

Commercial Application of Biotechnology in Male Reproduction

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Introduction

Commercial artificial insemination (AI) is one of the greatest animal biotechnologies introduced in the 20th century. The benefits of improved genetics and reduced disease transmission have been readily recognized and accepted by the dairy producer and at present, approximately 70% of U.S. dairy cattle are bred by AI. However, adaptation of this tremendous biotechnology in the beef cattle industry lags far behind with present estimates of less than 10% of the nation's 33 million beef cows bred by AI.

Why has implementation rates of this tremendously successful male biotechnology varied so greatly between the beef and dairy industries? A primary factor is likely difference in the ease of implementation. Dairy producers handle animals multiple times daily and thus the "hassle-factor" associated with estrus detection and AI is not as great compared to the beef producer who may only handle cattle 2 or 3 times yearly. Other factors may also include: 1) differential ability to measure genetic merit 2) the economic value of genetic differences, and 3) the ability of the cow-calf producer to directly harvest the economic returns afforded from improved genetics. In the dairy industry, herd improvement organizations measure genetic variation in milk production with high levels of accuracy and the producer is directly reimbursed for the increased pounds of milk sold. In contrast, data reporting systems necessary for accurate genetic evaluations are less utilized by commercial beef producers, while carcass quality premiums have historically benefited the packer and/or feedlot operator. Further mediocrity towards AI technology in the beef cattle may have been prompted by emphasis on individual performance and/or pedigree estimates in sire selection rather than progeny performance; either of which tends to encourage use of natural service and/or young sires over high reliability, proven sires. However, many of these historic roadblocks to beef AI appear to be deteriorating. Significant advancements in estrus synchronization technology have greatly enhanced success and ease of implementation. Education and marketing programs such as Certified Angus Beef (CAB) have heightened consumer awareness that "all beef is not created equal", resulting in greater demands for high-quality red meat that are stimulating economic spider veins from packer through the feedlot to the cow-calf operator. Pending initiatives for a national animal identification system may greatly enhance opportunities to improve the size, scope, and accuracy of beef cattle genetic evaluations, while facilitating economic recognition for genetic investments (or lack thereof) by the seed-stock producer. Nonetheless, despite more than 60 years of unprecedented success in the dairy industry which is rapidly being mimicked by the swine and poultry industries, AI can still be considered a novel biotechnology for much of the beef cattle industry.

Opportunities

New technology, in any industry, must demonstrate a return on investment before widespread implementation can be expected. Technologies that improve production efficiency with increased output per unit of input, or that lower production cost while maintaining output quantity and quality, are easy decisions for implementation. Other technologies that may increase production costs and/or lower production efficiency over existing technology must add value to the product that the consumer perceives as worthy of the additional cost before widespread adaptation can be expected.

For the natural service herd-sire, opportunities to introduce new technology primarily exist in areas of sire selection and sire management. However, adaptation of new technologies within the AI industry provides economics of scale allowing producers access to a tremendous wealth of additional technologies at little to no additional cost. For the purposes of this manuscript, the following discussion will focus on the expanded opportunities for introduction of male oriented biotechnologies within the AI industry.

The primary objectives of the AI center are to provide superior genetics in the form of highly fertile frozen semen. Each quality control program attempts to minimize variation in sire fertility by minimizing variation in the quality of semen made available to producers while optimizing efficiency of semen utilization. These objectives provide for 5 basic phases of production wherein new technology may be implored:

- 1) Sire selection
- 2) Sire management
- 3) Semen processing and cryopreservation techniques
- 4) Post-thaw semen evaluations and quality control
- 5) Semen storage and delivery

Sire Selection

Genetic selection

Sire sampling and progeny testing is the most expensive component in the production cost of frozen bovine semen. The simple procedures necessary to collect and extend semen, load it into a straw, and freeze it, are inconsequential compared to the cost associated with proving that the individual is truly of sufficient genetic merit to warrant propagation. Major AI centers with established progeny sampling programs usually operate at production cost disadvantage compared to on-farm or custom collected semen wherein little to no effort goes to ensure young sires are accurately sampled and evaluated. Presently, the ratio of proven sires marketed to young sires sampled is 1:9 in dairy breeds and 1:4 in beef breeds. In dairy breeds, most young sires are purchased through contract matings and sampling requires approximately 6 years from initial purchase with total sampling costs estimates at \$25,000 to \$30,000 per sire. Thereby each proven dairy sire represents an investment in excess of \$250,000. These costs are much reduced for beef breeds but are still significant influencers of straw production cost.

Tremendous opportunity exists for improved efficiency of the sire sampling process through incorporation of DNA marker assisted selection. Improved accuracy of young sire selection would result in more favorable ratios of sires marketed to sires sampled dramatically impacting sampling costs. Several DNA markers are routinely used in the

Holstein breed screen young AI sires for undesirable traits such as BLAD, CVM, and DUMPS prior to the expense of progeny sampling. Use of genetic markers for production traits has been initiated in several young dairy sire sampling programs however the impact of these applications is either preliminary or proprietary. Promising technologies for the beef industry include marker assisted selection for growth, carcass quality, meat tenderness, and disease resistance.

