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MANAGEMENT FACTORS INFLUENCING FERTILITY IN SYNCHRONIZED AND NATURAL BREEDING PROGRAMS

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Introduction

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

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

Reproductive failure is a major source of economic loss in the beef industry. The majority of this loss occurs because cows do not become pregnant during a defined breeding season. Therefore, the goal of any breeding program (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, but one of the best methods to look at factors that influence fertility is with 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 estrus detection is considered to be easy, it is the bulls' job. However, differences do 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 introducing him to a cow herd and determining his desire to detect cows in estrus.

For successful artificial insemination of cattle to occur, the producer (herd manager) must take the place of the herd bull in detecting the cows/heifers ready to be inseminated. Accurate detection of animals in standing estrus is the goal of good estrus detection and plays a vital role in the success of any artificial insemination program. In a study conducted at Colorado State University, animals were administered an estrus synchronization protocol, then monitored for standing estrus 24 hours a day or twice a day for 30 minutes. By day 5 after estrus synchronization, 95% of animals monitored 24 hours a day were detected in standing estrus, while only 56% of animals observed twice a day for 30 minutes were detected in standing estrus (Downing et al., 1998). With a 95% estrus 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 estrus detection rate on increasing pregnancy rate.

Estrus 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%**

The success of any artificial insemination program requires detecting the animals that are ready to be bred (standing estrus) and inseminating them at the correct time. Failing to detect estrus and mis-detection of estrus can result in significant economic losses (Heersche and Nebel, 1994). Accurate estrus detection can be a difficult and time-consuming activity. When estrus was detected in 500 Angus cows with Heat Watch estrus-detection aids (24 hour a day estrus detection), the length of estrus averaged 10 hours (ranged from 0.5 hours to 24 hours), and 26% of cows exhibited estrus for less than 7 hours and had fewer than 1.5 mounts per hour (Rorie et al., 2002).

To maximize detection of standing estrus, it is extremely important to visually monitor cattle as much as possible. Observations should occur as early and as late as possible as well as during the

middle of the day. Continuous observation of over 500 animals exhibiting natural estrus in 3 separate studies indicated 55.9% of cows initiated standing estrus from 6 p.m. to 6 a.m. (Table 2). Furthermore, when cows were observed for standing estrus every 6 hours (6 a.m., noon, 6 p.m., and midnight), estrus 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 estrus detection aids have been developed to assist with this time-consuming chore. These estrus-detection aids can effectively determine which cows are or have been in standing estrus, therefore relieving some of the time required to visually observe cattle for standing estrus. A comparison between visual estrus detection every 3 hours (8 times daily), a marker animal, and Estrus Alert 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 detecting the most possible number of animals ready to be inseminated and 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).

Estrus synchronization

Estrus synchronization simply implies the estrous cycles of a group of heifers/cows are manipulated to cause them to exhibit standing estrus around the same time. This can greatly reduce the number of days needed to detect a group of animals in standing estrus. However, the question is often asked, "Do estrus synchronization protocols increase or decrease fertility?"

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 ranges 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 estrus detection or pregnancy rates in the first 21 days of the breeding season provided the bulls were highly fertile and had large scrotal circumferences (Rupp et al., 1977).

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

A single injection of prostaglandin $F_{2\alpha}$ (**PG**) on day 4 of the breeding season (bulls introduced on day 1) resulted in more cycling cows becoming pregnant during days 5 to 9 of the breeding season compared to cycling cows not injected with PG (55.7 vs. 25.0%, respectively; (Whittier et al., 1991). In addition, pregnancy rates were similar ($P > 0.10$) for cows in which estrus was synchronized with a single injection of PG and exposed to a bull for 80 hours (19%) compared to non-synchronized cows exposed to a bull for 21 days (33%, Landivar et al., 1985). When cows were synchronized with a single injection of PG on day 4 of the breeding season, there were no differences in pregnancy rates over the first 25 days of the breeding season (1 cycle) between synchronized and non-synchronized cows (Whittier et al., 1991). Therefore, the greatest benefit of estrus synchronization (PG) with natural service is the ability to get more cows pregnant during the first 5 to 7 days of the breeding season (Table 3). Cows that exhibit estrus early in the breeding season will also have additional chances to conceive during a defined breeding season. The average estrous cycle is 21 days (range 18 to 23 days), allowing one chance every 21 days for a cow to conceive. During a 65-day breeding season, cows that cycle naturally have only three chances to conceive, but cows that are synchronized and show estrus the first few days of the breeding season have up to four chances to conceive.

