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EFFECTS OF TIMING OF ARTIFICIAL INSEMINATION AND SITE OF SEMEN DEPOSITION ON FERTILITY IN LACTATING DAIRY COWS AND GENDER RATIO OF RESULTING OFFSPRING

By

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ABSTRACT

EFFECTS OF TIMING OF ARTIFICIAL INSEMINATION AND SITE OF SEMEN DEPOSITION ON FERTILITY IN LACTATING DAIRY COWS AND GENDER RATIO OF RESULTING OFFSPRING

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This study was designed to determine the effect of prolonged subsistence of sperm in the female reproductive tract prior to ovulation in lactating dairy cows, with two different sites of AI, on pregnancy rates per AI (PR/AI) and resulting gender ratio. The Ovsynch program was utilized for precise timing of AI in relation to ovulation (24-32 h following final GnRH). Cows from three commercial dairies (n=1603) were assigned to one of four treatment groups (2 x 2) based on parity and days in milk (DIM). Cows were inseminated in the uterine body (B) or uterine horn (H) 8 h before (B or H - 8 h groups), or 16 h after (B or H 16 h groups), the final GnRH injection of Ovsynch. Ultrasound was used to diagnose pregnancy at 28 and 56 d after AI. There was no effect of site of AI on PR/AI, so B and H groups were combined within -8 and 16 groups. More female calves than male calves (65: 35 female: male ratio) were born in the -8 h group ($P < 0.01$). However, the 16 h group had a greater ($P < 0.01$) PR/AI (39 % vs. 29 %) than the -8 h group. To evaluate bull fertility at farm 3, service sires were selected based on Estimated Relative Conception Rate. There was an effect of service sire at -8 and 16 h on PR/AI and embryonic survival from 28 to 56 d ($P < 0.01$). In summary, prolonged subsistence of sperm in the female tract diminishes fertility and procreates more female offspring. Some service sires attenuated this diminished fertility and improved embryonic survival.

I dedicate this thesis to Bruce J. Macfarlane, my best friend

and partner for life. Together we can overcome

any challenge in life, love and research.

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AI	artificial insemination
am	morning
BCS	body condition score
bST	bovine somatotropin
CL	corpus luteum
CR	conception rate
d	day
DHIA	dairy herd improvement association
DIM	days in milk
DRMS	dairy records management system
ERCR	estimated relative conception rate
FSH	follicle stimulating hormone
GnRH	gonadotropin releasing hormone
h	hour
IVF	in vitro fertilization
LH	luteinizing hormone
pm	afternoon
PR	pregnancy rate
PR/AI	pregnancy rate per AI
RP	retained placenta
SCC	somatic cell count
SCS	somatic cell score
ZP	zona proteins

INTRODUCTION

A decrease in conception rates (CR) and reproductive efficiency has occurred in the dairy industry over the past 30 years. In the 1970s, CR from artificial insemination (AI) in multiparous lactating dairy cows was 66% (Spalding et al., 1974). The CR in multiparous lactating cows decreased to 40 % in the 1980s (Butler and Smith, 1989). In the last 10 years CR in lactating cows declined to approximately 37 % (Lopez-Gatius, 2003).

Decreased reproductive efficiency due to decreased CR reduce dairy farm profitability by increasing the days open and increases culling of potentially highly profitable cows (Gwazdauskas et al., 1981; Pecsok et al., 1994; Schindler, 1991). Decrease in CR also reduces the number of genetically superior replacement heifers born. To maintain herd size the number of cows culled from a herd must equal the number of replacement heifers needed. This becomes a challenge if numbers of replacement heifers produced do not equal numbers of cows leaving the herd.

The current average female: male gender ratio for calves born to multiparous dairy cows is 46: 54 (Ryan and Boland, 1991). The opportunity to increase the number of heifer calves born would allow dairy producers to raise more replacements and potentially increase sustainability and profitability. Pursley et al. (1998), and Vasconcelos et al. (1997) altered the gender ratio in favor of females when insemination occurred approximately 28 h prior to ovulation in lactating dairy cows. Unfortunately, about a 20% reduction in CR was found for cows inseminated at 28 h compared with AI

at approximately 12 h prior to ovulation. The reduced CR may be due to sperm residing in the uterus for 28 h prior to fertilization. The extended time in the uterus may challenge sperm survivability and reduce fertilization capability.

We speculate that producers would not use the 28 h protocol because of the reduced CR. Therefore, we chose to investigate site of semen deposition as a possible technique to increase CR. This is particularly important in the treatment groups where sperm presence in the female reproductive tract is extended. Deposition of semen within the uterine horn may reduce retrograde sperm loss and possibly decrease exposure of sperm to phagocytosis in the cervix and uterine body (Mattner, 1968). Senger et al. (1988) demonstrated a dramatic increase in CR from 45 to 70 % using deep uterine horn insemination. More recently, Pursley and co-workers (2003; unpublished) increased CR in lactating dairy cows from 35 to 50 % with deep uterine horn AI. In addition, Diskin and Pursley (2003; unpublished) increased CR in lactating dairy cows in Ireland from 45 to 50 % with deep uterine horn AI.

The objective of my research was to determine whether timing of AI and deep uterine horn insemination can be combined to produce an optimal program for dairy producers to obtain high CR and increase number of heifer calves born.

Additionally, my experiments assessed physiological and management factors associated with milk production, parity, somatic cell, previous calving information, body condition and lameness and their effect on fertility in multiparous lactating dairy cows.

The hypotheses for this thesis were:

1. Artificial insemination at approximately 36 h before the time of ovulation will increase the percentage of female calves.
2. Uterine horn artificial insemination will attenuate the decrease in CR in lactating dairy cows when inseminated approximately 36 h before ovulation.

Chapter 1

REVIEW OF LITERATURE

Timing of Artificial Insemination, Site of Semen Deposition and Management Factors that Influence Fertility in Lactating Dairy Cows and Gender Ratio of Offspring

Part I: Timing of Artificial Insemination (AI)

Optimal Time of AI in Relation to Estrus or Ovulation

During the development and validation of AI, several studies investigated the optimal time of AI in relation to estrus to achieve highest fertility (Aschbacher et al., 1956; Barrett and Casida, 1946; Hall et al., 1959; Trimberger, 1948; Trimberger and Davis, 1943). Optimal fertility was reached from mid-estrus until a few hours after the end of estrus. These data were responsible for the establishment of the am-pm or 12 h rule (if a cow is in estrus in the morning then AI in the evening). Later work suggested that inseminations during the middle to the end of estrus resulted in higher fertility than early estrus (Fields et al., 1975; Hall et al., 1959). Robbins et al. (1978) found that beef cows inseminated within 24 h following first observation of estrus did not have a reduction in fertility. However, cows inseminated more than 24 h after onset of estrus had a greater percent of degenerate embryos on d 6 or 7 (Dalton et al., 2001).

The variability in the interval between onset of estrus and the actual observation of estrus made it difficult to define the ideal time of AI in relation to ovulation (Foote, 1979; Gwazdauskas et al., 1986). However, development of Ovsynch (Figure 1) provided an effective protocol for determining the optimal time of AI (Pursley et al., (1995). Ovsynch effectively controls the time of ovulation, thus allowing for precise timing of AI in relation to ovulation. In two studies, greater than 95% of cows ovulated a dominant follicle 24 to 32 h after the final GnRH injection. This is a dramatic reduction in the variability of time to ovulation (8 h) compared to previous synchronization programs using $PGF_{2\alpha}$ in which the range of time to ovulation was 5 d (Pursley et al., 1997).

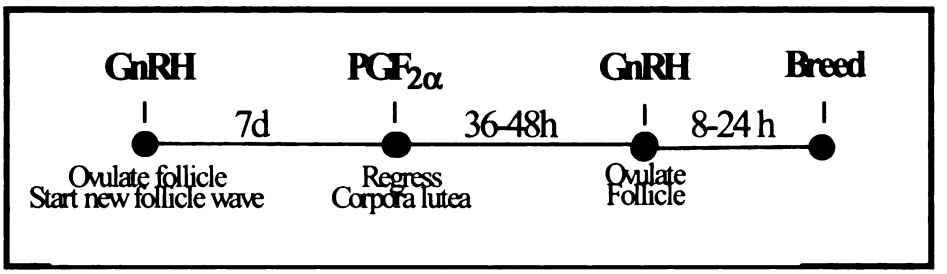

Figure 1. The timing and purpose of hormone injections in the Ovsynch program, adapted from (Pursley et al., 1995).

Pursley et al. (1998) showed that similar PR could be achieved following an Ovsynch program, with inseminations occurring at 0, 8, 16, and 24 h after final injection of GnRH. Yet, cows inseminated 16 h following GnRH of Ovsynch had the highest fertility, whereas cows in the 0 h group had the lowest embryonic loss. Interestingly, the 0 h group (inseminated approximately 24 to 32 h prior to ovulation) had the 2nd lowest CR but produced more female calves.

Importance of Increasing Female Offspring

The average female: male gender ratio in dairy calves born in the 1970s was 49: 51 (Foote, 1977). However, more current studies with large sample sizes, had outcomes of approximately 46: 54 females: males (Berry et al., 1995; Ryan and Boland, 1991). A lower percentage of heifers born poses a challenge because number of heifer calves raised must equal the number of cows culled to maintain herd size. Currently, the average culling rate in 12,000 herds (DRMS, Dairy Metrics June 2003) was approximately 39 % per year. In the U.S. total number of cows is slowly declining. This would insinuate there is insufficient numbers of heifers to replace the cows that are being culled. There are two types of culling, voluntary and involuntary. Voluntary culling

consists of cows leaving the herd because of a management decision. Involuntary culling is an unavoidable culling decision based on the health of the cow (Weigel et al., 2002). Involuntary culling of once productive cows reduces farm profitability and leads to the need for more replacement females. Increasing the number of replacement heifers would alleviate the pressure of both voluntary and involuntary culling of dairy cows.

Predetermining the gender of mammalian offspring with sperm sorting or embryo sexing is not a new idea (Bondioli et al., 1989; Pinkel et al., 1982). Johnson et al. (1989) produced the first live rabbit offspring using a sperm sorting technique. Rabbits inseminated with X-bearing sorted sperm had 94 % females. Since then, offspring of preselected sex have been produced with 90 % accuracy in pigs, cattle, sheep and most recently horses (Buchanan et al., 2000; Cran and Johnson, 1996; Seidel et al., 1999). However, sperm sorting has not become commercially accepted because of expensive equipment required, slow processing rates, and reduced sperm quality.

