Proceedings, Applied Reproductive Strategies in Beef Cattle
October 27 and 28, 2005, Reno, Nevada

AN UPDATE ON INDUSTRY APPLICATION OF TECHNOLOGY IN MALE REPRODUCTION

J. M. DeJarnette

Select Sires, Inc
Plain City, Ohio, 43064

INTRODUCTION

Commercial artificial insemination (AI) is arguably 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 the US dairy cattle population is bred by AI. Since the introduction of commercial AI in the early 1940s, the US dairy cattle population has declined from over 25.6 million to only 9.2 million head, while total annual milk production has increased from 53.1 billion kg to 70.8 billion kg in 1997 (USDA, 1961 & 1999) and represents a 369% increase in production efficiency. Although the dairy industry has readily adapted and benefited from this tremendous male biotechnology, less than 10% of the 33 million beef cows in the US are presently bred by AI.

Why have implementation rates of this tremendously successful male biotechnology varied so greatly between the beef and dairy industries? A primary factor is likely difference in ease of implementation. Dairy producers handle animals multiple times daily and thus the "hassle–factor" associated with implementation of estrus detection and AI programs is not as great compared to the beef producer who may only handle cattle two or three 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, most commercial beef producers do not contribute to genetic evaluations programs, while carcass quality premiums have historically benefited the packer and (or) feedlot operator. Further mediocrity towards AI technology in the beef cattle industry may have been prompted by an 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 of AI. Education, marketing, and branded beef 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 seedstock 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 but promising new male reproductive technology for much of the beef cattle industry.

Adaptation of male reproductive technologies by the AI industry

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.

The primary goals of the AI center are to identify superior genetics and maximize efficient utilization of these germplasma, while minimizing variation in both the quality and fertility of semen made available to producers. There are basically 4 phases of production wherein new technology may be implored to accomplish these objectives:

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

Sire selection and management

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, are inconsequential compared to the cost associated with proving the individual is of sufficient genetic merit to warrant such propagation. Thereby, major AI centers with established progeny sampling programs usually operate at a 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 though contract matings and sampling requires approximately 6 years from initial purchase with total sampling cost estimates at $25,000 to $30,000 per sire. Thereby each proven dairy sire represents an investment in the vicinity 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. This could increase the selection accuracy of young sires worthy of sampling and thereby result in more favorable ratios of sires marketed to sires sampled. Presently, several DNA markers are routinely used in the Holstein breed to screen young sires for undesirable recessive traits such as BLAD, CVM, and DUMPS prior to the expense of progeny sampling. To date, application of genetic markers for production traits has been limited and primarily implemented subsequent to progeny sampling rather than prior. 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 all available males that patiently await their opportunity with little to no exhibition of physical 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 (exaggerated) testis size and semen quality. This is in contrast to a monogamous species, such as the human or the highly inbred Cheetah, wherein no selection emphasis has been placed on male fertility and appear to be among the poorest semen quality producers in the animal kingdom.

Among domestic species, genetic selection for reproductive capacity in the male has been most intensely practiced in the dairy sire through more than 60 years of AI. Low AI adaptation rates 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).

Table 1. Comparisons of semen production characteristics of mature Angus & 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. 1st 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.
[d] Post-thaw estimate of percent intact acrosomal membranes after 3 hours of incubation at 37°C.
[fg] Row values with different superscripts differ at $P < 0.05$.

Semen production capacity in the bovine is most readily associated with the highly heritable trait of scrotal circumference (SC), which appears to have a genetic link to age at puberty in the female (Brinks, 1994). However, most selection for SC has employed a threshold approach (>30 cm at 1 year of age) with primary selection pressure focused on production traits. This approach does not appear to have influenced SC measures in the Holstein AI population, as present age adjusted measures are similar to those reported 40 years ago (DeJarnette et al., 2003). Technologies that may enhance 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.