Table 3. Comparison between synchronized and non-synchronized pregnancy rates when bred by natural service in cows and heifers.

Study	Cows/ Heifers	Period of Time	Synchronization Method	Pregnancy Rate Anestrual	Pregnancy Rate Estrual Unknown
Whittier et al., 1991	Cows	4 days	1 shot PG	13.6%	55.7%[a]
			Not synchronized	22.7%	25.0%[b]
Plugge et al., 1989	Heifers	7 days	MGA + PG	62%[a]	
			Syncro-Mate B	67%[a]	
			Not synchronized	23%[b]	
Lamb et al., 2006	Cows	10 days	CIDR	43%[a]	
			Not synchronized	35%[c]	
Landivar et al., 1985	Cows	80 hours	1 shot PG	19%	
		21 days	Not synchronized	33%	
Whittier et al., 1991	Cows	25 days	1 shot PG	59.1%	86.1%
			Not synchronized	59.1%	76.3%
Lamb et al., 2006	Cows	30 days	CIDR	64.4%	
			Not synchronized	64.7%	

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

Some estrus synchronization protocols that utilize progesterone (CIDR), norgestomet (Syncro-Mate B), or GnRH can initiate estrous cycles resulting in a shorter anestrous postpartum period or earlier onset of puberty (Lucy et al., 2001; Perry et al., 2004a; Yavas and Walton, 2000). In a small study, peripubertal heifers treated with melengestrol acetate (**MGA**, an orally active progestin) for 10 days resulted in a similar number of MGA treated heifers and control heifers attaining puberty by day 7 after MGA withdrawal, but by day 10 following MGA treatment, 50% more of the treated heifers attained puberty compared to the control animals (Imwalle et al., 1998). Synchronization with a progestin [norgestomet (Syncro-Mate B) or MGA] resulted in more ($P < 0.01$) heifers becoming pregnant (67% and 62%) during the first 7 days of the breeding season compared to non-synchronized heifers (23%, Plugge et al., 1989). Furthermore, when a CIDR was inserted 7 days before the start of the breeding season and removed the day the bull was introduced (no injections) more ($P < 0.05$; 43%) CIDR-treated cows became pregnant by day 10 compared to non-synchronized cows (35%; Lamb et al., 2006). However, when a single injection of PG was administered to a group of anestrous cows, no difference was

detected between synchronized and non-synchronized cows (13.6% and 22.7%, respectively, Whittier et al., 1991). Therefore, estrus synchronization protocols capable of inducing puberty and shortening the anestrous postpartum period can result in an even greater percentage of cows having a chance to become pregnant during the first few days of the breeding season.

Artificial insemination. Estrus synchronization makes AI more feasible due to the reduction in time and labor required for estrus detection. Therefore, it is also necessary to compare fertility between synchronized and non-synchronized females bred by AI (Tables 4 and 5). When AI is combined with estrus synchronization, the limitation on serving capacity of a single bull is removed, and a large number of females can be bred to a single sire during the first few days of the breeding season. This can result in a more uniform calf crop that is older and heavier at weaning.