Timing of AI in relation to ovulation is a comprehensible and economical method for altering gender. Work in cattle indicated that timing of insemination can alter gender ratio. Pursley et al. (1998), and Vasconcelos et al. (1997) altered the gender ratio in favor of females when insemination occurred ~ 28 h prior to ovulation in lactating dairy cows. However, CR was reduced approximately 20 %. A recent in vitro fertilization (IVF) study tested whether sperm incubation prior to oocyte fertilization affected sex ratio of blastocysts. Prolonged incubation of sperm for 24 h prior to fertilization reduced development and fertilization rates, but more female-hatched blastocysts were present on d 9 (Lechniak et al., 2003). However, Rorie et al. (1999) published data that conflicts with the previously described work. His work was based on time of insemination at

either 20 h before ovulation or 10 h after ovulation. No difference in gender ratio of calves born was found. Twenty hours may not be sufficient enough time for X and Y chromosome-bearing sperm to deviate. Additionally, small sample size or poor prediction of time of ovulation may explain the conflicting results.

Sperm Survival in the Female Reproductive Tract

Timing of AI that occurs farther from the time of ovulation may reduce sperm survivability and CR. Spermatozoa survivability in inseminated cows was tested as early as 1945 and determined that sperm survive for only 30 h (Laing, 1945). However, it was reported later that bovine sperm have a fertile life of 30 to 48 h in the uterus (Bazer et al., 1987). Sperm adherence to the epithelial lining of the ampulla extends sperm longevity. Although sperm can survive in the female reproductive tract for 2 d, fertility is reduced. Optimal sperm survival rate and subsequent fertility may be dependent on timing of insemination relative to time of ovulation.

Semen Quality and Quantity

Quality and quantity of sperm present at the site of fertilization following prolonged exposure in the uterus may also affect fertility. A number of studies have investigated effects of quality and quantity of the semen using traditional AI practices (insemination 12 h after observed estrus). The optimal sperm number to achieve maximal fertility varies among bulls. Therefore, AI companies use relatively high numbers of sperm (approximately 20 million) to compensate (Foote and Parks, 1993).

Bull fertility differences can be divided into two categories. Compensable traits, which can be compensated with semen quantity, or uncompensable traits, which cannot be compensated with increased quantity (Saacke et al., 2000). The number of accessory spermatozoa, fertility rate and embryo quality increase when sperm per insemination dose increased from $20x10^6$ to $100x10^6$ (Nadir et al., 1992). Accessory sperm number present in the zona pellucida of the oocyte increased from 3 to 27 (Saacke et al., 1994). Accessory sperm are sperm trapped in the zona pellucida by the zona reaction after penetration of the fertilizing spermatozoon. The authors suggested that quantity of sperm might stimulate competition among fertilizing sperm, which may be favorable to embryonic development as well as fertilization rates. A possible mechanism for this hypothesis is a greater number of sperm at the site of fertilization may include more highly competent sperm. Another study considered 75 d non-return rates using different semen extenders with various semen doses. Results indicated that low semen dose caused decreased fertility (Schenk et al., 1987). denDaas et al., (1998) found that sperm numbers needed to obtain 95 % of the maximal CR in dairy bulls ranged from 1 to 11 million. Different semen extenders in this study did not differ significantly. Taken together this literature indicates that a certain minimum number of sperm are necessary before fertilization can occur.

Sperm characteristics related to quality are motility, membrane integrity, acrosome integrity, and ability to bind the zona pellucida (den Daas, 1992). Successful penetration of the oocyte depends on quality of the sperm inseminated. This is also known as extrinsic sperm quality. Intrinsic quality is the paternal contribution to proper development of the zygote. This is dependent on the quality of sperm (den Daas, 1992;

Pace et al., 1981). Cows inseminated with above average quality semen had 21% more number 1 and 2 quality embryos and 27% fewer degenerated embryos and unfertilized ova than cows inseminated with below average semen (Nadir et al., 1992). Beef heifers inseminated with either high or low fertility semen had a fertilization rate of 89 % and 70 % respectively (Callaghan and King, 1980). Call and Stevenson (1985) suggested that greater emphasis should be placed on selection of genetically superior sires for both milk production and fertility.

Clay and McDaniel (2001) developed a model for calculating relative bull fertility called the Estimated Relative Conception Rate (ERCR). The ERCR provides producers a management tool for selecting high fertility bulls. Selection of higher fertility bulls could lead to improved reproductive efficiency. ERCR measures the 70 d non-return rate of AI service sires relative to other service sires used within the same herd. ERCR is predicted using only first service insemination data and only after a bull has had at least 300 inseminations. Environmental factors such as herd, month of insemination, parity of cow, days in milk (DIM) and milk production are taken into account when calculating ERCR. A bull with a ERCR of 4, means that the bull should have CR 4 % greater than contemporaries. Bulls with higher CR potential may give producers a viable tool to increase reproductive efficiency.

Sperm Capacitation and Binding

Capacitation is defined as the changes sperm undergo within the female reproductive tract to achieve fertilization capability. Timing of AI may affect the ability of sperm to become capacitated. Capacitation activities peak in bovine oviductal fluid

around the time of estrus and decline during the luteal phase of the estrous cycle (Didion and Graves, 1986; Lauderdale and Ericsson, 1970). Zona binding proteins on the sperm head must be exposed during capacitation in order for ZP_3 binding to occur (Senger, 1999). To determine the exposure time needed to capacitate sperm, Lauderdale and Ericsson (1970) labeled bull semen with tetracycline HCl (T-HCl) and placed it in uteri of cows and heifers. The absence of fluorescence was an indirect method to detect capacitation. Sixty percent of sperm recovered from females in estrus had capacitated sperm 5 h after AI. Only 2% of cows in the luteal stage of the estrous cycle had capacitated sperm. Didion and Graves (1986) supported these findings and also analyzed sites within the female reproductive tract that might differ in ability to affect capacitation. Most of the capacitated sperm was found in the ampulla, ipsilateral to the dominant follicle.

Sperm binding to the zona pellucida is the first gamete interaction in the fertilization process and lack of interaction may be a common defect in some bulls (Braundmeier et al., 2002). Later steps in fertilization such as zona penetration, membrane fusion, or oocyte activation, or even an earlier step prior to zona binding, may also contribute to reduced fertility.

Certain regions of the female reproductive tract regulate sperm function as they progress through the oviduct (Boquest et al., 1999). Oviductal proteins may extend the longevity of spermatozoa during preovulatory periods and promote the acrosomal reaction (Boquest et al., 1999). The acrosome reaction begins when the plasma membrane fuses with the acrosomal vesicle membrane. Without this reaction sperm cannot penetrate the oocyte. The acrosome reaction allows penetration of the oocyte by

releasing enzymes on the sperm head. This allows binding and digestion of the zona pellucida to occur. Boquest et al., (1999) found that proteins from bovine oviductal epithelium promote sperm viability, delay acrosome damage, and suppress sperm locomotion. Possible changes in osmotic pressure and temperature could cause irreversible damage to the sperm acrosome, and diminish sperm fertilizing capability (Senger, 1999).

Summary

AI 12 h following initial observation of estrus once was the optimal time to achieve greatest fertility. With the advent of Ovsynch, timing of AI in relation to ovulation is possible. Pursley et al., (1998) determined that the optimal insemination time to achieve highest fertility is 16 h following the LH surge (final GnRH injection of GnRH) of Ovsynch. Ovsynch allows precise timing of ovulation approximately 24 to 32 h following the LH surge. AI ~ 28 h before ovulation produces more female offspring than AI at 16 h before ovulation. This is particularly important to dairy producers, whose livelihood depends on replacement females. However, AI 28 h prior to ovulation is not a viable management practices because fertility is decreased. The decrease in fertility may be due to semen quality or quantity inseminated, or the ability for sperm to survive, capacitate and remain fertile in the female tract prior to fertilization of the oocyte.

Part II: Site of Semen Deposition

Determining the ideal site of semen deposition to gain greatest fertility is important to AI efficiency. In a review by (Lopez-Gatius, 2000), the author reported that

vaginal insemination was replaced with cervical insemination some time in the 1940s. Then in the 1950s and 1960s, experiments using fresh semen indicated little difference in fertility rates among the uterine horn, uterine body or mid-cervix insemination (Knight et al., 1951; Macpherson, 1968; Olds et al., 1953). However, Weeth and Herman (1951) indicated that cows inseminated in the uterine body had higher fertility than cows inseminated in the cervix. Furthermore, Macpherson (1968) used high concentrations of frozen-thawed sperm and determined that placement of semen in the uterine body instead of the cervix increased cow fertility. These data were responsible for establishment of current AI procedures. However, with the decline in fertility in lactating dairy cows, reproductive specialists are re-investigating site of semen deposition to achieve greater fertility. Presently, pregnancy following a single AI in lactating dairy cows rarely results in rates higher than 40 % (Pursley et al., 1997; Washburn et al., 2001). This is alarming considering that in the 1960s, it was common to have PR of 60 % or better (Spalding et al., 1974).

Two of the primary arguments for use of uterine horn insemination are: placement of semen nearer to the uterotubal junction (main sperm reservoir prior to ovulation) and reduction of incidental cervical deposition (Lopez-Gatius, 2000). There are two types of uterine horn insemination methods, bicornual and unicornual. Senger et al. (1988) demonstrated a dramatic increase in CR when using bicornual insemination compared with uterine body insemination. CR on 4000 cows were improved from 45 to 70 % when cows were inseminated by splitting the semen in one straw between each horn compared with the standard deposition of one straw in the body of the uterus (Senger et al., 1988). However, other investigators have failed to confirm above results (Graves et al., 1991;

McKenna et al., 1990; Williams et al., 1988). More recent work of Pursley and co-workers (2003; unpublished) demonstrated increased CR in lactating dairy cows (from 35 to 50 %) with deep uterine horn AI (n = 800) as compared with uterine body. In addition, Diskin and Pursley (2003; unpublished) observed increased fertility in 3200 lactating dairy cows in Ireland (from 45 to 50 %) using deep uterine horn insemination.

The unicornual insemination method deposits semen in the horn either ipsilateral or contralateral to the side of ovulation. As expected, higher fertility was observed in cows inseminated in the ipsilateral versus the contralateral horn (Dalton et al., 1999; Lopez-Gatius, 1996; Lopez-Gatius and Camon-Urgel, 1988; Zavos et al., 1985). Hawk and Tanabe (1986) and Momont et al. (1989) found no difference in fertilization or PR when uterine body insemination was compared with horn insemination ipsilateral to the side of impending ovulation.

The contradictory results among horn insemination studies may be attributed, in part, to various insemination depths and techniques of deposition within the horn, as well as inaccuracy of placement of semen in the uterine horn or uterine body. Inaccuracy of semen placement within the reproductive tract was first observed following a dye deposition technique (Wright, 1964). Years later, a more sophisticated radiographic technique was established to determine proper position of the syringe tip in the uterine body. It was established that inseminators failed 61 % of 586 attempts to correctly deposit semen in the uterine body and more than 20 % of those attempts were in the cervix (Peters et al., 1984).

