body condition prior to calving and the interval from calving to first estrus, thus complicating fertility (Odde, 1990). Changing the dietary intake after calving can alter conditions in cattle prior to breeding but these changes have less of an impact when BCS at calving is > 6 and the feed intake is high vs adequate rather than adequate vs low. There may be a level of reduced fertility in cattle when body conditions are too high. Anderson et al. (1987) reported reduced fertility in beef heifers that carried a condition score of greater than 6. It seems to be more of an issue in heifers than cows with too much condition at breeding. It remains to

be unknown whether this phenomenon is due to excess lipids acting to alter steroidogenesis or totally inhibiting hormone synthesis and release.

Several studies have reported changes in fertility when the condition of an animal is compromised. Richards et al. (1986) reported synchronized pregnancy rates of 61 % for cows with a BCS of 5 or greater compared to a 19 % pregnancy rate for cows with a BCS of 4 or less (Yelich et al., 1988), suggesting that cattle with body conditions of 5 or greater at time of synchronization is adequate for producing acceptable fertility rates. Work done by Stevenson et al. (2000) reported estrous response and first service conception rates were increased for beef cows with a body condition score of 5 or greater vs 4 or less synchronized with a Select Synch (73.8 and 67.1 %, 48.1 and 60 %), Select Synch + norgestomet (83.5 and 69.7 %, 52.5 and 51.9 %) and 2-shot PG protocol (54.4 and 68.8 %, 32.2 and 50 %). Stevenson's group concluded that for every 1 unit increase in BCS, there was a 10.6 to 27.8 % increase in the proportion of cows exhibiting estrus, thus allowing more animals the chance to conceive to AI. Estrous response and conception rates were also increased when cows of *Bos Indicus* breeding had a body condition score of 5 or greater vs cows with a body condition of 4 or less using a Hybrid Synch protocol (Lemaster et al., 2001). Lemaster proposed that poor estrous responses seen in low conditioned cows were likely due to average body conditions at the beginning of the breeding season thus causing a majority of the cattle to be anestrous at the start of the breeding season. Many reports have concluded that increasing early cyclicity in postpartum cows and maximizing pregnancy rates can be achieved if cows are in a minimum BCS of 5 at parturition and postpartum diets are fed at levels higher than the required nutrients needed.

Differences in body condition may play a role in some of the major fertility factors, but does it solve issues involving metabolics such as nutrient partitioning? Ciccioli et al. (2003) looked at fertility factors, as well as hormonal profiles that may be affected by nutrient intake for beef cows in different body conditions at calving and fed different levels of feed after parturition. Ciccioli's group noticed a considerable increase in body condition after supplementation which increased pregnancy rates at first estrus vs cows in lower body condition (Ciccioli et al., 2003). Postpartum interval was shorter and size of the ovulatory dominant follicle was larger for cows supplemented with a higher grain diet. As we now know, prepartum cows with poor BCS can cause detrimental effects on reproduction. Above normal body conditions prepartum can enhance secretions of LH, responsible for initiation of first postpartum ovulation and follicular growth (Perry et al., 1991a; Grimard et al., 1995), but these effects may be enhanced more on cattle of thinner body conditions with increased nutrient intake than cattle that are in moderate body condition (Richards et al., 1986; Spitzer et al., 1995). Wettemann et al. (1999) concluded that when nutrient intake was restricted for a prolonged period of time, decreases in LH secretion caused a reduction in estradiol release from the dominant follicle which ceases ovulation. When nutrient intake levels were reversed, levels of LH and estradiol increased and ovulation occurred (Wettemann et al., 1999). Looper et al. (2003) showed that cows in moderate body condition (BCS = 5) had shorter intervals to first estrus (53.3 d) and shorter intervals to first normal luteal activity (58.3 d) after parturition vs cows in thin body condition (BCS = 4; 89.3 and 93.3 d), but length of luteal activity was not influenced.

There seems to be a lack of consistency on specific reproductive outcomes in cattle with varying degrees of body condition, but these inconsistencies are likely due to various factors such as the amount of energy intake, age of cattle, BCS at calving, duration of feeding period and the time of year. Numerous studies have shown that thinner cattle, typically primiparous cows, respond with increased reproductive performance from increased nutrient intake (Richards et al., 1986; Spitzer et al., 1995). Stored fat deposition seems to play a role in the level of increased fertility (increased ovarian activity). Increased body energy reserves have been shown to stimulate early ovulation in beef cows (Bishop et al., 1994), thus decreasing the number of nutrient induced anestrous cattle (Richards et al., 1989; Bossis et al., 2000).

Environmental factors can play a role in not only affecting postpartum intervals but also forage conditions that will affect the condition of cattle. Colder conditions will decrease digestibility and increase feed intake, whereas an increase in temperature, mud or rain may cause a decrease in feed intake (Mass, 1987). Time of year may have some effect on estrous behavior in beef cattle based on weather and temperatures, although Trimberger (1948) reported no effects on estrous behavior based on season and Walker et al. (1996) found no effects on duration of estrous under extreme hot temperatures. Pennington et al. (1985) found that length of standing estrus was longer in hotter weather conditions. White et al. (2002) reported seasonal influences on estrous behavior in beef cows with standing estrus occurring longer in the summer (17.6 h) than in winter (15.5 h) and spring (13.9 h) months. Cows were mounted more times per estrus in the winter than in the spring or summer. Seasonal and thermal stress can be harmful on reproductive efficiency in dairy cattle (Cavestany et al., 1985) and may alter the endocrine functions

that affect follicular dynamics (Wilson et al., 1998). Breed of cattle, management practices, climate and geographical locations may sometimes cause these fertility differences seen in different climatic conditions.

Fat Supplementation. The use of supplementing dietary fat as an alternative feed source to improve fertility was initially researched in dairy cattle. Early scientists believed that incorporating a fat source into the diet of dairy cattle would improve fertility during the breeding season. Past research began to notice that fat supplementation may increase adipose tissue triglyceride hydrolysis, therefore allowing for certain fatty acids to be used in reference for reproduction to influence fertility (Grummer and Carroll, 1991). The amount of available dietary energy has been known to affect energy balance within 3 weeks postpartum by reducing or changing the interval from parturition to first ovulation and affecting follicular development and luteal function. Poor body conditions due to inadequate energy intake can lead to poor reproductive responses, although supplementing dietary fat may reduce these negative effects on reproduction. While some studies have shown an influence of dietary fat on energy balance, others have shown no effects on energy balance when supplementing various types of fatty acids due to increased milk production (Erickson, 1989) or reduced DMI (Jerred et al., 1990), which is commonly seen after parturition. However, Staples et al. (1990) suggested that energy balance may be a suitable indicator of reproductive status since anestrous cows produced less milk and were in greater negative energy balance, due to lower feed intake, than cycling dairy cows.

There are certain essential fatty acids in the body that are important for specific hormone production with certain effects on fertility. Linoleic acid (C18:2), a precursor

for prostaglandin $F_{2\alpha}$ production, is involved in follicular growth, fertility and uterine involution. With approximately 60 to 90 % of dietary linoleic acid being hydrolyzed in the rumen, it has been suggested that essential fatty acid deficiency during the postpartum period may be overcome by supplementing postruminal linoleic acid to improve reproductive performance in dairy cattle (Lucy et al, 1990) or by-pass fat. Scholljegerdes et al. (2001) concluded that quantity of unsaturated fatty acids reaching the duodenum increased with safflower seed diets high in both linoleic and oleic acid, and the availability of fatty acid metabolism is dependent upon dietary fat source.

Supplementing dietary fats has been shown to affect plasma concentrations of $PGF_{2\alpha}$ metabolites in circulation depending upon the lipid supplemented. Early studies reported soybean oil supplementation increased plasma $PGF_{2\alpha}$ in dairy heifers (Lucy et al., 1990) and ewes (Burke et al., 1996). This increase in $PGF_{2\alpha}$ was seen within a short period of time after olive oil (7 % linoleic acid) or safflower oil (70 % linoleic acid) was infused into the abomasum of lactating dairy cows (Lucy et al., 1990). Reports from Filley et al. (2000) indicated that proportions of major fatty acids, primarily linoleic acid, increased within 7 days and prostaglandin $F_{2\alpha}$ metabolites increased on days 7 and 9 in primiparous heifers supplemented with calcium soaps of fatty acids. Grant et al. (2003) reported higher concentrations of $PGF_{2\alpha}$ metabolites between days 25 to 80 postpartum in beef cows supplemented with safflower seeds high in C18:2.

Supplementing dietary fats high in linoleic acid have also played an inhibitory role in the synthesis of uterine PG (Staples et al., 1998) by either increasing the concentration of fatty acids that compete with arachidonic acid (C20:4) for binding of the enzyme cyclooxygenase, inhibiting arachidonic acid synthesis and reducing arachidonic

phospholipid pools (Bezard et al., 1994) or by inhibiting the activity of prostaglandin H synthase to synthesize new PG from arachadonic acid (Pace-Asciak and Wolfe, 1968; Thatcher et al., 1994). Poly-unsaturated fatty acids, especially eicosapentaenoic acid (C20:5) and docosahexaenoic acid (C22:6; n-3 family), are major inhibitors of desaturation and elongation of fatty acids which sometimes inhibits synthesis of C20:4 in the liver of cattle (Bezard et al., 1994). This process is due to the powerful competition linolenic acid (C18:3; precursors for C20:5 and C22:6) has with linoleic acid for the delta 6 and delta 5 desaturase enzymes. Supplementation of fishmeal into the diets of primiparous beef cows increased C22:6 levels during the first 5 weeks of supplementation and maintained C20:5 levels during supplementation (Bonneterre et al., 2001). Fishmeal supplementation high in C20:5 and C22:6 attenuated the release of PGF_{2α} from lactating dairy cows (Thatcher et al., 1997).

An experiment was done to determine the effects of supplementing diets high in either n-6 polyunsaturated fatty acids (protected soya) or n-3 polyunsaturated fatty acids (linseed) on synthesis of PG. Cheng et al. (2001) reported diets high in n-3 did not alter concentrations of PG, however cows fed diets high in n-6 showed a 50 % reduction in basal concentrations of PG and inhibited the ability of uterine tissues to respond to an oxytocin or calcium ionophore challenge. Cheng concluded that diets high in the n-6 family of fatty acids reduce endometrial synthesis of PG and may complicate the process of luteolysis an ovulation.

The effects of improved energy balance on fertility has been reported to reduce the postpartum interval in lactating cattle, however these effects on fertility may also be due to enhanced follicular development seen in cattle supplemented with dietary fat

(Grummer and Carroll, 1991). These changes in follicular development have been the result of modulating hormones that affect ovarian processes from fat supplementation. It is speculated that pulsatile LH patterns postpartum may be prolonged if energy balance is negative (Schillo, 1992), yet Morgan and Williams (1989) reported no effects on frequency and amplitude of plasma LH when lipid supplementation was administered. Supplementation of fatty acids may not stimulate increases in LH concentrations, but if energy balances fall below normal, delayed synthesis of LH pulses may occur. Animals consuming less than their maintenance requirements for lactation and receiving additional fat supplementation have reported increases in LH secretion (Hightshoe et al., 1991). Basal levels of LH were seen to increase when calcium soaps of palm oil fatty acids were supplemented to range beef cows (Hightshoe et al., 1991).

Changes in ovarian cellular responses have shown to be less affective if fat is supplemented at less than 4 % of total dry matter intake (Stanko et al., 1997). The use of supplemented fat from plant oils (whole cottonseed and soybean oil) have stimulated the largest increase in the number of medium-sized follicles during the end of the luteal phase within 3 to 7 weeks (Wehrman et al., 1991; Ryan et al., 1992; Thomas et al., 1997; De Fries et al., 1998) and size of the largest follicle (Lucy et al., 1990; De Fries et al., 1998). These increases in diameter of dominant follicles may be due to increases seen in LH synthesis from fat supplemented cattle. Increases in ovarian follicular growth between 3 to 7 weeks postpartum was highest for cows supplemented with fats high in polyunsaturated fatty acids (soybean oil) vs fats high in saturated (animal tallow) and highly polyunsaturated fatty acids (fish oil; Thomas et al., 1997). Alternative sources of dietary fats that are predominantly low in linoleic acid such as animal tallow (Thomas

and Williams, 1996), calcium salts of saturated fatty acids (Lucy et al., 1991) and fish oils (Thomas et al., 1997) have less of an effect on follicular development than fats high in linoleic acid.

There is strong evidence suggesting that consumption of fat, particularly polyunsaturated plant oils can positively influence luteal function and postpartum reproductive performance in beef cattle (Williams and Stanko, 1999). Improvements in fertility have been associated with increased circulating progesterone levels during (Fonseca et al., 1983) and after (Bulman and Lamming, 1978) insemination.

Progesterone levels were greater for postpartum beef cows supplemented with safflower seeds high in C18:2 prior to insemination vs cows supplemented with no dietary fat (Grant et al., 2003). Cholesterol levels in plasma typically increases from dietary fat supplementation in dairy cattle (Grummer and Carroll, 1991). Two main fatty acid transporters in the body, LDLs and HDLs, supply the source of cholesterol in cattle.

Because cholesterol levels increase in response to fat supplementation and cholesterol is the precursor for progesterone synthesis from luteal cells, the availability of extra cholesterol would result in an increase in the concentration of progesterone. These transporters have been reported to account for some of the regulation of bovine theca, granulosa and luteal cell growth and metabolism (Bao et al., 1995). Williams (1989) reported increased plasma cholesterol concentrations in lactating beef cows supplemented with whole cottonseed for three weeks after parturition and increased progesterone levels after 5 d of a GnRH injection to induce first ovulation three weeks later. Williams indicated that the average lifespan of a corpus luteum in fat supplemented beef cows was greater than the lifespan of non-fat supplemented beef cows. If dietary fat attenuates the

release of PG from the uterus, sensitivity is reduced and delayed luteolysis may occur, influencing the life of the CL. This induced lifespan of a CL has a confounding affect on reducing the incidence of short cycles (Williams and Stanko, 1999). Plasma cholesterol concentrations were seen to increase linearly from 3 to 12 weeks postpartum when dairy cows were fed for 100 days with prilled long-chain fatty acids, and plasma progesterone concentrations were also reported to increase during the luteal phases of the second and third postpartum estrous cycles (Carroll et al., 1990). Lammoglia et al. (2000) reported a higher percentage of heifers attaining puberty earlier with elevated serum cholesterol and progesterone concentrations from supplementing safflower seeds. Williams and Stanko (1999) concluded that dietary-mediated increases in plasma cholesterol could either change the rate of progesterone synthesis by the corpus luteum (Hawkins et al., 1995) or change its metabolism.

Early studies have shown promising work in a new area of production allowing managerial practices that may very well lead to improved reproductive efficiency through added fats. Such reproductive efficiency is seen in dairy cattle, with advances in beef cattle research. Wehrman et al. (1991) reported increasing the number of beef cows cycling at the start of the breeding season by up to 18 % when given supplemental whole cottonseed 30 d prior to breeding. Wehrman also suggested that these improvements were more evident when environmental conditions impacted the conditions seen in those cattle. Increased overall reproductive performance and reduced postpartum anovulatory periods were seen in cattle receiving calcium soaps of fatty acids (Espinoza et al., 1995). Beef cows supplemented with safflower seeds high in linoleic acid 40 to 50 days during the later part of gestation had higher pregnancy rates to AI (70 %) vs cows receiving no

additional fat (56 %; Bellows, 1999), which coincided with work done by Gambill et al. (1995) where pregnancy rates were increased by 50 % for range beef cows supplemented with a supplemental fat in the form of Alifet. Pregnancy rates from natural service were increased for first-calf heifers receiving three different fat sources (safflower – 94 %, sunflower – 91 % and soybean seeds – 90 %) for 65 days prior to parturition vs heifers receiving no additional fat (79 %), yet there were no differences in pregnancy rates between the three fat supplements (Lammoglia et al., 2000). First-service pregnancy rates in primiparous lactating beef cows increased 20 and 14 % when supplemented with fishmeal (Armstrong et al., 1990; Bonnette et al., 2001).

With the excitement fat supplementation brought to scientists for defining management practices that may improve fertility, other reports have shown negative effects on fertility in cattle. Days to first postpartum ovulation were not different for anestrus beef cows receiving a fat supplement (Megalac) vs no fat supplement with the addition of calf removal (Anderson et al., 1992). Feeding calcium soaps of fatty acids did not improve the postpartum interval and pregnancy rates in primiparous beef heifers (Filley et al., 2000). Work done by Lammoglia et al. (2000) reported no differences in pregnancy rates in beef heifers supplemented with fat for 162 days and no differences in pregnancy rates in beef heifers pubertal at the start of the breeding season for both diets suggesting that reducing the feeding period from 162 to 60 d just prior to the breeding season might have more of an impact on reproductive performance. A study was done to determine the length of different supplemental feeding periods using sunflower seeds as a source of dietary fat in beef heifers prior to the breeding season. Funston et al. (2001) reported no differences in pregnancy rates or estrous response in heifers fed sunflower

seeds for 30 or 60 days vs heifers fed no supplements, and heifers fed fat for 60 days in one location had 10 % lower pregnancy rates than heifers receiving no additional fat. Lactating beef cows supplemented with safflower seeds high in C18:2 reported lower first-service conception rates (33.3%) than cows supplemented with no dietary fat (66.7 %) and the interval to first conception was shorter for cows receiving no dietary fat (60 d) vs cows receiving safflower seeds (81 d; Grant et al., 2003). Feeding calcium soaps of long-chain fatty acids to dairy cows lengthened the interval to first service and reduced pregnancy rates (Lucy et al., 1992). First-calf heifers receiving fat supplements (Megalac) prior to parturition had longer postpartum intervals (90 d) vs heifers receiving no additional fat supplements (60 d) and length of the second and third estrous cycles were longer for heifers receiving fat with increased progesterone levels vs heifers receiving no additional fat (Oss et al., 1993). Several reports have shown that feeding diets high in saturated, unsaturated, polyunsaturated and highly polyunsaturated fats have resulted in positive (Armstrong et al., 1990; Gambill et al., 1995; Bellows, 1999; Bellows et al., 2001; Bonnette et al., 2001), negative (Lucy et al., 1992; Grant et al., 2003) and no effects (Burke et al., 1997; Filley et al., 2000; Lammoglia et al., 2000; Funston et al., 2001) on pregnancy rates in beef cattle. These reproductive responses may in fact all be due to type and amount of lipids fed, the time at which these lipids are supplemented and the age and stage of production the animal is in.

INFERTILITY

Infertility has become a challenge for producers in operations that rely on reproduction as a source of output for their business. The reason reproduction is so

important is because it is the number one factor that limits production efficiency in beef cattle (Dickerson, 1970) and to some extent, dairy cattle. There have been four primary causes of infertility listed in cattle: failure of uterine involution to occur, short estrous cycles, anestrous and general infertility; and the percentage loss on general fertility can range anywhere from 20 to 40 % if infertility is induced by any one of these four factors (Short et al., 1990). A primary reason infertility is high is due to cattle failing to become pregnant and the postpartum interval (PPI, interval from parturition to first estrus) is where the infertility issues begin (Wiltbank, 1970). Research in this area has occurred for over 60 years, but the majority of information known today on postpartum infertility has come from the past 20 years of research. Williams (1990) stated that after considering embryonic mortality and postnatal losses, approximately 15 to 20 % of the nation's cowherd fails to wean a calf annually and these losses are the result of cows failing to rebreed within 85 d after calving.

Uterine Involution. Uterine involution is a process that occurs physiologically in cattle just after calving that restores the natural quiescent state of the female reproductive tract in order for pregnancy to occur again year after year. During this state PG levels are high in order to prevent pregnancy from occurring while the female tract is recovering. Uterine involution does affect fertility in cattle when breeding occurs during this process, which is usually within 30 d after calving. Graves et al. (1968) found fertility to be compromised when cows were bred within 20 d of calving vs cows bred between 20 and 40 days after calving. Fertility can be compromised due to uterine involution, yet this compromise occurs due to producer error in timing of the breeding season relative to calving. Most breeding programs today do not begin the breeding season until 60 d

postpartum. Kiracofe (1980) concludes that length of an anestrous period in cattle has no correlation to uterine involution.

Postpartum Anestrus. Postpartum anestrus is commonly referred to as the postpartum interval, which is a time period in which an animal is not cycling. The end of the postpartum anestrous period occurs typically when first estrus occurs. In some occasions, this event may not be necessarily the end of the PPI, as a “silent” or “quiet” heat prior to first ovulation will occur and standing estrus may not be visually detectable. There have been several factors presented that can alter or lengthen the PPI in cattle. These factors include complications that may occur from the previous pregnancy, dystocia, presence of bull, season, breed, parity, suckling stimulus and nutrition (Short et al., 1990). While all of these factors can play an important role in lengthening PPI, suckling stimulus and nutrition are two main factors that affect the PPI. Many of them have more of an impact when two or more interact with one another.

Calving difficulty (dystocia) has been associated with increased PPI with delayed breeding and reduced fertility because of a longer recovery period for uterine involution to take place (Bellows and Short, 1978). Presence of bulls has been shown to be beneficial in shortening the length of postpartum intervals (Zalesky et al., 1984). Seasonal affects can alter the length of the PPI based on the amount of sunlight there is in a day. Research has indicated that calving cows during late spring and early fall seasons may shorten the PPI (Bellows and Short, 1978). These seasonal effects on PPI were reported to be caused by changes in pineal gland secretions of melatonin (Sharpe et al., 1986), although research has not been further pursued in this area to shorten PPI based on the impractical affects producers have on changing the duration of light in a day. Breed

of cattle (dairy and beef) have been shown to affect length of the PPI. Both dairy and beef cattle nursing calves will have an increase in the length of the PPI vs dairy cows that are milked. Age of cattle, for many years, have been managed to overcome these increased problems with long postpartum intervals. Younger cows (2 to 3 years of age) have a harder time recovering from parturition because of the maintenance requirements on a growing animal and calf. These younger females require more available nutrients during the last trimester of gestation and after parturition. After calving, these younger animals generally require a longer period for recovery, thus reducing the number of animals cycling at the beginning of an early breeding season. These factors are typically minor contributors to affecting the postpartum anestrous period, but many of these factors have synergistic effects with each other that can increase the PPI.

Two factors that play major roles in affecting postpartum anestrus are suckling and nutrition. Short et al. (1990) reported that suckling may have the most dramatic effect on PPI and research has shown that calves weaned at birth (Short et al., 1972) or early in the postpartum period prior to first estrus (Walters et al., 1982) will cause cows to have shorter PPI vs cows weaned after first estrus. With the effects that some of these factors have on PPI and fertility, suckling can be regulated more efficiently than most of the other factors by complete early weaning, short-term weaning (48 h) or partial weaning (short durations each day).

Calf Removal. Emphasis on calf removal has recently become popular due to the effect calf removal has on inducing cyclicity in non-cycling cattle. This period of anestrus in cattle can last as long as 50 to 100 days when poor body conditions exist. During this period of anestrus, pituitary stores and synthesis of LH is depleted, therefore

preventing those animals from ovulating. LH depletion that occurs at the end of gestation and parturition is due to negative feedback effects of placental steroids and estradiol, on the hypothalamo-hypophyseal axis (Moss et al., 1981), however two weeks after parturition, the pituitary gland can restore most of its LH stores (Nett et al, 1988) and has the ability to release normal amounts of LH when challenged with GnRH (Williams et al., 1982).

Past reports have suggested that suckling stimulus plays a major role in controlling the reproductive cycle in cattle (Short, 1976; Lamming, 1978). There have been some reports that suggest alternate endocrine factors may play a role in this delayed or long postpartum interval. It was reported that the suckling stimulus can affect concentrations of opioid peptides in neural tissue (Malven et al., 1986), therefore catecholamines, opioid peptides, may play a role in controlling these inhibitory effects of gonadal steroids on GnRH release (Dailey et al., 1987). Catecholamines may be partly responsible for this inhibition on LH during suckling intervals. There are also somatosensory pathways in the udders of cows that have some influence on the hypothalamo-hypophyseal axis. Forrest et al. (1980) reported an immediate decline in peripheral LH concentrations during the first hour after suckling stimulus was initiated. If short-term calf removal can block this inhibition to LH secretion and thus increase pituitary LH stores, then resumption of first estrus may occur earlier and the percentage of anestrous cattle from a single herd may be reduced. Williams et al. (1987) reported natural suckling stimulus from a calf inhibits pulsatile LH secretion vs calf removal, milking cows eight times a day, presence of a muzzled calf or both milking and presence of a muzzled calf. Based on these results, it is possible that the somatosensory input from

the nursing calf triggers an inhibitory effect on the hypothalamo-hypophyseal axis (Williams, 1990), thus delaying first postpartum estrus. Shively and Williams (1989) reported an increase in LH pulse frequency reaching a plateau at 96 h after calf removal, therefore suggesting that removing calves for shorter than 96 h may reduce the number of cows exhibiting an LH pulse frequency and reducing the number of cows ovulating. Shively and Williams also reported duration of calf removal for 6 d was required to ensure complete ovulation of all cows with calves removed. It was also reported that as few as 2 to 3 suckles per day for calves nursing their dams was adequate to delay first postpartum estrus in nursing cattle (Williams et al., 1984). If removing calves for a duration of 6 d is required to ensure 100 % ovulation rate, short term calf removal (24 – 72 hr) may not be viable. By implementing early weaning or temporary calf removal, it has been reported that there is no reduction in long-term growth or weaning weights on calves (Bellows et al., 1974; Beck et al., 1979).