Reproductive capacity

Polygamous species, such as the Macaque monkey, are sentinel examples of the capacity for intense selection for male reproductive capacity to influence a species. Female Macaques in estrus repeatedly copulate with available males that patiently await their opportunity with little to no exhibition of social competition for mating opportunities. The male that provides the greatest number and/or highest quality of sperm to the potential fertilizing pool has the greatest opportunity to sire the next generation. The result is a population of males with greatly enhanced testis size and semen quality. This is in contrast to a monogamous species such as the human or the highly inbred Cheetah, which are among the poorest semen quality producers in the animal kingdom.

Semen production capacity in the bovine is most readily associated with the highly heritable trait of scrotal circumference (SC), which has a genetic link to age at puberty in the female (Brinks, 1994). However, selection for SC employs a threshold approach (>30 cm at 1 year of age) with primary selection pressures placed on production traits. It is thereby perhaps not surprising SC measures in the present Holstein AI population are similar to those reported 40 years ago (DeJarnette et al., 2003). Low adaptation rates of AI have limited opportunities for genetic selection for male reproductive capacity in beef breeds. Additionally, traditional single sire mating in beef herds, wherein no social and/or gamete competition among males exists, has likely facilitated propagation of males with undesirable reproductive and/or semen quality traits. Although the genetic component of these differences is unclear, beef sires appear to produce lower quantities of sperm with lower and more variable semen quality characteristics than do dairy sires of similar age and scrotal circumference (Table 1). These differences imply tremendous opportunities may exist for genetic selection for improved reproductive capacity in the male of many beef breeds.

Table 1. Comparisons of semen production characteristics of mature Angus and Holstein AI sires.^a

| Item | Angus | Holstein |
|---|-------------------------------|-------------------------------|
| No. of sires | 28 | 166 |
| Age (yr) | 4.9±0.4 | 4.1±0.2 |
| SC | 40.7±0.5 | 39.8±0.2 |
| No. 1 st ejaculates ^b | 54±16 ^f | 72±7 ^g |
| Volume (mL) | 5.8±0.33 ^f | 6.6±0.15 ^g |
| Concentration (x10 ⁹ /mL) | 1.17±0.06 | 1.36±0.03 |
| Total cells/ejaculate (x10 ⁹) | 6.7±0.53 ^f | 8.7±0.24 ^g |
| Post-thaw semen quality (%) | | |
| Motility 0 h ^c | 74.5±0.63 | 76.6±0.26 |
| Motility 3 h ^d | 30.1±0.96 ^f | 35.8±0.39 ^g |
| Acrosomal integrity 3 h ^e | 73.0±1.14 ^f | 79.1±0.47 ^g |
| Normal morphology | 64.3±2.0 ^f | 77.6±0.8 ^g |
| Collections discarded for poor quality | 18.2% ^f (276/1514) | 3.5% ^g (420/11966) |

^a Data obtained from Select Sires semen production data for collections occurring in the years 2001 and 2002. Holstein sires were selected to have similar scrotal circumference (≥36 cm) to the available Angus population.

^b Average number of 1st ejaculates per bull upon which semen production and quality characteristics were based.

^c Subjective post-thaw estimate of percent motile cells after 0 hours of incubation at 37°C.

^d Subjective post-thaw estimate of percent motile cells after 3 hours of incubation at 37°C.

^e Post-thaw estimate of percent intact acrosomal membranes after 3 hours of incubation at 37°C.

^{f,g} Row values with different superscripts differ at P < 0.05.

Sire Management

Enhanced semen quantity

Technologies enhancing SC and/or semen production capacity are highly desired in AI sires wherein semen demand may often exceed supply. To these ends, active immunization against inhibin has demonstrated promising potential in the bovine and warrants further investigation (Martin et al., 1991; Bame et al., 1999). Induced, peri-natal hypothyroidism has been shown to dramatically increase testis size in several rodent species (Cooke et al., 1993); however, limited attempts to apply these techniques in the bovine have been unsuccessful (Kastelic et al., 1995). A limitation of these techniques to enhance testis size is they likely must be implemented prior to cessation of sertoli cell proliferation in order to be effective. This would have the distinct disadvantage of requiring treatment of all young sires prior to genetic evaluation, which in addition to costs, may mask the genetic component of SC and thereby facilitate propagation of undesirable SC genotypes.

Cloning

Cloning technology holds promise to increase the semen supply of high demand sires and/or extend marketing life of geriatric or deceased sires. Cloning's advantage is it could be selectively applied to mature, high demand sires. At present, the primary obstacle to commercial application of cloning in the bovine is the voluntary moratorium requested by the FDA. The potential impact and profitability of cloning resides in accurate prediction of the sires that should be cloned and doing so two years in advance of when the additional semen will be needed. The inherent error associated with pedigree estimates of young sires, rapid rates of genetic progress among proven sires, and constantly changing market conditions influencing genetic breeding objectives among producers will continue to make these decisions inherently problematic and perhaps "more art than science". Again, due to limited AI adoption rates in beef cattle, most bulls can presently meet market demands without the use of clones and the technology will likely have greater impact in dairy breeds. However, significant advances in cloning efficiency may open doors to commercial sale of cloned AI sires for use in natural service. Cloning may present an opportunity to salvage and/or expand marketing options by producing a "sero-negative copy" of a genetically superior animal sero-positive for a particular disease that restricts domestic and/or export opportunities.