Alternatively, cloning holds promise to increase the semen supply of high demand sires and (or) extend marketing life of geriatric or deceased sires and has the advantage that it can be selectively applied post-progeny test. The primary obstacle to the profitability of cloning will be 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. Rapid rates of genetic progress and constantly changing market conditions that influence genetic breeding objectives among producers will make the cloning selection process inherently problematic and perhaps “more art than science”. Due to limited AI adoption rates in beef cattle, any technology designed to enhance semen production capacity will have greater impact in dairy than in beef breeds. However, pending significant advances in cloning efficiency and legal contractual agreements to protect the AI center from competing sales of frozen semen, commercial sale of cloned copies (embryos or calves) of proven sires for use in natural service has been considered. In addition to doubling semen production, cloning may present an opportunity to salvage and (or) expand marketing options by producing a “negative copy” of a genetically superior animal that is seropositive for a particular disease that restricts domestic and (or) export opportunities. Should cloning of domestic animals receive FDA approval, successful implementation in the AI industry may still depend on consumer perceptions of this controversial technology.

Sire health. Reduced incidence of disease transmission is a 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 by all major AI organizations at the directive of CSS. Additional testing procedures may also 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 frozen semen produced by non-CSS participating

organizations may be equally safe, sire health status of the “on-farm” collected donor bull should be questioned and closely scrutinized in order to protect the cow herd from costly disease outbreaks and (or) sub-fertility. Similarly, the health status of a natural service sire can rapidly change upon exposure to an infected female.

Enhancing neat semen quality. Because post-collection treatments or freezing procedures have limited capacity to improve fertility potential of a poor quality ejaculate, 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 considerable variation in semen quality among males in many beef breeds (Table 1) may represent an opportunity 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). Opportunities to enhance sire selection for neat semen quality may reside in technologies that facilitate differentiation of testicular parenchyma from scrotal fat within SC measurements (Kastelic et al., 2001). 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, it’s unclear at present if such approaches have merit in the absence of a nutritional deficiency. Studies evaluating such approaches are also 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 novel feedstuffs 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 (or) infrared evaluation of thermoregulation capacity may further 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). Preliminary evaluation of FAA technology within the AI population however, has failed to demonstrate utility as more than 90% of sires appear to be positive for FAA with no obvious association between FAA presence and AI estimates of sire fertility (personal experience and communications with industry colleagues). An explanation for discrepancies in the utility of such technologies between natural service and AI is more thoroughly addressed in a subsequent section of this manuscript (Post-thaw semen evaluation). A limitation to implementation of any fertility prediction 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 an ability to sustain normal embryonic development once fertilization has occurred (Kidder et al., 1954; Bearden et al., 1956). The "threshold" number of sperm 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.