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

When estrus synchronization protocols are used that will initiate estrous cycles [progesterone (CIDR), norgestomet (Syncro-mate-B), and GnRH protocols], an even greater benefit can be realized. Cows treated with a CIDR for 7 days before the start of the breeding season and an injection of PG at time of CIDR removal resulted in 26% of anestrous and 46% of estrous-cycling cows becoming pregnant during the first 3 days of the breeding season compared to only 4% of anestrous and 11% of estrous-cycling control cows (Lucy et al., 2001). Cows synchronized with Syncro-Mate B (**SMB**) resulted in more cycling and anestrous cows pregnant ($P < 0.01$; 64% and 48%, respectively) during the first 5 days of the breeding season compared to cycling and anestrous non-synchronized cows (20% and 8% respectively, Miksch et al., 1978). Furthermore, when heifers were synchronized with SMB, a greater ($P < 0.05$) percentage became pregnant (36%) during the first 5 days of the breeding season compared to non-synchronized heifers (17%, Miksch et al., 1978). Estrus synchronization protocols that utilize GnRH are also able to initiate estrous cycles in anestrous cows. When a GnRH-based protocol (Ovsynch; 100 μg GnRH, i.m. on d -9; 25 mg PG, i.m. on d -2; 100 μg GnRH, i.m. on d 0 and timed AI on day 1) was compared to SMB with timed-AI, similar pregnancy rates were obtained ($P > 0.10$) following both protocols among anestrous cows (43% and 49% respectively, Geary et al., 1998). Therefore, estrus synchronization protocols capable of inducing puberty and shortening the anestrous postpartum period can result in anestrous cows having a chance to become pregnant during the first few days of the breeding season and more opportunities to conceive during the breeding season.

Table 4. *Comparison between synchronized and non-synchronized pregnancy rates when bred by artificial insemination during the synchronized period.*

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

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

Table 5. Comparison between synchronized and non-synchronized pregnancy rates when bred by artificial insemination during the first cycle of the breeding season.

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

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

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. Methods of inseminating cattle at a fixed-time with consistently high pregnancy rates may be a reality in the near future. Stevenson et al. (2000) reported higher pregnancy rates ($P < 0.05$) for cattle artificially inseminated following detection of standing estrus (44%; Select Synch - GnRH on day -9, PG on day -2 and detect estrus) compared to cattle bred by timed AI (33%; CO-Synch – Select Synch with timed insemination and a second injection of GnRH on day 0). However, Lemaster et al. (2001) reported higher ($P < 0.05$) pregnancy rates for timed AI following the CO-Synch protocol (31%) compared to AI following estrus detection with the Select Synch protocol (21%).

Currently, most fixed-time insemination protocols (ovulation synchronization protocols) utilize GnRH to ovulate a dominant follicle around the time of insemination. The Ovsynch (Pursley et al., 1998) and CO-Synch (Geary and Whittier, 1998) protocols include the same hormonal treatments to synchronize ovulation [on day -9, GnRH is administered, on day -2, PG is administered, and 48 hours later (day 0) GnRH is administered to induce ovulation around the time of insemination]. The MGA-Select timed-AI protocol (MGA is fed for 14 days, on day 26 GnRH is administered, on day 33 PG is administered and 80 hours later GnRH is administered to induce ovulation around the time of insemination, Perry et al., 2002b) also utilized GnRH to induce ovulation around the time of insemination. The use of GnRH at the time of insemination resulted in a wide range of follicle sizes being induced to ovulate (Perry et al., 2005), and although dominant bovine follicles ($\geq$ 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). Furthermore, 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). 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.

