FACTORS TO GET THE MOST COWS AND HEIFERS PREGNANT.

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Introduction

Research has indicated it takes the net revenue from approximately 6 calves to cover the development and production costs of each replacement heifer (E. M. Mousel Unpublished data). In addition, any cow that misses a single calving is not likely to recover the lost revenue of that missed calf (Mathews and Short, 2001). Therefore, longevity of a beef female is important to the sustainability and profitability of any beef operation. Considering the importance of longevity, an important question is as follows: Why are females culled from a beef herd? According to the 2007-08 NAHMS survey the greatest percentage of cows culled from the herd was due to pregnancy status (33.0%); other reasons for culling included age or bad teeth (32.1%), economic reasons (14.6%), other reproductive problems (3.9%), producing poor calves (3.6%), temperament (3.6%), injury (2.9%), udder problems (2.7%), bad eyes (1.8%), and other problems (1.8%). Therefore, understanding how management decisions impact pregnancy success and longevity will have an effect on the profitability and sustainability of an operation.

The majority of reproductive failure occurs because cows do not become pregnant during a defined breeding season. Therefore, the goal of any breeding program (AI or natural service; Synchronized or not) is to maximize the number of females that become pregnant. This means that fertility plays a major role in the success of any breeding program. This review will focus on the factors that affect pregnancy rates in both natural service and AI and synchronized and non-synchronized breeding programs. Fertility is influenced by many factors, and one of the best methods to look at these factors is with the use of the "Equation of Reproduction." The equation looks at 4 main topic areas: 1) Percentage of animals detected in standing estrus and inseminated, 2) Inseminator efficiency, 3) Fertility level of the herd, and 4) Fertility level of the semen.

Percentage of Animals Detected in Standing Estrus and Inseminated

For successful insemination of cattle to occur, animals must be detected in standing estrus. Detecting standing estrus (also referred to as heat detection or detecting standing heat) is simply looking for the changes in animal behavior associated with a cow/heifer standing to be mounted by a bull or another cow/heifer. With natural service, estrous detection is considered to be easy, as it is "the bulls' job." However, differences in estrous detection exist among bulls. Libido refers to a bull's desire to mate. Libido is thought to be a highly inherited trait with heritability ranging as high as 0.59 (Chenoweth, 1997). This is because there is more variation in libido between sons of different sires than between sons of the same sire. It is important to remember that scrotal circumference, semen quality, and physical confirmation (evaluated in a Breeding Soundness Evaluation) are not related to libido. Libido has a direct affect on pregnancy rate and, as such, it can influence the success of an entire breeding season. Libido can be practically evaluated by closely watching a bull after introduction into a cow herd and determining his desire to detect cows in estrus.

For successful artificial insemination of cattle to occur, the producer (herd manager, etc.) must take the place of the herd bull in detecting the cows/heifers that are ready to be inseminated. Accurate detection of animals in standing estrus is the goal of good estrous detection and plays a vital role in the success of any AI program. In a study conducted at Colorado State University, animals were administered an estrous synchronization protocol, then monitored for standing estrus 24 hours a day with a computer assisted estrus detection system (HeatWatch®) or twice a day for 30 minutes by visual observation. By day 5 after estrous synchronization, 95% of animals monitored 24 hours a day were detected in standing estrous, while only 56% of animals observed twice a day for 30 minutes were detected in standing estrus (Downing et al., 1998). With a 95% estrous detection rate and a 70% conception rate (95% X 70% = 67%), 67% of the animals will be pregnant; whereas, only a 39% (55% X 70% = 39%) pregnancy rate will occur with a 55% estrus detection rate (**Table 1**).

Table 1. Effect of estrous detection rate on increasing pregnancy rate

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

Therefore, the success of any artificial insemination program requires detecting the animals that are ready to be bred (standing estrus) and inseminating them at the correct time. Failing to detect estrus and inaccurate detection of estrus can result in significant economic losses (Heersche and Nebel, 1994). Accurate detection of estrus can be a difficult and time-consuming activity. When estrus was detected in 500 Angus cows with 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 extremely important to visually monitor cattle as much as possible. Continuous observation of over 500 animals exhibiting natural estrus in 3 separate studies indicated 55.9% of cows initiated standing estrus from 6 p.m. to 6 a.m. (**Table 2**). 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. Several estrous detection aids have been developed to assist with this time-consuming chore. These estrus-detection aids can effectively help determine which cows are or have been in standing estrus, therefore relieving some of the time required to visually observe cattle for standing estrus. 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). However, increased visual observation, in addition to the use of estrus-detection aids, could improve fertility by determining the most appropriate time for insemination.

Table 2. 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).

Estrous Synchronization on Fertility

Estrous Synchronization

Estrous synchronization simply implies the manipulation of heifers/cows to cause them to exhibit standing estrus around the same time. This can greatly reduce the number of days needed to detect a group of animals in standing estrus. However, the question is often asked, "Does estrous synchronization increase or decrease fertility?" To determine an answer to this question we need to compare both animals that are bred by natural service and AI.

Natural Service: *Nonsynchronized females:* When cows are bred by natural service, the serving capacity of the bull becomes a critical management consideration. Recommendations for the bull to female ratio in nonsynchronized cows range from 1:10 to 1:60. This range depends on the age, experience, and semen quality of the bull, as well as size and terrain of the breeding pasture. No differences were detected between a bull to female ratio of 1:25 and 1:60 for estrous detection or pregnancy rates in the first 21 days of the breeding season provided the bulls were highly fertile and had large scrotal circumferences (Rupp et al., 1977).