Sire health

Reduced incidence of disease transmission is major advantage of AI; however, AI is also a very efficient means of disease transmission if appropriate precautions are not exercised in selection and management of the donor sire. Therefore, sire and semen health status is of paramount importance to the AI center. Certified Semen Services (CSS), a division of the National Association of Animal Breeders (NAAB), establishes and monitors stringent sire health testing and management procedures for participating members to ensure a safe and disease free product. Relevant new disease screening technologies are researched and/or procedures implemented at the directive of these governing organizations. Additional testing procedures may be implemented in order to abide by the international import health requirements of various countries. The combined result (CSS testing + export testing) is that the average production bull in an AI center may receive approximately 30 "tests" per year while screening for more than 12 different diseases. Although semen produced by non-CSS participating organizations may be equally safe, sire health status of "on-farm" collected donor bulls should be questioned. Similarly, the health status of a natural service sire can rapidly change upon exposure to an infected female.

Enhancing neat semen quality

The greatest opportunity to enhance the fertility potential of a sire is to enhance the quality of the neat semen produced. Although the heritability of semen quality characteristics have generally been considered quite low, the greater variation in semen quality among males in many beef breeds (Table 1) may present a greater opportunities for genetic selection for semen quality and should be investigated. Similarly, much of the difference in semen quality between beef and dairy breeds may be a function of a genetic predisposition of beef breeds to deposit fat in the neck of the scrotum, which may diminish the animal's ability to thermoregulate the testis for normal spermatogenesis

(Vogler et al., 1993; Coulter et al., 1997). Sire housing and ventilation, seasonal use of air conditioning, and nutritional management to avoid over conditioning are effective means of influencing thermoregulation and optimum spermatogenesis, especially during the summer months. Supplemental feeding of various micro-minerals, lipids, antioxidants, or other compounds may enhance sperm membrane function and/or animals ability to tolerate high ambient temperatures and may be worthy of further investigation. However, its unclear at present if such approaches have merit in the absence of a nutritional deficiency. Studies evaluating such approaches are inherently problematic due to the latent effects of treatment on the 63-day duration of spermatogenesis, which often becomes confounded with changing environment and management during treatment. Toxic agents such as gossypol and endophytes have been associated with disruptions in normal spermatogenesis and the potential for anti-spermatogenic effects should be investigated in all new feed for the bull.

Identification of the subfertile sire

Whether bred by natural service or by AI, identification and elimination of the subfertile sire is essential to ensure optimum fertility potential is achieved. The breeding soundness exam with a thorough semen evaluation component is the foundation of sire selection for fertility. In addition to minimum standards for scrotal circumference and firmness, ultrasonic echotexture and infrared thermographic evaluation of thermoregulation capacity may augment selection of sires for reproductive capacity (Kastelic et al., 2001). The presence or absence of numerous fertility associated sperm membrane and/or seminal plasma proteins (Killian et al., 1993; Bellin et al., 1996; Amann et al., 1999) are promising areas of future research. A recently commercialized chute-side assay of one such protein has demonstrated a significant association with fertility in natural service mating sires (FAA; McCauley et al., 2004), however, preliminary evaluation of this technology within the AI population has not demonstrated utility but warrants further investigation. A limitation of implementing fertility technologies in the natural service scenario is the transient nature of many seminal quality characteristics, which may change from acceptable to unacceptable (or vice versa) subsequent to evaluation. In contrast, AI sires are monitored and screened for various semen quality and/or fertility attributes on a routine basis ensuring greater consistency in sire fertility potential.

Semen processing and cryopreservation.

To understand the value of technology introduced in semen processing, it is important to understand the principles of semen quality control and the relationship of compensable and uncompensable semen quality traits to fertility (Salisbury and VanDemark, 1961; Saacke, 1998; Figure 1). Compensable semen quality traits are those for which the female population will respond to increasing numbers of sperm per AI dose with increased fertility and are generally associated with measures of sperm viability (i.e., motility, acrosomal integrity, etc.). Uncompensable semen quality traits are those for which the female population will not display increased fertility in response to increased numbers of sperm per dose and appear to be associated with sperm morphology, DNA integrity, and the ability to sustain normal embryonic development (Kidder et al., 1954; Bearden et al.,

1956). The “threshold” is the value beyond which further increases in sperm numbers fail to increase fertility and may be achieved by satisfying the semen demand of the female population (Bulls A, B, & C) or when uncompensable semen quality traits become the limiting factor (Bull D). The rate at which individual bulls and/or ejaculates approach the threshold and the maximum level of fertility obtainable is a function of the severity and ratio of compensable and uncompensable sperm defects within the sample.