Sperm Transport in the Female

Site of semen deposition in the female reproductive tract may have an impact on sperm transport. Sperm deposited in the uterine body become widely dispersed throughout the reproductive tract, in a forward movement. Sperm are transported more efficiently in animals mated near the time of ovulation than those mated early in estrus due to uterine contractions and mucus flow (Hunter and Wilmut, 1983). Normal sperm transport in the female takes place in two phases: 1) Rapid transport phase in which sperm reach the oviduct within a matter of minutes, but do not have fertilization capability; 2) Sustained transport phase, where sperm capable of fertilizing ova reach the oviduct about 8 h after insemination (Wilmut and Hunter, 1984). Hunter and Wilmut established that sperm capable of fertilization were present in the oviduct 10-12 h after insemination. Sperm were held in the caudal end of the oviduct (isthmus) 18 h or more until ovulation at which point sperm proceeded toward the site of fertilization (Hunter and Nichol, 1986). Early research regarded the cervix as a sperm reservoir prior to ovulation (Mattner, 1966; Mattner, 1968). More recent work by Hawk (1987) and Hunter and Wilmut (1983) indicated that the isthmus is the primary pre-ovulatory reservoir at the time of ovulation. Thus, deposition of semen in the uterine horn would bring sperm closer to the pre-ovulatory reservoir. Lowered temperature within the isthmus of the oviduct at estrus reduces sperm motility, which allows sperm to reside longer in this reservoir prior to fertilization (Hunter and Nichol, 1986).

The effectiveness of sperm transport can be measured directly or indirectly (Hawk, 1983). An indirect measure of sperm transport is cow fertility. Counting sperm in various sections of the reproductive tract following insemination can be used as a

direct measure of sperm transport. Numerous investigators have used this direct measure of sperm transport (el-Banna and Hafez, 1970; Hafez and Dobrowolski, 1970; Hunter and Wilmut, 1983; Mitchell et al., 1985) to determine the fate of sperm in the female reproductive tract following AI. Direct measure of sperm transport has allowed investigators to determine that sperm capable of fertilization are present in the oviduct 10-12 h after insemination (Wilmut and Hunter, 1984). Other direct measures of sperm transport are fertilization rate and number of accessory sperm attached to the zona pellucida. However, there is controversy over whether accessory sperm represent increased fertility. Hawk and Tanabe (1986) determined that the number of accessory sperm in the zona pellucida following uterine horn insemination did not necessarily increase fertility. They found that zygotes developed with or without accessory sperm present. In contrast, Dalton et al. (1999) indicated that unicornual insemination provided greater sperm transport to the oocyte because of the number of accessory sperm present.

Uterine Environment and Fertility

Morrow et al. (1969) reported that slow involution of the uterus after calving was associated with inefficient reproduction. Uterine horn deposition may mitigate the effects of inferior uterine involution on sperm transport and fertility. Health of the uterine, cervical and oviductal environments are crucial to sperm transport and maintenance of a successful pregnancy (Lopez-Gatius, 1996). Lopez-Gatius investigated the effect of uterine horn insemination (first parity cows) in either the previously non gravid horn or gravid horn (pregnant horn) on sperm transport and PR. The investigators found that deposition of semen into the previously non-gravid horn increased PR when compared

with previously gravid horn insemination. This would indicate that previous pregnancy may negatively influence future reproductive efficiency.

Pitfalls of Uterine Horn Insemination

Although uterine horn insemination may increase CR, this technology has not been readily adopted by the dairy industry. One of the major concerns with uterine horn insemination is damage to the endometrium and introduction of bacteria. Larsson et al. (1986) discovered uterine horn insemination caused minor hemorrhage in the mucus membrane of heifers 2 d after insemination. Williams et al. (1987) addressed the effects of uterine horn insemination on health of the reproductive tract. Reproductive tracts were harvested 5 d after AI and evaluated for discoloration and luminal bacterial count. Neither uterine body nor cornual insemination increased bacterial population or trauma to the uterus. Either the uterine lumen was not damaged from AI or it had the capability to repair damage and eliminate bacteria within 5 d after AI.

In addition it was once believed that uterine horn insemination was very difficulty to teach and hard to execute. However, researchers have devised ways to train effective inseminators. McKenna et al. (1990) and Williams et al. (1988) trained inseminators on excised tracts using dye that stained the uterine lining at the site of deposition. Additionally, Beal et al. (1991) used an ultrasound to determine the location of a metal swivel attached to fishing line deposited into the uterine horn. These teaching procedures were 90 % effective in training inseminators to accurately place semen deep in the uterus (Pursley et al., 2003; unpublished).

Summary

Site of semen deposition to gain the greatest fertility has been investigated for many years. The most widely accepted site of insemination is the uterine body. The disadvantage to this site is increased incidence of cervical deposition. Uterine horn deposition is an alternative to uterine body insemination and is recommended as a way to limit cervical insemination. Additionally, uterine horn deposition may serve as a technique to by-pass uterine environments that hinder sperm transport and promote sperm loss. Skeptics argue that no advantage in fertility is gained when uterine horn insemination is used. Furthermore, concerns regarding damage to the uterus have been raised. However, the uterus seems to have the ability to repair any damage within 5 d following AI.

Part III: Physiological and Management Factors that may Affect Fertility

Efforts by dairy producers to remain profitable over the last two decades have resulted in increased herd size and more intense management practices. The increased demand on dairies means an increased demand on cows, and particularly production performance. It is important that dairy producers are aware of physiological and management factors that may reduce fertility and carefully manage cows to obtain higher fertility (Britt, 1985; Fonseca et al., 1983; Hillers et al., 1984; Oltenacu et al., 1984). Weigel (2002) reported that 96% of the variation in CR in lactating dairy cows is related to management and environmental factors. The following are some physiological and management factors that may affect cow fertility.

Genetics

Cow fertility is less than 3 % heritable (Hansen, 2000). Genetic control of cow fertility is extremely low, making environmental and management factors important to fertility (Hansen, 2000). Many studies attribute single trait selection for high milk producing cows as the cause for decreased fertility. Hansen (2000) suggests that in order for cows to become more reproductive efficient in the future, selection of traits that affect the longevity such as cow conformation and overall performance must be considered. In addition, genetic selection of bull fertility may offer the greatest opportunity to improve conception rates in lactating dairy cows.

Stress

The most important effect of stress on reproduction is disturbance of hypothalamic function. Acute stressors reduce normal pulsatile patterns of GnRH and LH (Dobson et al., 2001). Mastitis is one example of stress that may reduce fertility in cows. Infection of the udder can cause pain and stress for a cow, particularly severe cases. Somatic cell counts (SCC) are routinely used as an indicator of subclinical mastitis. Cows with high SCC have longer calving intervals than normal cows (Dobson et al., 2001).

Environmental heat stress is another major stressor for cows. Cows subjected to heat stress during the meiotic maturation process have increased numbers of abnormal or poor oocytes (Putney et al., 1989). Two experiments in two seasons evaluated fertilization rates and embryonic development in lactating and non-lactating dairy cows (Sartori et al., 2002). Experiment 1 (summer) compared lactating Holstein cows to

nulliparous heifers. Experiment 2 (winter) compared lactating cows to dry cows. Fertilization rates were reduced only in summer in lactating cows, which the author attributed to heat stress (Sartori et al., 2002). Embryos allocated from of lactating dairy cows in summer had a greater percentage of grades 3, 4 and 5 embryos (low quality) than non-lactating females. In winter, fertilization rates were similar in lactating and non-lactating cows.

Heat stress has a negative effect on oocyte quality both in vivo and in vitro, particularly in lactating dairy cows. Lactating dairy cows have higher body temperatures than non-lactating animals in response to increasing environmental temperature. This could be due to higher metabolic energy associated with feed intake and milk production, which makes them more prone to heat stress (Berman et al., 1985). Rivera and Hansen (2001) found that in vitro exposure of oocytes and sperm to 41° C during fertilization reduced the number of zygotes that cleaved to two cells. Lenz et al. (1983) also found that exposure of oocytes to high temperatures decreased oocyte maturation and subsequent fertilization rates.

Parity

Older cows have more reproductive disorders and lower reproductive performance (Hillers et al., 1984; Lerner et al., 1986). Cows in third and greater lactations had lower CR and longer intervals to first service than primiparous cows (Coleman et al., 1985; Hillers et al., 1984). The possibilities of more periparturiant diseases that occur with more calvings could be one possible explanation for lowered fertility (Erb and Martin, 1980).

CR from AI in first calf dairy heifers have remained close to 70 % over the past 10 years, whereas fertility in multiparous lactating cows has steadily declined (Foote, 1975; Pursley et al., 1997; Spalding et al., 1974). Given that heifers are often housed and fed a similar diet as cows, one could speculate that calving and (or) lactation may have some effect on cow physiology that reduces fertility. The condition of the uterus at parturition, or soon thereafter, is a major reason for low fertility in lactating dairy cows (Erb and Martin, 1980; Fonseca et al., 1983; Kay, 1978).

Retrograde Sperm Loss

Retrograde sperm loss is sperm discharged from the female tract. The effect of parturition on the uterus and retrograde sperm loss in dairy cows is unclear. However in non-lactating heifers we know the majority of retrograde sperm loss occurs 2 h following AI (Gallagher and Senger, 1989). Mitchell et al. (1985) recovered approximately 60 % of sperm inseminated in the mucus expelled from the vagina of multiparious dairy cows. The author also reported that retrograde sperm loss helped reduce the number of abnormal sperm present in the reproductive tract (Mitchell et al., 1985). Gallagher and Senger (1989) reported approximately 20 % of sperm were lost following insemination in the uterine body or horn in heifers. Although data do not exist that directly compare heifers with lactating cows for retrograde sperm loss, I speculate that sperm loss is greater in cows than heifers. This could be due to difference in the size and the physiological status of the uterus between cows and heifers. This may partially explain the difference in CR between dairy heifers and lactating dairy cows.

Phagocytosis

Phagocytosis is another way the female rids the reproductive tract of sperm. The presence of sperm in the female genital tract increases the number of leukocytes in the lumen of the uterus and cervix (Mattner, 1968). Sperm are foreign to the uterus and neutrophils attack "non-self" material upon entrance (Mattner, 1969).

During estrus when the uterine epithelium is under the influence of estradiol, leukocytes (specifically neutrophils) accumulate in the mucosa of the vagina and uterus (Senger, 1999). Mitchell et al. (1985) speculated that about 27 % of sperm loss after insemination was due to phagocytosis. However, this number may increase when estrus synchronization is used. Estrus synchronization may cause a "hyper-estrogenic" condition in cattle that triggers increased leukocytic activity, speeding up capacitation and phagocytosis of sperm (Lauderdale and Ericsson, 1970). Increased phagocytosis in lactating dairy cows may reduce number of sperm at the site of fertilization, thus reducing fertility.

Locomotion Scoring

Locomotion scoring is a method to assess the amount of lameness present in a herd. Lameness negatively affects the ability of cows to express estrus. Herds with prevalent lameness experience a decline in both the rate and accuracy of detection of estrus (Sprecher et al., 1996). Senger (1994) suggested that detection of estrus is the single most important limiting factor for high reproductive efficiency. Lameness can also lower first service CR and increase incidence of ovarian cysts (Melendez et al., 2003). Hernandez et al. (2001) found that the type of foot disorder impacted fertility. Cows with

abscesses/sole ulcers had 63 more days open than healthy cows. Cows with two or more foot disorders had 76 more days open when compared with healthy cows.