Natural Service: *Synchronized females:* When cows are synchronized and bred by natural service, management considerations should be made for the serving capacity of the bull. Healy et al. (1993) reported a tendency ($P < 0.10$) for pregnancy rates over a 28-day synchronized breeding season to be reduced when a bull to female ratio of 1:50 (77%) was used compared to a bull to female ratio of 1:16 (84%); however, no difference was detected between a bull to female ratio of 1:16 and 1:25 (84% and 83%, respectively). Synchronization with natural service will be discussed in detail in another chapter of this proceedings. However, when considerations for a bulls serving capacity are considered estrous synchronization protocols uses with natural service are capable of inducing puberty and shortening the anestrous postpartum period can result in an even greater percentage of cows having a chance to become pregnant during the first few days of the breeding season.

Artificial Insemination: Estrous synchronization makes AI more feasible due to the reduction in time and labor required for estrous detection. Therefore, it is also necessary to compare fertility between synchronized and non-synchronized females bred by AI. When AI is combined with estrous synchronization, the limitation on serving capacity of a single bull is removed, and a large number of females can be bred to a single sire or group of sires during the first few days of the breeding season. This can result in a more uniform calf crop that is older and heavier at weaning. Across several studies results indicate no reduction in fertility

and in fact some protocols can result in an increased chance to become pregnant during the first few days of the breeding season and more opportunities to conceive during the breeding season.

Fixed-Time Insemination

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

Currently, most fixed-time insemination protocols (ovulation synchronization protocols) utilize an injection of GnRH to ovulate a dominant follicle around the time of insemination. The Ovsynch (Pursley et al., 1998) and CO-Synch (Geary and Whittier, 1998) protocols include the same hormonal treatments to synchronize ovulation [on day -9, GnRH is administered, on day -2, PG is administered, and 48 hours later (day 0) GnRH is administered to induce ovulation around the time of insemination]. The use of GnRH at the time of insemination resulted in a wide range of follicle sizes being induced to ovulate (Perry et al., 2005), and although dominant bovine follicles (≥ 10 mm) have the ability to ovulate in response to a GnRH-induced gonadotropin surge, a larger dose of LH was required to induce ovulation of a 10 mm follicle compared to larger follicles (Sartori et al., 2001). A decrease in pregnancy rates occurred when small follicles were induced to ovulate following fixed-time AI in both heifers and cows (CIDR Protocol – Lamb et al., 2001; T.W. Geary unpublished data; CO-Synch protocol – Perry et al., 2005; Perry et al., 2004b; **Figure 1**). Furthermore, similar results were reported among *Bos indicus* cows. Evaluation of follicle size at time of fixed-time AI on 2388 Nellore and Nellore x Angus cross cows indicated that a follicle of at least 11.1 mm in diameter was needed to achieve maximum pregnancy success (Sa Filho et al., 2010). Therefore, the ovulatory follicle may affect fertility through the preparation of the oocyte for embryonic development, preparation of follicular cells for luteinization, and/or preparation of the uterine environment for the establishment and maintenance of pregnancy. However, when embryos of similar quality were transferred into cows induced to ovulate small (< 12 mm) or large (> 12 mm) follicles, cows induced to ovulate small follicles had significantly lower pregnancy rates compared to cows induced to ovulate large follicles (Mussard et al., 2003). The preceding study indicates the uterine environment is likely a major factor in decreased fertility following induced ovulation of small dominant follicles.

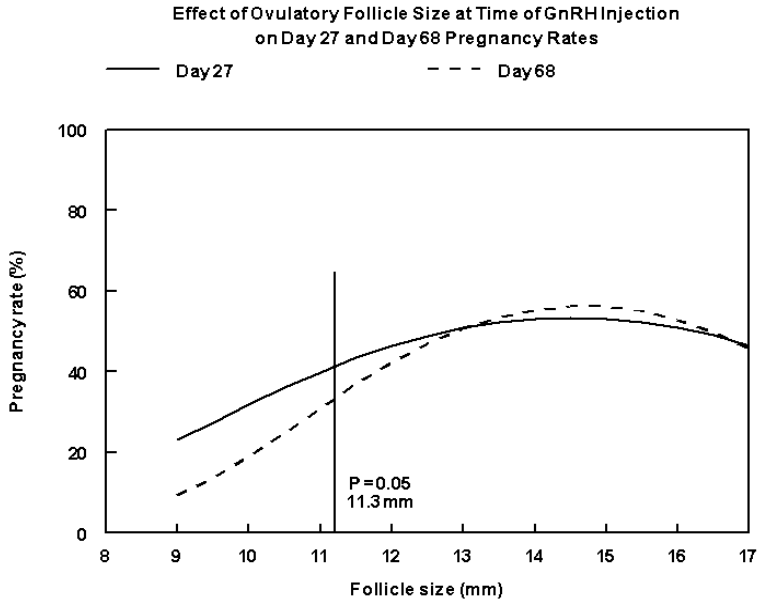

Figure 1. Regression analysis of the effect of ovulatory follicle size at time of GnRH injection/insemination on pregnancy rates 27 and 68 days after insemination. Follicle sizes at which pregnancy rates were decreased ($P < 0.05$) below the maximal pregnancy rates are indicated with vertical line. (Perry et al., 2005)

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

Pregnancy rates were also increased when animals were detected in standing estrus within 24 hours of fixed-time insemination regardless of follicle size induced to ovulate (Perry et al., 2005). Cows that initiate standing estrus around the time of fixed-time insemination had elevated preovulatory concentrations of estradiol compared to cows that did not exhibit standing estrus (Perry and Perry, 2008a). A recent study involving the reciprocal embryo transfer of embryos to and from cows induced to ovulate either a large or small follicle with GnRH revealed some interesting results about the factors affecting fertility (Atkins et al., 2013). While ovulatory follicle size and serum concentrations of estradiol were highly correlated ($r = 0.49$; $P < 0.0001$), both concentrations of estradiol and follicle size had independent positive effects on fertilization success. Furthermore, donors with greater concentrations of estradiol at the GnRH-induced ovulation were more likely to yield a fertilized embryo than an unfertilized oocyte (Jinks et al., 2013)

