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PHYSIOLOGICAL FACTORS THAT AFFECT PREGNANCY RATE TO ARTIFICIAL INSEMINATION IN BEEF CATTLE

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Introduction

Recent years have witnessed the rapid development of technologies utilized to increase reproductive efficiency and(or) improve the genetic merit of a herd. Some of these technologies include: estrus synchronization, artificial insemination, gender-selected semen, in vitro embryo production, embryo transfer, ultrasonography, transgenics, and cloning. Of the preceding reproductive technologies, estrus synchronization and artificial insemination (AI) are among the most powerful and applicable technologies for genetic improvement of beef herds (Seidel, 1995).

The development of effective methods of synchronizing estrus and ovulation has been based on our understanding of the physiological and hormonal mechanisms controlling the estrous cycle and the initiation of estrous cyclicity in prepubertal heifers and postpartum cows. Although estrus synchronization products (e.g. CIDR and GnRH) and protocols have changed over time, the basic physiological principles underlying how these products work have not. An understanding of the importance of estrus expression and the basic physiological factors affecting pregnancy rate following estrus synchronization and artificial insemination can facilitate the application of these technologies in cycling and anestrous females. The purpose of this paper is as follows: 1) review some management practices that increase the probability of a successful estrus synchronization and AI program, 2) emphasize the importance of estrus expression for the establishment of pregnancy, 3) discuss some basic physiological mechanisms that increase pregnancy rate following synchronization of estrus and(or) ovulation in cattle, and 4) provide guidelines for determining why the pregnancy rate to AI may have been lower than expected.

Important Considerations Before Implementing an Estrus Synchronization Program

Factors Affecting Pregnancy Rate. When it comes to reproductive management the things you do well will not compensate for the mistakes you make. Instead, the mistakes you make cancel out all the things you do well. This is best illustrated by examining the primary factors that affect pregnancy rate. In an AI program, pregnancy rate is the product of estrous detection rate and conception rate (Pregnancy rate = estrous detection rate x conception rate; see definitions below). The following definitions can be applied to an entire breeding season or to the synchronized period (period of time during which estrus is expressed after treatment with an estrus synchronization protocol [normally 5 to 7 days]).

Pregnancy rate – total number pregnant during the breeding season/ number of females exposed to breeding (expressed as a percent).

Estrous detection rate – total number of females detected in estrus/number of females exposed to breeding (expressed as a percent).

Conception rate – percentage of females that become pregnant to a designated insemination.

The effect of a decrease in estrous detection rate and/or conception rate on pregnancy rate can be seen in Table 1. Assume that 100% of the heifers have attained puberty and that you are able to detect 95% of the heifers in estrus during the synchronized period. With a conception rate of 70% the pregnancy rate would be: 95% estrous detection rate x 70% conception rate = 67% pregnancy rate! If we hold conception rate at 70% and decrease estrous detection rate to 75%, due to fewer animals cycling or less time spent detecting estrus, the pregnancy will be reduced to 53%. Alternatively, if estrous detection rate remains at 95% but conception rate is decreased to 50% due to compromised semen quality or poor insemination technique, the pregnancy rate would decrease to 48%. Finally, a decrease in both estrous detection rate and conception rate will decrease pregnancy rate from 67% to 38%. Therefore, understanding the effect of estrous detection rate and conception rate on pregnancy rate and the importance of attention to detail in every aspect of an estrus synchronization program is essential!

Table 1: Effect of estrous detection rate and conception rate on pregnancy rate in cattle.

Estrous detection rate	Conception rate	Pregnancy rate
95%	70%	67%
75%	70%	53%
95%	50%	48%
75%	50%	38%

What can I expect in terms of pregnancy rate? When beginning an AI program it is essential to have realistic expectations regarding the pregnancy rate. As discussed in the previous paragraph, pregnancy rate is the product of estrous detection rate and conception rate. It is important to remember that a pregnancy rate of 67% to a single insemination is good whether you are talking AI or natural service. For natural service, expected pregnancy rates are normally 60 to 70% during 21 days of breeding assuming the bulls are fertile and that 100% of the heifers and cows are cycling. However, a pregnancy rate of 60 to 70% over 21 days is unusually high for natural service since rarely are all the heifers and cows cycling at the start of the breeding season. In a FTAI program, all the cows are injected with GnRH (to synchronize ovulation) and inseminated at a predetermined time. Since there is no estrous detection with FTAI, estrous detection rate becomes the proportion of heifers and cows that ovulate in response to GnRH injection at insemination. It is normal for the pregnancy rate to be higher following FTAI compared to protocols that are dependent upon estrous detection since ovulation is induced and semen is deposited in all the cows in a FTAI protocol. In an estrous detection protocol only the females detected in estrus are inseminated and females that are anestrus or not detected in estrus are not inseminated.

Are my heifers and cows good candidates for an estrus synchronization protocol? The first question to ask is "Over the past few years what has been the pregnancy rate in my heifers or cows after a 60 to 80 day breeding season?" If the pregnancy rate at the end of the breeding season has been less than 85% there may be management issues that should be addressed before

initiating a synchronization and AI program. If the pregnancy rate in your herd over the past few years has been ≥ 85% then you need to evaluate whether your heifers and cows are good candidates for an estrus synchronization and AI program.

Criteria for heifers. Heifers that will be used for breeding purposes should not have received growth promoting implants. Previous studies report that implanting heifers within 30 days of birth impairs uterine function and decreases subsequent pregnancy rates. Heifers should have attained 65% of their mature body weight by the start of breeding. Do you know the mature weight of your cows? Knowing the mature weight of the cows in your herd can be helpful in calculating an appropriate target weight for your heifers. A minimum of 50% of your heifers should have a reproductive tract score of ≥ 4 approximately six weeks before the start of breeding. A reproductive tract score (RTS) is a subjective measurement of the sexual maturity of a heifer that is normally performed by a veterinarian approximately 4 to 6 weeks before the breeding season. The score is obtained by palpation per rectum and is based on the degree of uterine development and ovarian status (size of dominant follicle and presence or absence of a CL). Each heifer is assigned a score of 1 to 5 (1 = immature; 5 = presence of a corpus luteum) with a RTS of 1 referring to a prepubertal heifer, 2 or 3 referring to a peripubertal heifer (transitional stage), and 4 or 5 referring to a pubertal (cycling) heifer. The uterine and ovarian dimensions of heifers for each of the reproductive tract scores (RTS) are described in Table 2.