Body Condition Scores and Nutrition

Energy requirements of lactating dairy cows are met through a combination of dietary intake and mobilization of body reserves. Cows producing large quantities of milk cannot consume enough feed to maintain a positive dietary energy balance. During early lactation the cow must mobilize body stores to accommodate metabolic requirements (Nebel and McGillard, 1993). Body condition scoring (BCS) (based on a 5 point scale) is a method to evaluate the amount of body fatness a cows maintains (Wildman et al., 1982). BCS can be used to adjust dairy herd rations and herd health programs to achieve higher cow productivity, fertility, and longevity. Pulsatile LH secretion is suppressed, until negative energy balance reaches the lowest level and begins returning towards a positive energy balance (Butler and Smith, 1989; Canfield and Butler, 1990). Once energy balance is reached, LH secretion is reestablished and ovulation occurs. Body condition early in lactation is one of the best indicators for potential anestrous in a cow. Additionally, cows that are over or under-conditioned are more susceptible to metabolic problems, infections and reproductive difficulties (Gearhart et al., 1990). Cows with a BCS of 3.0 (on a 5 point scale) at AI may have the greatest likelihood for pregnancy success (Loeffler et al., 1999). In contrast, Waltner et al. (1993) indicated that BCS had no significant relationship to incidences of pyometra, metritis, RP, cystic ovaries or dystocia. However, the author cautions over interpretation of the data, as the study was conducted on one farm with little variation among BCS.

Herds with more variation in BCS may exhibit a significant relationship between BCS and reproductive diseases.

Proper nutrition is essential for energy balance. This balance is crucial to follicular growth, oocyte quality, embryo survival and overall fertility in the lactating dairy cow (O'Callaghan and Boland, 1999). Numerous studies of the nutritional needs and fertility in high producing cows have been published (Studer, 1998). Britt (1977) noted that mistakes in feeding dairy cows in late lactation and during the dry period caused the most damage to reproductive success. This coincides with greater embryo loss in dairy cows that have low or high BCS in early gestation (Lopez-Gatius et al., 2002; Silke et al., 2002). A recent review of nutritional effects on ovulation, embryo development and establishment of pregnancy in ruminants collectively indicated deleterious effects of high dietary intake and excess crude protein on fertility (O'Callaghan and Boland, 1999). The lack or overuse of vitamins and minerals was also detrimental to fertility (Swanson, 1989). The effects of nutrition can be found in early oogenesis even before the oocyte develops competency (Boland et al., 2001). Boland et al. (2001) reviewed effects of nutrition on follicular development, oocyte quality and embryonic development in ruminants. High dietary intake exerts a negative effect on developmental capacity of embryos. This may be due to increased blood flow and steroid metabolism in lactating dairy cows. (Butler and Smith, 1989; Sangsritavong et al., 2002; Wiltbank et al., 2000).

Bovine Somatotropin

Bovine somatotropin (bST) is a growth hormone in cattle. Administration of bST to lactating dairy cows increases milk production (Peel and Bauman, 1987). Producers that use bST can expect an 8 to 17 % increase in milk production. However, there are disadvantages to using bST. Cows treated with bST may have decreased energy balance (Lucy et al., 1993). Also, treated cows do not express estrus as intensely as untreated cows (Kirby et al., 1997). Therefore, bST-treated cows may have fewer opportunities to become pregnant.

Dalton and Marcinkowski (1993) evaluated the effect of bST on steroid hormones in the female and indicated no effects on basal GnRH during the breeding period. Yet, Kirby et al. (1997) reported that cows treated with bST had reduced FSH, and a faster turnover of dominant follicles. Other research also indicated that bST reduced FSH but increased initial development of the CL and extended its functional life span (Lucy et al., 1994).

A study was conducted to evaluate long-term effects of bST on fertility. Data from cows treated with bST over a 2-year period were collected. Multiparous cows treated with bST for 1 year had increased days to conception. The cows treated with bST for 2 years tended to have lower CR than control cows (71 vs 87% ; (Hansen et al., 1994). Additionally, increasing dose of bST decreased body condition linearly at the end of both years. Cows in their second year of treatment were slower to regain body condition. Another study reported that cows treated with bST had a significantly lower PR at 180 DIM than controls (Luna-Dominguez et al., 2000). In contrast, bST increased PR after a first Ovsynch synchronized insemination (Moreira et al., 2000). Given the

varied results of bST on fertility parameters, more research is needed to understand the true impact of bST on fertility.

Retained Placenta and Dystocia

Retained placenta (RP) is generally defined as failure to expel the placenta within 8 to 12 h after calving (van Werven et al., 1992). However, other work has defined RP as the failure to expel within 24 h and even up to 48 h after parturition (Halpern et al., 1985; Lee et al., 1989). Twins, abortions, and stillbirths have been associated with higher incidence of RP (Youngquist and Bierschwal, 1984). It is difficult to evaluate the effect of RP on fertility because there is no set standard for normal expulsion of the placenta. vanWerven (1992) studied the effect of RP retention time on fertility. RP did not alter reproductive performance nor culling rate of first, second or third lactation cows. However, cows in fourth or greater lactation had lower fertility as duration of RP increased.

A primary concern in cows with RP is increased susceptibility to uterine infections (Erb and Martin, 1980). Holt et al. (1989) investigated the effect of GnRH injection at 15 d postpartum in cows that were clinically normal versus cows with uterine discharge or RP (> 24 h). Effects on ovarian function, progesterone concentrations and overall fertility were examined. The results indicate that cows with RP had the lowest CR and most days to first service. Loeffler et al. (1999) pointed out that cows with cloudy uterine discharge after calving were only half as likely to conceive as females with a clear discharge.

Dystocia is defined as delayed and difficult parturition (Berger, 1994). Many producers use a calving difficulty score to quantify (on a 1 to 5 scale) difficulty of birth. Calving difficulty in Holsteins can impair subsequent reproductive performance by increasing days open, and increasing services per pregnancy (Mangurkar et al., 1984; Thompson et al., 1982; Thompson et al., 1983). Possible losses attributed to dystocia include reduced milk production and fertility, death of cow or calf and increased veterinary costs (Dematawewa and Berger, 1997).

Twinning

Multiple births are desirable in many species. However, birth of twins in dairy cows is associated with increased dystocia and health problems for the cow such as RP and uterine infections. Additionally, cows with twins have about 22 more days open than cows with singletons (Chapin and Van Vleck, 1980). Jones and Rouse (1920) associated twinning with older cows. Cole and Rodolfo (1924) associated twinning to time of year, and Lush (1925) to genetics. More recent work attributes increased twinning rates to increased parity and milk production (Kay, 1978; Wiltbank et al., 2000; Wood, 1975; Wood, 1984).

Most twin calves occur as a result of multiple ovulations (dizygotic twins) (Wiltbank et al., 2000). Wiltbank et al. (2000) suggested that multiple ovulations in high producing dairy cows are due to increased intake of dry matter. Increased dry matter intake increases liver blood flow and steroid metabolism. Normal dominant follicle selection usually results in an increase in circulating of estradiol, which suppresses follicle stimulating hormone (FSH). However, with increased estradiol metabolism the

concentration of FSH remains higher, allowing a second follicle to be recruited into dominant status. Therefore, higher producing cows may be more prone to double ovulations than lower producing cows (Fricke and Wiltbank, 1999). Wiltbank et al. (2000) reported that 20 % of high producing cows have double ovulations whereas only 7 % of low producing cows have double ovulations.

Milk Production, Days in Milk and Milking Frequency

Extensive research has been dedicated to understanding the relationship between milk production and fertility. Indeed, most studies reported that high milk production (herd average) had a negative effect on fertility (Faust et al., 1988; Fonseca et al., 1983; Laben et al., 1982; Ron et al., 1984; Spalding et al., 1974) while others found no effect or slightly positive results (Boyd et al., 1954; Everett et al., 1966; Gaines, 1927; Hillers et al., 1984). Some studies indicated that good management of metabolic needs of high producing cows could overcome the negative effect of milk production on fertility (Call and Stevenson, 1985; Laben et al., 1982; Nebel and McGillard, 1993). Call and Stevenson (1985) found (from DHIA herd summaries) that increased rolling herd averages were accompanied by an increased interval to first service and number of open cows. Hillers et al. (1984) examined the influence of management and environmental factors on reproduction in four large Washington dairy herds. Milk yield did not affect first service CR. However, others reported that cows with high milk yield had longer intervals to first service (Call and Stevenson, 1985). Other work indicates that selection for milk yield improved fertility in virgin heifers (Hansen et al., 1983). Recently, cows with above-average milk production within one high producing herd had greater PR/AI

(45.8 vs. 33.8%) compared with below-average herdmates (Peters and Pursley, 2002). An explanation for high fertility in higher producing cows may be that they have fewer health problems, such as lameness, mastitis, RP, or ketosis. The observation that poor-milk producing cows have more health problems and are less likely to conceive (Badinga et al., 1985) supports this explanation.

Days in milk (DIM) may also affect fertility. Loeffler et al. (1999) found a lowered pregnancy success in both early and late lactation females. Dairy cows achieved optimal fertility at approximately 82 d after parturition. This time following parturition allows cow to reach a positive energy balance and time for their uteri to properly involute. In addition to days in milk, milking frequency may also affect fertility in lactating dairy cows. A 15-16 % increase in milk production can be expected when milking frequency is increased. It has been documented that there are longer calving intervals and more services per pregnancy in cows milked 3 times per day (3X) than 2X (Smith et al., 2002).

Summary

Management practices that emphasize genetic selection for healthy efficient cows with good locomotion and body condition score may enhance reproductive efficiency. Retrograde sperm loss and phagocytosis are responsible for significant amounts of sperm lost from the reproductive tract following AI. Increased sperm loss may mean lowered numbers of sperm at the site of fertilization and thus lowered fertility. This may be confounded by age of cow. Older cows experience more reproductive disorders and lower fertility. Monitoring and managing mastitis, heat stress, and postpartum uterine

health may be beneficial to lessen the effects of parity, retrograde sperm loss and phagocytosis on fertility in the lactating dairy cow. The relationship of increased milk production and use of bST on the decline in fertility remains unclear.

Chapter 2

PAPER TO BE SUBMITTED TO JOURNAL OF DAIRY SCIENCE

Effect of Timing of Artificial Insemination and Placement of Semen on Fertility in Lactating Dairy Cows and Gender Ratio of Resulting Offspring

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MATERIALS AND METHODS

This study was conducted on two dairy farms in Michigan and one in Kansas. Primiparous and multiparous lactating dairy cows (n = 1603) were randomly assigned to one of four treatments based on lactation number (1, 2, ≥ 3) and DIM (< 150 and ≥ 150 DIM). Treatments were arranged as a 2 x 2 factorial experiment as illustrated in Table 1. All farms maintained cows in free-stall barns with sand bedding and headlocks. Research began March 2002 at Farm 1 (n = 133) located in mid Michigan (700 lactating cows) with a rolling herd average of 11,298 kg and ended May 2002. Cows were milked

Table 1. Sample size for each of the four treatment groups.