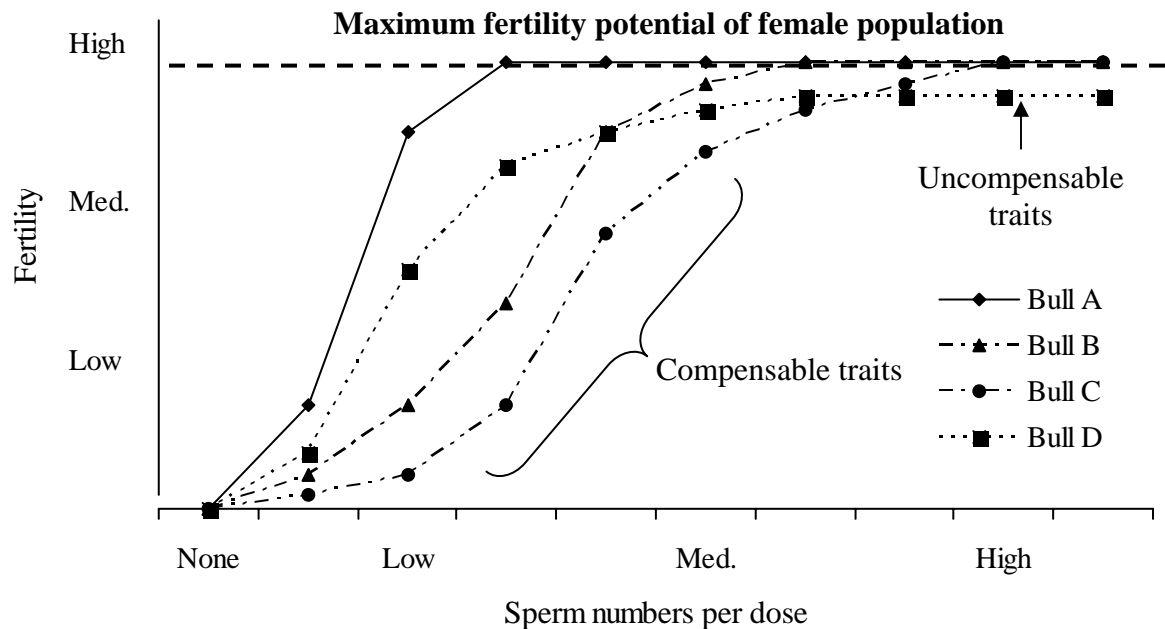


Figure 1. Relationship of sperm numbers per dose and fertility for bulls of varying semen quality (Adapted from Salisbury and VanDemark, 1961).

Most new semen processing technologies target enhancing post-thaw sperm survival (compensable attribute) as the primary measure of success and likely influencing the threshold numbers of sperm required per dose to achieve optimum fertility potential but not the affecting absolute level of fertility be achieved. Improved measures of compensable attributes will tend to shift dose titration curves to the left allowing for more efficient use of genetically valuable semen without compromise to conception rates. However, a recent global survey of semen processing practices at major AI organizations reported the average cryopreserved AI dose contains approximately 20×10^6 total spermatozoa (Range $10\text{-}40 \times 10^6$; Viswanath, 2003), which is from 2 to 20 times greater than estimates of the minimum threshold numbers required (Filseth et al., 1992; van Giessen et al., 1992; den Daas et al., 1998). These observations suggest the AI industry is not fully utilizing existing efficiencies and new technologies to increase the numbers of live sperm post-thaw (compensable trait) It is unlikely fertility will increase outside the research setting where extremely low cell number dosages are often used. If technology A delivers “enough” sperm to the cow to achieve optimum fertility potential, “more” sperm from technology B cannot be measured as “better”. Furthermore, extremely high cell number dosages often marketed as “double-strength” semen for improved conception are not supported as being effective by controlled dose titration research. To the contrary,

extremely high sperm concentrations ($>200 \times 10^6/\text{mL}$) will displace cryoprotectants, which may have deleterious effects on sperm survival. Thus, major AI organizations implore many variants of extender composition, freezing rates, level and type of cryoprotectants, etc., yet semen from all centers achieves comparable fertility as each organization provides significantly more sperm per dose than required to achieve optimum fertility potential.

Another example of a technology that influences compensable semen quality traits is sperm packaging method. Ampules, pellets, or 0.25 vs. 0.5 mL French straws interact with extender type and freezing rate to influence the number of sperm surviving the freeze/thaw process, yet all methods can achieve acceptable fertility as a function of sufficient cell numbers per dose. Adaptation becomes a function of production efficiency and/or marketing constraints. Therein the United States, Latin America, and much of Asia adopted the more “user friendly” 0.5 mL straw, while European countries capitalize on the production and storage cost efficiencies afforded by the smaller volume 0.25 mL straw.