Table 2. Description of uterine and ovarian measurements for different reproductive tract scores (RTS).

RTS	Uterine horns (diameter, mm)	Ovarian length (mm)	Ovarian height (mm)	Ovarian width (mm)	Ovarian structures
1	Immature, < 20 mm, no tone	15	10	8	No palpable follicles
2	20-25 mm no tone	18	12	10	8 mm follicles
3	20-25 mm slight tone	22	15	10	8-10 mm follicles
4	30 mm good tone	30	16	12	> 10 mm follicles, CL possible
5	> 30 mm	> 32	20	15	CL present

Criteria for postpartum cows. To increase the number of cows cycling at the beginning of the breeding season, they should have calved unassisted, be in good body condition at calving, disease-free, and allowed an adequate period of recovery from calving to the subsequent breeding season. Postpartum cows that are good candidates for an estrus synchronization program normally meet each of the following criteria: 1) body condition score at calving of ≥ 5 (1= emaciated; 9 = obese), 2) mean postpartum interval of the cows to be synchronized should be ≥ 40 days at the beginning of the protocol. This does not mean that each cow should be ≥ 40 days postpartum but that the mean of the entire group to be synchronized should be ≥ 40 days. If the estrus synchronization protocol you plan to use includes CIDR administration, each cow should be a minimum of 21 days postpartum at the time of CIDR insertion, and 3) low incidence of calving difficulty since dystocia will lengthen the postpartum interval.

Which estrus synchronization protocol should I choose? When choosing an estrus synchronization protocol there are a number of issues to consider including whether you want to detect estrus and inseminate according to the AM/PM rule, inseminate at a predetermined time, or detect estrus for 72 to 84 hr (depending upon the protocol) and inseminate any cows not detected in estrus at a fixed-time. There is an estrus synchronization protocol sheet for heifers and cows that appears in the catalogs of the major AI companies and there are protocols that fit each of the preceding approaches to estrus synchronization. Other items to consider include the proportion of females that are cycling as well as the time, labor, and cost of the protocol.

Implementation of an estrus synchronization protocol. Estrus synchronization protocols must be followed precisely. Each product must be administered at the correct dose on the correct day (refer to protocol sheet) and in some cases at the right time of day. For example, the interval from prostaglandin $F_{2\alpha}$ (PGF) to gonadotropin releasing hormone (GnRH) and insemination must be in accordance with what is recommended in the protocol sheet (e.g. 66 ± 2 hr for the CO-Synch + CIDR protocol). The recommended time of insemination relative to PGF injection is based on research trials and should be strictly adhered to (discussed further below). In addition, estrus synchronization products must be stored, handled, and administered correctly. For specific tips on handling estrus synchronization products see Figures 1 and 2. Should a mistake occur in product administration or the treatment timeline seek advice immediately from a veterinarian, an extension agent specializing in reproduction, or a representative from an AI company. To minimize the probability of making a mistake, a good practice is to write each of the days of treatment, the product name, dose to be administered, and the day of insemination on a calendar and ask a trusted veterinarian, extension specialist, or AI company representative to review it before beginning the protocol.

Understanding the basic principles of the bovine estrous cycle and how the products synchronize estrus and ovulation can be helpful in reducing the probability of administering the wrong product on a certain day. For more information on how estrus synchronization protocols synchronize estrus and ovulation refer to the web based course entitled "Fundamentals of Beef Reproduction and Management:Focus on Estrus Synchronization (http://animalsciences.missouri.edu/extension/beef/estrous_synch/).

Figure 1. Proper handling and administration of GnRH and PG products.

- All injections of GnRH and PG products should be given intramuscularly (IM)
- Wear latex gloves when administering GnRH and PG products
- An 18 gauge 1 ½ inch needle is recommended for these injections
- Change needles frequently
    - Make sure that injection sites are free of manure and dirt, which may cause infection
    - Injecting cattle during wet weather increases the potential for infection
- **Always** follow manufacturer's recommended storage, dosage and administration procedures

How do I choose an AI sire and where do I obtain the semen? Sire selection is of critical importance and can have a long term effect within a herd, particularly when heifers are retained as replacements. When choosing a sire the following questions need to be addressed: 1) Will I raise my own replacement heifers or purchase them?, and 2) How will I market my calves? Answers to the preceding questions will determine the traits that need to be emphasized. If a

producer raises his or her own replacement heifers then selection pressure should be placed on maternal traits such as milk, maternal calving ease, stayability, etc. However, if replacement heifers are purchased off the farm then emphasis on maternal traits in your herd would not be important. When selecting a sire, you need to think about how you will be paid (e.g. pounds of weaning weight, carcass weight, carcass quality) and let this affect your sire selection decisions. Producers that sell their calves at weaning need to place selection pressure on preweaning growth; whereas, producers that retain ownership and market their calves on a grid should emphasize carcass weight, marbling, and ribeye area.

Expected progeny differences (EPDs) are an effective selection tool and are reported to be 7 to 9 times more effective at generating a response to selection than focusing on measurements of individual performance, which is strongly affected by environment. Use AI sires with high accuracy EPDs and where the semen has been collected from a certified semen services (CSS) facility. Avoid using unproven bulls and do not be hesitant to seek advice from individuals in the AI industry to help make this important management decision.

Another consideration when selecting a sire is whether the bull's semen has worked in FTAI programs. Differences among sires in pregnancy rate to FTAI have been noted; however, the same differences in pregnancy rate may not occur when cows are detected in estrus and inseminated according to the AM/PM rule. Therefore, just because an AI sire has a good conception rate following estrous detection does not ensure he will perform equally well when ovulation is induced and insemination occurs at a predetermined time. It is a good idea to ask an AI representative if there is information available regarding how a bull has worked in a FTAI program.