Efficient transportation of sperm through the female reproductive tract requires that the female be in estrus or under the influence of estrogen (Hawk, 1983). Estrogen may influence fertilization rates through both sperm transport and fertilization efficiency by altering the uterine environment around the time of fertilization. Uterine pH decreased at the initiation of standing estrus (Elrod and Butler, 1993) to a pH similar to seminal plasma (Acott and Carr, 1984). Furthermore, uterine pH was decreased in animals that exhibited standing estrus at the time of fixed-time AI compared to animals not in standing estrus (Perry and Perry, 2008a; Perry and Perry, 2008b), and there was a linear relationship between uterine pH at time of fixed-time AI and pregnancy success (Lares et al., 2008). Therefore, cows with a lower uterine pH at time of fixed-time AI had greater pregnancy success compared to cows with a high pH at time of fixed-time AI.

Following fertilization, luteal secretion of progesterone during the subsequent estrous cycle is required for the survival of the embryo/fetus (McDonald et al., 1952), and has been associated with fertility in cattle by stimulating both uterine secretions (Geisert et al., 1992) and embryonic growth and development (Garrett et al., 1988; Mann et al., 1996). Uterine secretions including nutrients, growth factors, immunosuppressive agents, enzymes, ions, and steroids contribute to early conceptus growth/survival (Geisert et al., 1992; Gray et al., 2001). Cows with normal developing embryos had greater concentrations of progesterone on days 3 and 6 after insemination compared to cows with degenerating embryos (Maurer and Echternkemp, 1982). Following a timed-AI protocol, serum concentrations of progesterone were affected ($P < 0.04$) by the size of the dominant follicle induced to ovulate (**Figure 2**). More specifically, the rise of progesterone following GnRH-induced ovulation was decreased ($P < 0.01$) in cows that ovulated ≤ 12 mm follicles compared to cows that ovulated larger follicles. Furthermore, cows induced to ovulate ≤ 12 mm follicles had decreased ($P < 0.05$) pregnancy rates compared to cows induced to ovulate larger follicles (29% vs. 71%, respectively, Perry et al., 2005). This decrease in concentrations of progesterone is dependent on follicle size. In the reciprocal embryo transfer study mentioned above (Atkins et al., 2013), serum concentrations of progesterone on day 7 (at embryo collection) were greater in cows producing a more advanced embryo. The relationship between follicle size and progesterone production 7 days later was also positively correlated ($r = 0.31$; $P < 0.0001$). Furthermore, there was a correlation between follicle size and CL weight ($P = 0.01$; $R^2 = 0.51$); for every increase of 1 mm in follicle size, day 10 CL weight increased by 1.1 g (Fields et al., 2012). However, there was no effect of estrus, follicle size, or day 10 CL weight on expression of steroidogenic enzymes (StAR, CYP11A1, or 3β; Fields et al., 2012). Therefore, as follicle size increased, CL weight increased, and concentrations of progesterone increased.

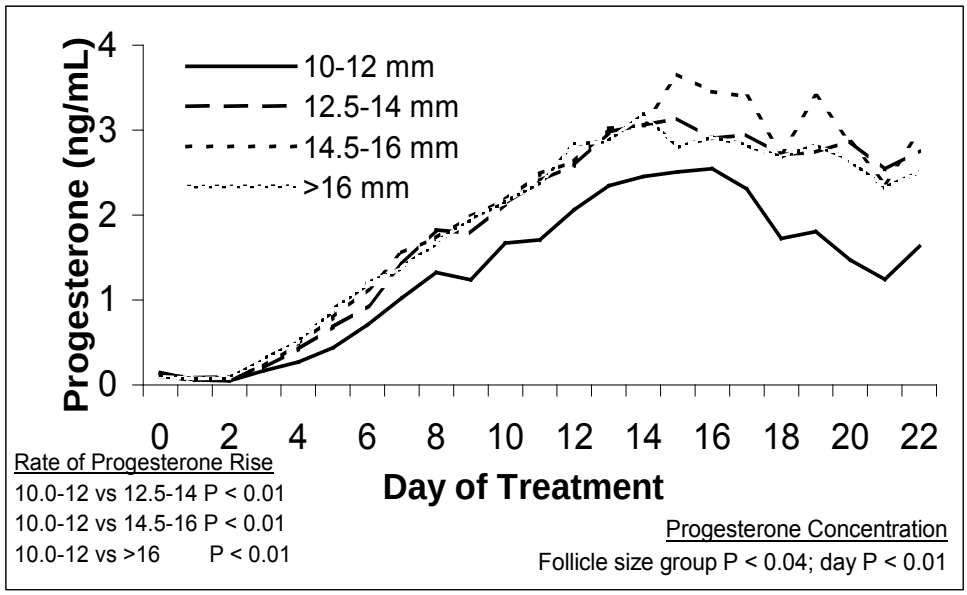

Figure 2. Effect of ovulatory follicle size, across both anestrous and cycling cows, on mean serum concentrations of progesterone from day 0 (second GnRH injection) through day 22, and rate of progesterone increase from day 0 to peak progesterone concentration. (Perry et al., 2005)

Inseminator Efficiency

Fertilization rates following natural service or artificial insemination in cattle range from 89 to 100% (Bearden et al., 1956; Diskin and Sreenan, 1980; Gayerie de Abreu et al., 1984; Kidder et al., 1954; Maurer and Chenault, 1983)**.** When pregnancy rates from 13,942 first service artificial inseminations were compared to 6,310 first services by natural service, no difference ($P > 0.10$) was detected between artificial insemination and natural service (Williamson et al., 1978).