Alternatively, processing technologies that impact uncompensable semen quality traits may enhance the fertility potential of sires that fail to achieve optimum levels (Bull D, Figure 1). As most uncompensable traits are believed to be associated with normal sperm morphology and/or DNA integrity, the probability of positively influencing these semen characteristics post-collection appear to be limited. However, sperm longevity could be argued to be a viability associated trait uncompensable in nature. Macmillan and Watson (1975) provided evidence variance in fertility among AI sires is largely a function of sperm longevity in the reproductive tract and thereby sensitive to deviations in insemination timing. In this study, the effects of interval from observed estrus to AI on non-return rates of sires with varying fertility levels were evaluated. Variance among sire fertility groups was greatest when AI was performed early in the estrus period and diminished as AI occurred closer to the time of ovulation (Figure 2). The change in variance was exclusively a function of improved conception at the later AI period for the average and below average fertility sires and non-return rates of above average fertility sires was not affected by time of AI. Thus, technologies that increase sperm longevity may reduce sensitivity to deviations in insemination timing and thereby improve fertility potential irrespective of sperm dosages. However, measuring fertility differences among males and/or treatments as a function of sperm longevity may require inseminations to occur very early relative to ovulation. Otherwise, short longevity semen may achieve identical conception to semen with greater longevity if semen deposition occurs at optimal timing relative to ovulation (Figure 2).

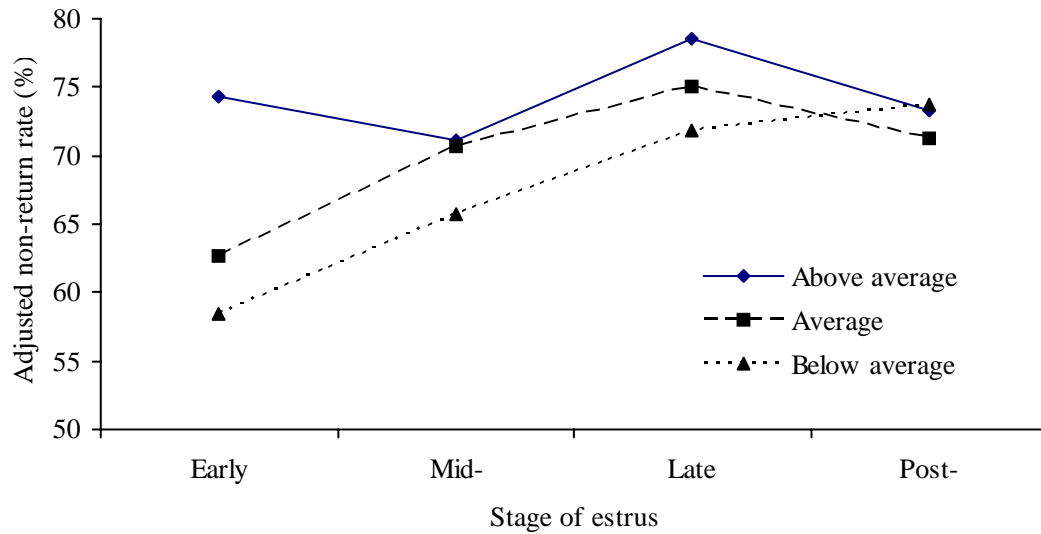


Figure 2. Effects of sire fertility group and stage of estrus at insemination on non-return rates. (Adapted from Macmillan and Watson, 1975)

Semen cryopreservation techniques that improve post-thaw sperm longevity, with or without increasing the percentage survival, are promising areas of research to improve fertility from the male or inseminate perspective. To these ends, microencapsulation of spermatozoa for sustained time release (Vishwananth et al., 1997) or techniques designed to reduce the magnitude of cryopreservation-induced capacitation (Watson, 1995), such as pre-freeze addition of cholesterol and/or antioxidants (Maxwell and Watson, 1996) warrant further investigation. Mixing samples of “early” and “late” capacitating sperm (Meyers et al., 1995) has been suggested as a method to improve fertility by accommodating a wider ovulation window (Elliott, 1974). However, with the exception of a single experiment (Elliott, 1974), most controlled studies indicate conception rates of heterospermic samples are comparable to the homospermic means but not greater than the fertility of highest individual in the mix (Elliott, 1974; Stahlberg et al., 2000; Vicente et al., 2004; DeJarnette et al., 2003). The success of this technique may be limited by accurate identification of the bulls and/or ejaculates that should be mixed.

Exposure of sperm to fertility associated proteins or antigens is also a promising arena of study that may increase fertility potential of the inseminate (Amann et al., 1999) perhaps in both a compensable and uncompensable manner. This might allow low dose inseminations of treated samples to achieve greater fertility than high dose inseminations of untreated semen and thereby greatly enhance efficiency of semen utilization. However, implementation of such technology must be carefully considered to ensure it is used to supplement normal fertility sires and not to compensate or mask the subfertile sire, which could lead to propagation of subfertility within the population.