It has been suggested that presence of mammary tissue, along with suckling stimulus, can delay first postpartum estrus in cows vs cows mastectomized independent of nutritional effects (Short et al., 1972). Even though body condition in suckling cows is adequate, the PPI can still be delayed if suckling occurs up to breeding. Earlier studies have reported no advantages to calf removal on pregnancy rates (Salverson et al., 1999) when synchronized with a Syncro-Mate B protocol and bred off of heats (Pace and Sullivan, 1980; Brown et al., 1986), yet the PPI was reduced (Bellows et al., 1974) and estrous response (Dowling et al., 1977) and pregnancy rates were improved (Kiser et al., 1980) in cows inseminated at a pre-determined time. Hoffman et al. (1996) reported reduced intervals to first ovulation and increased concentrations of mean LH in cows

with complete calf removal on day's 4 – 9 postpartum vs cows without calf removal or restricted calf contact with dam, although interval was in fact shorter and LH concentrations were higher in dams with restricted calf contact vs cows without calf removal. Lamb et al. (1997) reported that milk removal, either by the cows' own calf or an alien calf, will prolong anovulation. This study showed that complete calf removal or presence of calf without suckling reduced the interval to first ovulation vs no calf removal, introducing an alien calf after weaning of dam's own calf or introducing an alien calf while dam's own calf is restricted from nursing. While some studies have shown that presence of calf without suckling or mastectomized cows maintained with their own calves creates a slightly longer PPI (Stevenson et al., 1994) compared to cows with temporary or complete calf removal, PPI can significantly be reduced in cows with presence of natural calf that is not able to suck vs cows with continuous suckling stimulus either by their natural or alien calf. In a study done by Lamb et al. (1999), first postpartum ovulation was similar in cows with calves weaned, calves weaned while being milked twice a day, calf present without nursing or calf present without nursing while being milked twice a day, but when calf nursed ad libitum or even suckled twice daily, interval to first postpartum ovulation was doubled. Lamb also concluded that the number of times a cow is milked per day has no affect on interval to first postpartum ovulation. Geary et al. (2001a) reported improved pregnancy rates by 9 % in cows with 48 h calf removal vs cows without calf removal, yet the synchronization protocol used made no difference in pregnancy rates when calf removal was incorporated into both systems. It has been reported (Shively and Williams, 1989) that LH pulse frequency increases and interval to first postpartum ovulation decreases in cows with calves

temporarily removed for 48, 96 and 144 h. If LH concentrations and interval to first ovulation can be improved with longer calf removal, then pregnancy rates to AI may be improved if the duration of calf removal is longer than 48 h. The effects of growth and productivity on the calf in later months has not been studied, nor has the effects of calf growth and productivity been researched in calves that are temporarily removed for longer than 72 h.

By implementing temporary calf removal with a progesterone source (Mackey et al., 2000) or GnRH in cattle of adequate body condition, ovulation can be induced, but these practices may not work effectively in cattle of poor body condition (Williams, 1990). This system has been reported to improve fertility, as well as the economics of a managed breeding program. By implementing calf removal, not only is PPI reduced, estrous response increased and overall pregnancy rates increase, but birth date of calves come earlier in the season. Calves born earlier in the calving season will typically have heavier weaning weights, increase the number of replacement heifers that cycle earlier in the breeding season, therefore increasing the lifetime productivity of that animal (Williams, 1990).

Nutrition in cattle has become important for controlling postpartum anestrus. Because cattle and other ruminants have the ability to convert low quality forage into a marketable product and be able to store these edible nutrients as nutrient reserves in times of plentiful availability, nutrition and reproduction together have become a powerful tool for managing and marketing beef, yet both nutrition and reproduction can be detrimental on fertility if not managed right. The effects of nutrition on anestrus and fertility can be affected by nutrient reserves in the body, competition for nutrient reserves from other

physiological functions in the body that come first in order of importance for maintenance functions and quality and quantity of feed intake (Short et al., 1990).

The resumption of estrous cycles is controlled by actions of the hypothalamus, pituitary and ovary all acting on each other and incidences that prevent resumption of normal estrous cycles, as discussed earlier, affect those actions in some form or another. The period prior to first estrus is a period of hormone tranquility. Hypothalamus and pituitary activity is reduced for a period of 10 to 20 days after calving and the amount of pituitary LH is low with decreased LH release from the pituitary due to influence from either estradiol or GnRH. The hypothalamus acts as an ignition key that starts a vehicle (pulse generator) and turns on the pituitary to secrete various hormones that act on other parts of the body. This shutdown of the pulse generator is not sustained nor affects PPI (Short et al., 1979) if the generator is turned on after uterine involution. This pulse generator shutdown can be stimulated by exogenous injections of GnRH to stimulate release of LH and thus initiate early resumption of the estrous cycle. However, this stimulatory effect does not induce estrus in all animals, especially ones that are very early in their PPI or nutritionally restricted (Edwards et al., 1983; Peters et al., 1985). Early estrus can be initiated by supplementing exogenous progesterone to induce an early positive feedback pathway from the ovary to the pulse generator (Britt et al., 1974). Ovarian follicular involvement in anestrous animals is minimal, but there seems to be effects of follicle production and the switch from progesterone production to estradiol production that influences timing of the resumption of estrous cycles. Estradiol feedback to the hypothalamus is negative during the early and later part of anestrus and this negative feedback will gradually be overcome by a positive feedback pathway on the

pulse generator to turn on GnRH synthesis, allowing for increased release in LH, thus first ovulation.

Some management practices implemented today can affect the length of PPI. Monitoring nutrition during the later part of gestation is a simple practice that will improve fertility during postpartum anestrus. Body condition scoring prior to calving will allow a producer to manage cattle that are in a lower body condition. Optimal BCS of 5 to 7 at calving will ensure that the majority of those cows will have reduced postpartum intervals. Cows in a BCS of < 5 will increase the incidence of longer PPI and reduce fertility at breeding unless the availability of nutrients after calving is plentiful. Cows in a BCS of > 7 will often be too costly for the producer and is not necessary. These cattle have enough nutrient storage and the extra forage should be utilized in other ways. Not all breeds of cattle or genetics of one breed will BCS the same after calving. The amount of milk that one animal produces after parturition will affect nutrient partitioning and in turn will affect BCS. Taking into consideration those factors and adjusting BCS for the affects that lactation, depending upon breed, and even dystocia have on nutrient partitioning will ensure good body condition on cattle at time of breeding, which will allow for good management considerations.

Length of a breeding season can affect the number of animals cycling at the beginning of the next breeding season. If the breeding season is 60 days or longer, those cows conceiving later in the breeding season will calve later in the calving season and have a shorter period for postpartum recovery before the next breeding season begins. When cows calve in a 45 day breeding season, cows conceiving later in the calving season will have a longer period for recovery from uterine involution. By slowly shifting

a longer breeding season to a shorter season, the incidence of postpartum anestrus will be reduced and fertility during the breeding season will not likely be compromised.

Short Estrous Cycles. Short estrous cycles were first noted by Menge et al. (1962) and Morrow et al. (1966) and was later found to be caused by changes in follicular development, concentrations of gonadotropins before and after first ovulation and development of luteal receptors for LH (Inskeep, 1995). These cycles are quite common and generally occur within 30 to 40 days postpartum after first ovulation. Fertilization can be achieved from this first ovulatory follicle, but pregnancy may not be maintained in cattle that exhibit a short cycle after ovulation due to regression of this short-lived corpus luteum (Graves et al., 1968; Odde et al., 1980). Short cycles occur because of a CL that is not capable of functioning correctly due to its size, amount of progesterone secreted and stimulatory response (Odde et al., 1980). This stimulatory response, shortly after first ovulation, is due to high levels of premature PG being secreted from the uterus that aids in uterine involution and causes premature regression of that CL prior to the time maternal recognition occurs from the embryo. Although early PG release may be responsible for affecting fertility after short estrous cycles, it has been speculated that oocyte quality might also be hindered from ovulation prior to initiation of a short estrous cycle. Work done by Breuel et al. (1993) reported that cows pretreated with a progestagen supplement and with a larger preovulatory dominant follicle had lower conception rates and higher preovulatory concentrations of oestradiol. Inskeep (1995) attributes 50 % of the low fertility seen from cows with short luteal phases to events that may affect the oocyte or embryo before day 7 of pregnancy and the other 50 % to hostile uterine environments on or after day 7 of pregnancy. It was reported that contributions to

the hostile environment was a combination of PGF_{2α} release from the uterus and a CL from a short luteal phase after first ovulation, secreting an embryotoxin that causes early embryonic mortality (Inskeep, 1995). What makes up this embryotoxin compound is unknown, but Hu et al. (1990) found that CL's destined to be short lived produced more PGF_{2α} *in vitro* than cattle with a normal CL life span after first ovulation.

Another possible cause for embryonic mortality is the occurrence of persistent dominant follicles in synchronization systems that use long acting progesterone treatments. As persistent follicles continue to grow, increased concentrations of estradiol are found in circulation from these oversized follicles (Breuel et al., 1993) and fertility can be compromised due to embryonic mortality losses in cows with these persistent follicles (36 %) vs cows with normal size ovulatory follicles (91 %). McGuire et al. (1990) indicated that sufficient estrogen concentrations remained in circulation 9 days after Syncro-Mate B removal from an initial injection of estradiol valerate. It was also reported that estradiol concentrations were higher on the day of standing estrus and 3 to 5 days after insemination for cows with degenerating embryos vs cows with normal embryos (Ayalon, 1978). Thatcher et al. (1994) found increased levels of estradiol in circulation in correlation to decreased conception rates on days 14 to 17 of the estrous cycle after insemination, which is typically when maternal recognition occurs. It was concluded that increased concentrations of estradiol may result in the increased release of PG that occurs 4 to 9 days after mating in cows with short estrous cycles, thus causing early embryonic losses (Inskeep, 1995), or the early regressing CL, caused by premature PG release, setting up for increased secretions of estradiol during the time of maternal recognition.

These short cycles present a problem to producers that use estrous synchronization systems with gonadotropins and prostaglandins. Gonadotropins have been shown to induce cyclicity in non-cycling cattle, therefore causing the animal to exhibit a standing estrus and ovulate after PG administration prior to breeding, however the incidence of short cycles is possible and can affect fertility. The use of exogenous progestins has been implemented for inducing cyclicity and to maintain a fertile pregnancy (Ramirez-Godinez et al., 1981; Smith et al., 1987). A progestin that is given exogenously will reduce the levels of PG secreted from the uterus, allowing for the CL to function normally after first ovulation if induced.

Embryonic mortality has recently been suggested to have more of an impact on reproduction. Casida et al. (1968) stated that embryonic death contributes very little to postpartum infertility in beef cows, yet in analyzing all their postpartum interval data, pregnancy rates in cows slaughtered at 3 d after insemination was 52 % compared to cows slaughtered 15 d or palpated from 38-44 d after insemination (35 %). This data would show an estimated embryonic mortality rate of 17 %. The causes of embryonic mortality are not yet understood, but researchers have suggested that a number of factors may be responsible. These factors, reported by Hansen (2002), include defects within maturation of the oocyte and future embryo, poor maternal environment, timing difficulties between the embryo and mother or failure of maternal recognition. A combination of stress factors on the embryo and mother or physiological factors affecting normal functions of the ovary may all have influences on embryonic survivability.

One factor that can affect embryonic survival is nutrition, which includes diets high in degradable crude protein (Armstrong et al., 2001), cattle with low body

conditions and primiparity (Snijders et al., 2000). Intrinsic factors that occur within the maturation of an oocyte include premature meiosis in persistent dominant follicles that reduce the percentage of embryos developing into the blastocysts stage or the development of oocytes when released from smaller preovulatory follicles vs oocytes released from larger preovulatory follicles (Lonergan et al., 1994). If the age of a preovulatory dominant follicle can affect fertility, then cattle exhibiting two follicular waves vs three follicular waves may have reduced fertility due to the length in days that a dominant follicle is present. A study by Ahmad et al. (1997) showed that pregnancy rate differences were not seen in cattle with two follicular wave patterns (84 %) vs cows with three follicular waves (100 %).

Heat stress has been reported to be a problem in reducing the competency of an oocyte; however fertilization has not necessarily been a factor (Putney et al., 1989). Exposure of pregnant females during embryonic development to heat stress will result in embryonic loss. Exposing embryos to heat shock led to embryonic death due to reduced protein synthesis (Edwards and Hansen, 1996).

Maternal recognition, or the amount of interferon tau (IFN-T) released from the conceptus around day 15 to 17 has been reported to affect embryonic mortality. It was found that size of the fetus in utero could dictate the amount of interferon tau released from the conceptus, with bigger fetuses secreting more IFN-T (Geisert et al., 1988). It was also shown that cows secreting higher levels of progesterone earlier after ovulation have increased amounts of IFN-T secreted at maternal recognition (Mann et al., 1998). Studies done to increase levels of IFN-T in circulation around the time of maternal recognition by administering IFN- α to cows after breeding have shown a small decrease

in fertility along with some physiological side effects (Barros et al., 1992). The effects of administering GnRH around day 11 to 14 to attempt to delay CL regression in order to decrease embryonic mortality by allowing time to develop adequate IFN-T synthetic capacity illustrated mixed reviews from work done by Peters et al. (2000).

INTRODUCTION TO RESEARCH

With less than 25 % of all cattle producers having incorporated any of the basic principles that scientists have recommended to effectively maximize reproduction to further improve the economics of their business (Ladewig, 1986), there is a gradual decline in pregnancy rates in cattle over the last 40 years. Anestrus is a major contributor to infertility in beef cows and prepubertal heifers (Short et al., 1990), yet the importance of early cyclicity in both heifers and cows relative to reproductive outcomes cannot be overemphasized. Currently, there is no consistent TAI synchronization protocol that exists for controlling ovulation in beef heifers or beef cows. Using alternative feed resources, such as dietary fat supplementation, has been reported to improve fertility in beef and dairy cattle. Pregnancy rates to AI have been shown to increase when plasma progesterone concentrations were elevated during the luteal phase of an estrous cycle after insemination (Butler et al., 1996). There have been positive influences in progesterone concentrations observed from both exogenous hCG and fat supplementation (Schmitt et al., 1996a,b; Diaz et al., 1998).

Three objectives were studied to evaluate maximizing fertility by introducing dietary fat supplementation, exogenous hCG injections and synchronization protocols into management programs. Chapter 2 describes the first objective; to determine if

supplementing whole safflower seeds high in oleic acid (C18:1) for an average of 55 d beginning 35 d prior to AI with an hCG injection post AI would increase fertility in beef heifers. Chapter 3 describes the second objective; to determine if pregnancy rates improve by delaying time-insemination to 72 h after PG in a Co-Synch protocol and to determine if a second injection of GnRH would increase fertility by targeting the window of estrous synchrony with calf removal. Chapter 4 describes the third objective; to determine if a second injection of GnRH increases the percentage of induced ovulations and improves fertility in beef heifers synchronized with a CO-Synch + CIDR protocol and mass mated at 54 h post PG injection.

CHAPTER 2

Effects of Feeding Supplemental Safflower Seed with Human Chorionic Gonadotrophin Following AI on Pregnancy Rates in Beef Heifers¹

INTRODUCTION

Replacement heifer development has been a challenge for producers in their efforts for breeding season preparations. Meeting a heifer's nutrient requirements during pre-breeding development is of critical importance and current research has indicated that supplemental fat may be an additional nutrient needed in the diet (Bellows, 1999). Primiparous beef cows supplemented with safflower seeds high in both oleic and linoleic acid increased first-service conception rates to AI (Lammoglia et al., 1997). A higher percentage of heifers' attained puberty earlier with an increase in BCS and backfat thickness when supplemented with safflower seeds high in linoleic acid (Lammoglia et

al., 2000). Various reports indicate that feeding diets high in oleic and linoleic acid have had positive (Lammoglia et al., 1997; Bellows et al., 2001), negative (Grant et al., 2003), and no effect (Funston et al., 2001; Bottger et al., 2002) on pregnancy rates in beef cattle.

Human Chorionic Gonadotropin (hCG), given exogenously, can induce a secondary corpus luteum from ovulated follicles, resulting in elevated serum progesterone concentrations (Schmitt et al., 1996a,b; Diaz et al., 1998). By incorporating a post breeding injection of hCG, it has been reported that pregnancy rates may increase (Brown et al., 1973) or not be affected (Hansel et al., 1976) in beef cattle.

Plant oil supplementation has been shown to increase concentrations of serum progesterone (Williams, 1989; Staples et al., 1998). There have been positive influences in progesterone concentrations observed from both exogenous hCG and fat supplementation (Schmitt et al., 1996a,b; Diaz et al., 1998); therefore, the objective of this study was to determine if supplementing whole safflower seeds high in oleic acid (C18:1) for an average of 55 d beginning 35 d prior to AI with an hCG injection post AI would increase fertility in beef heifers.

MATERIALS AND METHODS

Animal management. During the same year, nulliparous crossbred Red Angus and Composite beef heifers from separate breeding seasons, early (EBH; n=48, BCS=5.3 ± 0.33, BW=326.1 kg ± 30.5, age=13.5 months) and late breeding heifers (LBH; n=48, BCS=5.6 ± 0.41, BW=338.1 kg ± 31.1, age=13.5 months), were stratified by BW, BCS and age within a breeding season and assigned to treatments in a 2 x 2 factorial arrangement with factors being a control diet (CON) consisting of mixed alfalfa/grass hay

and oat grain or a safflower seed diet (SAFF) consisting of the control diet plus safflower seed high in oleic (69.9%) acid with hCG or saline injections given 5 d post AI. Diets were formulated to be isocaloric and isonitrogenous for both groups (Table 2.1) and hCG was given at 3,300 IU (i.m.; Chorulon; Intervet, Inc., Millsboro, DE) and saline solution was given at 3 mL (i.m.). Experimental protocol and synchronization treatments are labeled in Figure 2.1. Heifers were fed their respective diets beginning 35 d prior to AI and continued between 11 to 28 d post AI. Heifers in the CON and TRMT groups were housed in 54.9 x 67.1 meter and 33.5 x 36.6 meter pens and group fed their respective grain diets in feed bunks. After complete consumption of the respective grain diets, heifers were fed mixed alfalfa/grass hay in 34 kg square bales in the same feed bunks. All heifers were given free choice water, cobalt iodized salt and trace mineralized solar salt blocks. All heifers were synchronized using the 7-11 MGA Select Synch protocol (Figure 2.1). Heifers received melengestrol acetate (MGA; Pharmacia and Upjohn, Kalamazoo, MI) at $0.5 \text{ mg}\cdot\text{hd}^{-1}\cdot\text{d}^{-1}$ for 7 d (day -18), 25 mg (i.m.) injection of PGF_{2α} (PG; In-Synch, Pro Labs, St. Joseph, MO) at MGA removal (day -11), 100 μg (i.m.) injection of GnRH (Fertagyl, Intervet, Inc., Millsboro, DE) on day -7, and a second injection of PG on day 0. Heifers were monitored for estrous behavior twice daily for 1 hour at 06:00 and 18:00 h for 13 d and were artificially inseminated by one of two experienced AI technicians approximately 12 h after the onset of standing estrus. All heifers were diagnosed for pregnancy via transrectal ultrasonography 45 d following AI.

Sample collection. Heifer BCS (1 to 9; 1=emaciated and 9=obese; Whitman, 1975) and BW were assessed on all heifers at the beginning of MGA supplementation. Blood samples collected at different times during the study are illustrated in Figure 2.1.

Prior to both breeding seasons, two jugular vein blood samples were collected with sterile vacutainer tubes (Sherwood Medical, St. Louis, MO) prior to last PG injection (-35 and -28) to evaluate serum progesterone concentrations for cyclicity. Heifers were assumed to be cyclical before the onset of treatments if any one of the two samples contained serum progesterone concentrations ≥ 1 ng/mL. Jugular vein blood samples for serum progesterone analysis were also collected on days 4, 5, 6, 7, and 8 post AI from each heifer from both breeding seasons to determine progesterone concentrations as a result of post AI injection of hCG or sterile saline solution.

Three jugular vein blood samples were collected with sterile vacutainer tubes (Sherwood Medical, St. Louis, MO) during both breeding seasons and analyzed for fatty acid composition during the 35 d pre-breeding supplementation period (Figure 2.1): Sample 1, beginning of dietary supplementation (day -35); sample 2 (day -20); sample 3, at PG injection prior to AI (day 0). Three blood samples (d -35, -20 and 0) from four randomly selected heifers (two heifers diagnosed as pregnant to AI and two heifers diagnosed as open to AI) from each supplement group were then used for further fatty acid composition analysis. Specific fatty acids analyzed from each heifer for differences of percent weight in serum were: palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidonic (20:4), eicosapentaenoic (20:5) and docosahexaenoic (22:6) acid.

Analytical procedure. Blood samples collected for progesterone analysis were allowed to clot on ice within a 12 h period. Samples were then centrifuged at 486 x g at 4°C for 15 min. Serum was collected and stored at -20°C until analyzed for progesterone concentrations using a double antibody RIA (Niswender, 1973). Serum samples were

assayed in duplicate and within and between assay CV for serum samples was 13.4 and 4.7 across three assays, respectively. Sensitivity of the assay was 322 fg/mL.

All blood samples collected for fatty acid analysis were allowed to clot on ice within a 12 h period. Samples were then centrifuged at 456 x g at 4°C for 15 min. Serum was collected and stored at -20°C until analyzed for fatty acid composition. Percent weight of specific fatty acids in blood serum was analyzed as described by Burns et al., (2002).

Statistical analysis. Effects of breeding group (EBH vs LBH), BW, BCS and age on cyclicity status and effects of dietary supplement (CON vs SAFF), breeding group, diet x group interaction, cyclicity status, BW, BCS and age on estrous response were analyzed using Proc GENMOD procedure in SAS, (1996). Effects of dietary supplement, treatment (saline vs hCG), group, cyclicity status, BW, BCS and age on final pregnancy rates were analyzed using Proc GENMOD. Estrous response, cyclicity status and final pregnancy rate were analyzed as dependent variables. Significance was determined using Chi-Square at $P < 0.05$.

Data from heifers exhibiting an estrous response that were artificially inseminated were analyzed for main effect differences on conception rates. Preliminary analysis revealed no significant differences in conception rates between AI technician, dietary supplement, treatment and dietary supplement x treatment interactions, therefore data from both EBH and LBH were pooled and AI technician was not included in the final model. Effects of dietary supplement, treatment, group, sire, cyclicity status, BCS, BW, age, dietary supplement x cyclicity status and treatment x cyclicity status interactions on conception rates were analyzed using Proc GENMOD with conception rates analyzed as

the dependent variable. Differences in conception rates based upon dietary supplement, treatment, group, sire, cyclicity status and dietary supplement x treatment interactions were analyzed using differences in least squares means. Significance was determined using Chi-Square at $P < 0.05$.

Effects of dietary supplement, treatment, sample day, and all two and three way interactions for serum progesterone concentrations were analyzed using the Proc Mixed procedure in SAS (1996) with repeated measures. Differences in concentration of serum progesterone between dietary supplement, treatment and sample days were analyzed using differences in least squares means. Significance was determined at $P < 0.05$.

Effect of dietary supplement, sample, and dietary supplement x sample interaction on total weight percentage of fatty acids, for both breeding seasons, were analyzed using the Proc Mixed procedure in SAS (1996) with repeated measures. Differences in fatty acid composition of serum for dietary supplement and sample were compared using differences in least squares means. Significance was determined at $P < 0.05$.

RESULTS AND DISCUSSIONS

The percent of heifers cycling, with serum progesterone concentrations $> 1 \text{ ng/mL}$, at the beginning of MGA supplementation was 24 % (23/96) for both breeding seasons (data not shown). Cyclicity rates were not different ($P > 0.10$) between EBH (22.9 %) and LBH (25 %); however, overall cyclicity rates were low. The percent of heifers cycling between the CON (25 %) and TRMT (22.9 %) groups for both breeding seasons combined did not differ ($P > 0.10$). Use of a shorter blood sampling interval (7 days apart) may have underestimated the proportion of heifers cycling due to either short

cycles or because of differences in stages of the estrous cycle between heifers. If a heifer was in her proestrus stage at first sample collection (progesterone < 1 ng/mL), they may not have had elevated progesterone at the time of second sample collection (7 d later).

Overall estrous response from both breeding seasons was 79.2 % (76/96). Estrous response was similar ($P > 0.10$) for EBH (81.3 %; 39/48) vs LBH (77.1 %; 37/48) heifers. Estrous response was similar ($P > 0.10$) across both dietary treatment (CON; 83.3 %, 40/48 vs SAFF; 75.0 %, 36/48) groups (Table 2.2). Lammoglia et al., (2000) reported supplementing safflower seeds high in C18:2 to prepubertal F₁ heifers tended to increase the percentage of heifers becoming pubertal by the beginning of the breeding season. Our hypothesis was to initiate cyclicity in those late prepubertal heifers by supplementing a source of plant oil high in oleic acid as a dietary fat source. In the present study, differences in estrous response were not detected between dietary treatment groups; however, 83.3 % of the heifers from the CON group responded to synchronization vs 75.0 % of the heifers from the SAFF group. With a 24 % cyclicity rate and 79.2 % estrous response rate, it was evident that prepubertal heifers were induced to ovulate and exhibit a standing estrus from a combination of the supplement fed, MGA and GnRH with MGA and GnRH likely responsible for the increase in LH secretion that stimulates follicular growth and development and causes induced ovulations (Anderson et al., 1996; Imwalle et al., 1998). The positive response that Lammoglia et al. (2000) reported on percentage of heifers becoming pubertal was in part due to an increase in BCS and backfat thickness for those heifers fed high linoleate safflower seed versus a control diet. Hall et al. (1995) reported an increase in backfat thickness and BCS in beef heifers 75 d before reaching puberty. While changes in BCS

were not measured in the previous study, estrous response was high, relative to measured cyclicity rates, for both diet groups at the beginning of synchronization.

Final pregnancy rates for heifers from both breeding seasons were 91.7 % (88/96). Heifers from the LBH group (95.9 %; 46/48) tended ($P = 0.09$) to have higher pregnancy rates than heifers from the EBH group (87.5 %; 42/48). Final pregnancy rates were lower ($P < 0.05$) for heifers receiving hCG 5 d post AI (88.4 %; 38/43) vs heifers not receiving hCG post AI (97.6 %; 41/42). The reason hCG injected heifers had lower final pregnancy rates is unknown at this time. Pregnancy rates for all synchronized heifers were higher ($P < 0.05$) for EBH (65.0 %; 31/48) vs LBH (40.0 %; 19/48) and first-service conception rates for heifers exhibiting a standing estrus were higher ($P < 0.05$) for EBH (79.5 %; 31/39) vs LBH (51.4 %; 19/37).