With natural service, inseminator efficiency is influenced by the ability of a bull to service a cow. The purpose of the physical examination portion of a breeding soundness evaluation is to determine a bull's mating ability. Mating ability can be described as the physical capabilities needed to successfully breed a cow. In addition to structural unsoundness, diseases or injuries to the penis or prepuce can result in an inability to breed via natural service. These abnormalities will only be detected by careful examination or observation of an attempted mating of a cow. A bull that has high quality semen but is unable to physically breed cows is unsatisfactory for natural service.

With AI, inseminator efficiency is influenced by semen handling and the ability of the technician to deposit semen in the correct location. A detailed inventory of semen should be easily accessible, so that straws may be located and removed from the tank quickly to avoid exposure of semen to ambient temperature. Sperm injury (as judged by sperm motility) occurs at temperatures as warm as -79 ^{o}C (-110 ^{o}F; Etgen et al., 1957; Bean et al., 1963; DeJarnette, 1999), and injury to sperm cannot be corrected by returning semen to the liquid nitrogen (Berndtson et al., 1976; Saacke et al., 1978). Proper semen handling has been discussed in another chapter of this proceedings.

When numerous cows must be inseminated on a given day, multiple straws of semen are routinely thawed simultaneously to facilitate AI. Dalton et al. (2004) conducted a trial to

determine: a) the effect of simultaneous thawing of multiple 0.5-mL straws of semen and sequence of insemination (1st, 2nd, 3rd or 4th) on conception rates, b) whether conception rates achieved following AI by professional AI technicians (PAI) and herdsman-inseminators (HI) differed, and c) the effect of elapsed time from initiation of thawing straws of semen to seminal deposition on conception rates. Average conception rate differed between PAI and HI (45% vs. 27%, respectively), but simultaneous thawing and sequence of insemination (1st, 2nd, 3rd or 4th), and elapsed time from initial thaw to completion of fourth AI had no effect on conception rate within inseminator group (Dalton et al., 2004). Conception rates are most likely maximized when personnel: a) accurately identify and administer the appropriate treatments to all cows to synchronize estrus or ovulation, b) accurately identify cows in estrus, c) follow the AI stud's recommendations for thawing semen, d) prevent direct straw-to-straw contact during thawing of multiple straws simultaneously to avoid decreased post-thaw sperm viability as a result of straws sticking together (Brown et al., 1991), e) use appropriate hygienic procedures, f) maintain thermal protection of straws during AI gun assembly and transport to the cow, and g) deposit semen in the uterus of the cow within approximately 15 minutes after thawing.

Using conventional semen, many studies have compared semen deposition near the greater curvature of the uterine horns with traditional deposition into the uterine body. Although Senger et al. (1988), López-Gatius (1996), and Pursley (2004) reported increased conception rates when semen was deposited in the uterine horns rather than the uterine body, Hawk and Tanabe (1986), Williams et al. (1988), and McKenna et al. (1990) found no difference in fertility when comparing uterine body and uterine horn inseminations. Furthermore, Diskin et al. (2004) reported an inseminator and site of semen deposition interaction, with evidence of either an increase, decrease, or no effect of uterine horn deposition on conception rate for individual inseminators.

Unfortunately, it is not clear why some studies have shown an advantage following uterine horn insemination while others have not. A possible explanation for the positive effect of uterine horn inseminations may be related to the minimization or elimination of cervical semen deposition. Cervical insemination errors account for approximately 20% of attempted uterine body depositions (Peters et al., 1984). Macpherson (1968) reported that cervical insemination resulted in a 10% decrease in fertility when compared with deposition of semen in the uterine body. Clearly, all AI technicians must develop sufficient skill to recognize when the tip of the AI gun remains in the cervix. To maximize conception rates, AI technicians must continue to manipulate the reproductive tract until the tip of the AI gun is past the cervix and deposition into the uterus can be accomplished.

Fertility Level of the Herd

Fertility level of the herd may be the hardest factor to evaluate. Herd fertility includes cycling status, compliance with protocols, embryonic mortality, body condition (nutrition level), and disease. Several of these topics (cycling status, and nutrition) are discussed in great detail in other chapters of these proceedings. This review will focus on embryonic loss and the management factors that can increase or reduce embryonic mortality.

Fertilization rates are reported to be between 89% and 100% when animals are detected in estrus and semen is present at the time ovulation occurs (Bearden et al., 1956; Diskin and Sreenan, 1980; Gayerie de Abreu et al., 1984; Kidder et al., 1954; Maurer and Chenault, 1983). While fertilization usually takes place, conception rates (number of animals

that conceive divided by number of animals inseminated) are usually around 60% to 70% for natural service or artificial insemination. Although nature (poor oocyte quality, disease, chromosomal abnormalities, etc.) contributes much of this loss, management practices can also increase embryonic mortality. Stress, particularly heat and shipping stress, can be detrimental to embryos and decrease pregnancy rates.