Sperm sorting for gender pre-selection using flowcytometry is presently a research-validated technology (Seidel et al., 1999) that adds value to the semen dose and is presently in the early phase of commercial application in the U.S. dairy industry. Although research confirms 90% of offspring produced from sexed-sorted sperm are of the desired sex, conception rates are typically 70 to 75% of conventional semen. Reduced

conception influences the producer breakeven value of implementation, which combined with high purchase cost of sorting equipment, annual maintenance, and low output, makes it difficult to calculate a return on investment for all participating parties (technology owner, the AI center and the end user). Price differentials between the value of male vs. female offspring in the dairy industry offer opportunities for a return on investment despite reduced conception rates and commercial application is well underway. However, the more modest differentials in calf values due to gender in the beef industry stifle incentives for commercial application. These constraints may be alleviated by: 1) improved conception, 2) reduced machine cost, 3) greater output efficiency or 4) greater price differentials for male vs. female offspring. Use of flow-sorted sperm in conjunction with IVF to produce frozen embryos of known sex may have synergistic effect on the application of these technologies. Other techniques to sort sperm based on sex-specific membrane proteins remain under investigation but as yet have not been validated as sufficiently repeatable and/or biased to support commercialization (Hendriksen, 1999).

Post-thaw semen evaluation

The greatest opportunity to alter fertility potential of cryopreserved semen likely resides in improved post-thaw semen quality control evaluations that identify subfertile samples for culling. Reputable AI organizations spare little expense in attempts to “minimize the variation in the fertility potential of the semen released for sale”. There are several lines of defense by which these objects may be accomplished. The first line of defense is obviously to cull and discard ejaculates with less than acceptable semen quality characteristics. The second line of defense is in the number of semen quality attributes that are evaluated. Most measures of semen quality known to be associated with fertility potential are highly correlated with each other (Linford et al., 1976; Saacke et al., 1980). Thus, selection and screening for one trait will typically enrich the retained population for multiple semen quality attributes. Screening and discarding collections based on multiple semen quality traits, significantly reduces the probability that semen of less than acceptable fertility would be retained for inventory. The third line of defense is feedback from semen evaluation to semen extension whereby compensatory increases in cell numbers per dose allow marginal quality samples to obtain acceptable levels of fertility albeit at reduced efficiency of utilization. A final line of defense is to simply remove sires from the collection schedule (temporarily or permanently) whose semen consistently fails to pass quality control standards.

In contrast to the research setting where variation is often intentionally introduced to test hypotheses and theories, the intense efforts of the AI center quality control program to minimize variation in the quality semen retained for inventory results in minimal variation in fertility potential as evidenced by multi-regional sire fertility estimates indicating 91% of Holstein AI sires are within $\pm 3\%$ of average fertility (Clay and McDaniel, 2001). Because variation is a prerequisite to a statistical correlation, the lack of correlation between semen quality and fertility estimates in the commercial setting (DeJarnette, 2005) is an artifact of the quality control program that should be considered a comforting confirmation the program is performing to standards. Otherwise, significant correlations imply the trait in question has not been fully accounted for and some

collections are being allowed to pass quality control that should have been discarded. The significant negative correlation between cell numbers per dose and fertility is also an artifact of quality control wherein bulls producing semen of marginal quality maintain somewhat below average fertility despite compensatory increases in cell numbers per dose. Similarly, bulls with above average semen quality characteristics often achieve above average fertility at below average cell numbers per dose. These observations imply within the highly selected population of AI sires, most bulls achieve acceptable levels of fertility and “below average” fertility should not be equated to “low fertility”. By definition, half the individuals in any population (screened or unscreened) will be “below average”. However, below average in the AI population will be skewed towards greater fertility compared to the population at large due to the previously described culling of both bulls and ejaculates.

Nonetheless, the ability of the AI center to enrich the fertility potential of the semen retained for sale is primarily limited by the number of sperm attributes associated with fertility and by reliable and efficient techniques to accurately measure these attributes. Additionally, as implied by Amann and Hammerstedt (1993), the relationships of semen quality to fertility should be investigated for degrees of “association” rather than for degrees of “correlation”. Fertile sperm possess sufficient levels of all known and unknown semen characteristics necessary to achieve fertilization and sustain embryo development. Semen samples that possess sufficient levels of all “known” traits must still be considered of questionable fertility because the sample could be deficient in other “unknown” or unmeasured traits. Thereby, a small but annoying population of subfertile semen may escape detection using existing technologies and opportunities for further enrichment may reside in identification of novel semen quality traits associated with fertility. In particular, the presence or absence of fertility associated sperm membrane and/or seminal plasma proteins (Killian et al., 1993; Bellin et al., 1996; Amann et al., 1999) are a promising area of research. Flow cytometric evaluation of semen quality has the potential to simultaneously evaluate numerous quantitative and qualitative semen attributes with high levels of precision and repeatability (Garner, 1997). Similarly, computer automated sperm-motion analysis (CASA) and perhaps computer automated sperm morphology analysis (Parrish et al., 1998) hold promise to improve efficiency and/or accuracy in the semen evaluation process. Additional studies of the relationship of post-thaw sperm capacitation status and *in vivo* fertility as well as efficient methods to measure these traits in the commercial setting are warranted.