	Time of insemination relative to the final GnRH of Ovsynch	
Site of Insemination	-8 h	16 h
Body	Group 1 (n=400)	Group 2 (n=417)
Horn	Group 3 (n=372)	Group 4 (n=414)

twice daily for the majority of the herd and four times per day for cows early in lactation (70 to 80 DIM for 2-yr olds and 50 to 60 DIM for mature cows). Cows were fed a corn and alfalfa silage based ration balanced for milk production. Research was conducted on Farm 2 (n = 360) between May and June 2002. Farm 2 was located in southwestern Kansas (7000 lactating cows) and had a rolling herd average of 9798 kg. Cows were milked twice or thrice daily depending on production and DIM. Cows were fed primarily

corn silage, alfalfa hay and corn diet balanced for milk production. Research was conducted at Farm 3 (n=1110) between October 2002 and April 2003. Farm 3 was located in southern Michigan (2500 lactating cows) and had a rolling herd average of 10,886 kg. Cows were milked three times daily and were fed a corn silage and haylage based ration balanced for milk production.

Cows received the following estrous synchronization protocol: Pre Synch (two injections of $PGF_{2\alpha}$ 14 days apart) followed on d 14 by the Ovsynch program. The Ovsynch program used in this study consisted of an intramuscular injection of 100 µg gonadotropin releasing hormone (GnRH; Cystorelin ®, Merial Ltd., Duluth, GI.) followed in 7d with 25 mg $PGF_{2\alpha}$ (Lutalyse ®; Pharmacia-Upjohn Co., Kalamazoo, MI) then, 40-48 h later, with 100 µg of GnRH (Figure 1). Insemination took place at either -8 or 16 h after the second injection of GnRH. Farm personnel administered all injections except the final GnRH, which investigators administered to assure precision and timing.

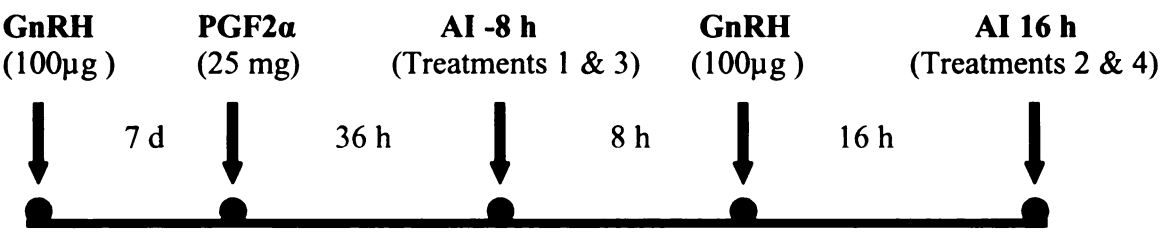

Figure 2. The timing for each injection used in the Ovsynch protocol and timing of AI for all cows on study.

One AI technician performed insemination for all cows, either within the uterine body or deep in the uterine horn ipsilateral to the ovary with the ovulatory follicle. The technician was trained for unilateral horn insemination. Beal et al. (1991) established the training protocol we used. The AI technician used an insemination gun with a metal

swivel attached to the end. The technician deposited the metal swivel into the uterine horn. With ultrasonography, proper placement was verified. This technique was effective in determining competency of the inseminator for proper placement of semen deep in the uterine horn. Proper uterine horn deposition was achieved when resistance was felt after slight straightening or manipulation of the horn.

Only cows responding to Ovsynch with a synchronized ovulation were included in the study. An Aloka 500V ultrasound machine with a 7.5 MHz linear array transducer probe (Corometrics Medical Systems Inc., Wallingford, CT) was used to evaluate ovaries and diagnose pregnancy. Ovarian structures were mapped using ultrasound immediately before insemination to determine size of the ovulatory follicle and presence of corpora lutea (CL). This information was used to establish which horn would be ipsilateral to the ovulatory follicle. Once the horn ipsilateral to the ovulatory follicle was determined, one straw of semen was placed either in the ipsilateral horn or uterine body depending on treatment. Two days after AI, cows were re-examined by ultrasound to determine a synchronized ovulation. A cow was considered synchronized if the ovulatory follicles (from the previous scan) had disappeared or a new CL was present. The scan was also used to confirm proper semen placement in the horn ipsilateral to the ovulatory follicle for cows inseminated in the uterine horn. Pregnancy diagnoses were determined between 28 to 33 d (hereafter referred to as 28 d) post- insemination for all treatment groups. Observation of a fetal heartbeat was required before pregnancy was confirmed and recorded. A second diagnosis was performed at 56 to 60 d (hereafter referred to as d 56) post insemination for cows previously confirmed pregnant at d 28.

The total number of cows inseminated in this study was 2347, but only 1603 cows

were included in the analyses. The synchronization rate for all cows was approximately 83%. Cows were excluded (n = 744) from the analysis if ovulation was not observed, if cows were bred < 5 days following experimental insemination, or the AI technician could not deposit semen in the designated location because of an impassible tract. Cows culled before both pregnancy diagnoses were also excluded.

Service sires for Farm 1 and Farm 2 were selected by farm management. However, on Farm 3 service sires were selected based on ERCR values and evaluated for fertility. Three bulls were selected that had +4 ERCR and two with -4. All bulls had a repeatability > 95 % from Select Sires, American Breeders Service (ABS), or CRI Genex Cooperative, Inc. Semen from each of the five sires was evaluated for concentration and motility post-thaw. We randomly selected 5 straws (0.05 ml) of semen to be evaluated from each of the bulls. Semen was diluted (1: 10) with modified Tyrode's (mTALP) media to maintain motility (Liu and Foote, 1998). Immediately after semen was diluted, a drop from the semen sample was placed on a slide and covered with a cover slip. The percentage of sperm moving progressively forward was estimated at 400x under a microscope. Concentration of sperm was determined by haemocytometry.

Semen from the five service sires were randomly allocated to cows in each of the four treatment groups by parity and days in milk. AI technician and ultrasound operator at pregnancy diagnosis were blind to bull selection and treatment.

Management factors that could affect fertility in the lactating dairy cows were also evaluated. These factors included lactation information, uterine health information, bST status, SCC near the time of AI, parturition data, BCS, and locomotion scores. For all farms, cows that failed to expel placental membranes within 24 h after calving were

diagnosed as a RP. Each of the three farms had a different regime for bST administration. Farm 1 started cows on bST at 63 DIM if cows exhibited a BCS of at least 2.3. Farm 2 started cows on bST 70 DIM and Farm 3 started cows at 65 DIM. Cows were either recorded as receiving bST at the time of insemination or not receiving bST; actual injection dates of bST were not recorded. Approximately 94 % of the cows on the study received bST by the time of AI.

Researchers scored cows at the time of the second ultrasound observation (2 d following inseminations) for both locomotion and body condition. Locomotion scores were based on a 1 to 5 scale adapted from Sprecher et al. (1996). A score of 1 is a normal cow that stands with a level top line and walks with a level top line. A score of 2 is a mildly lame cow that stands with a level top line but walks with an arched back. A score of 3 is a moderately lame cow that stands with an arched back and walks with an arched back. A score of 4 is a lame cow that stands and walks with an arched back and has a noticeable limp. A score of 5 is a severely lame cow that has an arched back, does not bear any weight on one leg and may have difficulty getting up from the lying down position.

BCS was evaluated on a five point scale modified from (Wildman et al., 1982). A cow with a score of 1 is emaciated with vertebrae prominent and hooks and pin bones sharply defined. The thurl region and thighs are sunken and in curving. The tail head depression is severely receded and the vulva appears prominent. A cow with a BCS score of 2 is thin, with less prominent vertebrae. The hook and pin bones are prominent but the depression of the thurl region between them is less severe than a cow with a BCS of 1. The area around the tail head is also less sunken and the vulva less prominent. A

cow with a BCS score of 3 is in average body condition. The backbone is a rounded ridge and hook and pin bones are round and smoothed over. The tail head area is filled out but there is no evidence of fat deposits. A cow with a score of 4 is in heavy condition. The ridge of the backbone is flattening over the loin and rump areas and rounded over the chine. The hook bones are smoothed over and the span between the hook bones over the backbone is flat. The area around the pin bones shows patches of fat deposits. A cow with a BCS score of 5 is extremely heavy conditioned. The bone structure of the top line, hook and pin bones and the short ribs are not visible. Fat deposits around the tailbone and over the ribs are obvious. The thighs curve out; the brisket and flanks are heavy and the chine very round.

Cow information such as parity, number of AI services (all services for current lactation, including experimental insemination), milking frequency, milk yield (nearest the time of insemination), SCC nearest the time of insemination (SCC and milk measurements taken every 2 weeks) and whether cows had received bST around at the time of breeding were retrieved from farm computer records. Somatic cells were recorded as a count on two of the farms record systems. Counts were converted to somatic cell score (SCS) using the following formula: $[\log_2 (SCC/100,000) +3]$. Cows with previous calving information such as RP or twins were recorded. Additionally, calving difficulty score (based on PCDART Protocol 1-5) for the previous calving was collected.

Statistical Analyses

Independent analyses were considered for the three response variables (pregnancy

diagnosis at 28 d, pregnancy diagnosis at 56 d, and pregnancy loss), using a generalized linear model technique (McCullagh and Nelder, 1989). The analyses were performed using the procedure GENMOD of the SAS System (SAS, 1999), with a binomial distribution and the canonical link function. Pregnancy rate per AI (PR/AI) is the number of cows diagnosed pregnant divided by the total number of cows AI. The difference in pregnancy rate between 28 and 56 d post insemination provided early pregnancy loss information. Preliminary analyses were performed including each management factor separately in addition to treatment effects (time, site of insemination, time x site of insemination). The final analysis started with a full model including all factors and first order interactions. Non-significant interactions were then removed from the model. Continuous data such as milk production, DIM, follicle size, CL size, and SCS were grouped into classes based on either a statistical distribution or biological factors established from known information or previous research practices. Some analyses were performed on selected cows. The effect of follicle size at the time of insemination on pregnancy outcome is one example of selection. We used only single ovulating cows because cows with multiple ovulations were found to have an advantage in fertility and would have introduced bias. When evaluating milk production and parity, we selected only first service cows 60 to 90 DIM. This was done to form a consistent set of cows to test the actual effect of milk production and parity on cows at the same stage of lactation with the same reproductive status. Finally, using a similar approach, the five selected sires were analyzed for PR/AI differences for time and site of insemination in cows at Farm 3. Tables were constructed for service sire x site and time of insemination to illustrate additional fertility information for each of the five sires. In addition to the field

fertility data, we also compared 5 straws of semen from each of the five bulls using the non-parametric ANOVA technique of Kruskal-Wallis (Zar, 1999), for concentration and motility differences.

A preliminary analysis of the effect of timing of AI on gender ratio was performed by comparing percentage of female calves using the chi-square goodness-of-fit test to an expected population value of 45.8% females and 54.2 % males (Ryan and Boland, 1991). A final analysis will be completed once all calves for this study are born.