In order to understand how stress may increase embryonic mortality, one must first understand the development of the embryo (**Table 3**). Just like the estrous cycle, embryo development begins on day 0, or the day of standing estrus. This is the day the female is receptive to the male and insemination occurs. Ovulation occurs on day 1 or about 30 hours after the first standing mount (Pursley et al., 1995; Vasconcelos et al., 1999; Wiltbank et al., 2000). If viable sperm is present, fertilization occurs inside the oviduct shortly after ovulation. The first cell division occurs on day 2, and by day 3 the embryo has reached the 8-cell stage (Shea, 1981). Between days 5 and 6 the embryo migrates into the uterine horn and by day 7 to 8 it forms into a blastocyst (Shea, 1981, Flechon and Renard, 1978, Peters, 1996). At this stage two distinct parts of the embryo can be seen: 1) the inner cell mass, which will form into the fetus and 2) the trophoblast, which will form into the placenta. Between days 9 and 11 the embryo hatches from the zona pellucida, a protective shell that has surrounded the embryo to this point (Shea, 1981, Peters, 1996). Then, on days 15 to 17, the embryo produces a chemical signal to prevent corpora lutea destruction and allow the cow to remain pregnant (Peters, 1996). The embryo attaches to the uterus beginning on day 19, and around day 25, placentation, an intricate cellular interface between the cow and the calf, begins. By day 42 the embryo has fully attached to the uterus of the cow (Peters, 1996).

Table 3. Time course of early bovine embryo development

Event	Day
Estrus	0
Ovulation	1
Fertilization	1
First cell division	2
8-cell stage	3
Migration to uterus	5-6
Blastocyst	7-8
Hatching	9-11
Maternal recognition of pregnancy	15-17
Attachment to the uterus	19
Adhesion to uterus	21-22
Placentation	25
Definitive attachment of the embryo to the uterus	42
Birth	285

Data adapted from: (Shea, 1981, Flechon and Renard, 1978, Peters, 1996, Telford et al., 1990)

Shipping Stress and Embryonic Mortality

With the knowledge of the critical time points in embryonic development, it is possible to completely understand how stress from shipping can result in increased embryonic mortality in cows (**Table 4**). When animals are loaded on a trailer and hauled to a new location, they become stressed and release hormones related to stress. These hormones lead to a release of different hormones that change the uterine environment in which the embryo is developing. During blastocyst formation, hatching, maternal recognition of pregnancy, and attachment to the uterus, the embryo is vulnerable to these changes. The most critical time points are between days 5 and 42 after insemination. Before day 5, the embryo is in the oviduct and is not subject to changes in the uterine environment. Therefore, stress does not influence embryo survivability at this time. The greater the length of time after day 42, the less severe the influence of shipping stress on embryonic loss appears to be. At the time of complete attachment of the embryo to the uterus the embryo is supported by the dam and appears to be not as easily affected by changes in its environment. On the other hand, in between these time points (5 – 42 days), the embryo is at greatest risk. Shipping during this time can cause detrimental changes to the uterine environment and may result in embryonic mortality. Administration of the prostaglandin inhibitor flunixin meglumine to cows and heifers 10 to 13 days after AI (when they were transported) reduced pregnancy losses about 9% (Merrill et al., 2007). However, administration of flunixin meglumine 10 to 15 d after breeding did not increase pregnancy establishment in cows. In another study, handling heifers to administer flunixin meglumine (compared to leaving them in the pasture) reduced pregnancy rates by 6% (Geary et al., 2010). Taken together, these studies provide evidence that some heifers are more susceptible to the stress of handling.

When should I not ship cows?

Shipping cows between days 5 and 42 can be detrimental to embryo survival and cause around a 10% decrease in pregnancy rates (**Table 4**). Critical time points such as blastocyst formation, hatching, maternal recognition of pregnancy, and adhesion to the uterus take place during this early time of pregnancy. If any of these time points are disturbed, then the result would lead to increased embryonic mortality and decreased pregnancy rates. Research has also demonstrated that shipping cattle 45 to 60 days after insemination can result in 6% of embryos being lost. Therefore, it is important to plan on transporting cattle before the breeding season or immediately after insemination.

When can I ship cows?

Shipping between days 1 – 4 is best. The embryo is still in the oviduct during this time; therefore, it is likely not subjected to uterine changes. Also after day 45, the embryo is well established and fully attached with the placenta; therefore it is less susceptible to the changes resulting from stress. Shipping at this point is less risky. However, embryonic loss from shipping has been reported up to 60 days after insemination. Care should always be taken to try to reduce the stress involved when animals are shipped. Do not overcrowd trailers and handle cattle as gently and calmly as possible.

Table 4. Effect of time of transport after insemination on pregnancy rates

	Days after insemination that transportation occurred			
	1 to 4	8 to 12	29 to 33	45 to 60*
Synchronized pregnancy rate	74%	62%	65%	
% pregnancy loss compared to transportation on days 1 to 4		12%	9%	6%*
Breeding season pregnancy rate	95%	94%	94%	

*Loss in heifers compared to percentage pregnant prior to transportation (pregnancy determined by transrectal ultrasonography)

Data adapted from Harrington et al., 1995, and T. W. Geary unpublished data

Heat Stress and Embryonic Mortality

The best time to ship cattle is during early stages of development. However, this is also the time point when the embryo is most susceptible to increased temperatures. Temperature, humidity, radiant heat, and wind all affect heat stress in cows. The rectal temperature of cattle is normally 102.2^{o}F, and an increase in rectal temperature but as little as 2^{o} F can result in decreased embryonic development (Ulberg and Burfening, 1967). When rectal temperatures reach 105.8^{o}F for as little as 9 hours on the day of insemination, embryonic development can be compromised (Rivera and Hansen, 2001). Heat stress has also been reported to change follicular waves, resulting in reduced oocyte quality (Wolfenson et al., 1995). Researchers have reported that heat stress 42 days prior to (Al-Katanani et al., 2001) and up to 40 days after breeding can affect pregnancy rates (Cartmill et al., 2001). This illustrates how important it is to plan ahead for the breeding season.