An often-overlooked consideration in new semen evaluation technologies is the potential for a high degree of correlation with existing measures of semen quality (Linford et al., 1976; Saacke et al., 1980). When possible, results of new techniques should be presented as the “additive” predictive value imparted over existing methodology. What does the newly identified attribute or procedure tell us over and above what we already knew? Is it more predictive or simply a different method to measure the same trait? If the latter, greater accuracy, sensitivity, or more efficient utility of implementation must be demonstrated if wide scale application is to be expected. Otherwise, the new technology may simply represent a more tedious and/or expensive method to measure what was already measured, which seems to be the primary hurdle that has limited application of many validated technologies such as flow cytometry (Christensen, 2002), CASA, and numerous *in vitro* fertilization assays of sperm function.

Limitations of sire fertility estimates

Most attempts to associate semen quality and fertility fail to acknowledge the accuracy and/or variance of the sire fertility estimate is typically the limiting factor. As mentioned previously, screening and culling of ejaculates and compensatory increases in cell numbers per dose minimizes variation in fertility of commercial semen released for sale. Additionally, sire fertility estimates are often confounded by the multitude of environmental and herd management factors modestly accounted for in the evaluation model (Saacke and White, 1972; Amann and Hammerstedt, 1993; Foote, 2003). A final consideration is most estimates of sire fertility are associated with large confidence intervals as a function of sample size and the inherent variance associated with a binomial distribution (Figure 3). Due to lack of large scale, organized methods to report and evaluate data, combined with questionable accuracy of data due to use of clean-up sires and delayed (if any) diagnosis of pregnancy, reliable estimates of beef sire fertility are limited but warrant consideration for development. In reality, methods of evaluating semen quality are likely much more sensitive than is our ability to accurately measure fertility with in the narrow range represented in the commercial AI population. Use of “early” AI in conjunction with controlled ovulation may provide a uniquely sensitive model to evaluate the fertility potential of sires and/or semen fertility (Macmillan and Watson, 1975; Saacke, 1998). Similarly, heterospermic insemination provides an extremely sensitive model to magnify differences in fertility potential of inseminates which should be exploited to enhance interpretation of the value of new fertility enhancing or diagnostic technologies (Saacke et al., 1980). However in all cases, researchers should abandon attempts to “correlate” semen attributes with fertility in lieu of more diagnostic approaches to simply identify the subfertile samples or sires that should be removed from the population (Amann and Hammerstedt, 1993).

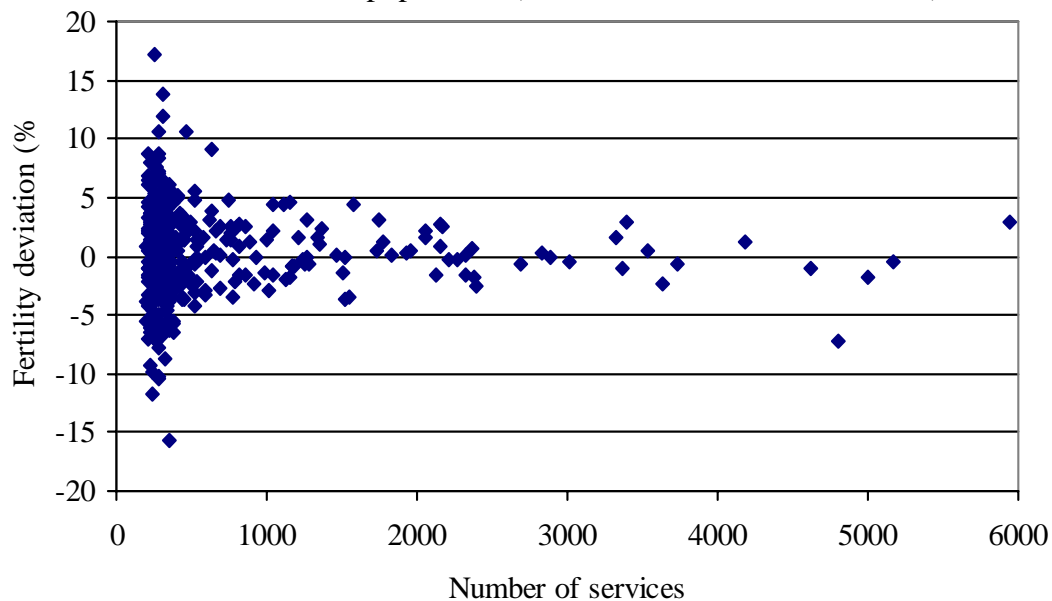


Figure 3. Relationship of sample size to variance in fertility estimates of Holstein AI Sires (n = 403). The fertility estimate is a Select Sires in-house, multi-service, non-return estimate calculated from insemination records obtained from progeny test herds that process data at Dairy Records Management Systems in Raleigh, NC and adjusted for effects of herd-month-year, lactation, days in milk, milk production and interval between AI services.

Semen Storage and Delivery

The final link in the male component of fertility potential is the technician's ability to maintain semen quality until deposited at the proper location in the female reproductive tract at a time conducive to optimum conception. Thereby, the dose response curves illustrated in Figure 1 may be equally applicable to technician proficiency. Highly proficient technicians achieve optimum fertility at relatively low numbers of sperm per dose, while poor proficiency will require extremely high cells numbers per dose to achieve optimum fertility. Technologies that minimize technician variance, and/or the sensitivity of the inseminate to technician variance, may enhance fertility potential from the male perspective. To these ends, novel semen preservation techniques diminishing the thermal sensitivity of sperm are worthy of study.