Estrus Expression and the Establishment of Pregnancy

Detection of estrus: In cattle, the estrous cycle normally varies from 17 to 24 days and the duration of standing estrus is generally 12 to 15 hrs; however, considerable variation exists among individual animals (range < 8 to > 30 hr; O'Connor and Senger, 1997). The primary sign of estrus in cattle is standing to be mounted and secondary signs of estrus include frequent mounting, watery mucus from the vulva, and restlessness. Maximizing the estrous detection rate is dependent upon accurate detection of animals in standing estrus. Estrus was synchronized in a group of animals at Colorado State University and monitored for standing estrus 24 hours a day with a computer assisted estrous detection system (HeatWatch®) or twice a day for 30 minutes by visual observation. By day 5 after estrus synchronization, 95% of animals monitored 24 hours a day were detected in standing estrus; whereas, only 56% of animals observed twice a day for 30 minutes were detected in standing estrus (Downing et al., 1998). With an estrous detection rate of 95% and a conception rate of 70% (95% X 70% = 67%) approximately 67% of the animals will be pregnant; whereas, only a 39% (56% X 70% = 39%) pregnancy rate will occur with a 56% estrous detection rate.

Therefore, the success of any estrus-based artificial insemination program requires detecting animals in standing estrus and inseminating them at the correct time relative to detection of estrus (see Table 5 and Figure 7). Failing to detect estrus or errors in accurately detecting estrus can result in significant economic losses. Accurate detection of estrus can be a difficult and time-consuming activity. When estrus was detected in 500 Angus cows with the HeatWatch® estrus-detection system, the length of estrus averaged 10 hours (range: 0.5 hours to

24 hours); however, 26% of cows exhibited estrus for less than 7 hours and had fewer than 1.5 mounts per hour (Rorie et al., 2002).

To maximize detection of standing estrus, it is important to visually monitor cattle as much as possible. Observations should occur as early and as late as possible as well as during the middle of the day. Continuous observation of over 500 animals exhibiting natural estrus in 3 separate studies indicated that 55.9% of cows initiated standing estrus from 6 p.m. to 6 a.m. (Table 3). Furthermore, when cows were observed for standing estrus every 6 hours (6 a.m., noon, 6 p.m., and midnight), estrous detection increased by 10% with the addition of a mid-day observation and by 19% when observed four times daily (every 6 hours) compared to detecting standing estrus at 6 a.m. and 6 p.m. alone (Hall et al., 1959). Therefore, detection of standing estrus can be one of the most time-consuming chores related to artificial insemination.

There are commercially available estrus detection aids that can be used in conjunction with visual observation to increase estrous detection efficiency in beef herds. The HeatWatch Estrus Detection System is probably the only tool that can replace visual observation, since this system provides precise data on the onset, intensity, and duration of estrus. Some of the more common estrus detection aids include tail chalk/paint, pressure mount detectors, gomer (spotter) bulls (teaser bulls; rendered sterile by vasectomy, epididectomy, and (or) penile deviation), and androgenized cows. Table 4 provides a list of common estrus detection aids, a description of how they work, some potential concerns, and relative cost. A comparison between visual estrous detection every 3 hours (8 times daily), a marker animal (a bull with a deviated penis), and Estrotect® patches resulted in a similar ($P > 0.79$) percentage of animals correctly identified in standing estrus (92%, 92%, and 91%, respectively; Perry, 2005). Increased visual observation, in addition to the use of estrus-detection aids, can improve pregnancy rates by determining the most appropriate time for insemination.

The number of mounts per estrus increases as the number of females in estrus increases (Helmer and Britt, 1985; Landaeta-Hernandez et al., 2002). This is likely due to the formation of sexually active groups of cattle which is known to increase the number of mounts per female (Hurnick et al., 1975; Galina et al., 1994). In nonsynchronized cattle there will be fewer sexually active groups (or fewer animals per group) and less mounting activity. Therefore, improved estrous detection efficiency is an advantage of an estrus synchronization program. However, it is also true that frequent animal handling and restraint are stressors (Dobson and Kamonpatana, 1986) and that increased handling and restraint of heifers during a synchronized estrus decreased the number of mounts per estrus (Lemaster et al., 1999). Depending upon the estrous synchronization protocol, a fixed-time insemination protocol should reduce the amount of animal handling associated with sorting estrual heifers at the time of insemination.

Effect of estrus expression on pregnancy rate: When insemination is performed at a fixed-time there will be heifers or cows that are in estrus and those that have not displayed estrus. Of those that are not in estrus some will show estrus if the GnRH injection (to initiate the ovulatory process) and insemination are delayed; whereas, some may not express estrus at all. It is still possible for some heifers and cows in the latter group to conceive since ovulation can be induced following GnRH injection. There is considerable data indicating that heifers and cows in estrus around the time of fixed-time AI have a higher pregnancy rate than those not in estrus (Figure 3).

Why does estrus expression at fixed-time AI increase pregnancy rate ?: Expression of estrus is stimulated by increasing concentrations of estradiol (a follicular hormone) at a time when

Figure 2. Proper handling and administration of progestins for estrus synchronization.	
Controlled Internal Drug Release (CIDR)	
1)	Be sure to wear protective (e.g. latex) gloves when handling CIDR inserts.
2)	The CIDR applicator should be rinsed in a disinfectant solution (Nolvasan or Chlorohexidine). There should be two buckets each containing a disinfectant solution. The applicator should be washed free of debris in the first bucket and then rinsed clean in the second. By keeping the same washing sequence the disinfectant in the second bucket will remain relatively clean for a sustained period of time. This sequence of events will improve sanitation from animal to animal and reduce the likelihood of infection.
3)	Fold the wings of the CIDR and insert it into a clean applicator. The CIDR will protrude approximately one inch from the applicator.
4)	Apply lube to the end of the applicator.
5)	Wipe the vulva clean before inserting the applicator.
6)	When inserting the CIDR make sure that the nylon tail is curved downward. If the tail is pointed upward it will be easier for other animals to pull out the CIDR thus reducing retention rate.
7)	Gently insert the applicator containing the CIDR in an upward manner similar to the insertion of an AI catheter.
8)	Push the applicator as far forward as possible, deposit the CIDR by pressing the plunger, and slowly remove the applicator.
9)	To prevent other animals from removing the CIDR, the nylon tail can be clipped such that only 2 ½ inches protrude from the vulva.
10)	At CIDR removal, gently but firmly pull on the nylon tail until it is removed. Be sure to dispose of the CIDR properly.
Melengestrol Acetate (MGA)	
1)	MGA is an orally active feed additive that should be fed once a day at the recommended dose - 0.5 mg in a 3 to 5 lb carrier. Do not top dress MGA on other feeds. Provide adequate bunk space - 18-24 inches per animal.
2)	Allow heifers to adjust to carrier prior to MGA administration.
3)	MGA is approved by the FDA for heifers intended for breeding (suppression of estrus) and for heifers fed in confinement for slaughter for increased rate of weight gain, improved feed efficiency, and suppression of estrus.
4)	Use of MGA as part of any estrus synchronization protocol in beef cows constitutes and extra label use of medicated feed that is prohibited by the Animal Medicinal Drug Use and Clarification Act and regulation 21 CFR 530.11(b).