There was no treatment (hCG vs saline) x dietary supplement (CON vs SAFF) x breeding season (EBH vs LBH) interaction for conception rates ($P > 0.10$), therefore data from both EBH and LBH were pooled. Conception rates for heifers in all four treatment groups from both breeding seasons were 65.8 % (50/76). Pregnancy rates were not affected by body condition score or BW ($P > 0.10$). The proportion of nulliparous heifers conceiving to AI were similar ($P > 0.10$) in the CON (67.5 %; 27/40) vs SAFF (63.9 %; 23/36) diet groups (Table 2.2). Bellows (1999) reported a positive response in fertility from lactating primiparous beef heifers when fed supplements high in linoleic acid during the last 60 to 75 days of gestation. This magnitude of response was, in part, due to quality of supplemental diet fed and avoiding the postpartum nutritional sink to initiate earlier postpartum cyclicity. Funston et al. (2001) reported no improvements on estrous response or pregnancy rates for nulliparous beef heifers in adequate body condition (BCS

5 to 6) supplemented with sunflower seeds. Due to the initial moderate body conditions of heifers in the present study (BCS: CON=5.4, SAFF=5.5), there seemed to be a lack of beneficial effect of oilseed supplementation on fertility which may be due to the lack of lactational stresses in virgin heifers.

The proportion of heifers pregnant to AI was similar ($P > 0.10$) for those receiving hCG (66.7 %; 26/39) or saline (64.9 %; 24/37) 5 d post AI. Breuel et al. (1989) reported increased pregnancy rates from heifers receiving hCG on day 4 of the pre-breeding cycle and 4 d post AI. Though estrous response was numerically higher for heifers receiving hCG in the current study, differences in conception rates were not seen. These results coincide with data reported by Schmitt et al. (1996b) who failed to observe an increase in pregnancy rates in fertile heifers when hCG was given 5 d post AI. Conception rates for heifers in the CON + hCG (68.2%; 15/22) group were numerically higher than heifers in the CON + saline (66.7%; 12/18), SAFF + hCG (64.7%; 11/17), and SAFF + saline groups (63.2%; 12/19), but were not statistically different ($P > 0.10$). As noted earlier, estrous response was higher for heifers in the CON group and for heifers that received hCG, which could have explained why CON + hCG heifers had numerically higher conception rates vs heifers from the other three treatment groups.

Jugular vein blood samples were collected from each heifer for five consecutive days beginning 5 d after AI to evaluate serum progesterone concentrations. Differences in progesterone concentrations averaged for all heifers over time between EBH and LBH were not detected ($P > 0.10$). Serum progesterone concentrations averaged for all heifers over time were not different for heifers in the SAFF (2.11 ng/mL) vs CON (1.93 ng/mL) groups ($P > 0.10$); however, progesterone concentrations were higher for hCG ($P < 0.05$)

vs saline injected heifers. This increase in serum progesterone from hCG injected heifers was expected due to hCG's ability to stimulate induction of secondary CL's on the ovary (Schmitt et al., 1996a,b; Diaz et al., 1998). Breuel et al. (1989) reported elevated serum progesterone concentrations from heifers given hCG on day 4 of the pre-breeding cycle and 4 d post AI. There was no treatment (saline vs hCG) x dietary supplement (CON vs SAFF) x time interaction for serum progesterone concentrations ($P > 0.10$); however, progesterone concentrations for heifers in the SAFF + hCG (2.03 ng/mL) group were higher ($P < 0.05$) over time than heifers in the CON + saline (1.37 ng/mL) and SAFF + saline (1.15 ng/mL) groups, but similar ($P > 0.10$) for heifers in the SAFF + hCG vs CON + hCG (1.63 ng/mL) groups (Figure 2.2). Progesterone concentrations increased ($P < 0.05$) from d 4 to d 8 post AI and serum progesterone concentrations for hCG injected heifers were approximately twice as high (2.61 ng/mL) as saline injected heifers (1.37 ng/mL). This rise in progesterone following hCG injection coincides with work from Schmitt et al. (1996b) and Diaz et al. (1998) where they reported a dramatic rise in plasma progesterone concentrations beginning by d 8 or 9 of the estrous cycle.

Jugular vein blood samples were collected three times during the 35 d feeding period prior to AI to determine percent weight of fatty acids with respect to dietary supplement fed. Serum fatty acid composition was similar over time ($P > 0.10$) between the randomly selected heifers from both breeding seasons; therefore data from heifers in both EBH and LBH groups were pooled. Serum fatty acids C16:0, C18:2, C20:4, C20:5 and C22:6 were similar ($P > 0.10$) for both diet groups over time. Percent weight of C16:0 and C18:2 in blood serum did not vary over time due to the low levels of C16:0 and C18:2 supplemented from the safflower seeds and high levels supplemented from the

oats. Changes in C20:4 not observed from both diets over time could have been due to the low levels of C20:4 observed in both diets supplemented. Changes in C20:5 and C22:6 from both diets were not observed because of the low percentage of omega 3 fatty acids supplemented in the diet (Mattos et al., 2000). Both C20:5 and C22:6 cannot be synthesized in the body and thus have to be supplemented in the diet due to the lack of desaturation of fatty acids at positions greater than the $\Delta 9$ positions (Cook, 1996). There was no dietary supplement (CON vs SAFF) x time interaction ($P > 0.10$) for percent C18:0 in blood serum. Percent weight of C18:0 increased ($P < 0.05$) over time in the blood. Percent weight of C18:0 tended ($P = 0.09$) to be higher for heifers fed the SAFF vs the CON diet over time. Composition of C18:0 in serum of heifers receiving CON or SAFF diets at the beginning of supplementation was low, but due to biohydrogenation of unsaturated fatty acids in the ruminant of cattle, C18:2 is typically hydrogenated to C18:0, a saturated fatty acid, by ruminal bacteria to alleviate toxicity in the rumen (Harfoot and Hazelwood, 1988), and therefore increases duodenal flow of C18:0. Therefore the increase in C18:0 is likely due to biohydrogenation of unsaturated fatty acids. There was a dietary supplement (CON vs SAFF) x time interaction ($P < 0.05$) on percent C18:1 in blood serum. Percent weight of C18:1 increased ($P < 0.05$) over time for heifers fed the SAFF vs CON diet, (Figure 2.3). Serum samples collected on d 0 in the SAFF diet were higher ($P < 0.05$) in C18:1 than samples collected on d -35, -20 and 0 in the CON diet, and samples collected on d -35 and -20 in the SAFF diet (Figure 2.3). Serum samples collected on d -20 in the SAFF diet were also higher ($P < 0.05$) in C18:1 than samples collected on d -35 in the CON and SAFF diets. Because oleic acid in safflower seeds fed is high (69.9 %), C18:1 increased significantly over time for the

SAFF fed heifers vs the CON fed heifers. There was no dietary supplement (CON vs SAFF) x time interaction ($P > 0.10$) on percent C18:3 in serum. Percent weight of C18:3 decreased ($P < 0.05$) over time in serum from heifers receiving CON or SAFF diets. A decrease in percent weight of C18:3 in serum for heifers receiving CON or SAFF diets over time was expected due to the percent of linolenic acid in the alfalfa/grass hay that was fed for both diets (Table 2.3). Hay and grain base diets typically have lower α -linolenic acid as compared to forages.

Anestrus is a major contributor to infertility in beef cows and prepubertal heifers (Short et al., 1990), but the importance of early cyclicity in both heifers and cows relative to reproductive outcomes cannot be overemphasized. Heifers in the previous study were in moderate body condition and estrous response was not influenced by dietary supplementation, so the lack of response in fertility is not surprising. Age of an animal dictates the timing and diet alters the amplitude of lipogenesis that can affect body condition over time (Smith et al., 1987). Age and diet play a major role in controlling the effects of supplementation of dietary fats on reproductive performance, and it seems that supplementing fats prior to breeding in beef heifers has little reproductive benefits. Although hCG positively influenced serum progesterone levels in heifers post AI, fertility was not affected.

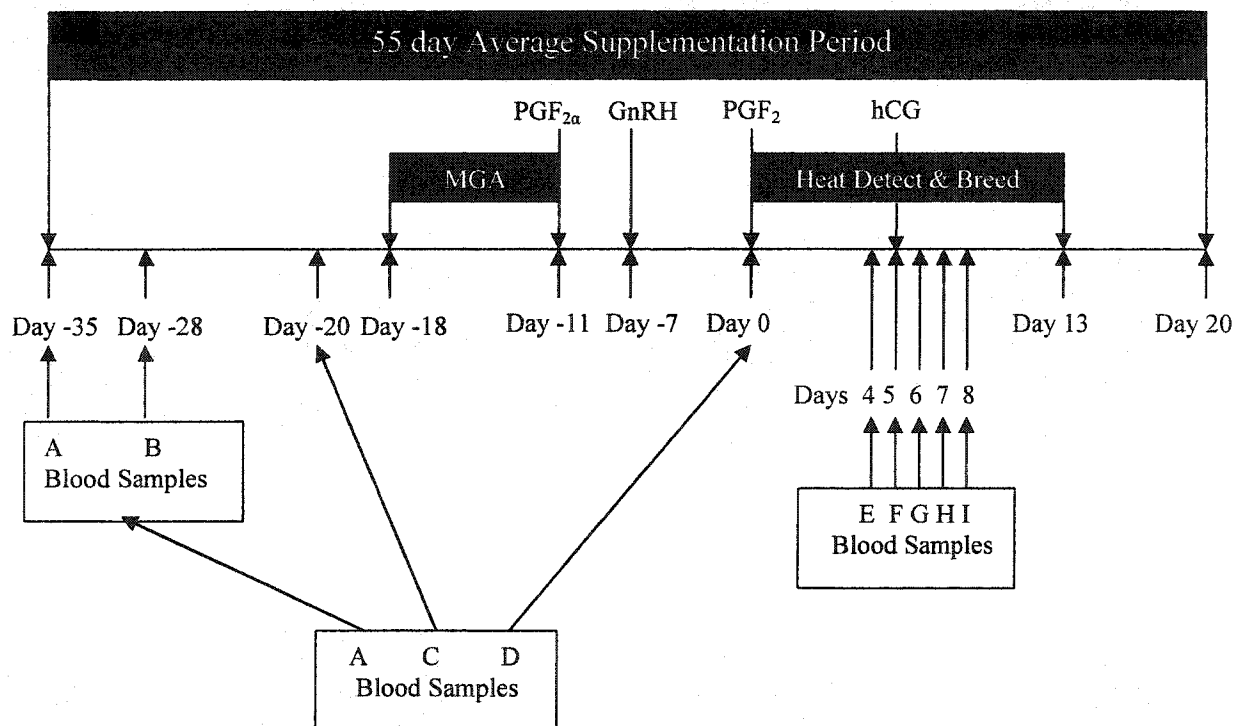


Figure 2.1. Experimental protocol for synchronization of beef heifers using the 7-11 MGA Select Synch protocol with dietary supplementation and hCG injection treatments. Blood samples A, B, E, F, G, H and I were collected for serum progesterone and blood samples A, C and D were collected for fatty acid composition. GnRH = gonadotropin-releasing hormone; PGF_{2α} = prostaglandin F_{2α}; hCG = human chorionic gonadotropin.

Table 2.1. Ingredients and chemical analysis of experimental diet.

Item	Diet	
	Control	Safflower
Ingredient	% DM ^a , (kg fed)	
Alfalfa/Grass Hay	78 ^a , (6.1) ^b	75.2 ^a , (5.9) ^b
Oat Grain	22, (1.7)	12.7, (1.0)
Safflower Seed	--	12.1, (0.96)
Proximal Analysis ^c		
Dry Matter, %	92.6	92.8
Crude Protein, %	18.3	18.2
Ether Extract, %	2.5	5.4
TDN, %	60.8	58.3
Calcium, %	0.9	0.9
Phosphorus, %	0.3	0.3

^aPercentage of diet; DM basis. Heifers fed alfalfa/grass hay in the form of square bales (34.1 kg) after grain was consumed. Water, salt and trace mineral were fed ad libitum.

^bAmount of ingredient fed in kilograms.

^cAnalysis of feed samples prior to dietary supplementation

Table 2.2: Characteristics of mean averages for treatment (control diet (CON), safflower seed diet (SAFF), hCG injection, or saline injection) and treatment interaction on estrous response (ER, %) and conception rate (CR, %) for both early (EBH) and late (LBH) breeding heifers.

	EBH			LBH			Total		
	ER (%) N=48 ^b	N ^a	CR (%)	ER (%) N=48 ^b	N ^a	CR (%)	ER (%) N=96 ^b	N ^a	CR (%)
Overall	81.3	39	79.5	77.1	37	51.4	79.2	76	65.8
CON	83.3	20	80.0	83.3	20	55.0	83.3	40	67.5
SAFF	79.2	19	78.9	70.8	17	47.1	75.0	36	63.9
Saline	----	19	78.9	----	18	50.0	----	37	64.9
hCG	----	20	80.0	----	19	52.6	----	39	66.7
CON + Saline	----	8	87.5	----	10	50.0	----	18	66.7
CON + hCG	----	12	75.0	----	10	60.0	----	22	68.2
SAFF + Saline	----	11	72.7	----	8	50.0	----	19	63.2
SAFF + hCG	----	8	87.5	----	9	44.4	----	17	64.7

^aNumber of heifers in each treatment (CON vs SAFF; saline vs hCG) group or treatment group combined within each breeding season and breeding seasons combined.

^bTotal number of heifers synchronized for estrus within breeding season and breeding seasons combined. No effect of dietary supplement on estrous response; or dietary supplement, hCG/saline or treatment interaction on conception rates ($P > 0.05$).

Table 2.3: Fatty acid composition in dietary supplements.

Fatty Acid	Oats	Safflower	Alfalfa/Grass
Ether Extract, %	5.6 ^a	31.1 ^a	---
C16:0	17.8 ^b	5.5 ^b	7.6 ^b
C18:0	2.8	1.8	1.4
C18:1	36.1	69.9	5.6
C18:2	40.2	19.5	7.5
C18:3	2.0	0.2	14.8
C20:4	0.0	0.1	0.0
C22:6	0.0	0.0	0.0

^aPercent of ether extract for each supplement fed based on 100 % dry matter.

^bPercent weight of fatty acid present in dietary supplement.

Palmitic (C16:0), Stearic (C18:0), Oleic (C18:1), Linoleic (C18:2), Linolenic (C18:3), Arachidonic (C20:4), and Docosaehaenoic (C22:6) acid analyzed in dietary supplement.

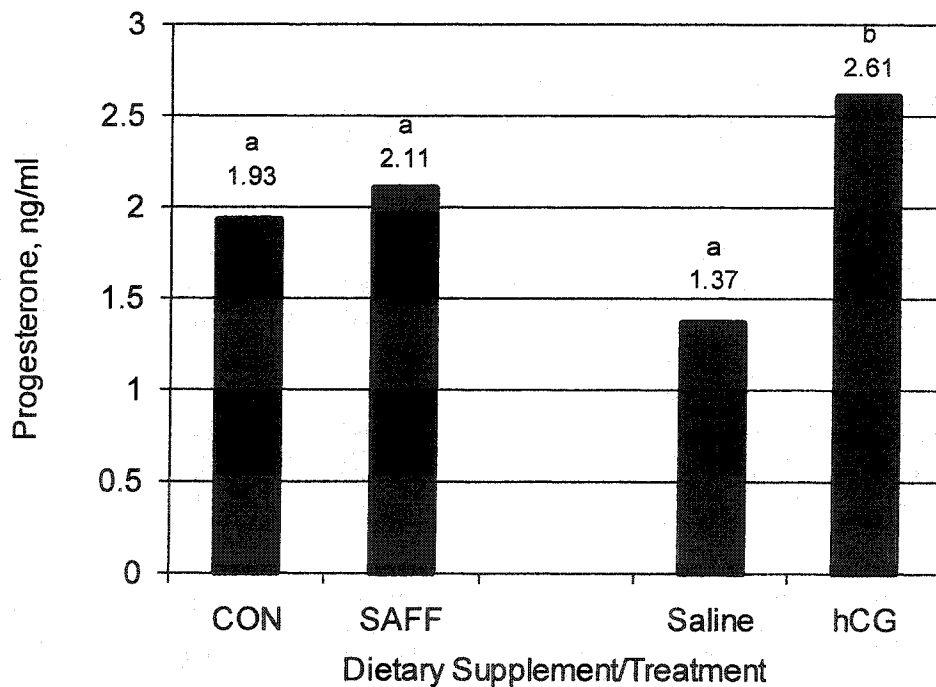


Figure 2.2: Effects of dietary supplement (CON vs SAFF) and treatment (saline vs hCG) groups on serum progesterone concentrations for all heifers in both breeding seasons over time. Blood samples were collected for 5 consecutive days starting 4 days post AI for each heifer and averaged together for all heifers within each treatment to look at main effects. Means within dietary supplement and treatment without a common letter (a,b) differ ($P < 0.05$)

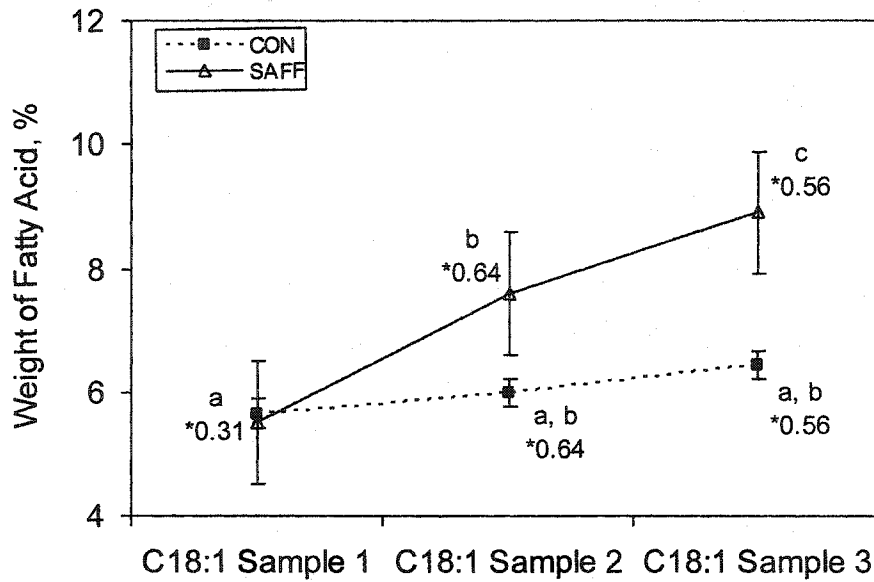


Figure 2.3: Effects of dietary supplement, control (CON) and safflower diet (SAFF), on percent weight of fatty acid in blood serum for Oleic (C18:1) acid over time from four randomly selected heifers in each supplemented group for both breeding seasons. Blood samples were averaged together for all heifers for each sample in each diet group for both breeding seasons and were collected at three different times during the 35 d pre-breeding supplementation period: Sample 1) beginning of dietary supplementation (day -35); Sample 2) 15 d later (day -20); Sample 3) at PG injection prior to AI (day 0). *Standard errors for each sample in each diet group. Means without a common letter (a, b, c) differ ($P < 0.05$).

CHAPTER 3

Synchronization of Estrus in Postpartum Beef Cows with or without GnRH at 48 or 72 H Timed Insemination after PGF using the Co-Synch Protocol¹

INTRODUCTION

Estrous synchronization has become a powerful tool in managing the breeding season, but can become labor intensive when utilizing estrous detection for artificial insemination (Hixon et al., 2001). However, research has shown that an alternative method, timed insemination, can produce pregnancy rates comparable to synchronization protocols that require detection of estrus (Geary and Whittier, 1998; Perry et al., 2002; Hixon et al., 2001). In order to utilize this alternative method, GnRH was incorporated with PGF_{2α} to coordinate follicular growth and luteal regression in order to synchronize ovulation (Select Synch; Geary et al., 2000). Administering GnRH induces an LH surge

that stimulates luteinization or ovulation of dominant follicles (Twagiramungu et al., 1992a; Pursley et al., 1995). A modified version of the Ovsynch protocol, Co-Synch (Geary and Whittier, 1998), incorporates a second injection of GnRH at 48 h TAI after PG to induce ovulation. Geary et al. (2001) indicated that incorporating TAI at 72 h post PG injection may be feasible if the 72 h estrous response is high with no improvements in fertility rates by incorporating GnRH at TAI. If the average interval to standing estrus is 70 h after PG injection (Geary et al., 2000), then GnRH may not be necessary to improve fertility at TAI. However, pregnancy rates from fixed-time AI were acceptable when GnRH was administered at 54 and 72 h TAI in beef cows (Twagiramungu et al., 1995; Perry et al., 2002).

Calf removal has become an important part of breeding season management for synchronization programs in many countries and the practice of calf removal in beef cows prior to breeding has increased in the US over the years. Geary et al. (2001a) suggested that 48 h calf removal with the Co-Synch protocol induces fertile ovulation in cyclic and non-cyclic cows and enhances fertility. Therefore, the objectives of this study were to determine if pregnancy rates improve by delaying timed-insemination to 72 h after PG in a Co-Synch protocol and to determine if a second injection of GnRH at TAI would increase fertility by targeting the window of estrous synchrony with calf removal.

MATERIALS AND METHODS

Animal Management. Suckling, multiparous Composite and Hereford beef cows (n=226, postpartum interval, PPI=67 ± 19.3 d, body condition score, BCS=5 ± 0.4) were synchronized for timed AI (TAI) with the Co-Synch protocol and calf removal. Cows

from the herd have been separated by calving season, two months apart, for an ongoing calving season study. Cows from both calving seasons were treated within the same year and analyzed equally. Early calving cows (ECC; n=97, PPI=67 ± 16.2 d, BCS=5.2 ± 0.4) and late calving cows (LCC; n=129, PPI=67 ± 21.3 d, BCS=4.9 ± 0.4) were given 100 µg (i.m.) of GnRH (Fertagyl, Intervet., Inc., Millsboro, DE) on day 0 and 25 mg (i.m.) of PGF_{2α} (PG; In-Synch, Pro Labs, St. Joseph, MO) on day 7. Calves from all cows were removed at PG injection and returned to their dams the day of TAI. In a 2 x 2 factorial arrangement, animals were randomly assigned to one of four treatment groups: 1) 48 h TAI post PG with GnRH (48-TAI-G); 2) 48 h TAI post PG without GnRH (48-TAI); 3) 72 h TAI post PG with GnRH (72-TAI-G); 4) 72 h TAI post PG without GnRH (72-TAI). Cows from both calving seasons were inseminated by one of three experienced AI technicians. Cleanup bulls were turned out 14 days post 72 h TAI and left in with all cows for 60 days. All cows were diagnosed for pregnancy via transrectal ultrasonography 33 days following 72-TAI.

Sample collection. Cow BCS (1 to 9; 1=emaciated and 9=obese; Whitman, 1975) were assessed on all cows at start of estrous synchronization.

Statistical analysis. Preliminary analysis revealed no significant differences in pregnancy rates between treatments (48 vs 72 h TAI and GnRH vs no GnRH at TAI), treatment interaction or AI technician for both calving seasons (ECC and LCC), therefore data from both ECC and LCC were pooled and AI technician was not included in the final model. Effect of treatment, treatment interaction, sire, BCS and PPI on pregnancy rates were analyzed using Proc GENMOD procedure in SAS (1996) with differences in

pregnancy rates between treatments, treatment interactions and sire analyzed using least squares means. Significance was determined using Chi-Square at $P < 0.05$.

RESULTS AND DISCUSSION

Final pregnancy rates for cows in the early and late calving seasons were 90.7 and 83.7 %, respectively. Timed AI pregnancy rates for ECC (43.3%) and LCC (43.4%) were similar ($P > 0.10$), therefore data from both calving seasons were pooled to analyze for main effects. Combined AI pregnancy rates for all four treatment groups were 43.4% (98/226). Treatment (day x GnRH) interaction, BCS and PPI among treatments on TAI pregnancy rates were not different ($P > 0.10$). The proportion of pregnant cows to TAI was lower ($P < 0.01$) at 48 vs 72 h (Figure 3.1). The increase in fertility for cows inseminated at 72 h was likely due to the majority of cows exhibiting a delayed response to PG (average interval to estrus with the Select Synch protocol was 70 h; Geary et al., 2000). Pregnancy rates were reported to be higher in cows synchronized with the Ovsynch protocol (72 h TAI) vs cows synchronized with the Co-Synch protocol (48 h TAI) with differences in fertility likely due to inseminating closer to the time of average interval to ovulation (Geary and Whittier, 1998).

Cows receiving a second injection of GnRH at both 48 and 72 h post PG had higher pregnancy rates to TAI (50.8%; $P < 0.05$) vs cows that did not receive a second injection of GnRH at TAI (34.9%; Figure 3.1), respectively. If the response to PG is delayed until 72 h or later, this delayed response could have allowed for the second injection of GnRH to initiate a pre-ovulatory LH surge (Twagiramungu et al., 1995) that induced ovulation in cows with a dominant follicle present at the time of GnRH injection

(Thompson et al., 1999; Wood et al., 2001). Twagiramungu et al. (1995b) suggested that synchronizing beef cows using a GnRH-PG-GnRH system with TAI eliminated the need for heat detection with an average interval from PG to standing estrus of 56.2 h. With respect to GnRH, 75% of anestrus cows formed new luteal tissue and more than 85% ovulated in response to a second injection of GnRH 48 h after the Select Synch protocol (Thompson et al., 1999).