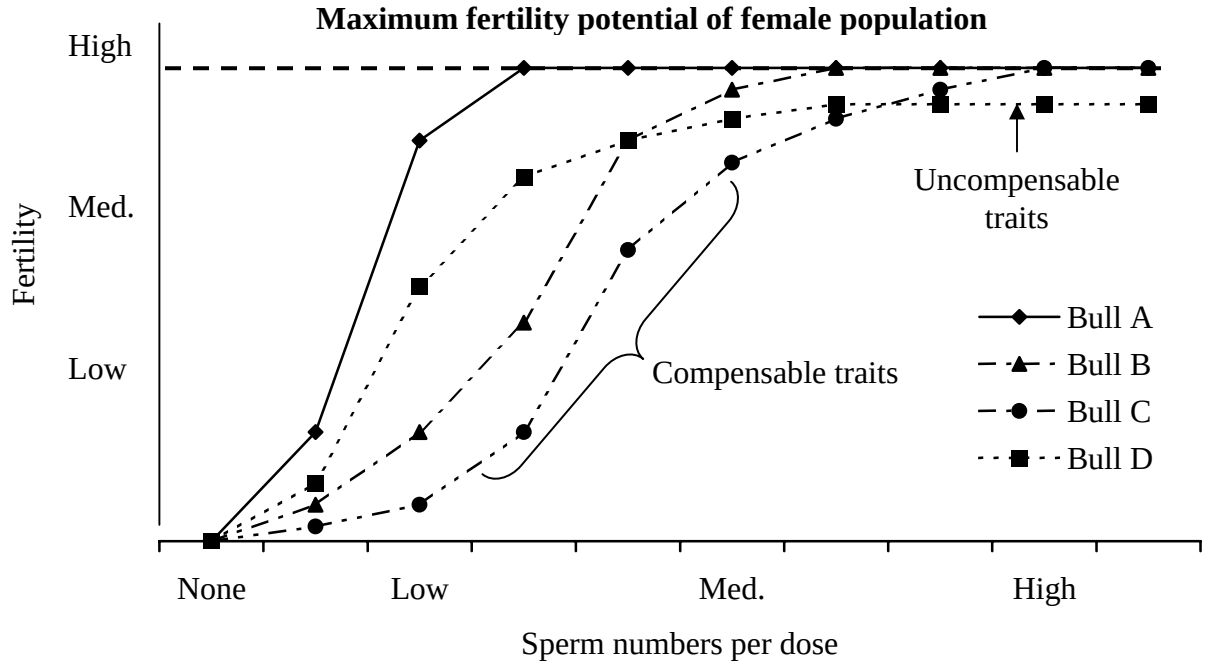

Figure 1. Relationship of sperm numbers per dose and fertility for bulls of varying semen quality (Adapted from Salisbury and VanDemark, 1961).

Even under the best of procedures, semen quality is not likely to be improved by the cryopreservation process. Novel semen processing technologies typically target a reduction in the magnitude of cryopreservation induced sperm damage and thereby enhance post-thaw sperm viability. As sperm viability appears to be a compensable semen quality attribute, such technologies would likely alter threshold numbers of sperm required per dose but have little to no effect on the absolute level of fertility achieved. However, technology-facilitated shifts in dose titration curves may allow for more efficient use of genetically valuable semen without compromise to conception rates. Although extensive dose titration studies indicate threshold sperm numbers using existing technologies range from 2 to 10 x 10^6 sperm per dose (Filseth et al., 1992; van Giessen et al., 1992; den Daas et al., 1998), a recent global survey of semen processing practices at major AI organizations reported the average cryopreserved AI dose contains approximately 20 x 10^6 total spermatozoa (Range 10 to 40 x 10^6; Viswanath, 2003). These observations imply the average AI dose contains from 2 to 20 times more sperm than are required for optimum fertility. As opposed to the research setting wherein below threshold cell number dosages are often employed to prove concept, fertility improvements are seldom realized in the commercial setting due to excessive numbers of sperm made available in all doses. In other words, if technology A delivers "enough" sperm to the cow to achieve optimum fertility potential, "more" sperm from technology B cannot be measured as "better". Thereby, 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 are required.

An 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. Similarly, extremely high cell number dosages or "double-strength" semen for improved conception are not supported by controlled dose titration research and in fact, may be counter productive to conception (Sullivan, 1970) as a function of polyspermy. In addition, extremely high sperm concentrations (>200 x 10^6/mL) displace cryoprotectants, which may have deleterious effects on sperm survival.

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 appears to be limited. However, sperm longevity could be argued to be a viability-associated trait that is uncompensable in nature. Macmillan and Watson (1975) provided evidence that 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. To these ends, microencapsulation of spermatozoa for sustained time release (Vishwanath 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. However, the ability to measure 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).