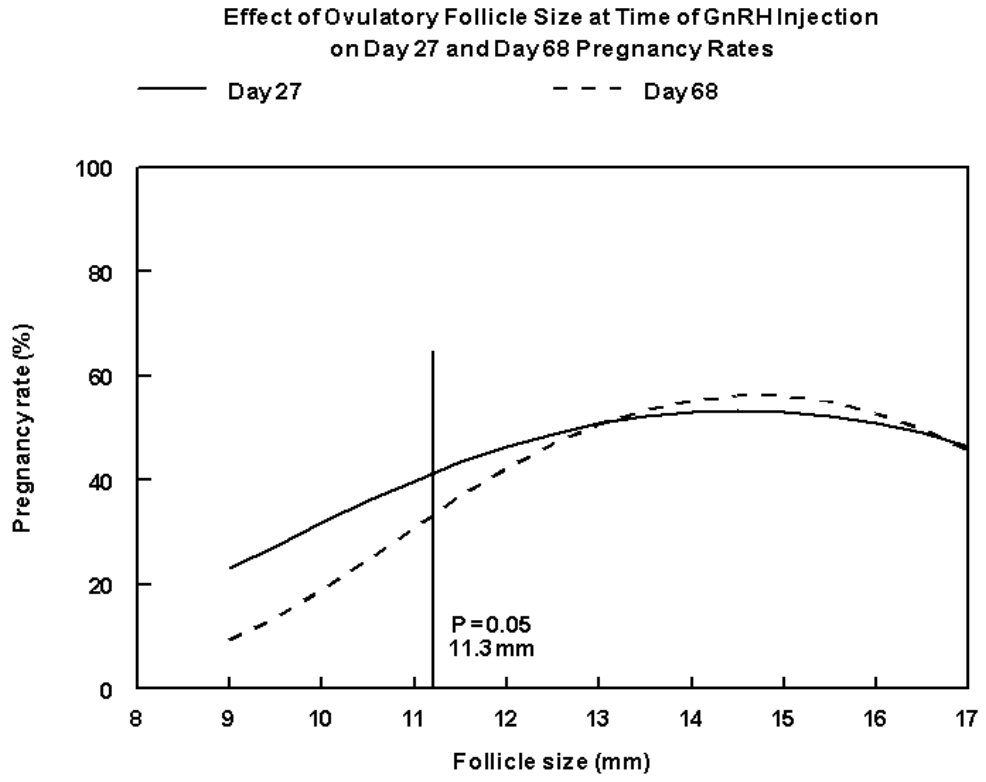

Figure 1. Regression analysis of the effect of ovulatory follicle size at time of GnRH injection/insemination on pregnancy rates 27 and 68 days after insemination. Follicle sizes at which pregnancy rates were decreased ($P < 0.05$) below the maximal pregnancy rates are indicated with vertical line (Perry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

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 and similar concentrations to cows that spontaneously initiated estrus and ovulation (Perry and Busch, 2005). Efficient transportation of sperm through the female reproductive tract

requires that the female be in estrus or under the influence of estrogen (Hawk, 1983). In a recent review by Santos et al. (2004) fertilization failure in lactating beef cows ranged from 0 to 25% and in lactating dairy cows from 12 to 45%. 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.

During final maturation, sperm lose their ability to biosynthesize, repair, grow, and divide, and become very simple in their metabolic function (Hammerstedt, 1993). This results in sperm becoming completely dependent on their external environment. While in the epididymis, sperm are stored for a long period of time in a relatively quiescent state, but upon ejaculation or dilution of caudal epididymis fluid, motility is increased (Acott and Carr, 1984; Carr and Acott, 1984). However, a consequence of the increased motility is a reduction in viability from several weeks to only several hours in the female tract (Austin, 1975). Medium pH influenced the motility of sperm collected from the caudal epididymis (Acott and Carr, 1984). Goltz et al. (1988) showed the motility of demembraned bull sperm increased as the pH of medium was raised from 6.6 to 7.1. An increase in sperm motility above basal levels appears to be necessary to assist the sperm in penetrating the viscous oviductal mucus and the cumulus matrix that surrounds the oocyte (Suarez and Dai, 1992) as well as the oocyte so fertilization can occur (Stauss et al., 1995). Therefore, changes in uterine pH from initiation of standing estrus (low pH) until ovulation may play a vital role in fertilization.

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 timed-AI protocols, 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 $\leq$ 12 mm follicles compared to cows that ovulated larger follicles. Furthermore, cows induced to ovulate $\leq$ 12 mm follicles had decreased ($P < 0.05$) pregnancy rates compared to cows induced to ovulate larger follicles (29% vs. 71%, respectively; Perry et al., 2002a).