Table 3. Time of day when cows exhibit standing estrus.

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

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

progesterone (secreted by the corpus luteum) is low. Estradiol secretion is higher in heifers and cows that show estrus compared to those that are not detected in estrus. Preovulatory secretion of

estradiol by a dominant follicle coordinates a number of physiological processes that are required for the establishment of pregnancy. Some of these effects occur during the preovulatory period (e.g. estrus expression, induction of the gonadotropin surge that induces ovulation, and sperm transport); whereas, other effects are manifested during the luteal phase (e.g. preparation of maternal environment for pregnancy). In general, the secretion of estradiol increases as the physiological maturity of a dominant follicle increases. Consequently, during the development of a fixed-time AI protocol emphasis is placed on maximizing the proportion of females that have a physiologically mature ovulatory follicle at insemination.

Fundamental Principles of Estrus Synchronization Protocols

Below are several basic principles that will help you understand how estrus synchronization protocols work. Understanding the following principles can be helpful in choosing an appropriate protocol for a group of heifers or postpartum cows.

Role of progestins in in initiating normal estrous cycles in anestrous females: At the start of a breeding season, most herds consist of a mixture of cycling and anestrous females. An effective estrus synchronization protocol must be able to induce a fertile estrus or ovulation in both anestrous and cycling heifers and cows. The first ovulatory estrus in prepuberal heifers and postpartum beef cows is commonly preceded by a short luteal phase (Perry et al., 1991; Werth et al., 1996). This short exposure to progesterone is believed to be necessary for reprogramming the reproductive axis to resume normal estrous cycles. Therefore, in herds that have a relatively large proportion of prepuberal heifers or anestrous cows, progestin pretreatment before induction of ovulation can initiate estrous cycling status and eliminate or at least reduce the occurrence of short estrous cycles. Two progestin products that are commercially available for estrus synchronization include Melengestrol Acetate (MGA) and the CIDR (Controlled Internal Drug Release).

Synchronization of follicular waves: To understand how FTAI protocols allow heifers or cows to be inseminated at a predetermined time with acceptable pregnancy rates, it is essential to understand "follicular waves" and how to synchronize them. In cattle, large (dominant) ovulatory sized follicles develop in sequential waves during the estrous cycle and consist of the following three stages: recruitment, selection, and dominance (Figure 4). The bovine estrous cycle usually consists of two to three follicular waves and each wave begins with the growth of a cohort of antral follicles from a pool of growing small follicles, which is referred to as recruitment. One of the recruited follicles is subsequently selected (i.e. selection) from the cohort for continued growth and becomes dominant (i.e. dominance) in terms of size and influence over smaller follicles. The remaining follicles in the cohort die by a process called atresia. During a nonovulatory follicular wave (e.g. first follicular wave in Figure 4), the dominant follicle eventually dies (atresia) and a new follicular wave is initiated. A viable dominant follicle present at corpus luteum regression will generally become the ovulatory follicle (e.g. second follicular wave in Figure 4). The estrous cycle length of cows that have three follicular waves is generally longer (20-24 days) compared to cows with two follicular waves (18-20 days).

Table 4. A list of estrus detection aids in beef cattle, a description of how they work, potential concerns, and relative cost.

Heat Detection Aid	How it Works	Potential Concerns	Relative Cost
Tail Chalk	Chalk is applied to tailhead. When animal is mounted the color will be rubbed off and hair will be ruffled.	Removal by trees, water, fences, or licking by other animals	$
Heat Mount Detectors	Detectors are applied to tailhead and turn a different color when mounted.	Partial activation or loss of detector requires interpretation, false activation (e.g. trees, fences, other animals)	$$
Heat Watch	Transmitters are attached to tailhead region. When transmitter is depressed a signal is sent to receiver.	Expensive to replace lost sensors, data interpretation, appropriate facilities/terrain	$$$
Gomer Bulls	Vasectomized, epididyectomized, and (or) penile-deviated animals are used as teaser animals and will mount females in estrus.	Feeding and maintenance expense, potential loss of desire to mate, and disease transmission by non penile-deviated animals	$$$
Chin Ball Marking Harness	Detector animal is fitted with harness leaving an inkmark on the back and neck of females that have been mounted.	Maintenance of equipment, feeding and maintenance of animal, ill-defined markings	$$
Androgenized cows	Testosterone injections before and during the breeding season or androgen implant causes cow to mount other females in heat.	Cost and labor of administering drug, variable response to hormone	$$

The development of effective protocols for fixed-time insemination is dependent upon the precise synchronization of follicular waves culminating in a fertile ovulation at a predetermined time. Initiation of a new follicular wave occurs following ovulation or turnover (atresia) of the dominant follicle. Injection of GnRH will induce a surge of LH within 2 to 4 hr and ovulation of a dominant follicle will occur 24 to 36 hr after injection (Figure 5). GnRH-induced ovulation has been utilized to synchronize follicular waves and to induce ovulation around the time of insemination in both heifers and cows. A single injection of a GnRH agonist is capable of ovulating dominant (≥ 10 mm) but not subordinate follicles (Figure 6; Ryan et al., 1998). However, the ability of a single injection of GnRH to induce ovulation and initiate a new follicular wave is dependent on the stage of follicular development (Geary et al., 2000; Atkins et al., 2005).