Pregnancy rates were higher ($P < 0.05$) for cows in the 72-TAI-G (54.9%) treatment group compared to cows in the 72-TAI (43.6%), 48-TAI-G (39.3%) and 48-TAI (25.5%) treatment groups; however, there were no differences ($P > 0.10$) in pregnancy rates for 72-TAI vs 48-TAI-G and 48-TAI, but cows bred at 48-TAI had the lowest overall mean pregnancy rate percentages (Table 3.1). The addition of a second injection of GnRH may have induced ovulation in cows not responding to PG because of low progesterone levels (regressing or developing CL) or cows that were anestrus. The first injection of GnRH either luteinized smaller growing follicles or induced ovulation of a dominant follicle present on the ovary and development of new growing follicles (Schmitt et al., 1996) with cows receiving a second injection of GnRH showing improved fertility because of induced ovulations in cows not responding to PG. Pregnancy rates were different for 72-TAI-G vs 72-TAI, 48-TAI-G, with 48-TAI having fewer cows conceiving to AI. These results would suggest that delaying TAI to 72 h post PG injection may improve pregnancy rates (Perry et al., 2002; Geary et al., 2001), and incorporation of a second injection of GnRH at TAI may increase the percentage of cows ovulating just after TAI (Wood et al., 2001).

Pregnancy rates for cows inseminated to sires B (51.8%) and C (54.3%) in all four treatment groups from both calving seasons were higher ($P < 0.05$) compared to cows inseminated to sire A (33.3%; Figure 3.2). The variation in pregnancy rates for the three sires used was consistent between the different treatment groups. Whether TAI occurred at 48 or 72 h or GnRH was or was not given at TAI did not affect the poor fertility seen from cows inseminated from sire A; however pregnancy rate percentages were slightly higher in cows inseminated from sire A when insemination occurred at 72 h (40%) or given GnRH at TAI (42%) vs insemination at 48 h (27%) or not given GnRH at TAI (26%; Figure 3.3). Fertility rates varied when several AI bulls were used for fixed time AI at 0, 12 or 24 h after first standing estrus in dairy cows (Dalton et al., 2001). These sire differences seen can impact pregnancy rates to a TAI synchronization protocol (Saacke, 2002) and affect consistency between producers that use the same protocols with different AI bulls.

Incorporating 48 h calf removal has shown to improve pregnancy rates in a TAI synchronization system (Geary et al., 2001a). Induction of ovulation in postpartum beef cows due to increased GnRH and LH pulse frequencies can occur by incorporating calf removal with a GnRH synchronization protocol (Williams, 1990). Beck et al. (1979) showed that 48 h calf removal does not affect weaning weights or long-term growth rates of weaned calves. Although the current study did not test removal vs no removal of calves for 48 or 72 h, there were no difficulties reported with pairs mothering up or calves nursing. Because synchronized pregnancy rates were higher for cows mass mated at 72 h, incorporating calf removal with 72 h TAI may provide alternative management practices that yield acceptable fertility levels with a tighter calving season.

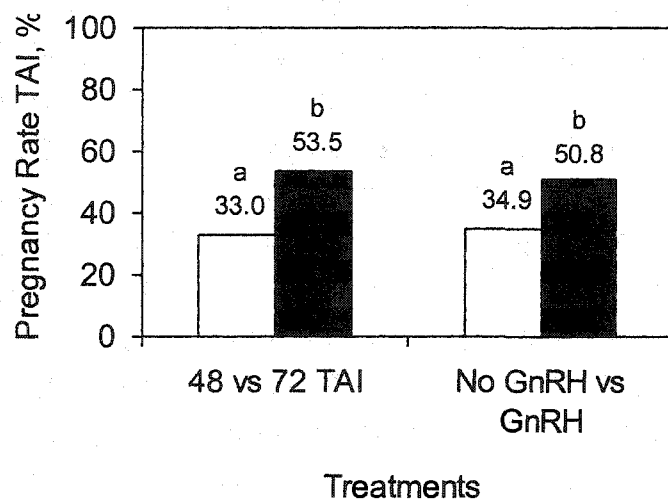


Figure 3.1: Effect of insemination time (48 h, open bar; 72 h, shaded bar) and GnRH injection (TAI; No GnRH, open bar; GnRH, shaded bar) on pregnancy rate to TAI. Means without a common letter within each treatment group (a, b) differ ($P < 0.05$).

Table 3.1. Characteristics of cattle bred TAI within each treatment group.

Characteristic	48-TAI	48-TAI-G	72-TAI	72-TAI-G
No. Head	51	61	55	59
Mean BCS	5.1 ± 0.5	5.0 ± 0.5	5.1 ± 0.4	5.1 ± 0.4
Mean PPI, d	67.4 ± 22.2	66.9 ± 17.0	68.2 ± 20.0	67.3 ± 18.6
Pregnancy rate, %	25.5 ^a	39.3 ^a	43.6 ^a	62.7 ^b

Means in a row for pregnancy rate, % without a common letter (a, b) differ ($P < 0.05$).

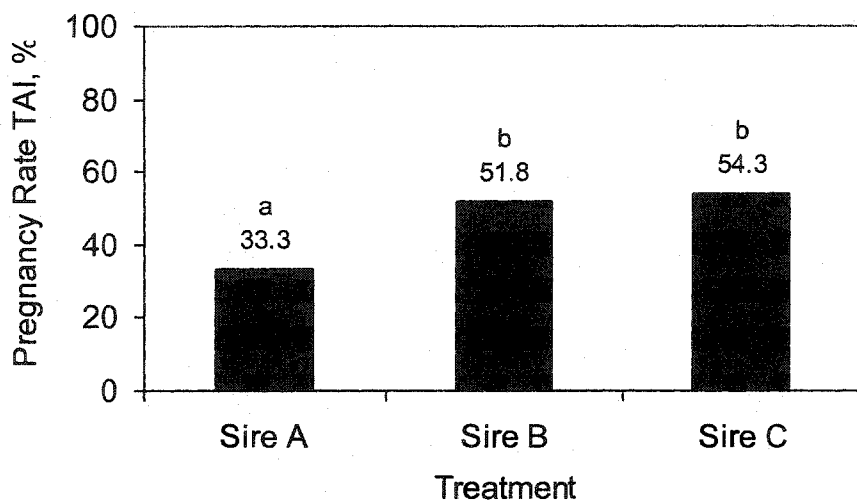


Figure 3.2: Pregnancy rates from cows inseminated to sires A, B and C for both seasons combined (ECC and LCC). Means without a common letter (a, b) differ ($P < 0.05$).

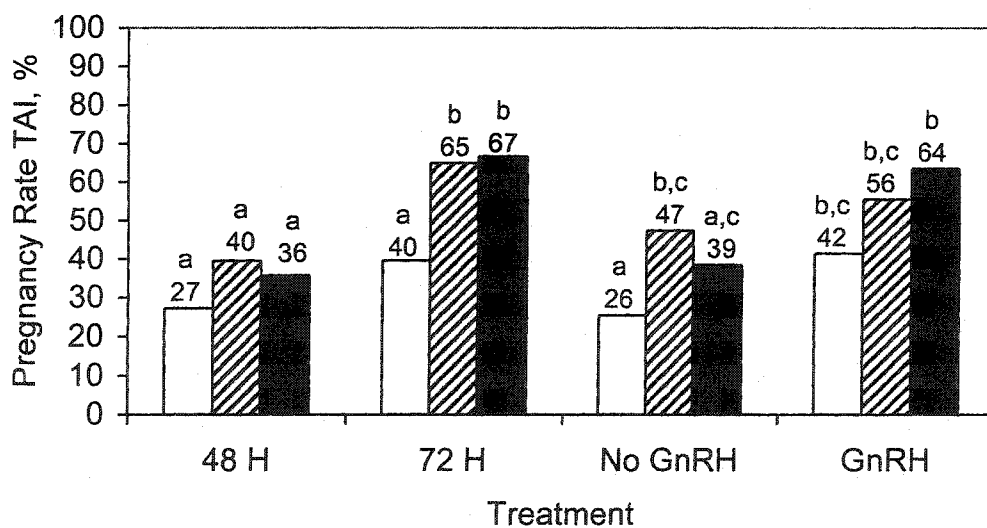


Figure 3.3: Pregnancy rates for cows inseminated to sires A (open bars), B (cross bars) and C (shaded bars) at 48 vs 72 h and for cows with or without receiving GnRH at TAI from both seasons combined. Means within each treatment (48 vs 72 h or no GnRH vs GnRH) without a common letter (a, b, c) differ ($P < 0.05$).

CHAPTER 4

Fertility in beef heifers synchronized using a modified Co-Synch plus CIDR protocol with or without GnRH at timed AI

INTRODUCTION

Estrous synchronization has become a powerful tool in managing breeding seasons, but may be labor intensive when utilizing visual observation for standing estrus (Hixon et al., 2001). Past approaches to synchronization have included the use of both gonadotropins and prostaglandins; however, variations in estrous response (Moreira et al., 2000), interval to standing estrus (Geary et al., 2001) and missed standing heats (Hixon et al., 2001) have influenced pregnancy outcomes from fixed-time AI protocols. Some of these responses are dependent upon the stage of the estrous cycle when GnRH and/or PG are given (Geary et al., 2000).

Schmitt et al. (1996) reported no differences in a 4-day estrous response period, but conception rates were reduced by 16 % when beef heifers were synchronized with a 9 d CIDR insert and PG without an initial injection of GnRH. Schmitt also reported that the average interval from PG to standing estrus was 47.3 h. Twagiramungu et al. (1995b) reported higher pregnancy rates in beef heifers synchronized with a modified Select Synch protocol with the addition of a second injection of GnRH at 54 h timed AI vs no GnRH at TAI. With respect to GnRH, 75 % of anestrous cows formed new luteal tissue and more than 85 % ovulated in response to a second injection of GnRH 48 h after a Select Synch protocol (Thompson et al., 1999).

When combining progestin with a gonadotropin and prostaglandin, estrous response was highest between 48 and 72 h after PG in pubertal heifers receiving MGA + PG or Select Synch (Wood et al., 2001). Martinez et al. (2002) reported a 29 % increase in pregnancy rates for beef heifers synchronized with a CO-Synch + CIDR protocol vs a CO-Synch protocol. The objective of this study were to determine if a second injection of GnRH increases the percentage of induced ovulations and improves fertility in beef heifers synchronized with a CO-Synch + CIDR protocol and mass mated at 54 h post PG injection.

MATERIALS AND METHODS

Animal management. Nulliparous crossbred beef heifers from one cooperator herd in South Dakota (SD; n = 211, BW = 392.3 ± 31.0 kg, body condition score, BCS = 5.7 ± 0.30) and two research station herds in Colorado (CO; n = 39, BW = 324.5 ± 29.5 kg, BCS = 5.7 ± 0.27) and Wyoming (WY; n = 125, BW = 325 ± 27.8 kg, BCS = 5.4 ±

0.50) were used to determine if a second injection of GnRH 54 h following CIDR removal increases the percentage of induced ovulations and improves timed AI (TAI) pregnancy rates. Heifers were synchronized with the Co-Synch plus EAZI BREED CIDR[®] (CIDR; 1.38 g of progesterone; Pharmacia Animal Health, Kalamazoo, MI) protocol and stratified by BW within BCS to be randomly allotted to one of two treatment groups. All heifers received 100 µg (i.m.) of GnRH (Fertagyl, Intervet., Inc.) with a CIDR insert on day 0, followed by CIDR removal and 25 mg (i.m.) of prostaglandin F_{2α} (PG; Lutalyse, Pharmacia Animal Health, Kalamazoo, MI) on day 7. At 54 hours post PG administration (d 9), heifers in the control (CON) and treatment (TRMT) groups were mass mated and heifers in the TRMT group were given a second injection of GnRH at that time. Heifers were assigned by weight class (90.9 kg increments) as either 1 (227.3 to 317.7 kg), 2 (318.2 to 408.6 kg) or 3 (409.1 to 499.5 kg) for each location to analyze for differences in pregnancy rates between weight classes. Heifers at the SD location were assigned to a time group (1 to 6; 1 = 54 h and 6 = 59 h) based on the hour bred at TAI beginning 54 h post PG injection to analyze for differences in pregnancy rates after AI over time. All heifers were diagnosed for pregnancy to AI via transrectal ultrasonography 45 d post TAI. Cleanup bulls were turned out 8-14 d after mass mating and left in for 45 d.

Sample collection. Body condition scores (1 to 9; 1 = emaciated and 9 = obese; Whitman, 1975) and body weights were assessed on all heifers at time of CIDR insertion (day 0). Ovaries from a subset of heifers at CO (n = 19) and WY (n = 49) were examined by transrectal ultrasonography to characterize diameter of the largest follicle and incidence of ovulation relative to the two treatment groups (GnRH vs no GnRH). Heifers

were scanned at time of CIDR removal (day 7) to determine the presence and location (left or right ovary) of a developing dominant follicle. While ultrasounding heifers at CIDR removal, follicular cysts were detected in 1 heifer from CO and 3 heifers from WY. These animals were removed from the study, leaving 18 (CO) and 46 (WY) heifers available for analysis. Follicles were classified as cystic if the diameter of the largest follicle was > 25 mm (Savio et al., 1990) at TAI. At 48 h (d 9), ovaries were scanned for a second time to determine diameter of the ovulatory follicle. Diameter of the ovulatory follicle was calculated as the mean of the horizontal and vertical diameters of the largest follicle, measured in mm. Follicles were classified as dominant if the diameter of that follicle was ≥ 10 mm (Ginther et al., 1989; Savio et al., 1990). Follicles classified as dominant were then sorted into 4 follicle size classes (1 = 10 to 11.9 mm, 2 = 12 to 13.9 mm, 3 = 14 to 15.9 mm and 4 = ≥ 16 mm; Lamb et al., 2001). At 40 h following TAI (d 11), ovaries were scanned for a third time to determine whether ovulation had occurred. Ovulation was defined as the disappearance of a large dominant follicle present on the ovary at time of insemination. Ovaries were scanned using transrectal ultrasonography (5-MHz intrarectal transducer, Aloka 500V, Corometrics, Wallingford, CT).

Heifers from CO (n = 39) were visually observed for 1 h three times daily from day 6 (24 h prior to PG) to 10 (72 h after PG) to characterize estrous response. Estrous response was used to calculate pregnancy rates for specific times of estrus relevant to TAI. Statistical analysis was not used to analyze these comparisons.

Prior to synchronizing, two jugular vein blood samples were collected with sterile vacutainer tubes (Sherwood Medical, St. Louis, MO) from all heifers at CO and WY 10 days apart (d -10 and 0) to determine cyclicity status. Heifers were assumed to be

cyclical before the onset of treatments if any one of the two samples contained concentrations of serum progesterone ≥ 1 ng/mL.

Analytical procedure. All blood samples collected for progesterone analysis were allowed to clot on ice within a 12 h period. Samples were then centrifuged at 486 x g at 4°C for 15 min. Several samples developed fibrin clots, so individual samples were stirred using a separate plastic pipette for each clotting sample and then re-centrifuged at 486 x g at 4°C for 15 min. Serum was collected and stored at -20°C until analyzed for progesterone concentration by solid-phase radioimmunoassay (RIA; Diagnostic Products Corp., Los Angeles, CA). Serum samples were assayed in duplicate and sensitivity of the assay was 0.08 ng/ml. Within and between assay CV for serum samples was 12.86 and 9.6 % across two assays, respectively.

Statistical analysis. Effects of treatment (GnRH vs no GnRH) and location (CO and WY) on final pregnancy rates were analyzed using the GENMOD procedure in SAS (1996). Effect of BW and BCS on final pregnancy rates were analyzed as covariates with significance determined using Chi-square at $P < 0.05$.

Preliminary analysis revealed no differences in pregnancy rates for AI technicians within and between locations or treatment x technician interaction; therefore AI technician was not included in the final model. Main effects were included as fixed effects with BW and BCS analyzed as covariates and pregnancy rate analyzed as the response variable. Preliminary analysis revealed a location effect on treatment, therefore data were not pooled and the main effects were evaluated within each location. Effects of treatment, sire, cyclicity rate and weight class on pregnancy rates were analyzed with Proc GENMOD procedure in SAS (1996) using differences in least squares means to

compare pregnancy rates between treatments, sire, cyclicity rate and weight class. AI sire was then included in all models as a random effect. Effects of BCS and BW on cyclicity rate were analyzed using Proc GENMOD. Effects of treatment, time of insemination (hour 1 to 6; 1 = 54 h and 6 = 59 h) and treatment x time interaction on pregnancy rates for the SD location was analyzed with Proc GENMOD using differences in least squares means to compare pregnancy rates between hour and within treatments. Significance was determined using Chi-square at $P < 0.05$.

All follicle data for heifers at CO and WY locations were analyzed using Proc GLM in SAS. The effects of treatment, location, treatment x location, BW and BCS on incidence of ovulation were analyzed. Pregnancy rate differences based on treatment, location, follicle diameter class, incidence of ovulation, BCS, BW, treatment x location, treatment x follicle diameter class, location x follicle diameter class and treatment x location x follicle diameter class interactions were analyzed for all heifers and for heifers that had ovulated at both locations. Both incidences of ovulation and pregnancy rates were analyzed as response variables while treatment, location, follicle diameter class and incidence of ovulation were analyzed as fixed effects. Significance was determined at $P < 0.05$.

RESULTS

Estrous response. Heifers at CO were heat detected three times daily 1 d prior to (d 6) and up to 3 d after PG injection (d 10) to determine the percent of standing estrus within a specific time period. There were no observed heifers in standing estrus until 36 h after CIDR removal and the average interval from PG to first observed standing estrus

was 51 h. (Figure 4.1). Estrous response was 64.1 % (25/39) within a 24 h period and 71.8 % (28/39) within a 36 h period beginning 36 h after CIDR removal. Pregnancy rates for heifers exhibiting estrus within 24 h was 76 % and heifers exhibiting estrus within 36 h was 67.9 %. The percentage of heifers pregnant that did not exhibit a standing estrus by 72 h after CIDR removal was 18.2 %.

Cyclicality status. Jugular blood samples collected twice prior to CIDR insertion (10 d apart) for CO and WY heifers indicated an average 58.5 % of the heifers were cycling prior to synchronization treatments (Table 4.1). The proportion of heifers cycling was lower ($P < 0.01$) for WY (46.4 %; 58/125) vs CO (97.4 %; 38/39) heifers. The proportion of heifers cycling was similar ($P > 0.10$; Table 4.2) between CON and TRMT heifers at both CO (100 and 95 %) and WY (47.6 and 45.2 %).

Fertility. The proportion of cycling and non-cycling heifers pregnant to AI was similar ($P > 0.10$) at both CO and WY locations (Table 4.1). Pregnancy rates for non-cycling heifers at the CO location was 0 %, however because of the high cyclicality rates seen at that location, there was only one heifer that was not cycling at the beginning of synchronization in which she failed to conceive. The proportion of cycling and non-cycling heifers pregnant to AI did not differ ($P > 0.10$) between CON vs TRMT groups at CO and WY (Table 4.2).

Pregnancy rates for the two treatment groups within all three locations are summarized in Figure 4.2. No CIDRs were lost between CIDR insertion and CIDR removal at all locations. Final pregnancy rates for heifers at Colorado (97.4 %; 38/39) and Wyoming (89.1 %; 106/119) were not different ($P > 0.10$; data not shown) and final pregnancy rates were not determined at SD because of management considerations.

Timed AI pregnancy rates for heifers from both treatments combined did not differ ($P > 0.10$) between CO (53.9 %; 21/39), SD (47.4 %; 100/211) and WY (55.2 %; 69/125).

There was a treatment effect on AI pregnancy rates at the SD location, therefore treatment data from all locations were not pooled and treatment effects were analyzed within each location. At SD, the proportion of pregnant heifers was lower ($P < 0.05$) in the CON (40.4 %) vs TRMT group (54.2 %), but differences in pregnancy rates were not observed between treatments ($P > 0.10$) for heifers at the CO and WY locations.

Effects of BCS and body weight on heifer pregnancy rates were not significant ($P > 0.10$; Table 4.1); however, average BW was heavier for SD heifers compared to CO and WY heifers at the beginning of the breeding season. Heifer body weights were sorted into three weight classes by 90.9 kg increments (1 = 227.3 to 317.7 kg, 2 = 318.2 to 408.6 kg and 3 = 409.1 to 499.5 kg) for all three locations and analyzed for differences in pregnancy rates as a fixed variable (Table 4.1). Treatment by wt class interaction did not affect pregnancy rates within location ($P > 0.10$); however, weight class tended ($P = 0.08$) to affect pregnancy rates for heifers at SD, but no differences ($P > 0.10$) in pregnancy rates were observed for heifers at CO and WY within weight class.

The number of service sires used varied within location. Pregnancy rate differences were not observed ($P > 0.10$) for CO heifers inseminated to sires A (57.9 %) and B (50 %; Figure 4.3), but 3 out of 13 AI sires used at SD and 1 out of 4 sires used at WY resulted in lower pregnancy rates within location ($P < 0.05$). Pregnancy rates ranged from 45.2 to 73 % from the four sires used at WY (Sires C, D, E and F; Figure 4.3). Heifers inseminated to sire E (45.2 %) had lower ($P < 0.05$) pregnancy rates than heifers inseminated to sire F (73 %), but pregnancy rates were similar ($P > 0.10$) between sires

C, D and E and C, D and F. Pregnancy rates ranged from 31.8 to 72.2 % from the 12 sires used at SD (Sires G, H, I, J, K, L, M, N, O, P, Q and R; Figure 4.4). Heifers inseminated to sire P (72.2 %) had higher ($P < 0.05$) pregnancy rates than heifers inseminated to sires J, M and N (33.3, 34.8 and 31.8 %), but pregnancy rates were similar ($P > 0.10$) between sires G, H, I, J, K, L, M, N, O, Q, and R and G, H, I, K, L, O, P, Q and R. The 4 sires from both SD and WY with lower pregnancy rates accounted for 37 % (37/100) of the fertility while the 13 other sires from both SD and WY with higher pregnancy rates accounted for 56 % (132/236) of the fertility.

Heifers at the SD location were assigned to a time group (1 to 6; 1 = 54 h and 6 = 59 h) based on the hour bred at 54 h TAI to look at pregnancy rate differences from TAI over time (Figure 4.5). There were no effects of hour or treatment x hour interaction on pregnancy rates ($P > 0.10$). Pregnancy rates were similar ($P > 0.10$) for heifers inseminated at hours 1, 2, 3, 4 and 6 (54.1, 53.6, 52.5, 48.6 and 53.3 %); however, pregnancy rates for heifers inseminated at hours 1, 2, 3 and 6 were significantly higher ($P < 0.05$) than heifers inseminated at hour 5 (26.8 %). Pregnancy rates for heifers inseminated at hour 4 tended to be higher ($P = 0.07$) than heifers inseminated at hour 5. Heifers in the CON group had numerically lower pregnancy rates vs heifers in the TRMT group that were bred at hour 1 (40.0 and 63.6 %), but similar ($P > 0.10$) pregnancy rates were observed for heifers bred at hour 6 for both the CON (52.9 %) and TRMT (53.8 %) groups.

Follicle size and ovulation after treatments. At both CO and WY locations, diameter of the ovulatory dominant follicle was determined and classified prior to TAI in which there were no treatment, follicle diameter class, location, treatment x follicle

diameter class, treatment x location, follicle diameter class x location and treatment x location x follicle diameter class interaction on pregnancy rates ($P > 0.10$) at both locations, therefore follicle diameter class data from both locations were pooled.

Pregnancy rate differences were not seen ($P > 0.10$) for follicle classes 1 (83.3 %), 2 (57.1 %), 3 (66.7 %) and 4 (42.9 %); however pregnancy rates tended to decrease ($P = 0.12$) as follicle size increased from class 1 to 4 (Figure 4.6).

At both CO and WY locations, the incidence of ovulation (number of heifers ovulating a dominant follicle by 40 h after TAI [d 11] vs the number of heifers treated) was not different ($P > 0.10$) between CO and WY heifers (66.7 and 73.9 %). Ovulation rates tended to be lower ($P = 0.10$) for heifers in the CON (62.5 and 66.7 %) vs TRMT (81.3 and 73.9 %) groups at CO and WY (Figure 4.7); however, there was no treatment x location interaction; therefore data from both locations were pooled. Although incidence of ovulation tended to be higher for heifers in the TRMT vs CON group at both locations, sample size likely affected statistical differences not seen. Heifers ovulating by 40 h after TAI at CO and WY, regardless of treatment, had higher ($P < 0.01$) pregnancy rates (58.7 %) than heifers that had not ovulated (16.7 and 16.7 %).

DISCUSSIONS

In the current study, our objective were to determine if a second injection of GnRH at 54 h TAI increases the percentage of induced ovulations and improves pregnancy rates in beef heifers synchronized with the CO-Synch + CIDR protocol. The proportion of heifers pregnant to AI at the SD location (47.4 %) were numerically lower than both CO (53.9 %) and WY (55.2 %) locations. Pregnancy rates at SD increased for

heifers in the TRMT vs CON group and pregnancy rate percentages were similar for both treatment groups at CO and WY. We did not determine cyclicity status for heifers at SD; however, it is unclear at this time as to why low fertility rates were observed at SD. Cyclicity did not affect the response to treatment from heifers at both CO and WY locations and thus, would not be expected to have contributed to fertility at SD. The high fertility rates seen from TAI in the TRMT heifers at SD were similar to recent reports of incorporating an additional injection of GnRH at 54 h TAI in beef heifers and 48 and 72 h TAI in beef cows synchronized with a modified Select Synch protocol (Twagiramungu et al., 1995b; Walker et al., 2003). Several SD heifers may not have responded to the initial GnRH injection and potentially had undergone follicular turnover just before removal of the CIDR. If such were the case, these heifers would be expected to have a smaller follicle at TAI which, without GnRH, did not ovulate until later. In beef heifers, estrous response rates were 65 % within a 72 h period when a 7 d CIDR insert was incorporated with PG given one day prior to CIDR removal (Lucy et al., 2001). With an initial injection of GnRH at CIDR insert preventing persistence of ovulatory follicles (Schmitt et al., 1996), average interval to standing estrus is shorter when incorporated with a CIDR+PG protocol (Martinez et al., 2002).