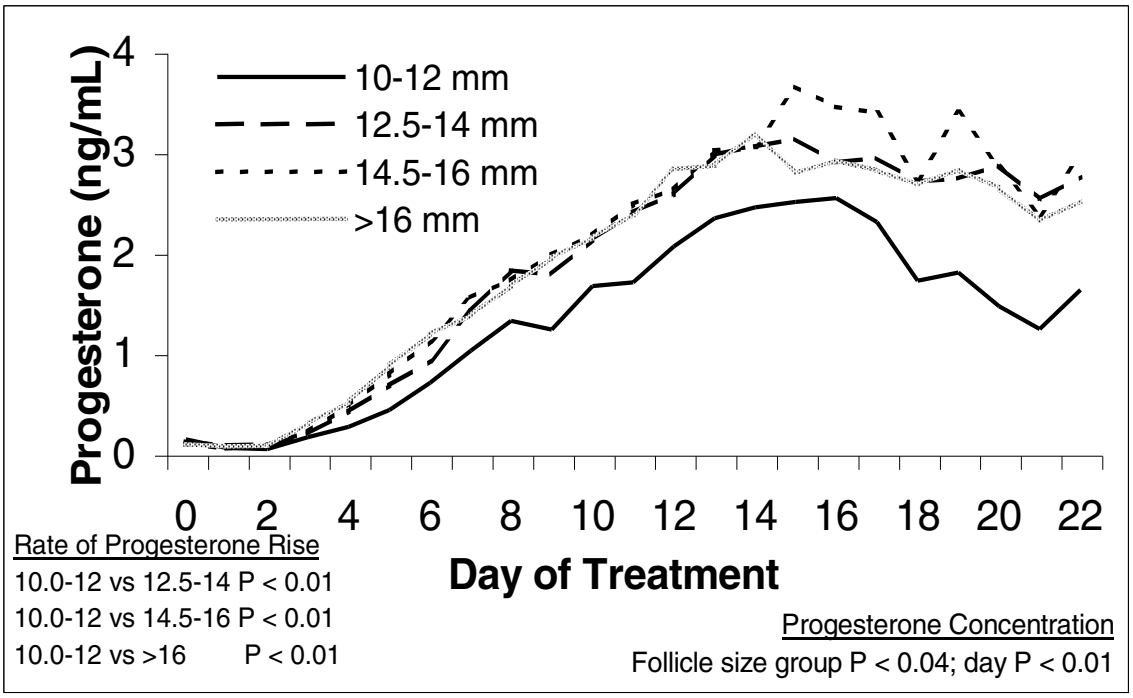

Figure 2. Effect of ovulatory follicle size, across both anestrous and cycling cows, on mean serum concentrations of progesterone from day 0 (second GnRH injection) through day 22, and rate of progesterone increase from day 0 to peak progesterone concentration (Perry et al., 2005).

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). Furthermore, no differences were detected between synchronized pregnancy rates when cows were bred by AI or natural service (Plugge et al., 1989).

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 observing 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. When removing a straw from a liquid nitrogen refrigerator, it is imperative that the technician keep the canister, cane and unused semen straws as low as possible in the neck of the tank. It is best to keep all unused straws below the frost-line in the neck of the tank. Sperm injury (as judged by sperm motility) occurs at temperatures as low as -79 degrees C (Etgen et al., 1957; Bean et al., 1963; DeJarnette, 1999). Furthermore, injury to sperm cannot be corrected by returning semen to the liquid nitrogen (Berndtson et al., 1976; Saacke et al., 1978).

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 (1^{st}, 2^{nd}, 3^{rd} or 4^{th}) 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 (1^{st}, 2^{nd}, 3^{rd} or 4^{th}), and elapsed time from initial thaw to completion of fourth AI had no effect on conception rate within inseminator group (Dalton et al., 2004). Nevertheless, a general recommendation as to the number of straws that may be thawed simultaneously detracts from the overall importance of proper semen handling for successful AI. 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 to avoid decreased post-thaw sperm viability as a result of straws freezing 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.

Many studies have compared semen deposition near the greater curvature of the uterine horns with conventional 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, nutrition, and disease) 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 mortility.

Fertilization rates are usually between 90% and 100% when semen is present at 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 6). 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 (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 sends a signal to the cow to tell her she is pregnant (Peters, 1996). This is the first signal that the cow gets to know if she is pregnant. 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 6. 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 7). 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. These 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 mother 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.

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 7). Research has also demonstrated that shipping cattle 45 to 60 days after insemination can result in 6% of embryos being lost. Therefore, even shipping cattle 45 to 60 days after insemination may increase embryonic mortality. 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. Therefore, it is important to plan on transporting cattle before the breeding season or immediately after insemination.