RESULTS

PR/AI for cows inseminated at all farms and all treatments combined at 28 and 56 d were 34.3 and 28.0 %, respectively (Table 2). The percent of embryo survival between 28 and 56 d after insemination was 81.9%. Interactions of farm x time of insemination and farm x site of insemination were not significant. Therefore, to evaluate the effect of farm, all treatments were combined. There was however, an effect of farm, with Farm 1 having the highest fertility ($P = 0.04$) at 28 d post insemination across treatments.

Pregnancy rate following insemination 8 h before or 16 h after the final injection of GnRH of the Ovsynch with semen deposition occurring in either the uterine body deep uterine horn is depicted in Table 3. The 16 h treatment groups had a greater percentage of cows pregnant than the -8 group at 28 d ($P < 0.01$) and 56 d ($P < 0.01$) following AI. There was less pregnancy loss in cows inseminated in the uterine body ($P < 0.05$) than the uterine horn.

Compared with an expected gender ratio of 46: 54 (female: male) ratio based on (Ryan and Boland, 1991) there were more ($P = 0.01$) female than male calves born after AI at -8 h than would be expected (Table 4). Gender ratio in the 16 h group did not significantly differ when compared with the expected ratio.

At Farm 3 there was a significant difference in fertility among the five service sires at 28 and 56 d ($P < 0.01$) following AI at -8 or 16 h (Table 5). However, there was no interaction of sire x time of insemination. Table 6 shows the difference in fertility or service sires based on site of insemination. Again, the percent cows pregnant at diagnosis differed among sires ($P < 0.01$). No interaction of sire x site of insemination for the five

sires at 28 and 56 d following AI was found. Finally, a comparison post-thaw semen analysis is depicted in Table 7. It appears that the 1 and 5 bulls have the lowest motility and the highest live concentration of sperm per straw. There was not significant variation between the five straws of semen evaluated for each bull.

PR/AI by parity for first service cows 60 to 90 DIM for either above or below mean milk production (40.4 kg/d) is depicted in Table 8. There was a trend for cows with milk production greater than the mean to have higher fertility at 28 ($P = 0.07$) and 56 d ($P = 0.09$) following AI than cows with below average milk production. The effect of parity on pregnancy rate at 28 and 56 d is depicted in Table 9. Parity for all cows regardless of stage of lactation or number of services had no significant difference in PR/AI at 28 or 56 d following AI.

PR/AI for single ovulating cows with follicles larger than 12 mm at the time of AI were greater at 28 d ($P = 0.01$) and 56 d ($P = 0.02$) compared to cows with < 12 mm follicles (Table 10). The effect of multiple ovulations per cow on PR/AI at 28 and 56 d is depicted in Table 11. PR/AI for cows with multiple ovulations were greater at 56 d following AI ($P = 0.02$) than single ovulating cows. There was a trend for greater fertility in multiple ovulating cows at 28 d post insemination ($P = 0.07$) and less embryonic loss ($P = 0.08$) than for single ovulating cows.

Table 12 depicts the relationship between CL size at the time of insemination and 28 d and 56 d PR/AI and pregnancy loss. Larger CL ($\geq$ 17 mm) at the time of insemination decreased the percent of cows pregnant at 56 d and increased pregnancy loss (Table 12). Additionally, PR/AI in cows with 3+ locomotion score were greater at 28 d following uterine body ($P = 0.05$) than uterine horn AI (Table 13).

PR/AI for all treatments combined by number of AI services for current lactation is depicted in Table 14. There was a clear trend for first service cows to have higher PR/AI than cows with multiple services, but data were not significant at ($P < 0.05$).

SCS near the time of AI did not affect the percent of cows pregnant at 28 or 56 d following AI (Table 15). There were no interactions for SCS x time or site of insemination. In addition, PR/AI and pregnancy loss between 28 d and 56 d was not different for cows in different stages of lactation (Table 17).

There was no effect of calving difficulty score from the previous calving on PR/AI (Table 16). Cows with or without prior calving difficulty had similar fertility. Additionally, the effect of BCS on percent pregnancy for all treatments combined is presented in Table 18. Cows with > 3.46 BCS tended to have higher pregnancy rate at 56 d and less pregnancy loss then cows with lower BCS.

Table 2. The effect of farm on percent of lactating dairy cows pregnant at 28 and 56 d following AI. [a]

	Farm 1	Farm 2	Farm 3	Total PR/AI	P-value [b]
n	133	360	1110		
28 d	44.1	33.8	33.5	34.3	0.04
56 d	34.8	26.4	27.9	28.0	0.11
Loss [c]	20.3	22.1	16.3	18.1	0.22

[a] There were no interactions for farm x site of insemination or farm x time, therefore all treatments were combined.

[b] P-value indicates differences between farms

[c] Percent pregnancy loss between 28 and 56 d.

Table 3. Effect of timing of AI relative to the final GnRH injection of Ovsynch and site of insemination on percent lactating dairy cows pregnant at 28 and 56 d.

Time of insemination	Site of insemination	n	28 d	56 d	loss [a]
-8 h	Body	400	30.4	25.7	15.6
	Horn	372	28.6	21.5	24.5
Total PR/AI -8 h			*29.3* *	*23.4* *	*20.0*
16 h	Body	417	40.4	34.3	14.9
	Horn	414	37.7	30.4	18.8
Total PR/AI 16 h			*39.0*	*32.3*	*16.8*
	Total PR/AI for body		*35.4*	*29.9*	*15.3* **
	Total PR/AI for horn		*33.2*	*26.0*	*21.3*

[a] Percent pregnancy loss between 28 and 56 d.

*Less than value at 16 h ($P < 0.01$)
**Less than value for horn insemination ($P < 0.05$)

Table 4. Percent female and male offspring born to lactating dairy cows at Farm 1 and 2, inseminated at -8 h or 16 h relative to final GnRH injection of Ovsynch.

Time of Insemination	Gender Ratio Female : Male	n	P-value [a]
-8 h	65.2 : 34.8	46	0.01
16 h	56.1 : 43.9	66	0.09
n	67 45	112	

[a] P-value indicates difference between -8 and 16 h to the expected female: male gender ratio (45.8: 54.2) obtained from Ryan and Boland (1991).

[b] There was no effect of site of insemenation on gender ratio, therefore all treatments were combined.

Table 5. Effect of service sire and timing of AI relative to final GnRH injection of Ovsynch on percent lactating dairy cows pregnant at 28 and 56 d following AI at Farm 3.

		28 d		56 d		Loss [a]	
Sire	**ERCR**	**-8 (n)**	**16 (n)**	**-8 (n)**	**16 (n)**	**-8**	**16**
1	**-4**	17.7 (68)	29.6 (98)	10.3 (68)	24.7 (97)	41.7	14.3
2	**-4**	34.1 (88)	46.8 (94)	27.3 (88)	42.6 (94)	20.0	9.1
3	**+4**	45.3 (95)	44.0 (116)	41.1 (95)	43.1 (116)	9.3	2.0
4	**+4**	35.4 (82)	38.5 (91)	26.8 (82)	31.9 (91)	24.1	17.1
5	**+4**	24.1 (87)	33.0 (88)	20.7 (87)	28.7 (87)	14.3	10.7
P-value [b]		<0.01		<0.01		0.01	
Interaction P-value [c]		0.44		0.33		0.66	

[a] Percent of pregnancy loss between 28 and 56 d

[b] P-value for difference among sires within -8 and 16 h and for the average of the two

[c] P-value for the interaction of sire x time of insemination

Table 6. Effects of service sire and site of semen deposition, either uterine body or deep uterine horn, on percent lactating dairy cows pregnant at 28 and 56 d following AI on farm 3.

		28 d		56 d		Loss [a]	
Sire	**ERCR**	**Body (n)**	**Horn (n)**	**Body (n)**	**Horn (n)**	**Body**	**Horn**
1	**-4**	26.8 (82)	22.6 (84)	22.2 (81)	15.5 (84)	14.3	31.6
2	**-4**	38.8 (85)	42.3 (97)	34.1 (85)	36.1 (97)	12.1	14.6
3	**+4**	42.5 (106)	46.7 (105)	40.6 (106)	43.8 (105)	4.4	6.1
4	**+4**	39.6 (91)	34.2 (82)	30.8 (91)	28.1 (82)	22.2	17.9
5	**+4**	26.7 (86)	30.3 (89)	25.6 (86)	23.9 (88)	4.4	19.2
P-value [b]		<0.01		<0.01		0.01	
Interaction P-value [c]		0.78		0.76		0.36	

[a] Percent pregnancy loss between 28 and 56 d

[b] P-value for difference among sires within -8 and 16 h and for the average of the two

[c] P-value for the interaction of sire x time of insemination

Table 7. Semen evaluation (for concentration and motility) for each of the five bulls used to AI cows at Farm 3.

Bull [a]	ERCR	Live sperm per straw	Percent motility	Total live motile sperm per straw
1	- 4	7,450,000 ± 539,965	0.49 ± 0.01	3,633,750 ± 207,379
2	- 4	4,000,000 ± 410,791	0.75 ± 0.02	3,000,000 ± 316,653
3	+ 4	4,525,000 ± 632,949	0.67 ± 0.03	2,978,750 ± 344,791
4	+ 4	4,150,000 ± 449,652	0.69 ± 0.02	2,840,000 ± 265,066
5	+ 4	8,825,000 ± 979,756	0.56 ± 0.02	4,883,125 ± 421,515
P-value		< 0.01	< 0.01	0.02

[a] Each estimate for semen evaluated per bull was based on (n = 5) randomly selected 0.05 cc. straws of semen.

Table 8. Effect of milk production (above or below the mean) for 1st service dairy cows 60-90 d in lactation on percent pregnant at 28 and 56 d following AI. [a]

	Milk Production (Mean=40.4 kg)					
	Above	Below	Above	Below	Above	Below
Parity	**28 d (n)**		**56 d (n)**		**Loss** [b]	
1	31.8 (151)	35.4 (178)	26.7 (150)	28.7 (178)	14.9	19.1
2	41.3 (63)	26.1 (46)	31.8 (63)	22.2 (45)	23.1	9.1
3+	51.1 (92)	39.7 (73)	45.1 (91)	31.5 (73)	10.9	20.7
P-value	0.07		0.09		0.99	

[a] There were no interactions for milk production and parity by treatment effects, so all treatment groups were combined.

[b] Percent pregnancy loss between 28 and 56 d

Table 9. Effect of parity on percent lactating dairy cows pregnant at 28 and 56 d following AI.