Cyclicity status in the current study was evaluated at both CO and WY locations; however, heifers at SD were not evaluated for cyclicity because of the commitment from a cooperator herd and management considerations. The percent of heifers cycling ranged from 46.4 to 97.4 % at both locations. Heifers from WY had low cyclicity rates with a 55.2 % pregnancy rate to TAI which indicated the effectiveness of GnRH and a CIDR insert to induce puberty in prepubertal heifers. This is consistent with a review

conducted by Odde (1990) reporting the advantage that a progestogen has on cattle not cycling at the beginning of the breeding season. The progesterone insert is known to increase LH secretion which increases follicular growth and development resulting in ovulation following its removal (Anderson et al., 1996; Imwalle et al., 1998). Lucy et al. (2001) reported 48 % synchronization rate in prepubertal beef heifers using a 7 d CIDR insert plus PG. Lamb et al. (2001) reported cows not cycling at the beginning of a CIDR + CO-Synch protocol produced pregnancy rates that were 36 percentage points higher than cows not synchronized with a CIDR insert. Based on previous reports indicating progestogen sources inducing cyclicity in non-cycling cows or prepubertal heifers, our results support the effectiveness of CIDR inserts to induce puberty in prepubertal heifers.

Short estrous cycles typically occur when anestrous or prepubertal cattle are induced to ovulate (Short et al., 1990). Fertility is compromised in suckled beef cows (Ramirez-Godinez et al., 1982) when short estrous cycles follow induced ovulations from GnRH administrations (Troxel and Kesler, 1984). Incorporating a norgestomet implant prevented short estrous cycles from naturally occurring after the first pubertal ovulation in beef heifers (Gonzalez-Padilla et al., 1975), and may be the reason differences in pregnancy rates were not observed between cycling and prepubertal heifers at both locations.

Variations in weight differences did not affect pregnancy rates within all three locations; however, body weights were heavier in heifers from SD at the time of CIDR insertion. While BCS did not influence pregnancy rates in the current study, heifers were conditioned as either 5, 5.5, 6 or 6.5 at all three locations. Anderson et al. (1987) reported reduced fertility in beef heifers that carried a condition score greater than 6. The

subjectivity of body condition scores, within locations, may have prevented us from detecting differences in pregnancy rates. Weight differences may explain more variation in pregnancy rates, so heifer body weights, at all three locations, were sorted into three weight classes. All heifers at CO and WY were classed as 1 (227.3 to 317.7 kg) or 2 (318.2 to 408.6 kg) while the majority of heifers at SD were classed as 2 or 3 (409.1 to 499.5) with 1 heifer falling into class 1. While 100 % of the heifers from CO and WY and 72 % of the heifers from SD weighed between 272 to 408 kg, the remaining 28 % from SD weighed between 409 to 499 kg. Transient decreases in pregnancy rates were observed as body weights of heifers from SD increased from 409 to 499 kg. Based on these weight breaks, there was a tendency for fertility levels to fall for heifers in class 3 at SD indicating that heifers may have been over-conditioned and may have become anestrus (after puberty had occurred). The issue of too much condition seems to be more influential in heifers rather than cows during the breeding season because of lactation stresses placed on a lactating cow. Whether this phenomenon is due to excess lipids acting to alter steroidogenesis or totally inhibit hormone synthesis and release is unknown.

Synchronizing ovulation for AI at a pre-determined time in cattle has been very controversial when it comes to predicting the interval to estrus and achieving acceptable fertility rates. In the current study, heifers at the SD location were assigned to a time group to determine if pregnancy rates vary over time from 54 to 59 h after CIDR removal. Lucy et al. (2001) reported a 65 % estrous response rate within 72 h of a CIDR plus PG protocol. Schmitt et al. (1996) and Martinez et al. (2002) reported that the average interval from PG to standing estrus in heifers was 47.3 and 47.8 h when a CIDR

insert or MGA feeding was incorporated with GnRH at the time of insert/beginning of MGA; however, Richardson et al. (2002) reported average interval to estrus was 68 h in beef heifers after a CIDR+ PG protocol. Our reports show that the average interval from PG injection to first observed standing estrus was 51 h in heifers from CO. These responses are largely dependent upon the stage of the estrous cycle when GnRH and/or PG is given (Geary et al., 2000). The biggest variation in estrous response seen is the interval from PG injection to detected standing estrus (Geary et al., 2001). With these variations seen using CIDR inserts, our results showed no differences in pregnancy rates for heifers bred over a 6 h time period. However, heifers inseminated at hour 5 (58 h post CIDR removal) had the lowest pregnancy rate vs heifers inseminated during hours 1, 2, 3, 4 and 6 with hour 6 having the highest combined (CON + TRMT) percentage of heifers pregnant. The reason for this low fertility seen in heifers inseminated at hour 5 is unknown. Twagiramungu et al. (1995b) reported higher pregnancy rates in beef heifers synchronized with a modified Select Synch protocol plus GnRH at 54 h timed AI and estrous response was highest between 48 and 72 h after PG in pubertal heifers receiving MGA+PG, MGA+Select Synch (Wood et al., 2001), or CIDR+PG protocols (Schmitt et al., 1996; Martinez et al., 2002; Richardson et al., 2002).

Percentages showed that 4 out of 17 sires used at SD and WY resulted in lower pregnancy rates. Although semen used from sires at all locations were not analyzed for motility and morphology, fertility levels from all sires used was assumed normal at that time. Dalton et al. (2001) reported significant differences between AI bulls on fertility rates when used for insemination at hours 0, 12 and 24 after first standing estrus in dairy cows. Saacke (2002) emphasized that the magnitude of sire differences can significantly

impact pregnancy rates in a timed AI synchronization program. Identifying sires that may be inferior for producing superior fertility rates in cattle that are synchronized for TAI might enhance overall pregnancy rates for various timed AI synchronization systems and increase producer acceptability in using these systems.

Diameter of the ovulatory follicle did not influence pregnancy rates in the current study; however, percentages showed that as follicle size increased, pregnancy rates tended to decrease. Previous work reporting that as diameter of the ovulatory follicle increased, pregnancy rates increased in beef cows (Lamb et al., 2001). However, pregnancy rates were not affected by follicle size in lactating beef cows synchronized with a CO-Synch protocol (Perry et al., 2002). Physiologically, the developing heifer may gradually be maturing as well as reproductive functions responsible for puberty and the estrous cycle. The developing dominant follicle from a developing heifer may not acquire diameters seen in beef cows to produce competent fertile oocytes. Growth rates, prior to ovulation, of growing follicles vary within cattle and do not seem to affect fertility (Perry et al., 2002), so results from the previous study would show that diameter of ovulatory follicles between individual heifers does not seem to affect fertility.

Ovulation rates were numerically higher for TRMT heifers, but differences were not significant due to the high percentage of ovulating heifers from the CON group that did not receive GnRH at TAI. Incorporating a second injection of GnRH at timed AI in dairy cattle successfully induced ovulation 24 to 32 h after GnRH (Pursley et al., 1994), but ovulation rates in the current study from heifers receiving a CIDR insert, without an additional injection of GnRH (62.5 %), resulted in pregnancy rates that were not different from heifers receiving additional GnRH at TAI. It would appear that incidence of

ovulation is high without incorporating a second injection of GnRH at TAI and suggest that a tight estrous response was in close proximity to the 54 h TAI period.

In summary, inseminating beef heifers for timed AI at 54 h in a modified CO-Synch protocol with a CIDR insert produced acceptable pregnancy rates for all locations (overall average 50.7 %). Lucy et al. (2001) reported synchronization rates of 64.7 % and conception rates of 60.1 % in beef heifers synchronized with a CIDR insert and PG given one day prior to CIDR removal without GnRH administration. Incorporating an initial injection of GnRH at CIDR insertion prevents development of persistent follicles and reduced fertility (Schmitt et al., 1996). However, incorporating a second injection of GnRH may not be feasible unless the conditions of heifers impede the percentage cycling at the start of the breeding season. Insemination of heifers from 54 to 59 hours seems to target peak estrous response so that increased fertility rates can be achieved. The data also suggest that some bulls are of lower fertility when used for timed AI and thus might explain the poor pregnancy responses seen when numerous AI sires are used with timed-insemination protocols. In the current study, pregnancy rates were not improved for heifers in the TRMT group at two locations, thus suggesting that the addition of a CIDR insert, PG and GnRH at CIDR insertion induced puberty in prepubertal heifers while producing acceptable pregnancy rates to TAI.

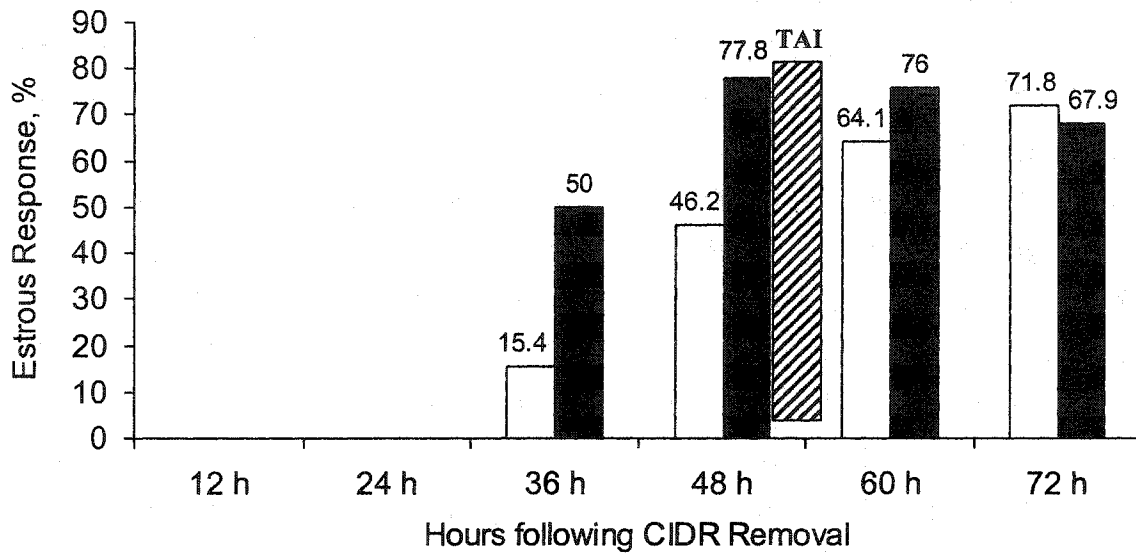


Figure 4.1: Estrous response (open bars) and pregnancy rates (shaded bars) for heifers detected in estrus at the CO location relative to CIDR removal (0 h) and TAI (54 h). Estrous response was calculated as the total number of heifers in estrus divided by the number of heifers synchronized for AI up to that time frame. Pregnancy rates were calculated as the total number of heifers pregnant divided by the total number of heifers in heat up to that time frame. Statistical Analysis was not performed on this data.

Table 4.1: Characteristics of heifers bred TAI by location

Item	Location						Overall
	Colorado (CO)		Wyoming (WY)		South Dakota (SD)		
	(no.) %						
Cyclicity^a							
% cycling	(38/39)*	97.4	(58/125)	46.4	n/a		(96/164) 58.5
PR, cycling	(21/38)	55.3	(32/58)	55.2	n/a		(53/96) 55.2
PR, noncycling	(0/1)	0.0	(35/67)	52.2	n/a		(35/68) 51.5
Body Condition							
5	(13/24)	54.2	(36/70)	51.4	(62/127)	48.8	(111/221) 50.2
6	(8/15)	53.3	(33/55)	60.0	(38/84)	45.2	(79/154) 51.3
Wt class, kg^b							
1 (227.3 – 317.7)	(8/16)	50.0	(30/51)	58.8	(0/1)	0.0	(38/68) 55.9
2 (318.2 – 408.6)	(13/23)	56.5	(40/74)	54.1	(77/151)	51.0	(130/248) 52.4
3 (409.1 – 499.5)	(23/59)	39.0	(23/59)	39.0			

^aPercentage of heifers cycling based on progesterone values ≥ 1 ng/ml taken on d -10 and 0. Cyclicity data was not available (n/a) for heifers at the SD location because they were a cooperator herd.

Pregnancy rates (PR) for the percent of heifers cycling and noncycling.

^bCombined weights separated into three classes for all three locations.

*Raw mean percentages within a row for % cycling for CO and WY lacking the common asterisks differ ($P < 0.05$).

Table 4.2: Cyclicity and pregnancy rates of heifers bred TAI within each treatment group at the CO and WY locations.

Item	Location											
	Colorado (CO)		Wyoming (WY)		Total							
	CON	TRMT	CON	TRMT	CON	TRMT						
	(no.) %											
% cycling ^a	(19)	100.0	(20)	95.0	(63)	47.6	(62)	45.2	(82)	59.8	(82)	57.3
PR, cycling	(19)	52.6	(19)	57.9	(30)	53.3	(28)	57.1	(49)	53.1	(47)	57.5
PR, noncycling	(0)	0.0	(1)	0.0	(33)	54.5	(34)	55.9	(33)	54.5	(35)	54.3

^aPercentage of heifers cycling based on progesterone values ≥ 1 ng/ml taken on d -10 and 0. Cyclicity data not available for heifers at SD because they were a cooperator herd.

Pregnancy rates (PR) for the percent of heifers cycling and noncycling.

Raw mean percentages within a row for each location and total do not differ ($P > 0.05$).

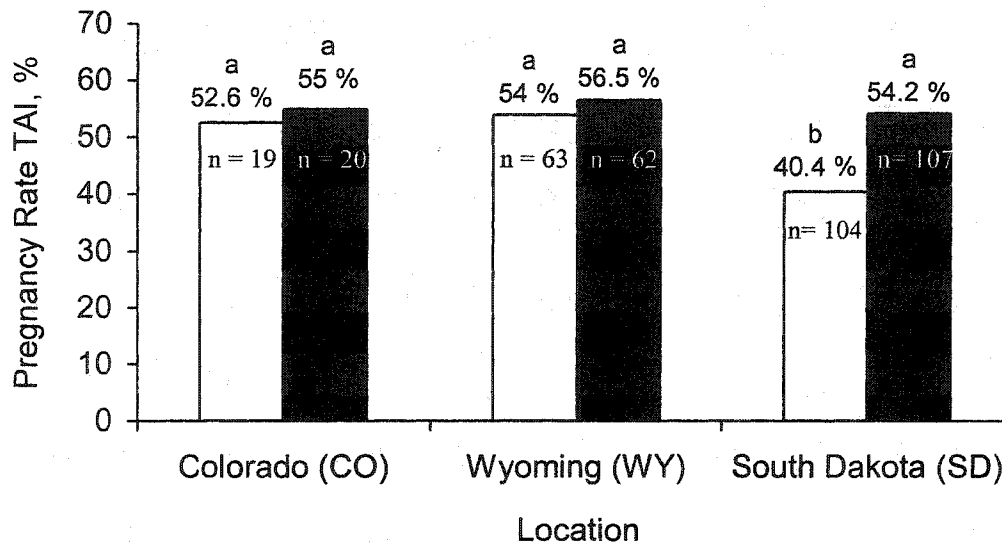


Figure 4.2: Effect of treatment (CON [open bars] vs TRMT [shaded bars]) on pregnancy rates to 54 h fixed time AI within location. Raw mean percentages within location without a common letter (a,b) differ ($P < 0.05$).

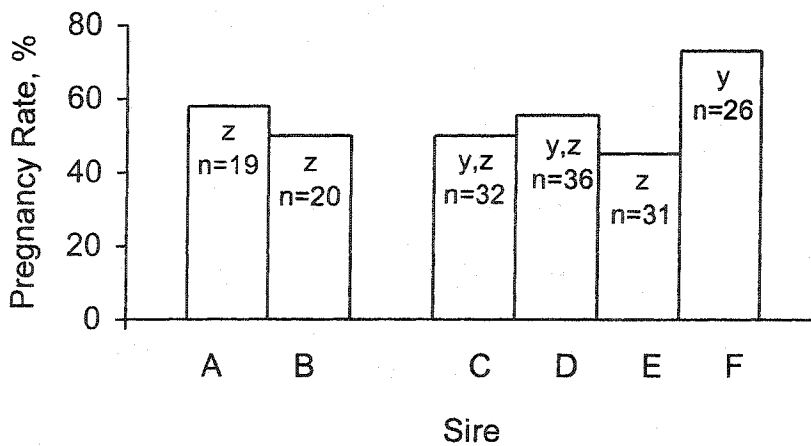


Figure 4.3: Effect of AI sire within location (CO = sires A and B; WY = sires C, D, E and F) on pregnancy rates when sire was analyzed as a fixed effect. Raw mean percentages within each location without a common letter (y,z) differ ($P < 0.05$).

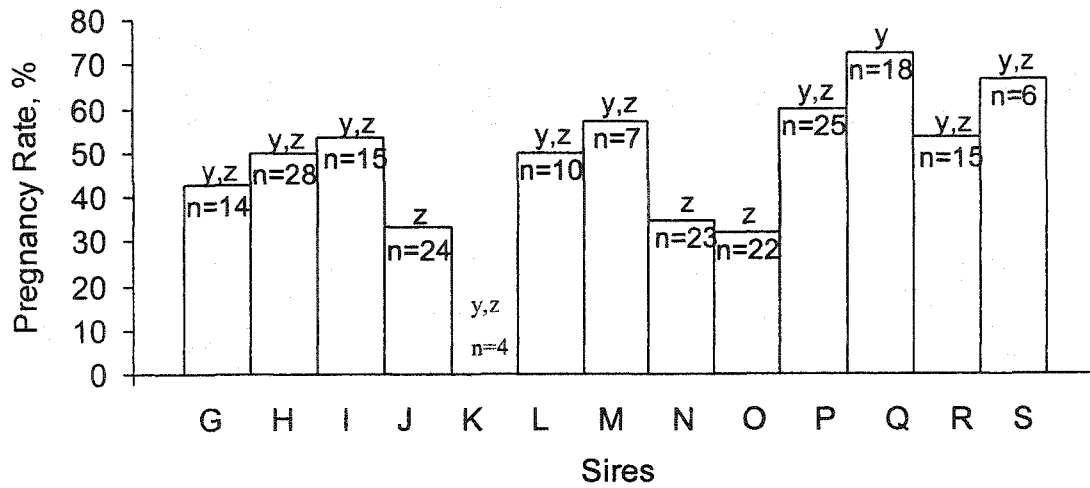


Figure 4.4: Effect of AI sires at the SD location (sires G to S) on pregnancy rates when sire was analyzed as a fixed effect. Raw mean percentages without a common letter (y,z) differ ($P < 0.05$).

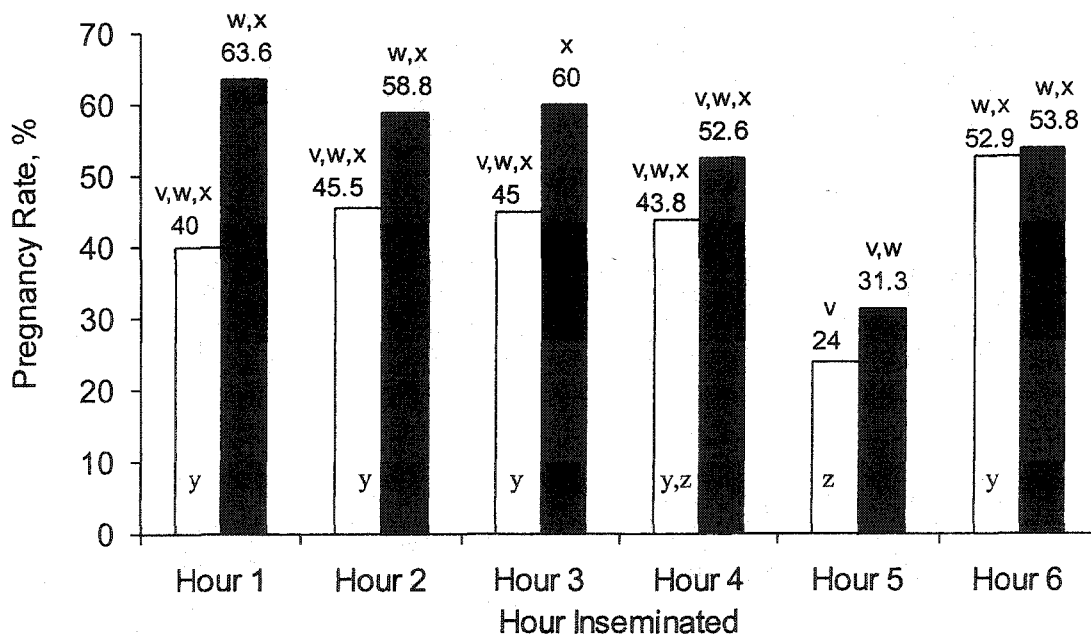


Figure 4.5: Effect of treatment (CON [open bars] vs TRMT [shaded bars]) and hour (1 = 54 h and 6 = 59 h) heifers were inseminated on pregnancy rates at the SD location. Raw mean percentages for both treatments combined across all hours inseminated without a common letter (y, z) differ ($P < 0.05$). Comparisons listed for pregnancy rate differences seen between treatment groups for different hours lacking a common letter (v, w, x) differ ($P < 0.05$).
 CON hour 5 vs CON hour 6 CON hour 5 vs TRMT hours 1, 2, 3, and 6
 TRMT hour 5 vs TRMT hour 3

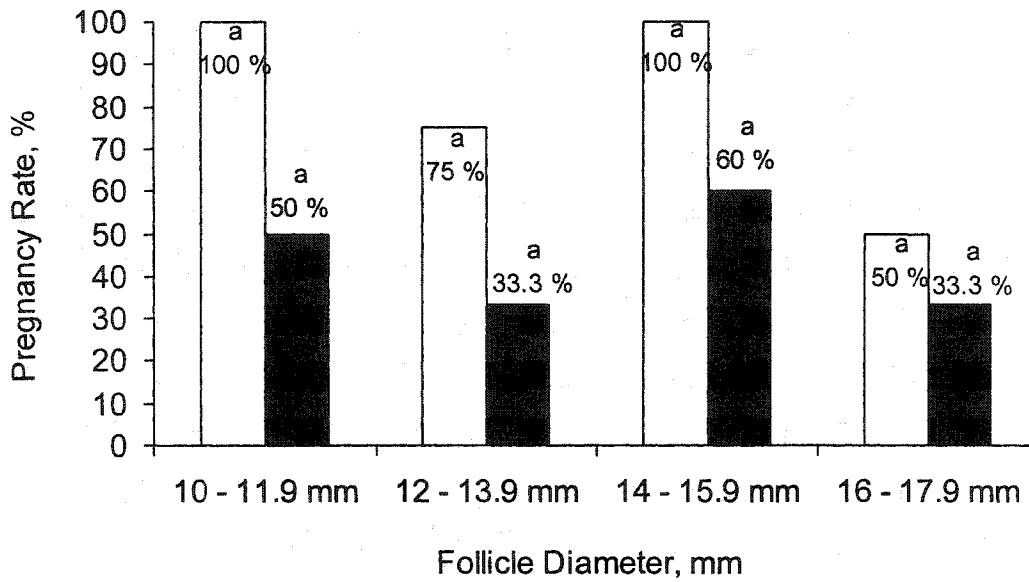


Figure 4.6: Effect of diameter of ovulatory follicle at timed AI (d 9) on pregnancy rates for heifers ultrasounded at CO and WY for both the CON (open bars) and TRTMT (shaded bars) groups. Raw mean percentages for pregnancy rates within and between treatment groups and within follicle size class did not differ ($P < 0.05$).

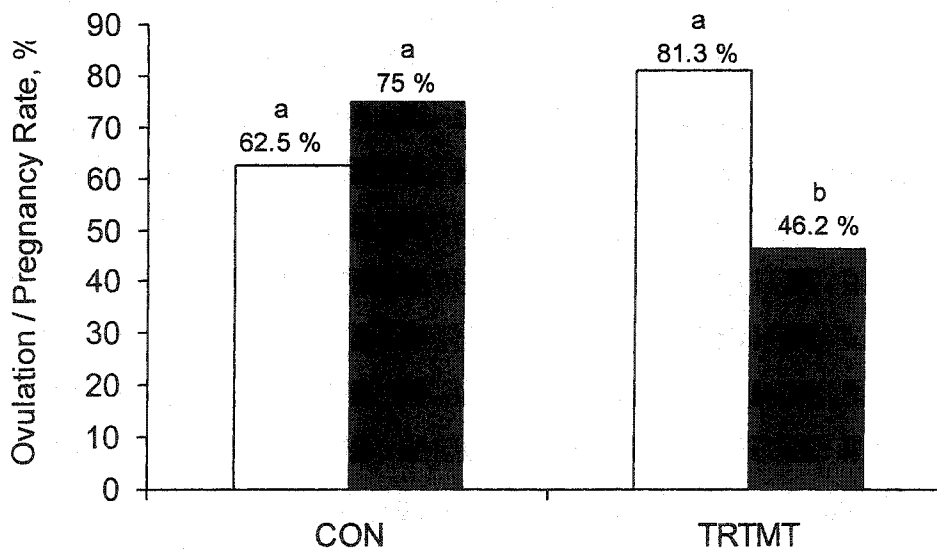


Figure 4.7: Effect of treatment (CON vs TRTMT) on ovulation rates (open bars) and pregnancy rates (shaded bars) for heifers ultrasounded at TAI (d 9) and 40 h after TAI (d 11) from CO and WY only. Raw mean percentages for ovulation rates between treatment and pregnancy rates between treatment lacking a common letter (a,b) differ ($P < 0.05$).

IMPLICATIONS

Dietary fat supplementation has been reported to improve fertility in beef and dairy cattle. In chapter 2, supplementing dietary fat in the form of whole safflower seed high in oleic acid, or post AI injection of hCG did not influence conception rates to AI. It is our understanding from the current study that the impact of fat supplementation on fertility may be limited to heifers with low body condition or primiparous lactating heifers that require additional nutrients to maintain positive postpartum body condition. Had the body condition of these heifers been impaired, a positive influence on fertility may have been detected with fat supplementation. Replacement heifer development has always been challenging for producers, and incorporating a dietary fat supplement to prepubertal heifers may have beneficial affects on fertility; however, feeding 0.96 kg of whole safflower seed 35 d prior to breeding, or post AI injection of hCG does not positively influence fertility for heifers in good body condition.