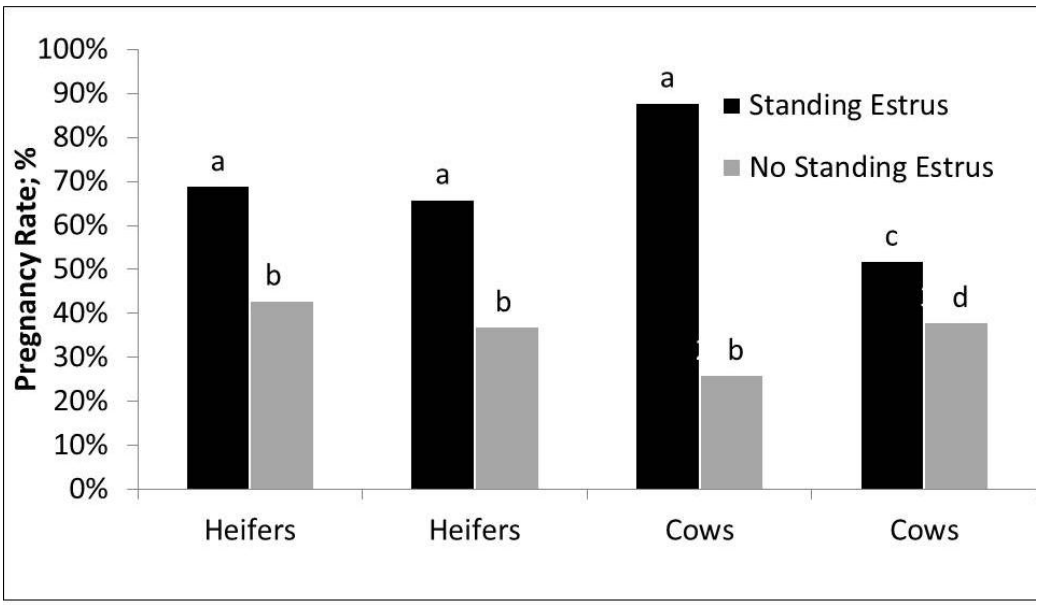

Figure 3. Effect of estrus expression around the time of fixed-time AI on pregnancy rate in beef heifers and postpartum cows ($^{ab}P < 0.01$; $^{cd}P < 0.05$). In each case animals that were detected in estrus around the time

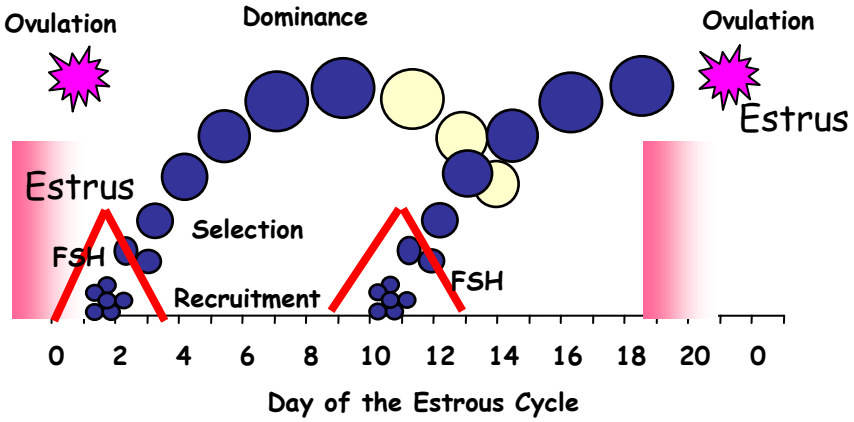

Figure 4. Relationship between circulating concentrations of follicle stimulating hormone (FSH) and stages of a bovine follicular wave (recruitment, selection, and dominance). A transient increase in FSH (solid line) initiates recruitment of a cohort of follicles, from which a single follicle is normally selected to become the dominant follicle. If the corpus luteum regresses in the presence of a viable dominant follicle ovulation will occur (second follicular wave). However, in the absence of luteal regression, the dominant follicle becomes atretic (regresses; light circles; Modified from Kojima and Patterson, 2003).

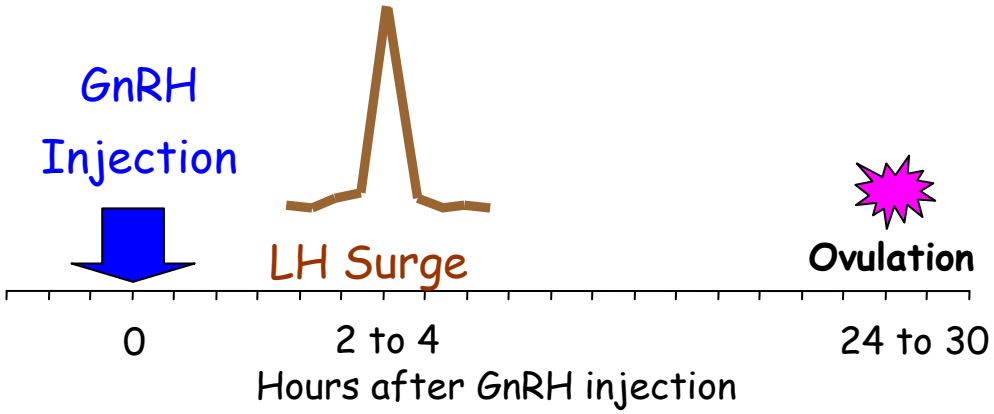

Figure 5. Injection (im) of GnRH will induce a surge of LH within 2 to 4 hr and ovulation of a viable dominant follicle (≥ 10 mm) will occur within 24 to 36 hr (Modified from Kojima and Patterson, 2003).