The influence of semen deposition site on fertility has been most extensively investigated. Approximately 90% of sperm deposited in the uterine body may be lost due to retrograde flow (Mitchell et al., 1985; Nelson et al., 1987). Although in theory deposition of semen in the uterine horns should reduce retrograde sperm loss, facilitate sperm transport to the oviducts, and improve pregnancy rates to AI; Gallagher & Senger (1989) observed no reduction in retrograde sperm loss following cornual deposition and a review of numerous studies comparing fertility after semen deposition in the uterine horns or uterine body have failed to yield consistent results (DeJarnette et al., 2003). However, most of these studies have been conducted at well above threshold cell number dosages. Perhaps the greatest advantage of horn breeding may not be in greater fertility per se but rather in simply lowering the threshold numbers of sperm required for optimum fertility and therein explain the significant technician by semen deposition site interaction observed in many horn-breeding studies.

Although estrus expression and ovulation are controlled by the female, reproductive success (AI or natural service) depends on detection of these events and timely delivery of semen by the male and/or technician. One of the most consistent and repeatable measures in bovine reproductive physiology is the 25 to 30 hour interval from initial standing estrus to ovulation. The founding studies upon which recommendations for insemination timing in cattle were developed (AM/PM Rule; Trimberger and Davis, 1943; Trimberger, 1948) have been reconfirmed by more recent data (Dransfield et al., 1998) and continue to indicate optimum conception is achieved when AI is performed 8 to 12 hours after the initial standing mount. However the primary limitation to proper AI timing is a function of distinguishing the difference between the "initial" standing mount and the "first observed" mount. Despite tremendous amounts of research and technology directed at this issue, heat detection remains a primary obstacle to successful AI (Senger, 1994). The simplest and most economical technologies (tail paint, KaMar, and other mounting aids) often increase the odds of detection of estrus but often lack accuracy due to false positives as a function of the visual evaluation. Automated systems to measure mounting activity such as HeatWatch™, can precisely identify the time of the initial mount and has the advantage that information is transmitted to a central computer to generate breeding lists. However, adaptation of this technology appears to have been limited by high initial set-up cost, labor associated with maintaining transponders on the appropriate animals for the appropriate length of time, and the high cost associated with loss of transponders. Numerous other electronic mounting technologies have been

researched and commercially developed to varying degrees. Many of these devices are compared to the HeatWatch system and promoted as having a lower set up cost. However, most of these devices do not identify the time of the first mount nor transmit information to a central computer, making the KaMar or tail paint the more appropriate controls. Video cameras have been successfully adapted for 24-hour surveillance of dairy cattle in confined, free-stall housing. Automated pedometer systems that measure increased physical activity associated with estrus have been implemented quite extensively in dairy herds with varying degrees of success. Other commercialized technologies such as progesterone testing and devices to measure electrical conductivity of vaginal mucous have had limited implementation due to accuracy limitations, ease of use, and/or expense.

Perhaps the greatest male reproductive technology introduced in recent years is the widespread implementation of synchronization protocols, such as Ovsynch (Pursley et al., 1997) and CO-synch (Geary and Whittier, 1998) that allow a fixed time AI to be precisely scheduled within a few hours of prior ovulation, diminishing the necessity of estrus detection programs. Likewise, as predicted by the data of Macmillan and Watson (1975; Figure 2), proper insemination timing may minimize or eliminate the effects of sperm longevity on conception and thereby minimize variance in fertility among sires and extender treatments compared to inseminations after detected estrus. However, fixed time AI protocols less precise in controlling the time of ovulation and/or schedule insemination at greater intervals prior to the expected time of ovulation may in fact magnify the importance of sperm longevity to conception. Thereby the timed AI protocol chosen may interact with sperm longevity to affect the magnitude of fertility difference among sires (Hiers et al., 2003). However at present, no evidence suggests re-ranking of sire fertility within heat detection or various timed AI protocols should be expected. In either case, increased use of estrus (ovulation) synchronization may hopefully facilitate implementation of one of the oldest, most highly proven, and most often over-looked male reproductive technologies in the beef cattle industry – artificial insemination using semen obtained from genetically elite proven sires.

Summary and Conclusion

Numerous opportunities exist to enhance the profitability of beef production through implementation of male oriented biotechnologies. However, many promising technologies are cost prohibitive to natural service herd-sire applications. Additionally, the transient nature of both semen quality and sire health status makes adaptation of many biotechnologies particularly problematic for the natural service sire. Adaptation of new technology by the AI industry provides economies of scale that allows beef producers to capitalize on these benefits at little to no additional cost. The most readily available and economically justifiable male oriented biotechnology available to beef producers is the largely underutilized technology of artificial insemination using highly fertile semen obtained from genetically superior donor sires of known health status. Perhaps the introduction of systematic ovulation control programs will facilitate greater utilization of AI by the beef industry and thereby better position the average producer to capitalize on other biotechnologies that may be introduced in the future.

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