Several methods have been researched to reduce the effects of heat stress. Shade, fans, and misters can all reduce the effects of heat stress in natural service or AI programs. These methods allow animals to stay cooler during the hottest parts of the day. In humid areas, misters may not actually benefit the animals. If the water cannot evaporate, it will not be effective at cooling the animal.

Producers that utilize AI can also implement timed AI (TAI) protocols to increase pregnancy rates during the hot summer months. Timed AI has increased pregnancy rates over animals inseminated 12 hours after estrous detection in conditions of heat stress (Aréchiga et al., 1998; de la Sota et al., 1998). This is most likely due to fewer animals showing signs of estrus when under heat stress. When the weather is too hot, animals tend not to move around as much and do not show signs of standing estrus. Heat detection is a vital part of getting more animals pregnant. Since fewer animals are seen in heat, fewer animals can be inseminated. In this case, TAI protocols that synchronize ovulation would be the best choice because of the lack of necessity for heat detection.

Using embryo transfer during times of heat stress can also increase pregnancy rates. High quality, fresh embryos have been proven to increase pregnancy rates over AI in heat stressed cows (Putney et al., 1989). Embryos at time of embryo transfer can adapt to the elevated temperatures. Therefore, use of embryo transfer during times of heat stress can improves pregnancy success.

Stress from Change in Diet

Changes in nutritional status can also have a tremendous influence on embryonic survival through many mechanisms. Heifers fed 85% maintenance requirements of energy and protein had reduced embryo development on day 3 and day 8 compared to heifers fed 100% maintenance (Hill et al., 1970) indicating decreased embryonic growth. Therefore, changes in nutrition can have a tremendous impact on embryo survival and the ability of heifers to conceive during a defined breeding season.

Previous research has indicated that grazing skills are learned (Flores et al., 1989a, b, c) early in life (Provenza and Balph, 1988). This learning resulted in the development of preferences or aversions to plants and in the development of the skills necessary to harvest and ingest forages efficiently (Provenza and Balph, 1987). Heifers that grazed forage from weaning to breeding rather than being placed in drylots appeared to retain better grazing skills and had increased average daily gains into the subsequent summer (Olson et al., 1992: Perry et al., 2014). A decrease in feed intake from 120% of maintenance to 40% of maintenance resulted in a loss of 56.3 lbs over 2 weeks (4.03 lbs/day; Mackey et al., 1999); similar to the losses reported by Perry et al., (**Figure 3**) when heifers that were developed in a feedlot from weaning until the next spring were moved from a feedlot to grass. However, heifers that were developed from weaning until the next spring on range with supplementation showed no weight loss the following spring. Furthermore, heifers that were kept in a drylot until AI (n = 214) had decreased (P = 0.04) pregnancy rates compared to heifers that had previous grazing experience (n = 207; 59.4% vs. 49.1%). Therefore, post-insemination nutrition may influence embryonic survival. Nutritionally mediated changes to the uterine environment can occur by changing components of uterine secretions or by influencing the circulating concentrations of progesterone that regulate the uterine environment (see review by Foxcroft, 1997).

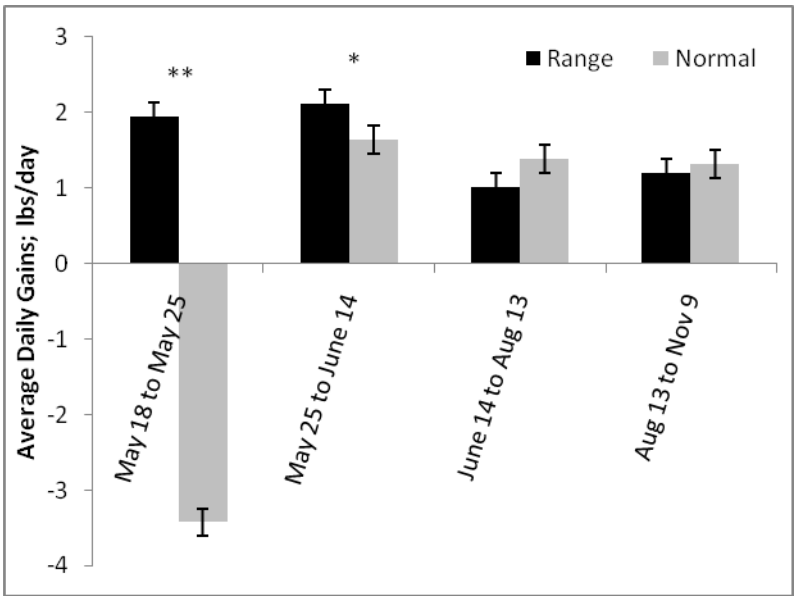

Figure 3. Average daily gain (lbs/day) of heifers weaned and developed on range (Range) compared to heifers weaned and developed in a drylot (Normal). All heifers were moved to the same pasture on May 18th (*P = 0.06; **P < 0.05)

In another recent study, beef heifers (n = 164) were developed in a feedlot from weaning to breeding. At time of insemination heifers were randomly allotted to one of two treatments: 1) heifers were moved from the feedlot to graze spring forage, or 2) heifers were moved to graze spring forage and supplemented with DDGS (5 lbs/hd/day) for 42 days.

Pregnancy success was determined 42 days after AI. Heifers that were grazing spring forage alone lost 37 ± 4 lbs, but heifers that were grazing spring forage and were supplemented gained 45 ± 3 lbs from AI to pregnancy determination ($P < 0.01$). Pregnancy success was different between treatments ($P = 0.02$). Heifers that were not supplemented after AI had decreased pregnancy success (61%) compared to heifers that were supplemented (76%). Therefore, when heifers were developed in a feedlot, pregnancy success tended to be influenced by supplementation and subsequent weight gain after moving heifers to grass.