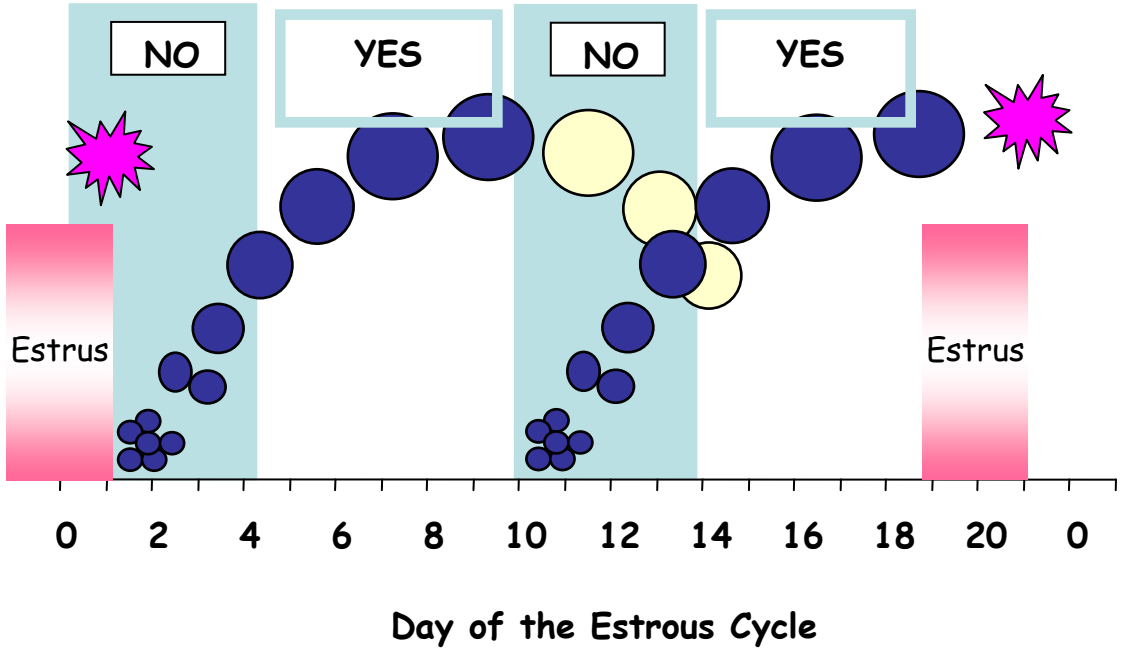

Figure 6. Injection of GnRH will induce ovulation of a dominant follicle (≥ 10 mm in diameter). Circles represent follicle development and atresia (light circles) during a wave. The above figure represents a "two-wave cow" and the shaded areas indicate when during a follicular wave follicles will ovulate (Yes) or not ovulate (No) in response to a single injection of GnRH (Modified from Kojima and Patterson, 2003).

Presynchronization for improved control of follicular waves: GnRH is injected to synchronize follicular waves in numerous estrus synchronization protocols. However, not all

heifers or cows will have dominant follicle capable of responding at the time GnRH is administered (Figure 6). As previously mentioned, a follicle needs to be approximately 10 mm in diameter to be capable of ovulating in response to GnRH. Approximately 60 to 65% of postpartum beef cows and less than 50% will have a follicle capable of responding to GnRH at any specific time. Therefore, some protocols will include a period of progestin treatment (CIDR or MGA) or an injection of PG prior to the GnRH-PG injection sequence as a means of increasing the proportion of animals that will respond to an injection of GnRH.

Timing of insemination following estrous detection or fixed-time AI: When utilizing an estrus synchronization protocol that requires estrous detection, insemination occurs approximately 8 to 12 hr following detection of estrus (AM/PM rule). In other words, if a cow is detected in estrus in the AM then AI should occur the following PM; whereas, if a cow is detected in estrus in the PM then AI should occur the following AM). It is essential that the presence of fertile sperm in the oviduct coincide with the time when the oocyte is viable (8 to 10 hr period following ovulation). Insemination (AI) too soon, following detection of estrus, can decrease the probability that viable sperm are present at ovulation. However, insemination too late, relative to detection of estrus, may result in the oocyte dying before the sperm complete capacitation (process, within the female tract, by which sperm gain the capacity to fertilize the egg) and are capable of fertilizing the oocyte. The time of insemination is based on an understanding of the relationship among the following biological parameters: duration of estrus, interval from the gonadotropin (LH) surge to ovulation, lifespan of the oocyte (egg), lifespan of frozen-thawed sperm in the female tract, and duration of capacitation. For pregnancy to occur it is essential that fertile sperm be present in the vicinity of the oocyte when it is still alive. The duration of the preceding factors are shown in table 5 below and relationship among these factors when insemination twelve hours after estrus detection (AM/PM rule) is depicted in Figure 7.

However, with FTAI protocols, time of insemination becomes a compromise between maximizing the proportion of females that show estrus before insemination and not waiting too long such that heifers or cows that were the first to show estrus end up being inseminated too late. There can be variation in the fertility of sires used in a FTAI protocol. Sires that achieve high fertility when insemination occurs approximately 12 hr after detection of estrus (AM/PM rule) do not always achieve high pregnancy success following fixed-time AI. Although the exact reasons for the difference are not known, it is likely that sperm longevity in the female tract is a primary reason.

Factors affecting Pregnancy Rate Following AI

In cattle, fertilization generally occurs following > 90% of inseminations, but pregnancy rate at the earliest possible detection (day 27) is generally < 70%. Cows induced to ovulate smaller follicles with GnRH have reduced pregnancy rates and experience greater embryonic loss, even after pregnancy has been established (Atkins et al 2012). These inefficiencies are likely due to either ovulation of an immature oocyte that compromises fertilization and embryo survival or ovulation occurs before the follicular cells have fully matured to produce sufficient estradiol during the preovulatory period and subsequently, progesterone to adequately prepare the uterus for pregnancy. The preceding study (Atkins et. al., 2012) was designed to differentiate between follicular effects on oocyte quality and uterine environment on pregnancy success in beef cattle and the primary results are summarized below.

Table 5. Duration of biological factors that affect the time of artificial insemination with frozen-thawed semen in cattle.

Biological factor	Duration
Duration of standing estrus	Highly variable but normally 12 to 15 hr
Time of the gonadotropin (LH) surge which initiates the ovulatory process	Begins around the onset of standing estrus and lasts a few hours
Time from the LH surge to ovulation	25 to 30 hr
Lifespan of the oocyte (egg)	8 to 10 hr
Lifespan of frozen-thawed semen in the female reproductive tract	Approximately 24 hr but can be variable among bulls
Duration of capacitation within the female tract	4 to 6 hr following insemination but may vary among bulls.
Lifespan of fertile (capacitated) sperm in the female tract	18 to 20 hr