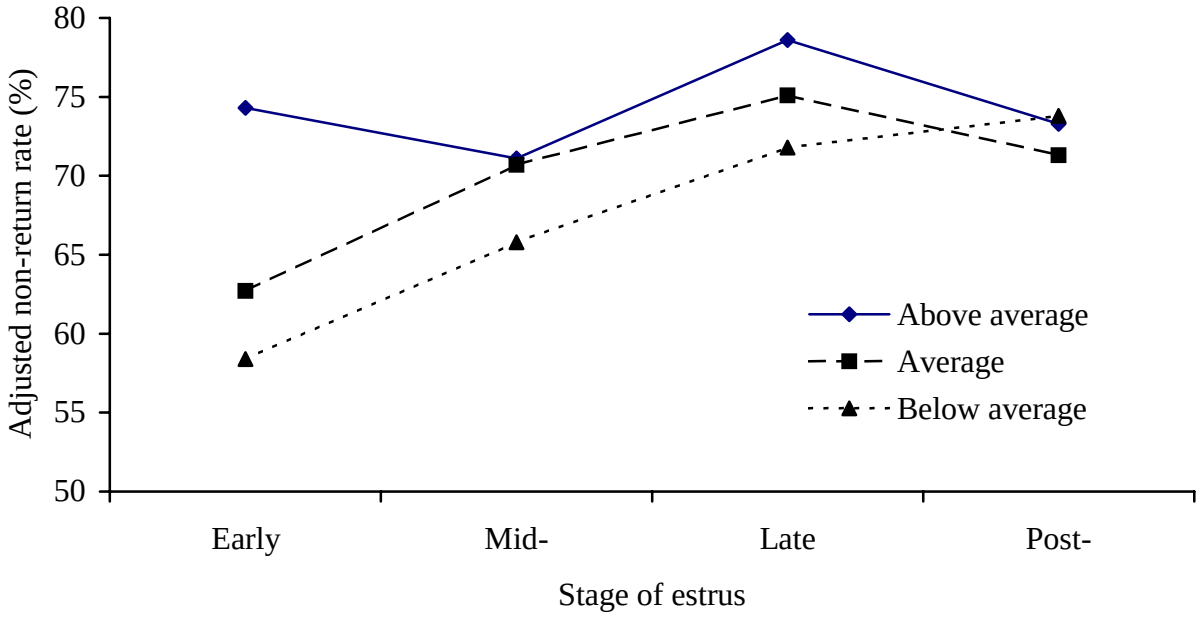

Figure 2. Effects of sire fertility group and stage of estrus at insemination on non-return rates. (Adapted from Macmillan and Watson, 1975)

Exposure of sperm to fertility associated proteins or antigens is also a promising arena of study that may increase fertility potential of the male (Amann et al., 1999 & 2005) 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 careful to ensure use to "supplement normal fertility" and not to compensate or mask the subfertile sire, thereby leading to propagation of subfertility within the population.

Sperm sorting for gender pre-selection using flow cytometry is presently a research-validated technology (Seidel et al., 1999) that adds value to the semen dose. Although several commercial sorting licenses have been granted and research confirms 85-90% of offspring produced from sexed-sorted sperm are of the desired sex, conception rates have typically been approximately 70% of those of the controls. Reduced fertility influences the producer breakeven value of implementation, which combined with high purchase cost of sorting equipment, annual maintenance, and low product output influences return on investment economics for the AI center and the technology owners. These constraints to commercialization 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 a 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 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, the intense efforts of the AI center quality control program to minimize variation in the quality semen retained for inventory, results in minimal variation fertility potential as evidenced by multi-regional sire fertility estimates that indicate 91% of Holstein AI sires are within ±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 (Table 2) 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 that produce 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 that, within

the highly selected population of AI sires, most bulls achieve acceptable levels of fertility and that "below average" fertility should not be equated to "low fertility". By definition, half the individuals in any normally distributed population (screened or unscreened) will be "below average". However, culling of bulls and ejaculates within the AI population tends to skew the fertility distribution towards a higher average compared to the population at large. Further selection and culling of "below average" could be practiced until only a single individual remained, who then, by definition, would only be of average fertility.

Table 2. Correlations of semen quality and fertility in a commercial AI population of Holstein sires[a].

		Fertility estimate			
		Select Sires[b] (n =136)		ERCR[c] (05/03; n = 35)	
Item	Mean±SEM	R	Prob.	R	Prob.
Motility 0 h	77 ± 0.27	0.13	0.15	0.11	0.52
Motility 3 h	38 ± 0.86	0.06	0.50	0.17	0.31
Acrosome integrity 3 h	81 ± 0.43	0.08	0.33	0.07	0.71
Normal morphology	82 ± 1.18	0.03	0.71	0.19	0.27
Sperm per dose	20 ± 0.77	-0.17[d]	0.05	-0.09	0.63

[a] Semen production data were obtained from AI center database for the years 2001 and 2002. Only ejaculates passing quality control and released for sale were included in averages and fertility estimates were matched to correspond with use of this semen (i.e., fertility estimates based exclusively on insemination occurring during the year 2002.

[b] In-house, multi-service, non-return sire fertility 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.

[c] Estimated Relative Conception Rate (ERCR) is a first-service, non-return sire fertility estimate calculated by Dairy Records Management Systems in Raleigh, NC from data obtained from all available herds and adjusted for the effects of herd-month-year, lactation, days in milk, and milk production. Sires included were restricted to those that were commercially introduced at or subsequent to the Feb. 2001 genetic evaluation to ensure appropriate matching of the semen production data base and the sire fertility estimate.

[d] Negative correlation between cell numbers per dose and fertility is an artifact of semen quality control and the negative correlation between normal sperm morphology and cell numbers per dose (R = -0.60, P = 0.001).