The results from chapter 3 suggest synchrony of ovulation may be tighter and that improved pregnancy rates are feasible if TAI is delayed until 72 h post PG injection with a second injection of GnRH. Identifying sires that may be inferior for producing superior fertility rates in cattle that are synchronized for TAI might enhance overall pregnancy rates for various timed AI synchronization systems and increase producer acceptability in using those systems. This study warrants further investigation as to why 72 h TAI

increased fertility rates in suckled beef cows and if a second injection of GnRH will consistently yield a 20% increase in pregnancy rates to TAI.

Currently, there is no consistent TAI synchronization protocol that exists for controlling ovulation in beef heifers. In the chapter 4 study, synchronization with GnRH+CIDR+PG induced cyclicity and ovulations in prepubertal heifers, while pregnancy rates were not improved for heifers receiving additional GnRH at 54 h TAI for two locations, but were improved at the third location. Producers may be able to achieve acceptable pregnancy rates in beef heifers using a synchronization protocol that utilizes 54 h TAI, without estrous detection, with a CIDR insert plus GnRH at CIDR insertion and PG. Administering the second GnRH injection at timed AI may not improve pregnancy rates to TAI, but may guard against low pregnancy rates.

REFERENCES

- Adams, G. P., R. L. Matteri, J. P. Kastelic, J. C. H. Ko, and O. J. Ginther. 1992. Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. *J. Reprod. Fertil.* 94:177.
- Ahmad, N., E. C. Townsend, R. A. Dailey, and E. K. Inskeep. 1997. Relationships of hormonal patterns and fertility to occurrence of two or three waves of ovarian follicles, before and after breeding, in beef cows and heifers. *Anim. Reprod. Sci.* 49:13-28.
- Anderson, K. J., J. S. Brinks, D. G. LeFever, W. C. Asbury, D. W. Schafer, and J. L. Moon. 1987. Reproductive performance of Angus heifers. *Colorado State Univ. Beef Program Rep.*, p. 71. Fort Collins.
- Anderson, K. A., D. M. Kniffen, J. P. Sonderman, J. W. Young, M. D. Holland, and K. E. Odde. 1992. Effect of a hyperlipidemic diet on postpartum beef cows. *Proc. WSAS Anim. Sci.* 43:16-18.
- Anderson, L. H., and M. L. Day. 1994. Acute progesterone administration regresses persistent dominant follicles and improves fertility of cattle in which estrus was synchronized with melengestrol acetate. *J. Anim. Sci.* 72:2955-2961.
- Anderson, L. H., C. M. McDowell, and M. L. Day. 1996. Progestin-induced puberty and secretion of luteinizing hormone in heifers. *Biol. Reprod.* 54:1025-1031.
- Armstrong, J. D., E. A. Goodall, F. J. Gordon, D. A. Rice, and W. J. McCaughey. 1990. The effects of levels of concentrate offered and inclusion of maize gluten or fish meal in the concentrate on reproductive performance and blood parameters of

dairy cows. *Anim. Prod.* 50:1-10.

- Armstrong, D. G., T. G. McEvoy, G. Baxter, J. J. Robinson, C. O. Hogg, K. J. Woad, R. Webb, and K. D. Sinclair. 2001. Effect of dietary energy and protein on bovine follicular dynamics and embryo production in vitro: Associations with the ovarian insulin-like growth factor system. *Biol. Reprod.* 64:1624-1632.
- Ayalon, N. 1978. A review of embryonic mortality in cattle. *J. Reprod. Fert.* 54:483-493.
- Bao, B., M. G. Thomas, M. K. Griffith, R. C. Burghardt, and G. L. Williams. 1995. Steroidogenic activity, insulin-like growth factor I production, and proliferation of granulosa and theca cells obtained from dominant preovulatory and nonovulatory follicles during the bovine estrous cycle. Effects of low-density and high-density lipoproteins. *Biol. Reprod.* 53:1271-1279.
- Barrett, G. R., and L. E. Casida. 1946. Time of insemination and conception rate in artificial breeding. *J. Dairy Sci.* 29:556-557.
- Barros, C. M., G. R. Newton, W. W. Thatcher, M. Drost, C. Plante, and P. J. Hansen. 1992. The effect of bovine interferon- α 1 on pregnancy rate in heifers. *J. Anim. Sci.* 70:1471-1477.
- Beal, W. E., J. R. Chenault, M. L. Day, and L. R. Corah. 1988. Variation in conception rates following synchronizing of estrus with melengestrol acetate and prostaglandin F_{2 α} . *J. Anim. Sci.* 66:599.
- Beard, A. J., R. J. Castillo, B. J. McLeod, R. G. Glencross, and P. G. Knight. 1990. Comparison of the effects of crude and highly purified bovine inhibin (M, 32000) on plasma concentrations of FSH and LH in chronically ovariectomized prepubertal heifers. *J. Endocrinol.* 125:21.
- Beck, T. W., R. P. Wettemann, E. J. Turman, and R. Totusek. 1979. Influence of 48 hour calf removal on milk production and calf growth in range cows. *Theriogenology* 11:367.
- Bellows, R. A., R. E. Short, J. J. Urick, and O. F. Pahnish. 1974. Effects of early weaning on postpartum reproduction of the dam and growth of calves born as multiples or singles. *J. Anim. Sci.* 39:589.
- Bellows, R. A., and R. E. Short. 1978. Effects of precalving feed level on birth weight, calving difficulty, and subsequent fertility. *J. Anim. Sci.* 46:1522.
- Bellows, R. A., R. E. Short, and G. V. Richardson. 1982. Effect of sire, age of dam and gestation feed level on dystocia and postpartum reproduction. *J. Anim. Sci.* 55:18.

- Bellows, R. A. 1999. Some effects of feeding supplemental fat to beef cattle. Proc. RBC Symposium XVI. P 81. Greeley, Colorado.
- Bellows, R. A., E. E. Grings, D. D. Sims, T. W. Geary, and J. W. Bergman. 2001. Effects of feeding supplemental fat during gestation to first-calf beef heifers. Prof. Anim. Sci. 17:81-89.
- Bezard, J., J. P. Blond, A. Bernard, and P. Clouet. 1994. The metabolism and availability of essential fatty acids in animal and human tissues. Reprod. Nutrit. Dev. 34:539-568.
- Bishop, D. K., R. P. Wettemann, and L. J. Spicer. 1994. Body energy reserves influence the onset of luteal activity after early weaning of beef cows. J. Anim. Sci. 72:2703-2708.
- Bogacz, V. L., J. E. Huston, D. E. Grum, and M. L. Day. 1999. Identification of the optimal dose of estradiol benzoate in combination with a progestin to program follicular turnover in cyclic cattle. J. Anim. Sci. 77(Suppl. 1):27.
- Bonnette, T. R., J. C. Whittier, T. E. Engle, and P. D. Burns. 2001. Effects of fishmeal supplementation on fertility in primiparous, lactating beef cows. Proc. WSAS Anim. Sci. 52:74-78.
- Bossis, I., R. P. Wettemann, S. D. Welty, J. A. Vizcarra, and L. J. Spicer. 2000. Nutritionally induced anovulation in beef heifers: ovarian and endocrine function during realimentation and resumption of ovulation. Biol. Reprod. 62:1436-1444.
- Bottger, J. D., B. W. Hess, B. M. Alexander, D. L. Hixon, L. F. Woodard, R. N. Funston, D. M. Hallford, and G. E. Moss. 2002. Effects of supplementation with high linoleic or oleic cracked safflower seeds on postpartum reproduction and calf performance of primiparous beef heifers. J. Anim. Sci. 80:2023-2030.
- Breuel, K. F., J. C. Spitzer, and D. M. Henricks. 1989. Systemic progesterone concentration following human chorionic gonadotropin administration at various times during the estrous cycle in beef heifers. J. Anim. Sci. 67:1564-1572.
- Breuel, K. F., P. E. Lewis, F. N. Schrick, A. W. Lishman, E. K. Inskip, and R. L. Butcher. 1993. Factors affecting fertility in the postpartum cow: role of the oocyte and follicle in conception rate. Biol. Reprod. 48:655-661
- Britt, J. H., D. A. Morrow, R. J. Kittok, and B. E. Seguin. 1974. Uterine involution, ovarian activity, and fertility after melengestrol acetate and estradiol in early postpartum cows. J. Dairy Sci. 57:89-92.
- Brown, H., J. F. Wagner, R. P. Rathmacher, J. W. McAskill, N. J. Elliston, and R. F.

- Bing. 1973. Effect of human chorionic gonadotropin on pregnancy rate of heifers, when used under field conditions. *J. Am. Vet. Med. Assoc.* 162:456.
- Brown, J. L., and J. J. Reeves. 1983. Absence of specific luteinizing hormone releasing hormone receptors in ovine, bovine and porcine ovaries. *Biol. Reprod.* 29:1179.
- Brown, L. N., K. G. Odde, D. G. LeFever, M. E. King, and C. J. Neubauer. 1986. Norgestomet-Alfaprostol or Syncro-Mate B for estrus synchronization in beef cows. *J. Anim. Sci. (Suppl. 1)*:383 (Abstr.).
- Brown, L. N., K. G. Odde, M. E. King, D. E. Lefever, and C. J. Neubauer. 1988. Comparison of melengestrol acetate-prostaglandin F_{2α} to Syncro-Mate B for estrous synchronization in beef heifers. *Theriogenology.* 30:1.
- Bulman, D. C., and G. E. Lamming. 1978. Milk progesterone levels in relation to conception, repeat breeding and factors influencing acyclicity in dairy cows. *J. Reprod. Fertil.* 54:447.
- Burke, J. M., D. J. Carroll, K. E. Rowe, W. W. Thatcher, and F. Stormshak. 1996. Intravascular infusion of lipid into ewes stimulates production of progesterone and prostaglandin. *Biol. Reprod.* 55:169-175.
- Burke, J. M., C. R. Staples, C. A. Risco, R. L. De La Sota, and W. W. Thatcher. 1997. Effect of ruminant grade Menhaden fish meal on reproductive and productive performance of lactating dairy cows. *J. Dairy Sci.* 80:3386-3398.
- Burns, P. D., T. R. Bonnette, T. E. Engle, and J. C. Whittier. 2002. Effects of fishmeal supplementation on fertility and plasma W-3 fatty acid profiles in primiparous, lactating beef cows. *Prof. Anim. Sci.* 18:373.
- Butler, W. R., J. J. Calaman, and S. W. Beam. 1996. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. *J. Anim. Sci.* 74:858-865.
- Carroll, D. J., M. J. Jerred, R. R. Grummer, D. K. Combs, R. A. Pierson, and E. R. Hauser. 1990. Effects of fat supplementation and immature alfalfa to concentrate ratio on plasma progesterone, energy balance and reproductive traits of dairy cattle. *J. Dairy Sci.* 73:2855.
- Casida, L. E., W. E. Graves, E. R. Hauser, J. W. Lauderdale, J. W. Riesen, S. Saiduddin, and W. J. Tayler. 1968. Studies on the postpartum cow. *Research Bulletin 270.* University of Wisconsin, Madison.
- Cavestany, D., A. B. El-Wishy, and R. H. Foote. 1985. Effect of season and high environmental temperature on fertility of Holstein cattle. *J. Dairy Sci.* 68:1471-1478.

- Cavaliere, J., G. Hepworth, K. I. Parker, P. J. Wright, and K. L. Macmillan. 2003. Effect of treatment with progesterone and oestradiol when starting treatment with an intravaginal progesterone releasing insert on ovarian follicular development and hormonal concentrations in Holstein cows. *Anim. Reprod. Sci.* 76:177-193.
- Chegini, N., Z. M. Lei, Ch. V. Rao, and W. Hansel. 1991. Cellular distribution and cycle phase dependency of gonadotropin and eicosanoid binding sites in bovine corpora lutea. *Biol. Reprod.* 45:506.
- Chenault, J. R., D. D. Kratzer, R. A. Rzepkowski, and M. C. Goodwin. 1990. LH and FSH response of Holstein heifers to fertirelin acetate, gonadorelin and buserelin. *Theriogenology* 34:81.
- Cheng, Z., R. S. Robinson, P. G. A. Pushpakumara, R. J. Mansbridge, and D. C. Wathes. 2001. Effect of dietary polyunsaturated fatty acids on uterine prostaglandin synthesis in the cow. *J. Endocrinology* 171: 463-473.
- Christian, R. E., and L. E. Casida. 1948. The effects of progesterone in altering the estrous cycle of the cow. *J. Anim. Sci.* 7:540 (Abstr.).
- Ciccioli, N. H., R. P. Wettemann, L. J. Spicer, C. A. Lents, F. J. White, and D. H. Keisler. 2003. Influence of body condition at calving and postpartum nutrition on endocrine function and reproductive performance of primiparous beef cows. *J. Anim. Sci.* 81:3107-3120.
- Conn, P. M., and W. F. Crowley, Jr. 1990. Gonadotropin-releasing hormone and its analogues. *N. Eng. J. Med.* 323:56.
- Cook, H. W. 1996. Fatty acid desaturation and chain elongation in eukaryotes. In *Biochemistry of Lipids, Lipoproteins and Membranes*. Pp 129-152. Ed. D. E. Vance and J. Vance. Elsevier, Amsterdam.
- Custer, E. E., W. E. Beal, S. J. Wilson, A. W. Meadows, J. G. Berardinelli, and R. Adair. 1994. Effect of melengestrol acetate (MGA) or progesterone-releasing intravaginal device (PRID) on follicular development, concentrations of estradiol-17 β and progesterone, and luteinizing hormone release during an artificially lengthened bovine estrous cycle. *J. Anim. Sci.* 72:1282-1289.
- Dailey, R. A., D. R. Deaver, and R. L. Goodman. 1987. Neurotransmitter regulation of luteinizing hormone and prolactin secretion. In: G. D. Niswender (Ed.) *Reproduction in Domestic Ruminants*. The Dorset Press, Dorchester, UK.
- Dalton, J. C., S. Nadir, J. H. Blame, M. Noftsinger, R. L. Nebel, and R. G. Saacke. 2001. Effect of time of insemination on number of accessory sperm, fertilization rate, and embryo quality in nonlactating dairy cattle. *J. Dairy Sci.* 84:2413-2418.

- Day, M. L., C. R. Burke, V. K. Taufa, A. M. Day, and K. L. Macmillan. 2000. The strategic use of estradiol to enhance fertility and submission rates of progestin-based estrus synchronization programs in dairy herds. *J. Anim. Sci.* 78:523-529.
- DeBois, C. H. W., and C. J. Bierschwal, Jr. 1970. Estrous cycle synchronization in dairy cattle given a 14-day treatment of melengestrol acetate. *Am. J. Vet. Res.* 31:1545.
- De Fries, C. A., D. A. Neuendorff, and R. D. Randel. 1998. Fat supplementation influences postpartum reproductive performance in Brahman cows. *J. Anim. Sci.* 76:864-870.
- DeJarnette, M. 1999. Estrous synchronization in cattle using GnRH and PGF. Select Sires Reproduction Management Tools. http://www.selectsires.com/news_menu.html.
- Diaz, T., E. J.-P. Schmitt, R. L. de la Sota, M.-J. Thatcher, and W. W. Thatcher. 1998. Human chorionic gonadotropin-induced alterations in ovarian follicular dynamics during the estrous cycle of heifers. *J. Anim. Sci.* 76:1929-1936.
- Dickerson, G. 1970. Efficiency of animal production-molding the biological components. *J. Anim. Sci.* 30:849.
- Dowling, D. W., J. E. Pexton, and P. T. Fagerlin. 1977. Methods of bovine estrus control: Calf separation and 7-vs. 9-day treatments. *J. Anim. Sci.* 45(Suppl. 1):151 (Abstr.).
- Downing, E. R., D. G. LeFever, J. C. Whittier, J. E. Bruemmer, and T. W. Geary. 1998. Estrous and ovarian response to the Select Synch protocol. *J. Anim. Sci.* 76(Suppl. 1):373 (Abstr.).
- Echternkamp, S. E., and R. R. Maurer. 1983. Conception, embryonic development and corpus luteum function in beef cattle open for two consecutive breeding seasons. *Theriogenology* 20:627.
- Eduvie, L. O., and B. E. Seguin. 1982. Corpus luteum function and pregnancy rate in lactating dairy cows given human chorionic gonadotropin at midestrus. *Theriogenology* 17:415.
- Edwards, S., J. F. Roche, and G. D. Niswender. 1983. Response of suckling beef cows to multiple, low-dose injections of Gn-RH with or without progesterone pretreatment. *J. Reprod. Fertil.* 69:65.
- Edwards, J. L., and P. J. Hansen. 1996. Elevated temperature increases heat shock protein 70 synthesis in bovine two-cell embryos and compromises function of maturing oocytes. *Biol. Reprod.* 55:340-346.

- Erickson, P. J. 1989. Niacin-lipid interactions in lactating cows. Ph.D. Dissertation. Univ. of Illinois, Urbana-Champaign.
- Espinoza, J. L., J. A. Ramirez-Godinez, J. A. Jimenez, and A. Flores. 1995. Effects of calcium soaps of fatty acids on postpartum reproductive activity in beef cows and growth of calves. *J. Anim. Sci.* 73:2888-2892.
- Estergreen, V. L., O. L. Frost, W. R. Gomes, R. E. Erb, and J. F. Bullard. 1968. Effect of ovariectomy on pregnancy maintenance and parturition in dairy cows. *J. Dairy Sci.* 50:1293.
- Fike, K. E., M. L. Day, E. K. Inskeep, J. E. Kinder, P. E. Lewis, R. E. Short, and H. D. Hafs. 1997. Estrus and luteal function in suckled beef cows that were anestrous when treated with an intravaginal device containing progesterone with or without a subsequent injection of estradiol benzoate. *J. Anim. Sci.* 75:2009-2015.
- Fike, K. E., M. E. Wehrman, B. R. Lindsey, E. G. Bergfeld, E. J. Melvin, J. A. Quintal, E. L. Zanella, F. N. Kojima, and J. E. Kinder. 1999. Estrus synchronization of beef cattle with a combination of melengestrol acetate and an injection of progesterone and 17 β -Estradiol. *J. Anim. Sci.* 77:715-723.
- Filley, S. J., H. A. Turner, and F. Stormshak. 2000. Plasma fatty acids, prostaglandin F₂ metabolite, and reproductive response in postpartum heifers fed rumen bypass fat. *J. Anim. Sci.* 78:139-144.
- Fonseca, F. A., J. H. Britt, B. T. McDaniel, J. C. Wilk, and A. H. Rakes. 1983. Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. *J. Dairy Sci.* 66:1128.
- Foote, R. H. 1975. Estrus detection and estrus detection aids. *J. Dairy Sci.* 58:248-256.
- Foote, R. H. 2002. The history of artificial insemination: Selected notes and notables. *Am. Soc. of Anim. Sci.*
- Forbes, W. L., L. R. Corah, and J. S. Stevenson. 1997. Synchronized follicular growth and luteal regression before insemination of suckled beef cows. *J. Anim. Sci.* 75(Suppl. 1):90 (Abstr.).
- Ford, S. P., and F. Stormshak. 1978. Bovine ovarian and pituitary responses to PMS and GnRH administered during metestrus. *J. Anim. Sci.* 46:1701.
- Forrest, P. K., R. C. Rhodes III, and R. D. Randel. 1980. Effect of variable suckling intensity and estrogen administration upon serum luteinizing hormone in Brahman cows. *Theriogenology* 13:333.

- Fortune, J. E., J. Sirois, and S. M. Quirk. 1988. The growth and differentiation of ovarian follicles during the bovine estrous cycle. *Theriogenology* 29:95.
- Fricke, P. M., L. P. Reynolds, and D. A. Redmer. 1993. Effect of human chorionic gonadotropin administered early in the estrous cycle on ovulation and subsequent luteal function in cows. *J. Anim. Sci.* 71:1242-1246.
- Funston, R. N., T. W. Geary, R. P. Ansotegui, J. J. Lipsey, M. D. MacNeil, and J. A. Paterson. 2001. Supplementation of whole sunflower seeds before AI in beef heifers. *Proc. WSAS Anim. Sci.* 52:381-383.
- Gambill, D. M., M. K. Perterson, D. E. Hawkins, I. T. Luna, J. S. S. Corona, D. Dunlap, and K. M. Havstad. 1995. Post-partum anestrus and fall pregnancy in two-year-old range cows supplemented with protein and fat. *J. Anim. Sci.* 73(Suppl. 1):255 (Abstr.).
- Garverick, H. A., R. G. Elmore, D. H. Vaillancourt, and A. J. Sharp. 1980. Ovarian response to gonadotropin-releasing hormone in postpartum dairy cows. *Am. J. Vet. Res.* 41:1582-1585.
- Geary, T. W., J. C. Whittier, E. R. Downing, D. G. LeFever, R. W. Silcox, M. D. Holland, T. M. Nett, and G. D. Niswender. 1998. Pregnancy rates of postpartum beef cows that were synchronized with Syncro-Mate B or the Ovsynch protocol. *J. Anim. Sci.* 76:1523-1527.
- Geary, T. W., and J. C. Whittier. 1998. Effects of a timed insemination following synchronization of ovulation using the Ovsynch or Co-Synch protocol in beef cows. *Prof. Anim. Sci.* 14:217-220.
- Geary, T. W., E. R. Downing, J. C. Bruemmer, and J. C. Whittier. 2000. Ovarian and estrous response of suckled beef cows to the Select Synch estrous synchronization protocol. *Prof. Anim. Sci.* 16:1-5.
- Geary, T. W., J. C. Whittier, R. G. Mortimer, J. W. Young, and R. R. Salverson. 2001. Synchronization of estrus in beef cows using GnRH and PGF with estrus AI or timed AI 72 h after PGF with or without a second GnRH injection. *Proc. Western Section Am. Soc. Anim. Sci.* 52:369-371.
- Geary, T. W., J. C. Whittier, D. M. Hallford, and M. D. MacNeil. 2001a. Calf removal improves conception rates to the Ovsynch and Co-Synch protocols. *J. Anim. Sci.* 79:1-4.
- Geisert, R. D., M. T. Zavy, B. G. Biggers, J. E. Garrett, and R. P. Wettemann. 1988. Characterization of the uterine environment during early conceptus expansion in the bovine. *Anim. Reprod. Sci.* 16:11-25.

- Ginther, O. J., J. P. Kastlic, and L. Knopf. 1989. Composition and characteristics of follicular waves during the bovine estrous cycle. *Anim. Reprod. Sci.* 20:187.
- Ginther, O. J., M. C. Wiltbank, P. M. Fricke, J. R. Gibbons, and K. Kot. 1996. Selection of the dominant follicle in cattle. *Biol. Reprod.* 55:1187-1194.
- Gonzalez-Padilla, E., R. Ruiz, D. LeFever, A. Denham, and J. N. Wiltbank. 1975. Puberty in beef heifers. III. Induction of fertile estrus. *J. Anim. Sci.* 40:1110-1118.
- Grant, M. H. J., B. W. Hess, D. L. Hixon, E. A. Van Kirk, B. M. Alexander, T. M. Nett, and G. E. Moss. 2003. Effect of feeding high-linoleate safflower seeds on reproductive endocrine dynamics in postpartum beef females. *Proc. WSAS Anim. Sci.* 54:36-39.
- Graves, W. E., J. W. Lauderdale, E. R. Hauser, and L. E. Casida. 1968. Relation of postpartum interval to pituitary gonadotropins, ovarian follicular development and fertility in beef cows (Effect of suckling and interval to breeding). *Univ. of Wisconsin Res. Bull. No. 270*, pp 23-26.
- Grieger, D. G., G. C. Lamb, T. G. Rozell, K. E. Thompson, and J. S. Stevenson. 1998. Site of semen deposition and fertility in beef cows inseminated according to estrus or at a fixed time after synchronization with GnRH-PGF_{2α}. *J. Anim. Sci.* 76 (Suppl. 1) / *J. Dairy Sci.* 81 (Suppl. 1):1088.
- Grimard, B., P. Humblot, A. A. Ponter, J. P. Mialot, D. Sauvant, and M. Thibier. 1995. Influence of postpartum energy restriction on energy status, plasma LH and oestradiol secretion and follicular development in suckled beef cows. *J. Reprod. Fertil.* 104:173-179.
- Grummer, R. R., and D. J. Carroll. 1991. Effects of Dietary fat on metabolic disorders and reproductive performance of dairy cattle. *J. Anim. Sci.* 69:3838-382.
- Guilbault, L. A., P. Rouiller, P. Matton, R. G. Glencross, A. J. Beard, and P. G. Knight. 1993. Relationships between the level of atresia and inhibin contents (α subunit and α - β dimer) in morphologically dominant follicles during their growing and regressing phases of development in cattle. *Biol. Reprod.* 48:268.
- Guthrie, H. D., D. R. Lamond, D. M. Henricks, and J. F. Dickey. 1970. Ovarian follicular changes in heifers treated with melengestrol acetate. *J. Reprod. Fertil.* 22:363.
- Hall, J. B., R. B. Staigmiller, R. A. Bellows, R. E. Short, W. M. Moseley, and S. E. Bellows. 1995. Body composition and metabolic profiles associated with puberty in beef heifers. *J. Anim. Sci.* 73:3409-3420.