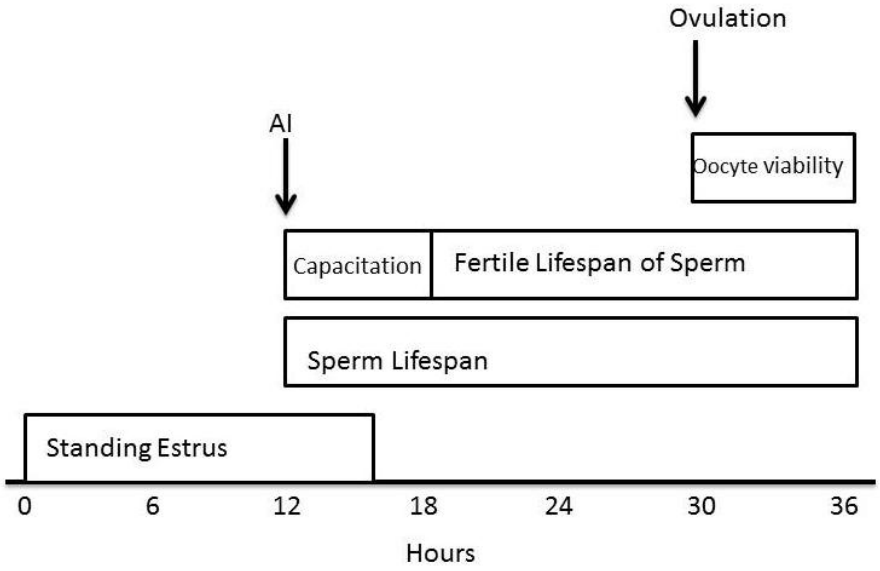

Figure 7. Illustration of the relationship among duration of estrus, duration of the sperm lifespan, length of capacitation, duration of fertile lifespan of sperm, time of ovulation, and duration of oocyte lifespan. Time periods are based on data from table 5.

Mechanisms Associated With Fertilization: Fertilization rate in beef cattle is normally high (≥ 90%) and the results of this study indicate that fertilization rate was decreased when ovulatory follicle size, serum estradiol at insemination, cow body weight, and days postpartum were decreased. Management strategies to increase fertilization rate at FTAI should include the following: 1) Utilize FTAI protocols that increase the physiological maturity (e.g. estradiol secretion) of the ovulatory follicle, 2) Increase the proportion of females that conceive early in

the breeding season which will shorten the calving season and thereby increase the number of days postpartum at the subsequent insemination, and 3) Ensure cows have adequate body weight at the time of estrus synchronization.

Mechanisms Associated With Pregnancy Establishment: After examining the effect of twelve or more factors on pregnancy rate at day 27, Atkins et al., (2012) were only able to account for about 10% of the variation in pregnancy rate. Therefore, much of the biology underlying establishment and maintenance of pregnancy in cattle remains to be determined. The establishment of pregnancy by day 27 was positively affected by serum progesterone at day 7 (d0 = insemination), and serum estradiol at insemination. The positive effects of estradiol and progesterone were independent and likely aid in the establishment of a maternal environment that is conducive to pregnancy establishment (Inskeep 2004).

Mechanisms Associated With Pregnancy Maintenance: GnRH-induced ovulation of small dominant follicles resulted in increased late embryonic/early fetal mortality in postpartum beef cows (Perry et. al., 2005). The majority of the preceding late embryonic/fetal loss occurred around the time of embryo uterine attachment (day 27 to 41; Inskeep 2004). This is a time when late embryonic/early fetal mortality has been reported by others and might be due to improper placentation. Pregnancy maintenance was directly affected by embryo quality and cow age. Consequently, late embryonic/fetal mortality was associated with poorer quality embryos and younger cows.

Summary: The follicular environment affected fertilization rate and embryonic survival prior to day 7 but survival after day 7 was primarily dependent on estradiol production, and subsequent progesterone production. The data emphasize that numerous variables contribute to successful establishment of pregnancy. However, increased estradiol at the time of insemination in cattle and probably all species is critically important to fertility and embryo survival.

How do I determine what may have gone wrong during a FTAI program? Occasionally the pregnancy rate following FTAI is much lower than expected. Trying to identify the root cause of a decreased pregnancy rate can be a daunting task due to the countless factors that can impact pregnancy rate following AI. When trying to trouble shoot what went wrong you should systematically work through the possibilities and not assume anything was done correctly – evaluate all the possibilities! A list of questions that may provide a systematic approach to identifying the problem is provided in Figure 8. Additional points to consider are included below.

What are the most common mistakes when implementing an estrus synchronization and AI program? One of the most common problems accounting for reduced pregnancy rates following FTAI is that the heifers or cows do not meet the criteria for being good candidates for an estrus synchronization and AI program (see previous section). The second problem is poor choice of an estrus synchronization protocol and(or) protocol compliance. If you have a mixture of cycling and anestrus animals at the beginning of estrus synchronization treatment, you need to use a protocol that includes a progestin (e.g. CIDR or MGA).

Figure 8. Questions to ask when the pregnancy rate to FTAI is lower than expected.

- What was the pregnancy rate following estrus synchronization and fixed-time AI (FTAI)?

• Was the pregnancy rate low or do you have unrealistic expectations (see Table 1)? Consider asking the following questions to an AI company representative, your veterinarian, or a beef reproduction specialist to identify potential causes of the reduced pregnancy rate.
1) What was the pregnancy rate in your heifers or cows after 60 to 80 days over the past few years? If less than 85% there may be other issues that should be addressed before initiating an estrus synchronization and AI program.
2) What was the nutrition (protein, energy, phytoestrogens, sulphates, etc) and mineral program before and after FTAI?
3) Did the animals meet the criteria for being good candidates for an estrus synchronization protocol (see earlier section)?
4) Did you use fixed-time AI or did you breed following detection of estrus? If you inseminated following detection of estrus, how frequently did you detect estrus (when did you begin and when did you end), what criteria did you use for detecting estrus, and when did you inseminate relative to detecting estrus?
5) What bull did you use and is there evidence that semen from this sire has resulted in acceptable pregnancy rates when using fixed-time AI or AI following estrous detection?
6) What protocol did you use and exactly when did you administer each of the products? You will need to confirm that the correct products were administered at the correct dosages and at the correct times. It is helpful to record on a calendar which product was administered on a particular day so you can check back to see if a mistake was made.
7) Was the biological activity of the various products compromised? You will need to verify that the products were not out of date and were stored and administered properly.
8) If using fixed-time AI, when did you inseminate the heifers or cows? Did you record who inseminated each animal? This will be helpful in identifying if there is a technician problem.
9) Where did you obtain the semen, how was it stored, and was the semen thawed correctly?