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 that can be 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 are those that 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 that the accuracy and (or) variance of the fertility estimate is typically the limiting factor. Sire fertility estimates are often confounded by a multitude of environmental and herd management factors that are only modestly accounted for in the evaluation model (Saacke and White, 1972; Amann and Hammerstedt, 1993; Foote, 2003). As a function of sample size and the inherent variance associated with a binomial distribution, most estimates of sire fertility are associated with large confidence intervals (Figure 3). Reliable estimates of beef sire fertility are all but non-existent due to 1) lack of adaptation of AI in the beef industry, 2) questionable accuracy of available data due to confounding use of clean-up herds sires and delayed (if any) diagnosis of pregnancy, and 3) lack of large-scale, organized methods to report and evaluate available data. 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. However, 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 temptations to "correlate" semen attributes with fertility in lieu of diagnostic approaches to simply identify the subfertile samples or sires that should be removed from the population (Amann and Hammerstedt, 1993).

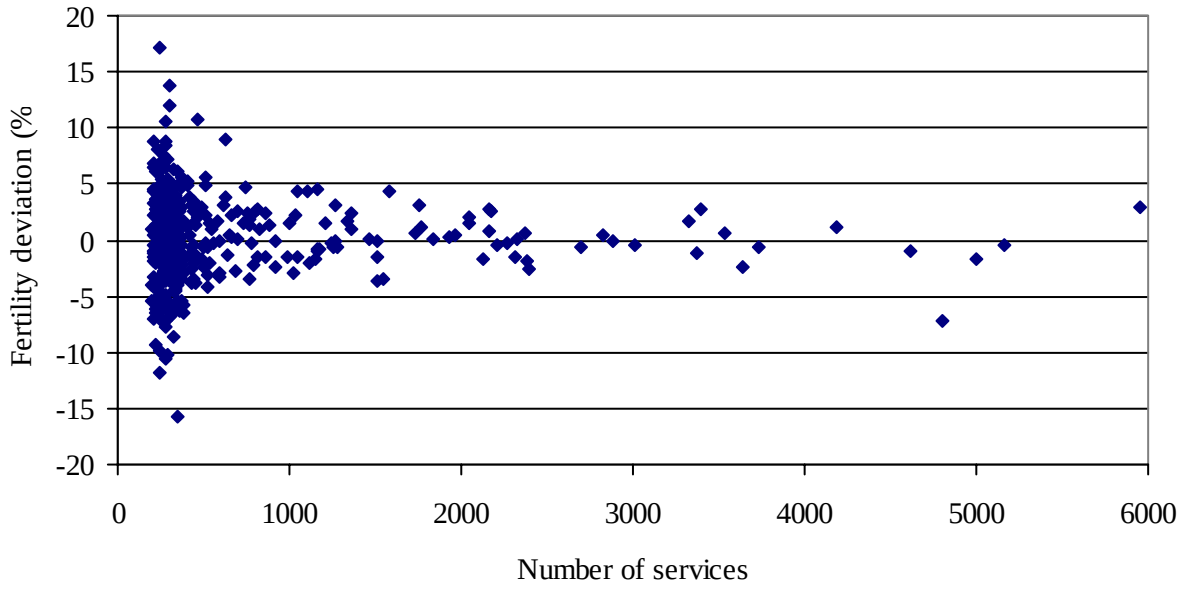

Figure 3. Relationship of sample size to variance in fertility estimates of Holstein AI Sires (n = 403). The fertility estimate is a Select Sires in-house, multi-service, non-return estimate calculated from insemination records obtained from progeny test herds that process data at Dairy Records Management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

Semen 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 cell 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 that diminish the thermal sensitivity of sperm are worthy of study.

The influence of site of semen deposition 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 explains the significant technician by site of semen deposition 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. Thus, 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 that 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 lack in accuracy due to false positive and as a function of the manual evaluation interval. Automated systems to measure mounting activity such as HeatWatch™, can precisely identify the time of 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: 1) identify the time of the first mount nor 2) 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 that are less precise in controlling the time of ovulation and (or) that 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, there is no evidence to suggest a 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