- Hansel, W., P. V. Malven, and D. L. Black. 1961. Estrous cycle regulation in the bovine. *J. Anim. Sci.* 20:621.
- Hansel, W., L. E. Donaldson, W. C. Wagner, and M. A. Brunner. 1966. A comparison of estrous cycle synchronization methods in beef cattle under feedlot conditions. *J. Anim. Sci.* 25:497.
- Hansel, W., R. W. Spalding, L. L. Larson, D. B. Laster, J. F. Wagner and R. K. Braun. 1976. Influence of human chorionic gonadotropin on pregnancy rates in lactating dairy and beef cows. *J. Dairy Sci.* 59:751.
- Hansel, W., J. Lukaszewska and W. Beal. 1978. Maintenance of the bovine corpus luteum of early pregnancy. *Biol. Reprod.* 1:(Suppl.18):27A (Abstr.).
- Hansen, P. J. 2002. Embryonic mortality in cattle from the embryo's perspective. *J. Anim. Sci.* 80(Suppl. 2):E33-E44.
- Harfoot, C. G., and G. P. Hazelwood. 1988. Lipid metabolism in the rumen. In: P. N. Hobson (Ed.). *The Rumen Microbial Ecosystem.* pp. 285-322. Elsevier Applied Science, New York, NY.
- Hawkins, D. E., K. D. Niswender, G. M. Moss, C. L. Moeller, K. G. Odde, H. R. Sawyer, and G. D. Niswender. 1995. An increase in serum lipids increase luteal lipid content and alters the disappearance rate of progesterone in cows. *J. Anim. Sci.* 73:541-545.
- Heape, W. 1897. The artificial insemination of mammals and subsequent possible fertilization or impregnation of their ova. *Proc. R. Soc. Lond. B* 61:52-63.
- Higgins, C. K., J. G. Berardinelli, D. K. Han, R. P. Ansotegui, and E. L. Moody. 1986. Estrus synchronization systems involving prostaglandin F_{2α} and progesterone pretreatment in beef heifers. *Theriogenology* 25:249-261.
- Hightshoe, R. B., R. C. Cochran, L. R. Corah, G. H. Kiracofe, D. L. Harmon, and R. C. Perry. 1991. Effects of calcium soaps of fatty acids on postpartum reproductive function in beef cows. *J. Anim. Sci.* 69:4097-4103.
- Hixon, D. L., R. D. Landis, B. M. Alexander, D. W. Moore, D. D. Carr, and G. E. Moss. 2001. A MGA-Prostaglandin-GnRH estrus synchronization protocol suitable for timed artificial insemination. *Proc. Western Section Am. Soc. Anim Sci.* 52:372-373.
- Hoffman, D. P., J. S. Stevenson, and J. E. Minton. 1996. Restricting calf presence without suckling compared with weaning prolongs postpartum anovulation in beef cattle. *J. Anim. Sci.* 74:190-198.

- Hu, Y., J. D. H. Sanders, S. G. Kurz, J. S. Ottobre, and M. L. Day. 1990. *In Vitro* prostaglandin production by bovine corpora lutea destined to be normal or short-lived. *Biol. Reprod.* 42:801-807.
- Hughes, T. L., A. Villa-Gody, J. S. Kesner, and R. L. Fogwell. 1987. Destruction of bovine ovarian follicles: effects on the pulsatile release of luteinizing hormone and prostaglandin F_{2α} induced luteal regression. *Biol. Reprod.* 36:523.
- Imwalle, D. B., D. J. Patterson, and K. K. Schillo. 1998. Effects of melengestrol acetate on onset of puberty, follicular growth, and patterns of luteinizing hormone secretion in beef heifers. *Biol. Reprod.* 58:1432-1436.
- Imwalle, D. B., D. L. Fernandez, and K. K. Schillo. 2002. Melengestrol acetate blocks the preovulatory surge of luteinizing hormone, the expression of behavioral estrus, and ovulation in beef heifers. *J. Anim. Sci.* 80:1280-1284.
- Inskeep, K. E. 1995. Factors that affect fertility during oestrous cycles with short or normal luteal phases in postpartum cows. *J. Reprod. Fert.* 49:493-503.
- Ireland, J. J., R. A. Milvae, T. L. Martin, R. F. Aten, and H. R. Behrman. 1990. Effect of histone H2a on progesterone production by bovine luteal cells. *Biol. Reprod.* 43:1058.
- Irvin, H. J., A. A. Zaied, B. N. Day, and H. A. Garverick. 1981. GnRH induced LH release in suckled beef cows. I. The effect of days post-partum and oestradiol-17β concentrations on the release of LH following administration of GnRH. *Theriogenology* 15:443.
- Ivanow [Ivanov], E. I. 1907. De la fecundation artificielle chez les mammiferes. *Arch. Sci. Biol.* 12:377-511.
- Jablonka-Shariff, A., L. P. Reynolds, and D. A. Redmer. 1994. Effect of FSH-P treatment and withdrawal on follicular growth and atresia in ewes. *J. Anim. Sci.* 72(Suppl. 1) / *J. Dairy Sci.* 77(Suppl. 1):76 (Abstr.).
- Jerred, M. J., D. J. Carroll, D. K. Combs, and R. R. Grummer. 1990. Effects of fat supplementation and immature alfalfa to concentrate ratios on nutrient utilization and lactation. *J. Dairy Sci.* 73:2842.
- Johnson, C. T. 1978. Time to onset of oestrus after the injection of heifers with cloprostenol. *Vet. Rec.* 103:204.
- Kesler, D. J., H. A. Garverick, R. S. Youngquist, R. G. Elmore, and C. J. Bierschwal. 1978. Ovarian and endocrine responses and reproductive performance following GnRH treatment in early post-partum dairy cows. *Theriogenology* 9:363.

- King, M. E., G. H. Kiracofe, J. S. Stevenson, and R. R. Schalles. 1982. Effect of stage of the estrous cycle on interval to estrus after PGF_{2α} in beef cattle. *Theriogenology*. 18:191.
- Kiracofe, G. H. 1980. Uterine involution: Its role in regulating postpartum intervals. *J. Anim. Sci.* 51(Suppl.II):16.
- Kiser, T. E., S. E. Dunlap, L. L. Benyshek, and S. E. Mares. 1980. The effect of calf removal on estrous response and pregnancy rate of beef cows after Syncro-Mate B treatment. *Theriogenology* 13:381.
- Ko, J. C. H., J. P. Kastelic, M. R. Del Campo, and O. J. Ginther. 1991. Effect of a dominant follicle on ovarian follicular dynamics during the oestrous cycle in heifers. *J. Reprod. Fertil.* 91:511.
- Kojima, F. N., T. T. Stumpf, A. S. Cupp, L. A. Werth, M. S. Roberson, M. W. Wolfe, R. J. Kittok, and J. E. Kinder. 1992. Exogenous progesterone and progestins as used in estrous synchrony regimens do not mimic the corpus luteum in regulation of luteinizing hormone and 17β-Estradiol in circulation of cows. *Biol. Reprod.* 47:1009-1017.
- Kojima, F. N., B. E. Salfen, J. F. Bader, W. A. Ricke, M. C. Lucy, M. F. Smith, and D. J. Patterson. 2000. Development of an estrus synchronization protocol for beef cattle with short-term feeding of melengestrol acetate: 7-11 Synch. *J. Anim. Sci.* 78:2186-2191.
- Ladewig, H. 1986. Characteristics and production practices of beef cattle producers in south and central Texas. *Texas Agric. Exp. Sta. Tech. Rep.* 86-2.
- Lamb, G. C., J. M. Lynch, D. M. Grieger, J. E. Minton, and J. S. Stevenson. 1997. Ad Libitum suckling by an unrelated calf in the presence or absence of a cow's own calf prolongs postpartum anovulation. *J. Anim. Sci.* 75:2762-2769.
- Lamb, G. C., B. L. Miller, J. M. Lynch, K. E. Thompson, J. S. Heldt, C. A. Löest, D. M. Grieger, and J. S. Stevenson. 1999. Twice daily suckling but not milking with calf presence prolongs postpartum anovulation. *J. Anim. Sci.* 77:2207-2218.
- Lamb, G. C., J. S. Stevenson, D. J. Kesler, H. A. Garverick, D. R. Brown, and B. E. Salfen. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F_{2α} for ovulation control in postpartum suckled beef cows. *J. Anim. Sci.* 79:2253-2259.
- Lamming, G. E., 1978. Reproduction during lactation. In: D. B. Crighton, N. B. Haynes, G. R. Foxcroft, and G. R. Lamming. (Ed.). *Control of Ovulation*. Butterworth, London.

- Lammoglia, M. A., R. A. Bellows, E. E. Grings, J. W. Bergmen, R. E. Short, and M. D. MacNeil. 1997. Effects of dietary fat composition and content, breed, and calf sex on birth weight, dystocia, calf vigor, and postpartum reproduction on first calf beef heifers. *Proc. Western Section Am. Soc. Anim. Sci.* 48:81-83.
- Lammoglia, M. A., R. E. Short, S. E. Bellows, R. A. Bellows, M. D. MacNeil, and H. D. Hafs. 1998. Induced and synchronized estrus in cattle: Dose titration of estradiol benzoate in peripubertal heifers and postpartum cows after treatment with an intravaginal progesterone-releasing insert and prostaglandin F_{2α}. *J. Anim. Sci.* 76:1662-1670.
- Lammoglia, M. A., R. A. Bellows, E. E. Grings, J. W. Bergman, R. E. Short, D. M. Hallford, and R. D. Randel. 2000. Effects of dietary fat and sire breed on puberty, weight, and reproductive traits of F1 beef heifers. *J. Anim. Sci.* 78:2244-2252.
- Lamond, D. R., J. F. Dickey, D. M. Henricks, J. R. Hill, Jr., and T. M. Leland. 1971. Effect of progestin on the bovine ovary. *J. Anim. Sci.* 33:77.
- Lauderdale, J. W. 1972. Effects of PGF_{2α} on pregnancy and estrous cycle of cattle. *J. Anim. Sci.* 35:246 (Abstr.).
- Lauderdale, J. W. 1979. Efficacy of Lutalyse sterile solution. In: J. W. Lauderdale and J. H. Sokolowski (Ed.) *Proceedings of the Lutalyse Symposium*. Pp 17-32. Upjohn Co., Kalamazoo, MI.
- Lauderdale, J. W., J. F. McAllister, E. L. Moody, and D. D. Kratzer. 1980. Pregnancy rate in beef cattle injected once with PGF_{2α}. *J. Anim. Sci.* 51(Suppl. 1):296 (Abstr.).
- Lavoit, M. C., and J. E. Fortune. 1990. Follicular dynamics in heifers after injection of PGF_{2α} during the first wave of follicular development. *Theriogenology* 33:270 (Abstract).
- Lemaster, J. W., J. V. Yelich, J. R. Kempfer, J. K. Fullenwider, C. L. Barnett, M. D. Fanning, and J. F. Selph. 2001. Effectiveness of GnRH plus prostaglandin F_{2α} for estrus synchronization in cattle of *Bos indicus* breeding. *J. Anim. Sci.* 79:309-316.
- Lonergan, P., P. Monaghan, D. Rizos, M. P. Boland, and I. Gordan. 1994. Effect of follicle size on bovine oocyte quality and developmental competence following maturation, fertilization and culture in vitro. *Mol. Reprod. Dev.* 37:48-53.
- Looper, M. L., C. A. Lents, and R. P. Wettemann. 2003. Body condition at parturition and postpartum weight changes do not influence the incidence of short-lived

- corpora lutea in postpartum beef cows. *J. Anim. Sci.* 81:2390-2394.
- Louis, T. M., H. D. Hafs, and D. A. Morrow. 1972. Estrus and ovulation after uterine prostaglandin $F_{2\alpha}$ in cows. *J. Anim. Sci.* 35:247 (Abstr.).
- Lucy, M. C., and J. S. Stevenson. 1986. Gonadotropin-releasing hormone at estrus: Luteinizing hormone, estradiol, and progesterone during the periestrual and postinsemination periods in dairy cattle. *Biol. Reprod.* 35:300-311.
- Lucy, M. C., T. S. Gross, and W. W. Thatcher. 1990. Effect of intravenous infusion of a soybean oil emulsion on plasma concentration of 15-keto-13, 14-dihydro-prostaglandin $F_{2\alpha}$ and ovarian function in cycling Holstein heifers. In: *Livestock Reproduction in Latin America*. pp 119-132. Int. Atomic Energy Agency, Vienna, Austria.
- Lucy, M. C., R. C. Staples, F. M. Michel, and W. W. Thatcher. 1991. Effect of feeding calcium soaps to early postpartum dairy cows on plasma prostaglandin $F_{2\alpha}$, luteinizing hormone, and follicular growth. *J. Dairy Sci.* 74:483-489.
- Lucy, M. C., C. R. Staples, W. W. Thatcher, P. S. Erickson, R. M. Cleale, J. L. Firkins, J. H. Clark, M. R. Murphy, and B. O. Brodie. 1992. Influence of diet composition, dry-matter intake, milk production and energy balance on time of post-partum ovulation and fertility in dairy cows. *Anim. Prod.* 54:323-331.
- Lucy, M. C., H. J. Billings, W. R. Butler, L. R. Ehnis, M. J. Fields, D. J. Kesler, J. E. Kinder, R. C. Mattos, R. E. Short, W. W. Thatcher, R. P. Wettemann, J. V. Yelich, and H. D. Hafs. 2001. Efficacy of an intravaginal progesterone insert and an injection of PGF $_{2\alpha}$ for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers, and dairy cows. *J. Anim. Sci.* 79:982-995.
- Mackey, D. R., J. M. Sreenan, J. F. Roche, and M. G. Diskin. 2000. The effect of progesterone alone or in combination with estradiol on follicular dynamics, gonadotropin profiles, and estrus in beef cows following calf isolation and restricted suckling. *J. Anim. Sci.* 78:1917-1929.
- Macmillan, K. L., and J. D. Watson. 1975. Fertility differences between groups of sires relative to the stage of oestrus at the time of insemination. *Anim. Prod.* 21:243-249.
- Macmillan, K. L. 1983. Prostaglandin responses in dairy herd breeding programs. *N.Z. Vet. J.* 31:110.
- Macmillan, K. L., and H. V. Henderson. 1984. Analyses of the variation in the interval from an injection of prostaglandin $F_{2\alpha}$ to oestrus as a method of studying patterns of follicle development during dioestrus in dairy cows. *Anim. Reprod. Sci.* 6:245.

- Macmillan, K. L., A. M. Day, V. K. Taufa, M. Gibb, and M. G. Pearce. 1985. Effects of an agonist of gonadotrophin-releasing hormone in cattle. I. Hormone concentrations and oestrus cycle length. *Anim. Reprod. Sci.* 8:203.
- Malven, P. V., J. R. Parfet, D. W. Gregg, R. D. Allrich, and G. E. Moss. 1986. Relationships among concentrations of four opioid neuropeptides and luteinizing hormone releasing hormone in neural tissues of beef cows following early weaning. *J. Anim. Sci.* 62:723.
- Mann, G. E., G. E. Lamming, and P.A. Fisher. 1998. Progesterone control of embryonic interferon tau production during early pregnancy in the cow. *J. Reprod. Fertil. Abstr. Ser. 21: Abstr. 37 (Abstr.)*.
- Martinez, M. F., J. P. Kastelic, G. P. Adams, and R. J. Mapletoft. 2002. The use of a progesterone-releasing device (CIDR-B) or melengestrol acetate with GnRH, LH, or estradiol benzoate for fixed-time AI in beef heifers. *J. Anim. Sci.* 80:1746-1751.
- Mass. J. 1987. Relationship between nutrition and reproduction in beef cattle. *Vet. Clinics N. Amer.: Food Anim. Practice* 3:633-645.
- Mattos, R., C. R. Staples, and W. W. Thatcher. 2000. Effects of dietary fatty acids on reproduction in ruminants. *J. Reprod. and Fertility.* 5:38-45.
- Mauck, H. S., M. D. Holland, M. E. King, D. G. LeFever, and K. G. Odde. 1988. Effectiveness of two MGA-PGF_{2α} combinations for estrous synchronization of beef heifers. *Proc. Western Section Am. Soc. Anim. Sci.* 39:283.
- McDowell, C. M., L. H. Anderson, R. P. Lemenager, D. A. Mangione, and M. L. Day. 1998. Development of a progestin-based estrus synchronization program: II. Reproductive response of cows fed melengestrol acetate for 14 days with injections of progesterone and prostaglandin F_{2α}. *J. Anim. Sci.* 76:1273-1279.
- McGuire, W. J., R. L. Larson, and G. H. Kiracofe. 1990. Syncro-mate B induces estrus in ovariectomized cows and heifers. *Theriogenology* 34:33-37.
- Mee, M. O., J. S. Stevenson, B. M. Alexander, and R. G. Sasser. 1993. Administration of GnRH at estrus influences pregnancy rates, serum concentrations of LH, FSH, estradiol-17β, pregnancy-specific protein B, and progesterone, proportion of luteal cell types, and in vitro production of progesterone in dairy cows. *J. Anim. Sci.* 71:185.
- Menge, A. C., S. E. Mares, W. J. Tyler, and L. E. Casida. 1962. Variation and association among postpartum reproduction and production characteristics in Holstein-Friesian cattle. *J. Dairy Sci.* 45:233-241.

- Milovanov, V. K. 1938. *Isskusstvenoye Ossemenebie Selsko-Khoziasvennykh Jivotnykh* [The Artificial Insemination of Farm Animals]. Seljhozgiz, Moscow.
- Moody, E. L. 1979. Studies on Lutalyse use programs for estrus control. In: J. W. Lauderdale and J. H. Sokolowski (Ed.) *Proceedings of the Lutalyse Symposium*. pp 33-52. Upjohn Co., Kalamazoo, MI.
- Moreira, F., R. L. de la Sota, T. Diaz, and W. W. Thatcher. 2000. Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. *J. Anim. Sci.* 78:1568-1576.
- Morgan, A. R., and G. L. Williams. 1989. Effects of body condition and postpartum dietary lipid intake on lipid metabolism and pituitary function of beef cows. *J. Anim. Sci.* 67(Suppl. 1):385 (Abstr.).
- Morrow, D. A., S. J. Roberts, K. McEntee, and H. G. Gray. 1966. Postpartum ovarian activity and uterine involution in dairy cattle. *J. Ameri. Vet. Med. Ass.* 149:1596-1609.
- Moss, G. E., M. E. Crowder, and T. M. Nett. 1981. GnRH-receptor interaction. VI. Effect of progesterone and estradiol on hypophyseal receptors for GnRH, and serum and hypophyseal concentrations of gonadotropins in ovariectomized ewes. *Biol. Reprod.* 25:938.
- National Animal Health Monitoring System (NAHMS). 1997. Part 1: Reference of 1997 Beef Cow-Calf Management Practices. P. 38, USDA-APHIS-VS, Fort Collins, CO.
- Nett, T. M., D. Cermak, T. Branden, J. Manns, and G. Niswender. 1988. Pituitary receptors for GnRH and estradiol, and pituitary content of gonadotropins in beef cows. II. Changes during the postpartum period. *Domest. Anim. Endocrinol.* 5:81.
- Niswender, G. D. 1973. Influence of the site of conjugation on the specificity of antibodies to progesterone. *Steroids* 22:413-424.
- Niswender, G. D., R. H. Schwall, T. A. Fitz, C. E. Farin, and H. R. Sawyer. 1985. Regulation of the luteal function in domestic ruminants: New concepts. In: R. O. Greep (Ed.) *Recent Progress in Hormone Research*. Vol 41:101. Academic Press, New York.
- Odde, K. G., H. S. Ward, G. H. Kiracofe, R. M. McKee, and R. J. Kittok. 1980. Short estrous cycles and associated serum progesterone levels in beef cows. *Theriogenology* 14:102.

- Odde, K. G., D. G. LeFever, H. S. Mauck, and R. E. Taylor. 1987. Calving distribution of estrous synchronized beef cows. Colorado State Univ. Beef Program Rep. P 89. Fort Collins.
- Odde, K. G. 1990. A review of synchronization of estrus in postpartum cattle. *J. Anim. Sci.* 68:817-830.
- Oss, G. M., D. N. Schutz, and K. G. Odde. 1993. Effects of a high fat diet on reproductive performance in pre- and postpartum beef heifers. *Proc. WSAS Anim. Sci.* 44:44-47.
- Pace-Aciak, C., and L. S. Wolfe. 1968. Inhibition of prostaglandin synthesis by oleic, linoleic, and linolenic acids. *Biochimica et Biophysica Acta.* 152:784-787.
- Pace, M. M., and J. J. Sullivan. 1980. Effect of Syncro-Mate B (SMB) and calf separation on beef cattle estrus and pregnancy rates. *J. Anim. Sci.* 51(Suppl. 1):312 (Abstr.).
- Patterson, D. J., G. H. Kiracofe, J. S. Stevenson, and L. R. Corah. 1989. Control of the bovine estrous cycle with melengestrol acetate (MGA): A Review. *J. Anim. Sci.* 67:1895-1906.
- Patterson, D. J., and L. R. Corah. 1992. Evaluation of a melengestrol acetate and prostaglandin F_{2α} system for the synchronization of estrus in beef heifers. *Theriogenology.* 38:441-447
- Patterson, D. J., J. B. Hall, N. W. Bradley, K. K. Schillo, B. L. Woods, and J. M. Kearman. 1995. Improved synchrony, conception rate, and fecundity in postpartum suckled beef cows fed melengestrol acetate prior to prostaglandin F_{2α}. *J. Anim. Sci.* 73:954-959.
- Pennington, J. A., J. L. Albright, and M. A. Diekman. 1985. Sexual activity of Holstein cows: Seasonal effects. *J. Dairy Sci.* 68:3023-3030.
- Perry, R. C., L. R. Corah, G. H. Kiracofe, J. S. Stevenson, and W. E. Beal. 1991. Endocrine changes and ultrasonography of ovaries in suckled beef cows during resumption of postpartum estrous cycles. *J. Anim. Sci.* 69:2548.
- Perry, R. C., L. R. Corah, R. C. Cochran, W. E. Beal, J. S. Stevenson, J. E. Minton, D. D. Simms, and J. R. Brethour. 1991a. Influence of dietary energy on follicular development, serum gonadotropins, and first postpartum ovulation in suckled beef cows. *J. Anim. Sci.* 69:3762-3773.
- Perry, G. A., M. F. Smith, and D. J. Patterson. 2002. Evaluation of a fixed-time artificial insemination protocol for postpartum suckled beef cows. *J. Anim. Sci.* 80:3060-3064.

- Peters, A. R., M. G. Pimental, and G. E. Lamming. 1985. Hormone responses to exogenous GnRH pulses in post-partum dairy cows. *J. Reprod. Fertil.* 75:557.
- Peters, A. R., T. A. Martinez, and A. J. Cook. 2000. A meta-analysis of studies of the effect of GnRH 11-14 days after insemination on pregnancy rates in cattle. *Theriogenology* 54:1317-126.
- Prado, T. M., R. P. Wettemann, L. J. Spicer, J. A. Vizcarra, and G. L. Morgan. 2002. Influence of exogenous gonadotropin-releasing hormone on ovarian function in beef cows after short- and long-term nutritionally induced anovulation. *J. Anim. Sci.* 80:3268-3276.
- Prescott, R. E., R. W. Silcox, D. J. Byerley, A. B. Caudle, and T. E. Kiser. 1992. Effect of GnRH on the dominant follicle of the first follicular wave in beef cows. *J. Anim. Sci.* 70(Suppl. 1):254 (Abstr.).
- Price, C. A., and R. Webb. 1989. Ovarian response to hCG treatment during the oestrous cycle in heifers. *J. Reprod. Fertil.* 86:303.
- Pursley, J. R., M. O. Mee, M. D. Brown, and M. C. Wiltbank. 1994. Synchronization of ovulation in dairy cattle using GnRH and PGF_{2α}. *J. Anim. Sci.* 72(Suppl. 1) / *J. Dairy Sci.* 77(Suppl. 1):230 (Abstr.).
- Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using PGF_{2α} and GnRH. *Theriogenology* 44:915-923.
- Pursley, J. R., R. W. Silcox, and M. C. Wiltbank. 1998. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. *J. Dairy Sci.* 81:2139-2144.
- Putney, D. J., M. Drost, and W. W. Thatcher. 1989. Influence of summer heat stress on pregnancy rates of lactating dairy cattle following embryo transfer or artificial insemination. *Theriogenology* 31:765-778.
- Ramirez-Godinez, J. A., G. H. Kiracofe, R. M. McKee, R. R. Schalles, and R. J. Kittok. 1981. Reducing the incidence of short estrous cycles in beef cows with norgestomet. *Theriogenology* 15:613.
- Ramirez-Godinez, J. A., G. H. Kiracofe, D. L. Carnahan, M. F. Spire, K. B. Beeman, J. S. Stevenson, and R. R. Schalles. 1982. Evidence of ovulation and fertilization in beef cows with short estrous cycles. *Theriogenology* 17:409.
- Randel, R. D., C. J. Callahan, R. E. Erb, H. A. Garverick, and B. L. Brown. 1972. Effect of melengestrol acetate on plasma progesterone, luteinizing hormone and total

- corticoids in dairy heifers. *J. Anim. Sci.* 35:389.
- Rawlings, N. C., L. Weir, B. Todd, J. Manns, and J. H. Hyland. 1980. Some endocrine changes associated with the postpartum period of the suckling beef cow. *J. Reprod. Fertil.* 60:301.
- Refsal, K. R., and B. E. Seguin. 1980. Effect of stage of diestrus and number of cloprostenol (ICI 80,996) injections on intervals to estrus, LH peak and ovulation in heifers. *Theriogenology* 14:37.
- Rettmer, I., J. S. Stevenson, and L. R. Corah. 1992. Endocrin responses and ovarian changes in inseminated dairy heifers after an injection of a GnRH agonist 11 to 13 days after estrus. *J. Anim. Sci.* 70:508.
- Richards, M. W., J. C. Spitzer, and M. B. Warner. 1986. Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. *J. Anim. Sci.* 62:300.
- Richards, M. W., R. P. Wettemann, and H. M. Schoenemann. 1989. Nutritional anestrus in beef cows: body weight change, body condition, luteinizing hormone in serum and ovarian activity. *J. Anim. Sci.* 67:1520-1526.
- Richardson, A. M., B. A. Hensley, T. J. Marple, S. K. Johnson, and J. S. Stevenson. 2002. Characteristics of estrus before and after first insemination and fertility of heifers after synchronized estrus using GnRH, PGF_{2α}, and progesterone. *J. Anim. Sci.* 80:2792-2800.
- Roberson, M. S., M. W. Wolfe, T. T. Stumpf, R. J. Kittok, and J. E. Kinder. 1989. Luteinizing hormone secretion and corpus luteum function in cows receiving two levels of progesterone. *Biol. Reprod.* 41:997-1003.
- Robinson, N. A., K. E. Leslie, and J. S. Walton. 1989. Effect of treatment with progesterone on pregnancy rate and plasma concentrations of progesterone in Holstein cows. *J. Dairy Sci.* 72:202.
- Rodger, L. D., and F. Stormshak. 1986. Gonadotropin-releasing hormone-induced alteration of bovine corpus luteum function. *Biol. Reprod.* 35:149.
- Roussel, J. D., and J. F. Beatty. 1969. Effects of melengestrol acetate on synchronization of estrus, subsequent fertility, and milk constituents of lactating dairy cows. *J. Dairy Sci.* 52:2020.
- Rowson, L. E. A. 1971. The role of reproductive research in animal production. *J. Reprod. Fertil.* 26:113-126.
- Rowson, L. E. A., R. Tervit, and A. Brand. 1972. The use of prostaglandin for