To investigate the idea that the decrease in AI pregnancy success may be due to grazing behavior and not just a change in diet, we conducted an experiment where heifers were moved from a grazing environment to a drylot following AI. Beef heifers at one location (n= 333) were developed on a forage diet from weaning to breeding. All heifers were brought into a feedlot and synchronized with a 7-d CO-Synch + CIDR protocol. At time of insemination heifers were randomly allotted to one of three treatments: 1) heifers were moved to graze spring forage, 2) heifers were moved to graze spring forage plus supplemented with DDGS (5 lbs/hd/day) for 42 days, or 3) heifers were returned to the feed lot for 42 days. Pregnancy success was determined 42 days after AI. Body condition increased ($P < 0.01$) from the day synchronization began (day -7; 5.4 ± 0.05) to day 42 in both the heifers that were supplemented on pasture and the heifers that were kept in the feed lot (5.9 ± 0.04 and 5.8 ± 0.04, respectively; Table 4). Body condition did not change from day -7 to day 42 among the heifers that were on grass alone (5.4 ± 0.05 and 5.4 ± 0.04 for day -7 and day 45, respectively; Table 4). Pregnancy success did not differ among treatments [59% (65/111), 57% (63/111), and 56% (62/111) for heifers on grass alone, heifers on grass plus supplemented, and heifers in the feed lot, respectively). Therefore, when heifers were developed on grass, there was no effect on pregnancy success whether they were returned to grass with or without supplementation or even kept in the feed lot.

To further investigate if method of heifer development could impact grazing behavior, we conducted an experiment to measure daily activity between drylot developed heifers that had been moved to grass before AI compared to heifers that were moved to grass on the day of AI. Sixty-nine drylot developed heifers were randomly allotted to one of two treatments 42 days before AI: 1) heifers remained in the drylot until AI, or 2) heifers were moved to graze spring forage for the 42 days prior to AI. Daily activity was measured by a pedometer. Prior to AI, heifers that were grazing spring forage took more ($P < 0.01$) steps per day compared to heifers in the drylot (**Figure 4**). However; following AI, heifers that had remained in the drylot until AI had increased activity compared to heifers that had previous experience grazing spring forage (**Figure 5**). When activity is increased energy requirements are also increased. Cows that were forced to walk 3.2 km per day had a greater than 30% increase in energy requirements compared to cows that were held in a drylot (Bellows et al., 1994). Hence, heifers switched from a drylot to pasture are not accustom to grazing, forced to eat a novel diet, and exert increased energy during the period following AI. These factors combined may be the reason some heifers developed in a drylot and moved to forage after insemination have reduced conception rates. Therefore, keeping consistency in management during the breeding season is important to achieving optimum pregnancy success.

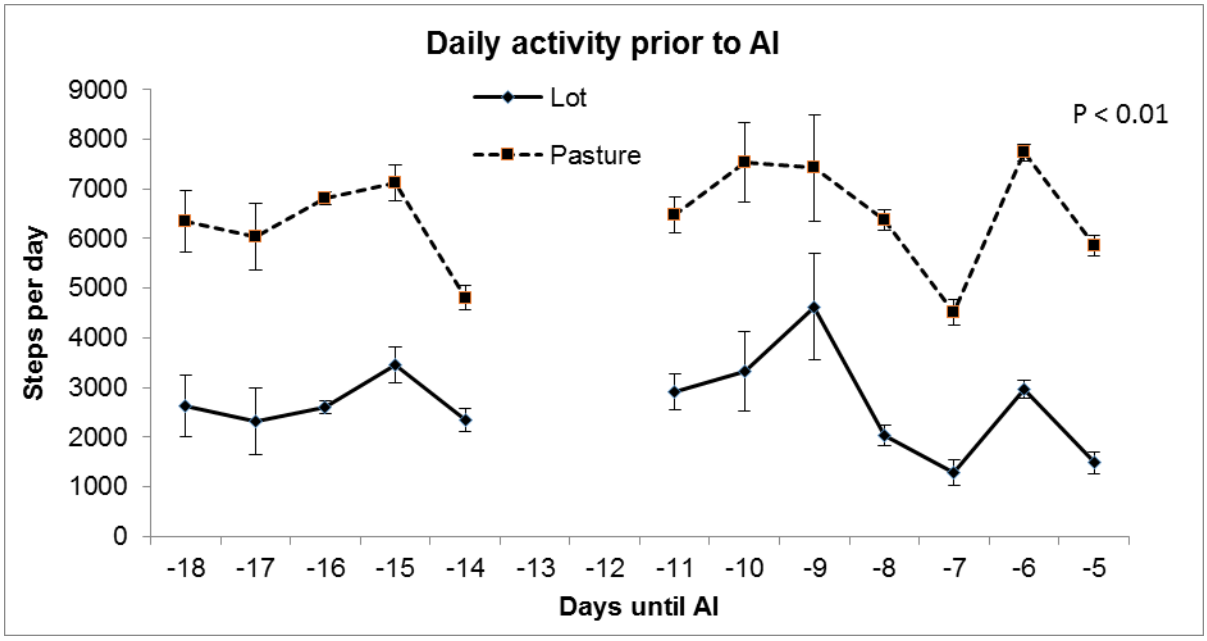

Figure 4. Daily activity for heifers that remained in the drylot until AI (LOT), and heifers that were moved to graze spring forage for the 42 days prior to AI (Pasture).