	Parity			
	1	**2**	**3+**	**P-value**
28 d	34.2	31.8	36.9	0.30
56 d	28.6	24.4	29.7	0.26
Loss [a]	16.4	22.8	18.8	0.42
n	916	321	366	

[a] Percent pregnancy loss between 28 and 56 d

Table 10. Effect of follicle size at time of insemination on percent single ovulating lactating dairy cows pregnant at 28 and 56 d following AI. [a]

	Follicle size (mm)		
	≤12	>12	P-value
28 d	27.2	35.6	0.01
56 d	21.5	28.7	0.02
Loss [a]	20.9	18.7	0.90
n	480	944	

[a] Percent pregnancy loss between 28 and 56 d

Table 11. Effect of multiple ovulations on percent lactating dairy cows pregnant at 28 and 56 d following AI. [a]

Multiple Ovulations	n	28 d	56 d	Loss [b]
Yes	179	40.4	36.1	10.8
No	1424	33.7	27.1	19.1
P-value		0.07	0.02	0.08

[a] There were no interactions for multiple ovulations x time or site of inseminantion, so all treatments were combined.

[b] Percent pregnancy loss between 28 and 56 d.

Table 12. Effect of corpus luteum (CL) size at time of insemination on percent lactating dairy cows pregnant at 28 and 56 d following AI.[a]

	CL Size (mm)					
	< 11	**11-13.9**	**14-15.9**	**16-16.9**	**> 17**	**P-value**
28 d	35.7	31.8	36.1	31.9	22.5	0.13
56 d	29.6	27.3	29.7	23.5	12.4	0.01
loss [b]	17.1	12.8	16.7	23.8	45.0	0.05
n	1175	151	119	69	89	

[a] There was no interaction for CL size x time or site of insemination, therefore all treatments were combined.

[b] Percent pregnancy loss between 28 and 56 d.

Table 13. Percent lactating dairy cows pregnant at 28 and 56 d following AI by site of insemination and scored for locomotion. [a]

Variable	Site of insemination	Locomotion Score			Interaction P-value
		1 (n)	2 (n)	3+ (n)	
28 d	Body	34.3 (560)	33.6 (155)	44.1 (84)	0.05
	Horn	33.0 (533)	35.8 (162)	26.7 (75)	
56 d	Body	29.3	27.1	36.2	0.14
	Horn	25.9	26.9	22.7	
loss [b]	Body	14.6	19.2	16.7	0.69
	Horn	21.1	23.2	15.0	

[a] There was no interaction for time x locomotion score ($p > 0.05$)

[b] Percent pregnancy loss between 28 and 56 d

Table 14. Effect of number of services on percent lactating dairy cows pregnant at 28 and 56 d following AI.[a]

	Number of AI services			
	1	**2**	**≥ 3**	**P-value**
28 d	37.2	31.7	32.6	0.08
56 d	30.6	25.4	26.5	0.09
Loss [b]	17.0	19.8	18.7	0.76
n	672	382	546	

[a] There were no interactions of AI services x time or site of insemination, so all treatments were combined.

[b] Percent pregnancy loss between 28 and 56 d.

**AI Services include experimental insemination in addition to previous services within current lactation.

Table 15. Effect of somatic cell score (SCS) near the time of insemination on percent lactating dairy cows pregnant at 28 and 56 d following AI.[a b]

	SCS				
	< .6	**.6 - 1.7**	**1.7 - 3.3**	**> 3.3**	**P-value**
28 d	34.7	32.9	32.1	37.5	0.38
56 d	27.8	27.7	26.9	29.6	0.86
loss [c]	19.3	15.8	16.3	20.7	0.59
n	409	389	402	403	

[a] The somatic cell score was recorded based on the test day that was nearest day of insemination.

[b] There were no interactions for SCS x time or site of insemination ($P < 0.05$), therefore all treatments were combined.

[c] Percent pregnancy loss between 28 and 56 d.

Table 16. Effect of previous calving difficulty score on percent lactating dairy cows pregnant at 28 and 56 d following AI. [a]

	Calving Difficulty Score		
	0 - 1	**> 1**	**P-value**
28 d	36.4	38.4	0.76
56 d	28.2	31.3	0.59
loss [a]	22.4	18.4	0.48
n	396	95	

[a] Percent pregnany loss between 28 and 56 d.

Table 17. Effect of days in milk (DIM) on percent lactating dairy cows pregnant at 28 and 56 d following AI. [a]

	DIM			
	< 75	**75 - 150**	**> 150**	**P-value**
28 d	35.8	34.4	33.5	0.63
56 d	29.1	28.4	26.7	0.54
loss [b]	18.6	17.0	20.0	0.66
n	316	912	375	

[a] There were no interactions for DIM x time or site of insemination, therefore treatments were combined.

[b] Percent pregnancy loss between 28 and 56 d

Table 18. Effect of body condition score (BCS) on percent lactating dairy cows pregnant at 28 and 56 d following AI. [a]

	BCS				
	1.0 - 2.35	**2.36 - 3.45**	**3.46 - 4.0**	**> 4.0**	**P-value**
28 d	30.3	33.2	37.2	31.3	0.25
56 d	23.2	25.7	31.5	28.1	0.07
Loss [a]	21.9	22.2	15.1	10.0	0.08
n	109	754	592	128	

[a] Percent pregnancy loss between 28 and 56 d

DISCUSSION

This study was designed to determine the effect of prolonged subsistence of sperm in the female reproductive tract prior to ovulation in lactating dairy cows, with two different sites of AI, on PR/AI and resulting gender ratio. Precise synchronization of ovulation (Ovsynch) allowed for the opportunity to test timing of AI in relation to ovulation. In a previous study (Pursley et al., 1995), Ovsynch synchronized ovulation 24 to 32 h following the final GnRH induced LH surge. Previous studied demonstrated that AI at the time of the final GnRH of Ovsynch (~ 28 h prior to ovulation) decreased the PR/AI but increased the number of female calves born. In the current study time of AI prior to ovulation to ~ 36 h (-8 group) was extended to determine if female to male ratio could be improved from previous work and if fertility could be maintained by deep uterine horn AI. Controls received AI 16 h following the GnRH induced LH surge (16 h group; ~ 12 h prior to ovulation). This time was previously proven to be the ideal time of AI prior to ovulation (Pursley et al., 1998). Since there was no effect of site of deposition, we will discuss the effects of timing of AI with the two sites of AI combined within each group.

Effect of Extending Sperm Subsistence in the Uterus / Oviduct Prior to Ovulation

Our results indicated that the female: male ratio was substantially shifted in favor of females born from cows in the -8 h group. Our results, 65 % females, differed from the expected value of 46 % females reported by Ryan and Boland (1991). These results are consistent with data from Pursley et al. (1998) that reported more heifers when AI

occurred ~28 h prior to ovulation. There are phenotypic differences between X- and Y-bearing sperm including plasma membrane surface charge, density, morphology (nucleus and head), and motility, and the degree of motility measured in effective velocity was greater in X-bearing sperm than in Y-bearing sperm (Johnson, 1995; Windsor et al., 1993). These differences may allow the X-chromosome-bearing sperm to have greater longevity than the Y-chromosome-bearing sperm. Thus, extending the time sperm are present in the uterus before ovulation may result in selected death of the Y-chromosome bearing sperm. However, it appears that the potential deviation in X vs. Y sperm takes a minimum amount of time. Prolonged pre-incubation of sperm for 20 h prior to in vitro fertilization increased the number of female hatched blastocysts (Lechniak et al., 2003). Substantial differences in sex ratio with regard to the relationship of time of insemination or intercourse and ovulation have been reported in other species (Guerrero, 1974; Hart and Moody, 1949; Hedricks and McClintock, 1990; Pratt et al., 1987; Verme and Ozoga, 1981). From these studies along with the current study, it appears that insemination around $\geq$ 1 d prior to ovulation results in more female offspring compared with a preponderance of males when insemination occurs around the time of ovulation. Some previous studies in cattle (Ballinger, 1970; Foote, 1977; France et al., 1984; Rorie et al., 1999) indicated that there is no relationship between time of AI and sex ratio. However, the design of the current study allowed for a more accurate prediction of time of ovulation, therefore extending the time that sperm were forced to subsist from AI to ovulation. The current study also had greater numbers of births to evaluate gender ratio in comparison to previous studies in cattle.

It is not clear from results to date if semen from bulls with a significant difference in fertility will perform similarly with regard to female: male ratio when inseminated into cows at various times from ovulation. Calving data from the 5 bulls on Farm 3 will be analyzed separately and compared to the expected gender ratio for dairy cows once all calves are born.

When sperm resided in the reproductive tract for ~36 h before fertilization, there was only a 9 % decrease in PR/AI at 56 d post-AI compared to controls. This decrease in PR/AI was not as dramatic as expected, based on previous findings that sperm may only survive in the female reproductive tract for approximately 30 h (Laing 1945). However, more recent studies suggested that frozen/thawed sperm may live up to 48 h following AI (Bazer et al., 1987). It is not clear if fertilization rates were similar between -8 and 16 groups and more embryonic losses occurred from fertilization to the 28 d pregnancy diagnosis, or if the difference in fertility was due to differences in fertilization rates. However, when Lechniak et al. (2003) incubated sperm for 20 h prior to in vitro fertilization there were reduced development and cleavage rates compared to no pre-incubation.

Pregnancy loss was similar between the -8 h and 16 h groups. Pursley et al.(1998) found fewer pregnancy losses when cows were inseminated at the time of GnRH injection. The 18 % pregnancy loss between 28 and 56 d post-AI found in this study appears to be similar to other studies (Pursley et al., 1998; Smith and Stevenson, 1995) when ultrasound was used to detect pregnancy at 28 d after AI. Vasconcelos et al. (1997) found an 11 % loss between 28 and 42 d and an additional 6 % loss between 42 and 56 d after AI.

It is well documented that service sires differ in their ability to fertilize oocytes in vitro (Marquant-Le Guienne et al., 1990; Ward et al., 2001b). Highly fertile beef bulls can be identified by the fertility associated antigen (FAA). Approximately, 60 % of beef bulls screened tested positive for this antigen. In a field study by Sprott et al. (2000), bulls that tested positive for FAA had increased PR/AI compared to bulls that tested negative for the antigen (68 vs. 47 %). However, nearly all dairy bulls thus far screened have tested positive for this antigen (Dr. Roy Ax, personal communication). To our knowledge, the current trial is the first in vivo study that compares service sire fertility following prolonged subsistence of sperm in the female reproductive tract for ~ 36 h prior to ovulation. We used the ERCR as a guide to select high and low fertility bulls. The ERCR is based on 70 d non-return rate, which is currently the best estimate of fertility available for selection of dairy bulls. However, it is not clear how accurately ERCR accounts for fertility in dairy bulls. Therefore, we did not base our analysis on the ERCR but rather on the individual bull.

The two insemination times chosen for the current study (-8 and 16 h) provide a novel way to evaluate bull fertility when sperm are inseminated in the female reproductive tract for an extended period of time prior to ovulation. Bull 3, the bull with the greatest PR/AI for this trial, had similar PR/AI at -8 compared to the 16 h treatment. In addition, this bull had fewer pregnancy losses between 28 and 56 d following AI. In contrast, Bull 1 had the lowest PR/AI, the greatest abatement in PR/AI in the -8 group, and the greatest pregnancy loss between 28 and 56 d post-AI. Other investigators (Callaghan and King, 1980; Nadir et al., 1992) also demonstrated a significant deviation in high vs. low fertility bulls. Ward et al. (2001a) studied the effect of bull fertility on the

kinetics of early embryonic development and found that embryos sired by high fertility bulls had a faster rate of early embryonic development.