- synchronization of oestrus in cattle. *J. Reprod. Fertil.* 29:145.
- Ryan, D. P., R. A. Spoon, and G. L. Williams. 1992. Ovarian follicular characteristics, embryo recovery, and embryo viability in heifers fed high fat diets and treated with follicle-stimulating hormone. *J. Anim. Sci.* 70:3505-3515.
- Ryan, D. P., S. Snijders, T. Condon, M. Greal, J. Sreenan, and K. J. O'Farrell. 1994. Endocrine and ovarian responses and pregnancy rates in dairy cows following the administration of a gonadotropin releasing hormone analog at the time of artificial insemination or at mid-cycle post insemination. *Anim. Reprod. Sci.* 34:179-191.
- Ryan, D. P., S. Snijders, H. Yaakub, and K. J. O'Farrell. 1995. An evaluation of estrus synchronization programs in reproductive management of dairy herds. *J. Anim. Sci.* 73:3687-3695.
- Saacke, R. G. 2002. Factors affecting fertilization in estrous synchronized cattle. *Proc. The Applied Reproductive Strategies in Beef Cattle Workshop*. Manhattan, KS. p.163-172.
- Salverson, R. R., J. C. Whittier, E. R. Downing, and T. W., Geary. 1999. Synchronization of ovulation using hCG or GnRH with the Co-Synch protocol in suckled beef cows. *Western Section Amer. Soc. Anim. Sci.* 77(Suppl. 1):3(Abstr.).
- Sanchez, T., M. E. Wehrman, E. G. Bergfeld, K. E. Peters, F. N. Kojima, A. S. Cupp, V. Mariscal, R. J. Kittok, R. J. Rasby, and J. E. Kinder. 1993. Pregnancy rate is greater when the corpus luteum is present during the period of progestin treatment to synchronize time of estrus in cows and heifers. *Biol. Reprod.* 49:1102.
- Sanchez, T., M. E. Wehrman, F. N. Kojima, A. S. Cupp, E. G. Bergfeld, K. E. Peters, V. Mariscal, R. J. Kittok, and J. E. Kinder. 1995. Dosage of the synthetic progestin, norgestomet, influences luteinizing hormone pulse frequency and endogenous secretion of 17 β -estradiol in heifers. *Biol. Reprod.* 52:464.
- SAS. Institute Inc., SAS/STAT(R) Version 8.1, Cary, NC: SAS Institute Inc., 1996.
- Savio, J. D., M. P. Boland, N. Hynes, and J. F. Roche. 1990. Resumption of follicular activity in the early post-partum period of dairy cows. *J. Reprod. Fertil.* 88:569.
- Savio, J. D., W. W. Thatcher, G. R. Morris, K. Entwistle, M. Drost, and M. R. Mattiacci. 1993. Effects of induction of low plasma progesterone concentrations with a progesterone-releasing intravaginal device on follicular turnover and fertility in cattle. *J. Reprod. Fertil.* 98:77-84.
- Schillo, K. K. 1992. Effects of dietary energy on control of luteinizing hormone secretion in cattle and sheep. *J. Anim. Sci.* 70:1271-1282.

- Schmitt, E. J.-P., T. Diaz, R. L. de la Sota, and W. W. Thatcher. 1993. Differential responses of the luteal phase in cattle following ovulation of the first wave follicle with a GnRH agonist versus hCG. *J. Anim. Sci.* 71(Suppl. 1):212 (Abstr.).
- Schmitt, E. J.-P., T. C. Diaz, M. Drost, and W. W. Thatcher. 1994a. Use of a GnRH-agonist for a timed-insemination protocol in cattle. *J. Anim. Sci.* 72(Suppl. 1) / *J. Dairy Sci.* 77(Suppl. 1):292 (Abstr.).
- Schmitt, E. J.-P., M. Drost, T. C. Diaz, C. Roomes, and W. W. Thatcher. 1994b. Effect of a GnRH agonist on follicle recruitment and pregnancy rate in cattle. *J. Anim. Sci.* 72(Suppl. 1) / *J. Dairy Sci.* 77(Suppl. 1):230 (Abstr.).
- Schmitt, E. J.-P., M. Drost, T. Diaz, C. Roomes, and W. W. Thatcher. 1996. Effect of a gonadotropin-releasing hormone agonist on follicle recruitment and pregnancy rate in cattle. *J. Anim. Sci.* 74:154-161.
- Schmitt, E. J.-P., T. Diaz, C. M. Barros, R. L. de la Sota, M. Drost, E. W. Fredriksson, C. R. Staples, R. Thorner, and W. W. Thatcher. 1996a. Differential response of the luteal phase and fertility in cattle following ovulation of the first-wave follicle with human chorionic gonadotropin or an agonist of gonadotropin-releasing hormone. *J. Anim. Sci.* 74:1074-1083.
- Schmitt, E. J.-P., C. M. Barros, P. A. Fields, M. J. Fields, T. Diaz, J. M. Kluge, and W. W. Thatcher. 1996b. A cellular and endocrine characterization of the original and induced corpus luteum after administration of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin on day five of the estrous cycle. *J. Anim. Sci.* 74:1915-1929.
- Schmitt, E. J.-P., T. Diaz, M. Drost, and W. W. Thatcher. 1996c. Use of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin for timed insemination in cattle. *J. Anim. Sci.* 74:1084-1091.
- Scholljegerdes, E. J., B. W. Hess, K. R. Hightower, G. E. Moss, D. L. Hixon, and D. C. Rule. 2001. Biohydrogenation, flow and disappearance of fatty acids in beef cattle fed supplemental high-linoleate or high-oleate safflower seeds. *Proc. WSAS Anim. Sci.* 52.
- Senger, P. L. 1999. *Pathways to Pregnancy and Parturition*. Current Conceptions, Inc. Washington State University Research & Technology Park.
- Sharpe, P. H., D. R. Gifford, P. F. Flavel, M. G. Nottle, and D. T. Armstrong. 1986. Effect of melatonin on postpartum anestrus in beef cows. *Theriogenology* 26:621.
- Shively, T. E., and G. L. Williams. 1989. Patterns of tonic luteinizing hormone release

- and ovulation frequency in suckled anestrous beef cows following varying intervals of temporary weaning. *Domest. Anim. Endocrinol.* 6:379-387.
- Short, R. E., R. A. Bellows, E. L. Moody, and B. E. Howland. 1972. Effects of suckling and mastectomy on bovine postpartum reproduction. *J. Anim. Sci.* 34:70.
- Short, R. B. 1976. Lactation-The central control of reproduction. In: *Breast-feeding and the Mother*. Ciba Foundation Symp. No. 45 p. 73. Elsevier, Excerpta Medica, Amsterdam and Oxford.
- Short, R. E., R. D. Randel, R. B. Staigmiller, and R. A. Bellows. 1979. Factors affecting estrogen-induced LH release in the cow. *Biol. Reprod.* 21:683.
- Short, R. E., R. A. Bellows, R. B. Staigmiller, J. G. Berardinelli, and E. E. Custer. 1990. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. *J. Anim. Sci.* 68:799-816.
- Sipher, E. 1991. *The Gene Revolution: The History of Cooperative Artificial Breeding in New York and New England, 1938-1940*. Eastern A. I. Cooperative, Inc., Ithaca, NY.
- Sirois, J., and J. E. Fortune. 1990. Lengthening the bovine estrous cycle with low levels of exogenous progesterone: A model for studying ovarian follicular dominance. *Endocrinology* 127:916-925.
- Smith, V. G., J. R. Chenault, J. C. McAllister, and J. W. Lauderdale. 1987. Response of postpartum beef cows to exogenous progestogens and gonadotropin releasing hormone. *J. Anim. Sci.* 64:540.
- Snijders, S. E. M., P. Dillon, D. O'Callaghan, and M. P. Boland. 2000. Effect of genetic merit, milk yield, body condition and lactation number on in vitro oocyte development in dairy cows. *Theriogenology* 53:981-989.
- Spallanzani, L. 1784. *Dissertations relative to the natural history of animals and vegetables*. Trans. by T. Beddoes in *Dissertations Relative to the Natural History of Animals and Vegetables*. Vol. 2:195-199. J. Murray, London.
- Spicer, L. J., and S. E. Echtenkamp. 1986. Ovarian follicular growth, function and turnover in cattle: A review. *J. Anim. Sci.* 62:428.
- Spitzer, J. C., 1986. Influences of nutrition on reproduction in beef cattle. *Current Therapy in Theriogenology*. Philadelphia, WB Saunders Co. p.320.
- Spitzer, J. C., D. G. Morrison, R. P. Wettemann, and L. C. Faulkner. 1995. Reproductive responses and calf birth and weaning weights as affected by body condition at parturition and postpartum weight gain in primiparous beef cows. *J.*

Anim. Sci. 73:1251-1257.

- Sprott, L. R., J. N. Wiltbank, W. N. Songster, and S. Webel. 1984. Estrus and ovulation in beef cows following use of progesterone-releasing devices, progesterone and estradiol valerate. *Theriogenology*. 21:349-356.
- Stanko, R. L., P. Fajersson, L. A. Carver, and G. L. Williams. 1997. Follicular growth and metabolic changes in beef heifers fed incremental amounts of polyunsaturated fat. *J. Anim. Sci.* 75(Suppl. 1):223 (Abstr.).
- Staples, C. R., W. W. Thatcher, and J. H. Clark. 1990. Relationship between ovarian activity and energy status during the early postpartum period of high producing cows. *J. Dairy Sci.* 73:938.
- Staples, C. R., J. M. Burke, and W. W. Thatcher. 1998. Influence of supplemental fats on reproductive tissues and performance of lactating cows. *J. Dairy Sci.* 81:856-871.
- Stevenson, J. S., M. K. Schmidt, and E. P. Call. 1984. Stage of estrous cycle, time of insemination, and seasonal effects on estrus and fertility of Holstein heifers after prostaglandin F_{2α}. *J. Dairy Sci.* 67:1798.
- Stevenson, J. S., A. P. Phatak, I. Rettmer, and R. E. Stewart. 1993. Postinsemination administration of Receptal: follicular dynamics, duration of cycle, hormonal responses, and pregnancy rates. *J. Dairy Sci.* 76:2536.
- Stevenson, J. S., E. L. Knoppel, J. E. Minton, B. E. Salfen, and H. A. Garverick. 1994. Estrus, ovulation, luteinizing hormone, and suckling-induced hormones in mastectomized cows with and without unrestricted presence of the calf. *J. Anim. Sci.* 72:690-699.
- Stevenson, J. S., D. P. Hoffman, D. A. Nichols, R. M. McKee, and C. L. Krehbiel. 1997. Fertility in estrus-cycling and noncycling virgin heifers and suckled beef cows after induced ovulation. *J. Anim. Sci.* 75:1343-1350.
- Stevenson, J. S., K. E. Thompson, W. L. Forbes, G. C. Lamb, D. M. Geiger, and L. R. Corah. 2000. Synchronizing estrus and (or) ovulation in beef cows after combinations of GnRH, norgestomet, and prostaglandin F_{2α} with or without timed insemination. *J. Anim. Sci.* 78:1747-1758.
- Stevenson, J. S., S. M. Tiffany, and M. C. Lucy. 2002. Incidence and timing of estrus, LH surge, and ovulation in cows treated with the Ovsynch protocol with estradiol cypionate (ECP) substituting for GnRH. *J. Anim. Sci.* 80(Suppl. 1) / *J. Dairy Sci.* 85(Suppl. 1):394.
- Stevenson, J. S., G. C. Lamb, S. K. Johnson, M. A. Medina-Britos, D. M. Grieger, K. R.

- Harmoney, J. A. Cartmill, S. Z. El-Zarkouny, C. R. Dahlen, and T. J. Marple. 2003. Supplemental norgestomet, progesterone, or melengestrol acetate increases pregnancy rates in suckled beef cows after timed inseminations. *J. Anim. Sci.* 81:571-586.
- Stock, A. E., and J. E. Fortune. 1993. Ovarian follicular dominance in cattle: relationship between prolonged growth of the ovulatory follicle and endocrine parameters. *Endocrinology* 132:1108.
- Taft, R., B. Sayre, and E. K. Inskeep. 1999. Early increases in estradiol in follicular fluid from cattle with low peripheral concentrations of progesterone. *J. Anim. Sci.* 77(Suppl. 1):23.
- Thatcher, W. W., K. L. Macmillan, P. L. Hansen, and M. Drost. 1989. Concepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. *Theriogenology* 31:149.
- Thatcher, W. W., C. R. Staples, G. Danet-Desnoyers, B. Oldick, and E. P. Schmitt. 1994. Embryo health and mortality in sheep and cattle. *J. Anim. Sci.* 72(Suppl. 3):16-30.
- Thatcher, W. W., M. Binelli, J. M. Burke, C. R. Staples, J. D. Ambrose, and S. Coelho. 1997. Antiluteolytic signals between conceptus and endometrium. *Theriogenology* 47:131-140.
- Thomas, M. G., and G. L. Williams. 1996. Metabolic hormone secretion and FSH-induced superovulatory responses of beef heifers fed dietary fat supplements containing predominantly saturated or polyunsaturated fatty acids. *Theriogenology* 45:451-458.
- Thomas, M. G., B. Bao, and G. L. Williams. 1997. Dietary fats varying in their fatty acid composition differentially influence follicular growth in cows fed isoenergetic diets. *J. Anim. Sci.* 75:2512-2519.
- Thompson, K. E., J. S. Stevenson, G. C. Lamb, D. M. Grieger, and C. A. Löest. 1999. Follicular, hormonal, and pregnancy responses of early postpartum suckled beef cows to GnRH, norgestomet, and Prostaglandin F_{2α}. *J. Anim. Sci.* 77:1823-1832.
- Trimberger, G. W., and H. P. Davis. 1943. Breeding efficiency in dairy cattle bred at various stages of estrus by artificial insemination. *J. Dairy Sci.* 26:757-759.
- Trimberger, G. W. 1944. Conception rate in dairy cattle by artificial insemination at various intervals before and after ovulation. *J. Dairy Sci.* 27:659-660.
- Trimberger, G. W. 1948. Breeding efficiency in dairy cattle from artificial insemination at various intervals before and after ovulation. *Nebraska Agric. Exp. Sta. Bull.*

Lincoln. No. 153:26.

- Trimberger, G. W., and W. Hansel. 1955. Conception rate and ovarian function following estrus control by progesterone injections in dairy cattle. *J. Anim. Sci.* 14:224.
- Troxel, T. R., and D. J. Kesler. 1984. The effect of progestin and GnRH treatments on ovarian function and reproductive hormone secretions of anestrous postpartum suckled beef cows. *Theriogenology* 21:699-711.
- Troxel, T. R., L. C. Cruz, R. S. Ott, and D. J. Kesler. 1993. Norgestomet and gonadotropin-releasing hormone enhance corpus luteum function and fertility of postpartum suckled beef cows. *J. Anim. Sci.* 71:2579-2585.
- Twagiramungu, H., L. A. Guilbault, J. G. Proulx, P. Villeneuve, and J. J. Dufour. 1992a. Influence of an agonist of gonadotropin-releasing hormone (buserelin) on estrus synchronization and fertility in beef cows. *J. Anim. Sci.* 70:1904-1910.
- Twagiramungu, H., L. A. Guilbault, J. Proulx, and J. J. Dufour. 1992b. Synchronization of estrus and fertility in beef cattle with two injections of buserelin and prostaglandin. *Theriogenology* 38:1131.
- Twagiramungu, H. 1994. Dynamique folliculaire et synchronisation de l'oestrus des vaches traitées avec l'agoniste de la gonadolibérine (buserelin). Ph.D. Thesis. Université Laval, Québec, Canada.
- Twagiramungu, H., L. A. Guilbault, J. G. Proulx, and J. J. Dufour. 1994a. Influence of corpus luteum and induced ovulation on ovarian follicular dynamics in postpartum cyclic cows treated with buserelin and cloprostenol. *J. Anim. Sci.* 72:1796.
- Twagiramungu, H., L. A. Guilbault, J. Proulx, R. Ramkumar, and J. J. Dufour. 1994b. Histological populations and atresia of ovarian follicles in postpartum cattle treated with an agonist of gonadotropin-releasing hormone. *J. Anim. Sci.* 72:192.
- Twagiramungu, Herménégilde, Louis A. Guilbault, and Jacques J. Dufour. 1995. Synchronization of ovarian follicular waves with a gonadotropin-releasing hormone agonist to increase the precision of estrus in cattle: A Review. *J. Anim. Sci.* 73:3141-3151.
- Twagiramungu, H., L. A. Guilbault, J. G. Proulx, and J. J. Dufour. 1995a. Buserelin alters the development of the corpora lutea in cyclic and early postpartum cows. *J. Anim. Sci.* 73:805.
- Twagiramungu, H., G. L. Roy, G. Laverdière, and J. J. Dufour. 1995b. Fixed-time insemination in cattle after synchronization of estrus and ovulation with GnRH

and prostaglandin. *Theriogenology* 43:341 (Abstr.).

- Ulberg, L. C., R. E. Christian, and L. E. Casida. 1951. Ovarian response in heifers to progesterone injections. *J. Anim. Sci.* 10:752-759.
- Walker, W. L., R. L. Nebel, and M. L. McGilliard. 1996. Time of ovulation relative to mounting activity in dairy cattle. *J. Dairy Sci.* 79:1555-1561.
- Walker, R. S., P. D. Burns, G. E. Sides, and D. D. Zalesky. 2003. Evaluation of time to AI with a modified Co-Synch protocol and calf removal in postpartum beef cows. *Proc. Western Section Ameri. Soci. Anim. Sci.* 54:87-89.
- Walters, D. L., R. E. Short, E. M. Convey, R. B. Staigmiller, T. G. Dun, and C. C. Kaltenbach. 1982. Pituitary and ovarian function in postpartum beef cows. II. Endocrine changes prior to ovulation in suckled and nonsuckled postpartum cows compared to cycling cows. *Biol. Reprod.* 26:647.
- Watts, T. L., and J. W. Fuquay. 1985. Response and fertility of dairy heifers following injection with prostaglandin F_{2α} during early, middle and late diestrus. *Theriogenology* 23:655.
- Web, R., G. E. Lamming, N. B. Haynes, H. D. Hafs, and J. G. Manns. 1977. Response of cyclic and post-partum suckled cows to injections of synthetic LH-RH. *J. Reprod. Fertil.* 50:203-210.
- Wehrman, M. E., T. H. Welsh, Jr, and G. L. Williams. 1991. Diet-induced hyperlipidemia in cattle modifies the intrafollicular cholesterol environment, modulates ovarian follicular dynamics and hastens the onset of postpartum luteal activity. *Biol. Reprod.* 45:514-523.
- Wehrman, M. E., M. S. Roberson, A. S. Cupp, F. N. Kojima, T. T. Stumpf, L. A. Werth, M. W. Wolfe, R. J. Kittok, and J. E. Kinder. 1993. Increasing exogenous progesterone during synchronization of estrus decreases endogenous 17β-estradiol and increases conception in cows. *Biol. Reprod.* 49:214.
- Werth, L. A., J. C. Whittier, S. M. Azzam, G. H. Deutscher, and J. E. Kinder. 1996. Relationship between circulating progesterone and conception at the first postpartum estrus in young primiparous beef cows. *J. Anim. Sci.* 74:616-619.
- Wettemann, R. P. 1980. Postpartum endocrine function of cattle, sheep, and swine. *J. Anim. Sci.* 51(Suppl. 2):2.
- Wettemann, R. P., I. Bossis, M. L. looper, F. J. White, N. H. Ciccioli, and T. D. Ridgway. 1999. Nutritional regulation of ovarian function in beef cattle. *J. Anim. Sci.* 77(Suppl. 1):19.

- White, F. J., R. P. Wettemann, M. L. Looper, T. M. Prado, and G. L. Morgan. 2002. Seasonal effects on estrous behavior and time of ovulation in nonlactating beef cows. *J. Anim. Sci.* 80:3053-3059.
- Whitman, R. W. 1975. Weight changes, body condition and beef cow reproduction. Ph.D. dissertation. Colorado State University, Fort Collins.
- Williams, G. L., J. Kotwica, W. D. Slinger, D. K. Olson, J. E. Tilton, and L. J. Joanson. 1982. Effect of suckling on pituitary responsiveness to gonadotropin-releasing hormone throughout the early postpartum period of beef cows. *J. Anim. Sci.* 54:594.
- Williams, G. L., R. G. Osborn, J. D. Kirsch, and J. E. Tilton. 1984. Suckling, milking and calf presence as regulators of tonic gonadotropin release and postpartum interval. 10th Int. Congr. Anim. Reprod. Artif. Insem. Univ. of Illinois, Urbana-Champaign. P 410.
- Williams, G. L., M. Kosiorowski, R. G. Osborn, J. D. Kirsch, and W. D. Slinger. 1987. The postweaning rise of tonic luteinizing hormone secretion in anestrus cows is not prevented by chronic milking or the physical presence of the calf. *Biol. Reprod.* 36:1079.
- Williams, G. L. 1989. Modulation of luteal activity in postpartum beef cows through changes in dietary lipid. *J. Anim. Sci.* 67:785.
- Williams, G. L. 1990. Suckling as a regulator of postpartum rebreeding in cattle: A Review. *J. Anim. Sci.* 68:831-852.
- Williams, G. L., and R. L. Stanko. 1999. Dietary fats as reproductive nutraceuticals in beef cattle. *Proc. American Society of Anim. Sci.* Available at: <http://www.asas.org/jas/symposia/proceedings/0915.pdf>.
- Williams, S. W., M. Amstalden, R. L. Stanko, D. L. Vallejo, and G. L. Williams. 1999. Influence of size of dominant follicle, serum estradiol, and exogenous GnRH on luteal sensitivity to prostaglandin F_{2α} in beef heifers. *J. Anim. Sci.* 77(Suppl. 1):22.
- Williams, S. W., R. L. Stanko, M. Amstalden, and G. L. Williams. 2002. Comparison of three approaches for synchronization of ovulation for timed artificial insemination in *Bos indicus*-influenced cattle managed on the Texas gulf coast. *J. Anim. Sci.* 80:1173-1178.
- Wilson, S. J., C. J. Kirby, A. T. Koenigsfeld, D. H. Keisler, and M. C. Lucy. 1998. Effects of controlled heat stress on ovarian function of dairy cattle. 2. Heifers. *J. Dairy Sci.* 81:2132-2138.

- Wiltbank, J. N., W. W. Rowden, J. E. Ingalls, K. E. Gregory, and R. M. Koch. 1962. Effect of energy level on reproductive phenomena of mature Hereford cows. *J. Anim. Sci.* 23:1049.
- Wiltbank, J. N., W. W. Rowden, J. E. Ingalls, and D. R. Zimmerman. 1963. Influence of post-partum energy level on reproductive performance of Hereford cows restricted in energy intake prior to calving. *J. Anim. Sci.* 23:1049.
- Wiltbank, J. N., R. P. Shumway, W. R. Parker, and D. R. Zimmerman. 1967. Duration of estrus, time of ovulation and fertilization rate in beef heifers synchronized with dihydroxyprogesterone acetophenide. *J. Anim. Sci.* 26:764.
- Wiltbank, J. N. 1970. Research needs in beef cattle reproduction. *J. Anim. Sci.* 31:755.
- Wood, S. L., M. C. Lucy, M. F. Smith, and D. J. Patterson. 2001. Improved synchrony of estrus and ovulation with the addition of GnRH to a melengestrol acetate-prostaglandin F_{2α} synchronization treatment in beef heifers. *J. Anim. Sci.* 79:2210-2216.
- Yelich, J. V., H. S. Mauck, M. D. Holland, M. E. King, and K. G. Odde. 1988. Synchronization of estrus in suckled beef cows with melengestrol acetate (MGA) and PGF_{2α}. *Proc. Western Section Am. Soc. Anim. Sci.* 39:302.
- Yelich, J. V., R. P. Wettemann, H. G. Dolezal, K. S. Lusby, D. K. Bishop, and L. J. Spicer. 1995. Effects of growth rate on carcass composition and lipid partitioning at puberty and growth hormone, insulin-like growth factor I, insulin, and metabolites before puberty in beef heifers. *J. Anim. Sci.* 73:2390-2405.
- Zalesky, D. D., M. L. Day, M. Garcia-Winder, K. Imakawa, R. J. Kittok, M. J. D'Occhio, and J. E. Kinder. 1984. Influence of exposure to bulls on resumption of estrous cycles following parturition in beef cows. *J. Anim. Sci.* 59:1135.
- Zeroual, A. 1994. Approche biotechnologique pour améliorer la synchronisation de l'oestrus chez le bovin de boucherie et intensifier l'utilisation de l'insémination artificielle. M. Sci. Thesis. Université Laval, Québec, Canada.
- Zimbleman, R. G., and L. W. Smith. 1966. Control of ovulation in cattle with melengestrol acetate. I. Effect of dosage and route of administration. *J. Reprod. Fertil.* 11:185.