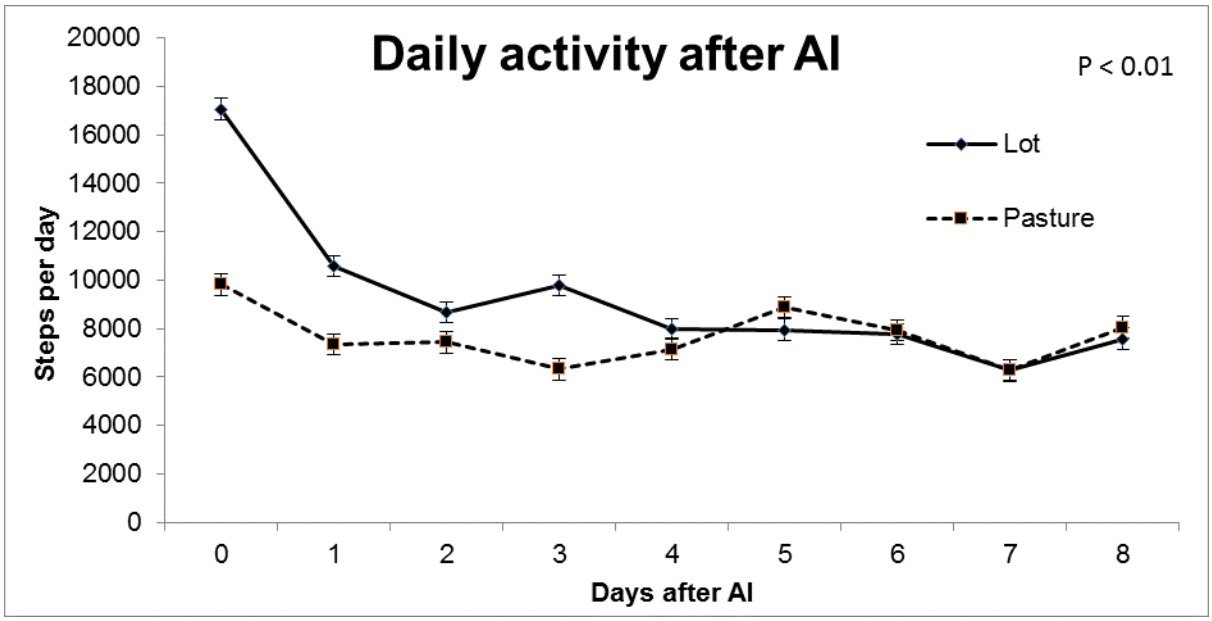

Figure 5. Daily activity for heifers that remained in the drylot until AI (LOT), and heifers that were moved to graze spring forage for the 42 days prior to AI (Pasture).

Impact of Timing of Vaccination on Pregnancy Success

Several studies have reported negative impacts on pregnancy success by vaccinating naïve heifers with a modified live vaccine (MLV) around time of breeding (Miller et al., 1989; Chiang et al., 1990; Miller, 1991). Among pre-vaccinated heifers, conception rates did not differ between heifers vaccinated 3 days before peak AI or 40 days before peak AI

(Bolton et al, 2007). However, when naïve heifers were synchronized with two injections of PGF and vaccinated with an MLV vaccine on the day of the 2nd PGF injection, heifers had a 30% first service conception rate and a 57% second service conception rate, as compared with control heifers that had a 78% first service conception rate and a 100% second service conception rate (Chiang et al., 1990). Furthermore, when seronegative heifers were vaccinated with a MLV at the first GnRH injection of a fixed-time AI protocol (PG 6-day CIDR protocol), they not only had decreased timed-AI conception rates, but 38% of them had abnormal estrous cycles and decreased conception rates at their first return to estrus (Perry et al., 2013). When seronegative heifers were vaccinated with BVDV, virus was isolated from white blood cells up to 10 days post-vaccination and from the ovary up to 12 days post-vaccination; furthermore, BVDV antigen was detected in the ovary up to 30 days post-vaccination (Grooms et al., 1998). Heifers experimentally infected with IBR at or near estrus had disrupted luteal function, but in most heifers the next estrous cycle was normal; however, in some heifers normal estrous cycles could be delayed for up two months (Miller and Van Der Matten, 1985). Therefore, general recommendations for vaccination of replacement heifers include: before and at weaning, with both heifers and cows receiving a booster vaccine at least 30 days before breeding. If it is absolutely necessary to give a modified live vaccine less than 30 days prior to breeding, the vaccine should be administered as soon as possible and only to animals that were vaccinated both before and at weaning. Animals that have not previously been vaccinated (naïve animals) should not be vaccinated near the time of breeding.

Fertility Level of the Semen

Clearly there are differences among bulls in the ability to achieve pregnancy success. For several decades seminal traits have been studied to try to predict reproductive success. Nevertheless, the determination of fertility differences between bulls requires the insemination of several thousand animals under the same management practices. All natural service bulls should have a comprehensive breeding soundness evaluation approximately 60 days prior to each breeding season. Whether natural service or AI is used, two of the most important indicators of bull fertility currently available are sperm motility and morphology. The influence of these different traits on the likelihood of pregnancy is discussed in great detail in another chapter of these proceedings.

Conclusions

This review has focused on some of the many factors that affect pregnancy rates in both natural service and AI and synchronized and non-synchronized breeding programs. One of the most comprehensive methods to look at factors that influence fertility is the "Equation of Reproduction." The equation looks at 4 main topic areas: 1) Percentage of animals detected in standing estrus and inseminated; 2) Inseminator efficiency; 3) Fertility level of the herd; and 4) Fertility level of the semen.

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