Table 7. 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 compared to percent pregnant prior to transportation (pregnancy determined by transrectal ultrasonography)

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

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 8. Time Points for Shipping Pregnant Cattle

	Day
When to ship	1 – 4 or after 45 to 60
When not to ship	6 - 42

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 by as little as 2^{o}F can result in decreased embryonic development (Ulberg and Berfening, 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 (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 artificial insemination (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 estrus 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 would be the best protocol to use, because it eliminates 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 young ruminants learn grazing skills from mothers and other adults (Flores et al., 1989a, b, c), and these behaviors are learned early in life (Provenza et al., 1988). 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: Salverson et al., 2009: Figure 3). Weaning is the period of time during which animals increased their consumption of forage (Lyford, 1988) to transition from maternal care to independence (Galef, 1981; Martin, 1984). This learning resulted in the development of preferences or aversions to plants and in the development of the motor skills necessary to harvest and ingest forages efficiently (Provenza et al., 1987). Furthermore, during the 1st year of life willingness to try novel food declined (Lobato et al., 1980). Thus young livestock ingest small amounts of novel food and gradually increase the amount ingested if no adverse effects occur (Burritt et al., 1987; Chapple et al., 1986). Therefore, when introduced to novel food livestock may spend significantly more time and energy foraging (Osuji, 1974), but ingest less (Arnold et al., 1977; Curll et al., 1983; Hodgson et al., 1981). Livestock with experience foraging have better skills and therefore, ingest more food per unit of time (Flores et al., 1989a; Hodgson, 1971). 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 Salverson et al. (2009; Figure 1) when heifers were moved from a feedlot to grass. (Ayalon, 1978; Bellows et al., 2002; Maurer et al., 1983; Peters, 1996; Silke et al., 2002). Therefore, post-insemination nutrition may influence embryonic survival through many mechanisms. 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).

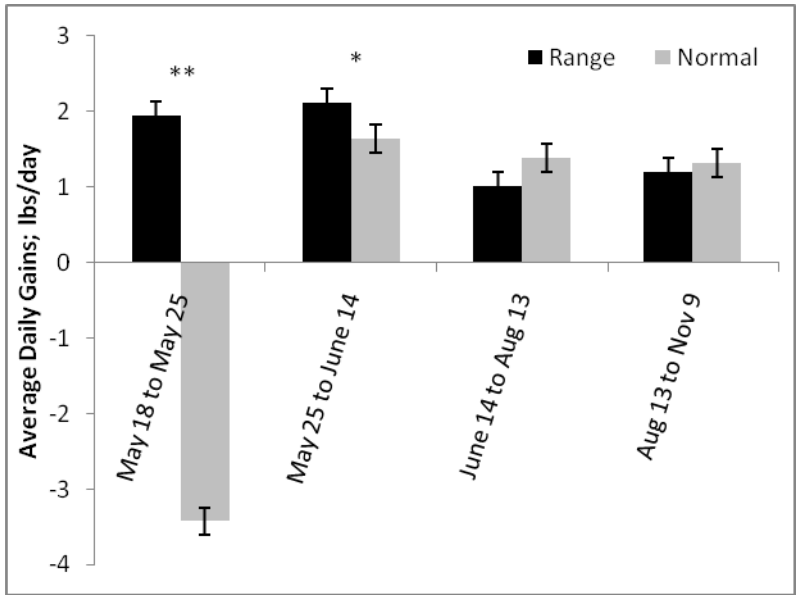

Figure 3. Average daily gain (lbs/day) of heifers weaned and developed on range (Range) compared to heifers weaned and developed in a drylot (Normal). All heifers were moved to the same pasture on May 18th (*P = 0.06; **$P < 0.05$)

Fertility Level of the Semen

Clearly there are differences among bulls in the ability to achieve great pregnancy success. For several decades seminal traits have been studied to try to predict reproductive success. The influence of these different traits on the likelihood of pregnancy is discussed in great detail in another chapter of these proceedings.

Conclusion

There are a tremendous number of factors that can influence fertility in a synchronized breeding program. This review has focused on some of the factors that affect pregnancy rates in both natural service and AI and synchronized and non-synchronized breeding programs. One of the best methods to look at factors that influence fertility is with the "Equation of Reproduction". The equation looks at 4 main topic areas: 1) Percent 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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