The difference in performance amongst bulls in our study is probably not attributed to concentrations of live motile sperm inseminated. Numbers of live motile sperm were similar amongst Bulls 2, 3 and 4, and greater in Bull 1 and 5. The only sperm characteristic we evaluated that appeared to be associated with fertility was percent motility. The two bulls with the lowest motility, Bull 1 and 5, also were the lowest fertility bulls.

Perhaps the most interesting aspect of the bull fertility data is the effect of bull on pregnancy loss between 28 and 56 d post-AI. It was clear that the highest fertility bull had the fewest pregnancy losses and the lowest fertility bull had the greatest losses. This is the first study that we are aware of that associates pregnancy loss subsequent to AI to the fertility outcomes of the service sire. From these data it appears that pregnancy loss may be partially predetermined at the time of fertilization.

Effect of Site of Semen Deposition on Fertility

It appears that improving percentage of female offspring by timing AI ~36 h before ovulation may come with a price. We hypothesized that deep uterine horn insemination would maintain PR/AI in lactating dairy cows inseminated in the -8 h group compared to the 16 hour group. However, there was no difference in PR/AI in uterine body vs. deep uterine horn inseminated cows. In four unpublished studies from our lab, we have detected a significant increase in fertility using deep uterine horn AI. In two of those studies, we detected an interaction of site of AI and AI technician. Unfortunately,

differences in AI techniques between these technicians have not been identified. The current study used one AI technician for all cows. This technician was trained for unilateral horn insemination prior to the study using a novel training technique that Beal et al. (1991) established using ultrasound. To minimize variability and strengthen our analysis we decided to use only one AI technician. This was an advantage when evaluating sire fertility. One technician reduces the variability, allowing the focus to be on the effect of the sire. However, a disadvantage is that we were unable to determine whether inseminator technique affected fertility based on site of semen deposition.

The proposed advantage of uterine horn insemination (bicornual or unicornual) when compared with body insemination is a greater likelihood of deposition of semen nearer the uterotubal junction and reduced incidence of cervical deposition (Lopez-Gatius, 2000). Many investigators have studied bicornual insemination, depositing half the semen in one uterine horn and half in the contralateral horn. However, varying results have been noted (Gallagher and Senger, 1989; Graves et al., 1991; Knight et al., 1951; McKenna et al., 1990; Senger et al., 1988; Williams et al., 1988). Senger et al. (1988) showed a dramatic increase in CR using deep bicornual insemination. CR were improved from 45% to 70% in 4000 cows. In the current study we used unicornual insemination. Similar to Zavos et al. (1985), we administered a full dose of semen deep into the uterine horn ipsilateral to the side of impending ovulation. Uterine horn insemination did not increase fertility when compared to uterine body insemination. This is in conflict with other unilateral insemination data previously reported (Lopez-Gatius, 1996; Lopez-Gatius and Camon-Urgel, 1988; Zavos et al., 1985) that showed marked improvement in fertility. Additionally, we observed significantly higher pregnancy loss

between 28 and 56 d post insemination for cows inseminated unilaterally in the uterine horn.

The Effect of Other Physiological and Management Factors on Fertility

Reproductive performance is an intricate part of a successful dairy management program. Reproductive performance is determined by the relationship of management, environment and physiological factors. In this study we also examined the relationship between some specific physiological and management factors and reproductive performance.

Parity is a physiological factor that affects fertility. Hillers et al. (1984) found that cows in third and greater lactations had lower CR and longer intervals to first service than first and second lactation cows. However, we did not find a difference in fertility among 1, 2 or >3 parity cows.

Multiple ovulations are also associated with parity and high production of milk in lactating dairy cows (Fricke and Wiltbank, 1999). However, our results did not indicate a higher likelihood of multiple ovulations in high producing or older cows. We did discover that cows with multiple ovulations (12.6 % of all cows) had a higher pregnancy rate at 56 d with a trend for less pregnancy loss. Although we do not have information on number of CL at 56 d post AI, multiple CL present on the ovary may be an explanation for lower pregnancy loss. Lopez-Gatius et al. (2002) found that cows carrying single calves with two CL were less likely to lose the pregnancy.

Along with parity, we also evaluated the relationship between stage of stage of lactation and fertility. Previous studies reported a higher risk of pregnancy failure for

cows that conceived in early and in late lactation (Loeffler et al., 1999). However, in the current study with a large sample size, we saw no difference in PR/AI for cows in different stages of lactation.

Suckled beef cows with follicles > 12 mm on d 2 following insemination had greater PR than those with follicles ≤ 12 mm (Lamb et al., 2001). Our results indicate a similar relationship between follicle size and fertility. Single ovulating cows with follicles > 12 mm at the time of insemination had a greater PR/AI at 28 and 56 d post insemination than cows with ≤ 12 mm follicles. A by-product of the Ovsynch program may be the synchronization and ovulation of smaller less competent oocytes. This may explain why cows with < 20 mm follicles have reduced fertility.

We are unaware of any data on CL size at the time of insemination and PR/AI and pregnancy loss in lactating dairy cows. Cows in current trial with a CL > 17 mm at the time of insemination had the lowest PR/AI. Perhaps, low fertility in cows with a large CL may be an indication of poor synchronization. Unfortunately, we did not collect CL regression information. Nonetheless, it is difficult to understand why some cows with a large CL at insemination conceived but consequently had an increased chance of pregnancy loss. The cause of this is unclear, but it may potentially be attributed to hormone levels at the time of fertilization and subsequent effects on development.

There was also a trend for first AI service cows to have a slightly higher percent pregnancy at 28 and 56 d post insemination. This could be attributed to cows that have fewer reproductive disorders or responded better to the Ovsynch program.

Novel information on potential management factor effects on fertility can be gained from the current study. One management factor that could directly affect fertility

is cow lameness. Locomotion scoring provides producers with a way to assess the amount of lameness in their herd. Previous studies indicate lameness can have a detrimental affect on fertility (Hernandez et al., 2001; Melendez et al., 2003). Our data indicated an unexpected interaction between locomotion score x site of insemination. Cows with >3 locomotion scores inseminated in the uterine horn had the lowest fertility. However, over interpretation of these data is discouraged because cows with a three locomotion score were not severely lame. Cows with a locomotion score of 3 may be predisposed to lameness in the future or recovering from an injury. However, a true evaluation of effects of severe lameness on fertility would require more animals with >4 locomotion score.

BCS is another management tool that producers can use to better manage cows. BCS can be used to adjust dairy herd rations and herd health programs to achieve the most cow productivity, fertility and longevity. Butler and Smith (1989) reported that, on average, cows resumed estrous cyclicity 10 d following the nadir in energy balance. One mechanism controlling estrous cyclicity in cows is LH pulsatility. LH secretion is suppressed until negative energy balance returns to balance. Similar to our findings, Loeffler et al. (1999) reported cows with a BCS of 3.0 had a trend for increased PR. It appeared that cows with extreme body condition scores either (very thin or fat) tended to have a lower PR/AI. One explanation for this is that cows over or under conditioned are more susceptible to metabolic problems, infections and reproductive difficulties (Gearhart et al., 1990).

Previous calving difficulty scores (Dematawewa and Berger, 1997) for each cow included in the study were recorded and evaluated for potential impact on subsequent

fertility. We did not discover a significant difference in fertility in cows without calving difficulty when compared with cows with slight to severe difficulty. However, numbers of animals with extreme calving difficulty were too few to accurately determine the effect of extreme calving difficulty on fertility.

Considerable work has been dedicated to studying the relationship of milk production and fertility and contradictory evidence is available in the literature. Some studies report a negative effect of high milk production on fertility (Faust et al., 1988; Fonseca et al., 1983; Laben et al., 1982; Ron et al., 1984; Spalding et al., 1974) while others found no effect or slightly positive results (Boyd et al., 1954; Eckles, 1929; Everett et al., 1966; Gaines, 1927; Hillers et al., 1984; Peters and Pursley, 2002). Our results would favor the latter. Data from current study is consistent with findings that cows with above-average milk production had a greater PR compared with lower producing cows (Peters and Pursley, 2002). In the current study, second lactation cows with above average milk production had a 15 % difference in PR/AI over their below average contemporaries. This is different from the results of Peters and Pursley (2002) who reported that first parity cows with above average milk production had the greatest advantage in fertility over below average contemporaries. Badinga et al. (1985) observed poor-producing cows to be less likely to conceive and more susceptible to health-related problems. Thus, high producing cows may have fewer health problems, such as lameness, mastitis, RP or ketosis.

SCS are routinely used as an indicator of subclinical mastitis. Mastitis can cause severe pain, loss of milk production and stress on the cow. Mastitis has previously been associated with poor reproductive performance (Dobson et al., 2001). However, in the

current study we saw no difference in PR in cows with low SCS compared to cows with high SCS. Again, over interpretation of these results are discouraged as we did not have a lot of variability in SCS for cows, with very few showing clinical signs of mastitis.

CONCLUSION

In conclusion, more female calves were generated with timed insemination at 32 to 40 h before ovulation with a 10 % decrease in fertility. Site of semen deposition did not provide a viable technique to maintain fertility for inseminations that occurred at 32 to 40 h before ovulation. More effort needs to be dedicated to establishment of an effective regime that incorporates AI prior to ovulation and maintains fertility. Selection of high fertility sires may be a viable option. The sire in the current study with the highest PR/AI was unaffected by the extended time of his semen in the uterus before fertilization. Surprisingly, this sire had higher fertility in the -8 h groups, which presumably challenged sperm survivability to a greatest extent than the 16 h group. Timing of AI prior to ovulation may be a novel field test to determine fertilization capacity of service sires.

Some of the physiology and management factors evaluated in this study proved to have an impact on PR/AI in lactating dairy cows. We discovered that multiple ovulations, large follicle size and small CL size at the time insemination are significantly associated with increased fertility. Additionally, pregnancy loss appeared to be more closely associated with physiological factors rather than management. It was discovered that cows with CL > 17 mm at the time of insemination had more pregnancy loss than cows with smaller CL. Cows with a single ovulation had more pregnancy loss than cows with multiple ovulations. Future work to determine mechanisms involved in pregnancy

loss should be pursued. Pregnancy loss may be an important link to the decline in fertility in multiparous lactating dairy cows.

BIOGRAPHY

Melissa Wolf Macfarlane is the fourth generation of her father's family to be born and raised in southern Oregon. She graduated from Crater High School in Central Point, Oregon in 1996. Following graduation she left her roots and moved to California. In the fall of 2000 she earned a Bachelor of Science degree from California State University, Chico. In the summer of 2001 she accepted an assistantship with the Department of Animal Science Michigan State University. In the fall of 2003 she received her Master of Science Degree. Following degree completion she returned to California to pursue a career in post-secondary agriculture education.

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