Progestin treatment will increase the proportion of prepuberal heifers and anestrus cows that will respond to the protocol. Furthermore, it is essential that each heifer or cow receives the correct estrus synchronization product, at the correct dose, and on the appropriate day. A third problem is that the facilities don't allow the cattle to be inseminated properly within a 2 to 3 hr time period and(or) cause undue stress on the cattle. With a FTAI protocol you have to carefully consider how many animals you can inseminate properly within the designated time period (e.g. 66 ± 2 hr for CO-Synch + CIDR protocol) with a minimum of stress. As previously mentioned, renting a breeding barn (Figure 1) or contracting with an AI company that has breeding barns available can alleviate the problems associated with marginal facilities.

Biological activity of the estrus synchronization products. It is not uncommon to hear someone blame a particular estrus synchronization product or the protocol for poor results. The commercially available products are effective when properly stored and administered. Furthermore, the protocols have been shown to consistently work in a variety of environments. The estrus synchronization protocols listed in the AI catalogs published by Select Sires, ABS Global, Genex, and Accelerated Genetics have been thoroughly tested in the field in a number of herds by numerous investigators in many states.

Rarely does one find that the biological activity of a particular product has been compromised provided the product has been stored properly, administered at the appropriate dose on the correct day of the protocol, and that the expiration date has not been exceeded. It is not uncommon for PGF or GnRH products to be administered at the wrong dose or to be injected subcutaneously instead of in the muscle. Intramuscular injections should be administered using an eighteen-gauge, 1.5 inch needle to minimize the possibility of back flow.

Potential problems associated with feeding melengestrol acetate (MGA). Occasionally there can be problems with feeding melengestrol acetate (MGA) if you don't pay attention to a few simple guidelines (Figure 3). The most common problem is that a heifer does not receive the correct dose (0.5 mg/hd/day). If a heifer does not receive enough MGA she may express estrus during the period of MGA feeding. Therefore, it is a good idea to watch the heifers for estrous activity as they come to the bunk. Alternatively, if a heifer receives more than the appropriate dose, expression of estrus may be delayed following the end of MGA feeding. To ensure that each heifer has an opportunity to receive the correct dose, MGA should be fed once daily in 3 to 5 pounds of carrier and each heifer should have 18 to 24 inches of bunk space. To be confident there is adequate bunk space and to train the heifers to come to the bunk it is a good idea to feed the carrier without MGA for a few days before the start of MGA treatment. At the end of 14 days of MGA feeding, heifers will express estrus within 2 to 5 days; however, heifers should not be inseminated at this estrus since pregnancy rates will be reduced. Be sure to inseminate the heifers at the designated time specified in the protocol.

Potential problems associated with CIDRs. Controlled Internal Drug Release (CIDR) is an intravaginal device that contains progesterone and acts like an artificial corpus luteum. Information on the proper handling and administration of CIDRs is provided in Figure 3. Normally there are few problems associated with CIDR treatment. CIDRs should not be inserted in cows that are less than 21 days postpartum because the probability of inducing cyclicity is low. CIDR insertion should be performed as cleanly as possible in order to reduce the risk of spreading disease (see Figure 3). When removing CIDRs it is not uncommon to detect a whitish discharge which is due to vaginal irritation from the wings of the CIDR and does not necessarily mean the animal has a vaginal infection. A difference in conception rate or pregnancy rate has not been detected between CIDR-treated animals that do or do not have a discharge.

Summary

Artificial insemination in beef cattle is more practical than ever due to advances in estrus synchronization protocols, identification of sires with highly accurate EPDs, and a market structure that can identify and reward producers for the quality of their cattle. Above all, a successful estrus synchronization and AI program is dependent upon being proactive, well organized, and attention to detail. The success of these systems hinges on many factors. A check list of management tips that should be implemented before, during, and after estrus synchronization and AI is provided in Figure 9.

Figure 9. Check list of tips for a successful estrus synchronization and AI program.

Things to do before fixed-time artificial insemination

- Keep accurate calving, breeding, and pregnancy records.
- Animal identification should be clear and easily readable.
- Ensure herd health and disease prevention with a well designed prebreeding vaccination protocol. Vaccinate females a minimum of 30 days before the breeding season begins.
- Decide which estrus synchronization protocol best fits your breeding program, facilities, and personnel (see protocol sheets in AI catalogs).
- Ensure all products are purchased and on-hand prior to initiation of the protocol.
- Prepare the calendar of actions to ensure protocol compliance.

Sire selection

- Determine if you will purchase or raise replacement heifers.
- Decide how you will market your calves.
- Select proven AI sires with high-accuracy EPDs that match performance goals.
- Purchase semen from a Certified Semen Services (CSS) collection facility.
- Prepare or update your semen inventory.
- Make sure females meet the criteria for being good candidates for estrus synchronization.

Heifer criteria

- Heifers should weigh 65% of their mature body weight by the start of breeding.
- At least 50% of heifers should have a reproductive tract score (RTS) ≥ 4 by two weeks prior to the start of synchronization or 6 to 8 weeks prior to the breeding season.

Cow criteria

- Synchronize and inseminate only cows with BCS at calving of ≥ 5 (1 = emaciated; 9.0 = obese).
- The average days postpartum of the group of cows to be synchronized should be ≥ 40 by the start of estrus synchronization and experience a minimum of dystocia.

Things to do at the time of estrus synchronization and artificial insemination

- Meticulously follow the estrus synchronization protocol!
- If detecting estrus, spend as much time observing the animals as possible.
- Use a minimum of one person to detect estrus per 100 head of cattle.
- Use estrous detection aids to facilitate visual observation of estrus.
- Use a properly trained technician for AI.

Things to do after fixed-time artificial insemination

- To distinguish between AI and bull bred pregnancies at pregnancy diagnosis, you should wait approximately 10 days to turn in clean up bulls after AI.
- Pregnancy check by 75 days after AI via ultrasound or 80 to 90 days after AI via rectal palpation to distinguish AI from bull bred pregnancies.
- If cattle need to be shipped do so between days 1 to 4 after AI and avoid shipping cattle between days 5 to 42 after AI.
- Maintain breeding females on an adequate nutrition and mineral program.

PAY ATTENTION TO DETAILS!

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Notes