Commercial application of artificial insemination is unquestionably one of the most cost effective and successful male related biotechnologies ever developed. Though readily adapted by the dairy industry, the commercial beef cattle industry has been much slower to capitalize on the benefits afforded by AI. Numerous opportunities exist for novel technologies to enhance the efficiency of sire selection and management for improved reproductive capacity. New technologies in semen processing and cryopreservation may interact with semen quality to influence threshold cell numbers per dose and efficiency of semen utilization but are not likely to improve fertility potential beyond that of the neat semen sample. Semen evaluation technologies that provide more accurate identification of the sub-fertile sire and (or) ejaculates are warranted, however, before widespread adaptation can be expected, techniques must be validated to either: 1) provide information regarding sire fertility that is not accounted for using existing technology or 2) provide a more efficient means of measuring the semen quality attribute than does existing technology. Such validation attempts may be enhanced by greater recognition of the accuracy limitations of most sire fertility estimates due to small sample sizes, binomial variation, and confounding environmental factors. Unfortunately, the transient nature of both semen quality and sire health status makes adaptation of many male oriented technologies particularly problematic for the natural service sire. However, producers readily capitalize on all technologies incorporated by the AI industry with each semen purchase. The introduction of more effective systematic ovulation control programs in recent years appears to be facilitating greater utilization of AI in the beef cattle industry. Adaptation of AI will in turn better position the beef producer to capitalize on other male oriented technologies that may be introduced in the future.

Literature Cited

Amann, R. P., and R. H. Hammerstedt. 1993. In Vitro evaluation of semen quality: An opinion. J. Andrology 14:397-406.

Amann, R. P., J. M. DeJarnette and C. E. Marshall. 2005. Could biotechnology improve outcome after IUI? J. Andrology 20(Suppl.):67 (Abstr.).

Amann, R. P., G. E. Seidel, Jr., and Z. A. Brink. 1999. Exposure of thawed frozen bull sperm to a synthetic peptide before artificial insemination increase fertility. J. Andrology 20:42-46.

Bame, J. H., J. C. Dalton, S. D. Degelos, T. E. M. Good, J. L. H. Ireland, F. Jimenez-Krassel, T. Sweeney, R. G. Saacke, and J. J. Ireland. 1999. Effect of long-term immunization against inhibin on sperm output in bulls. Biol. Reprod. 60:1360-1366.

Bearden, H. J., W. M. Hansel, and R. W. Bratton. 1956. Fertilization and embryonic mortality rates of bulls with histories of either low or high fertility in artificial breeding. J. Dairy Sci. 39:312-318.

Bellin, M. E., H. E. Hawkins, J. N. Oyarzo, R. J. Vanderboom, and R. L. Ax. 1996. Monoclonal antibody detection of heparin-binding proteins on sperm corresponds to increased fertility of bulls. J. Anim. Sci. 74:173-182.

Brinks, J. S. 1994. Relationships of scrotal circumference to puberty and subsequent reproductive performance in male and female offspring. Pages 363-370 *in* Factors Affecting Calf Crop. M. J. Fields and R. S. Sand, CRC Press, Boca Raton, FL.

Christensen, P. 2002. Danish semen analysis: fertility vs. quality tests. Proc. 19th Tech. Conf. Artif. Insem. Reprod., Natl. Assoc. Anim. Breeders, Columbia MO. pp 96-101.

Clay, J. S., and B. T. McDaniel. 2001. Computing mating bull fertility from DHI nonreturn data. J. Dairy Sci. 84:1238-1245.

Cooke, P. S., J. D. Kirby, and J. Porcelli. 1993. Increased testis growth and sperm production in adult rats following transient neonatal goitrogen treatment: optimization of the propylthiouracil dose and effects of methimazole. J. Reprod. Fert. 97:493-499.

Coulter, G. H., R. B. Cook, and J. P. Kastelic. 1997. Effects of dietary energy on scrotal surface temperature, seminal quality, and sperm production in young beef bulls. J. Anim. Sci. 75:1048-1052.

DeJarnette, J.M., C.E. Marshall, R.W. Lenz, D.R. Monke, W.H. Ayars, and C. G. Sattler. 2003. Sustaining the fertility of artificially inseminated dairy cattle: The role of the artificial insemination industry. J. Dairy Sci. 87(E. Suppl.): E93-E104.

den Daas, J. H. G., G. de Jong, L. M. T. E. Lansbergen, and A. M. van Wagtendonk-de Leeuw. 1998. The relationship between the number of spermatozoa inseminated and the reproductive efficiency of individual dairy bulls. J. Dairy Sci. 81:1714-1723.

Dransfield, M. B. G., R. L. Nebel, R. E. Pearson, and L. D. Warnick. 1998. Timing of insemination for dairy cows identified in estrus by a radiotelemetric estrus detection system. J. Dairy Sci. 81:1874-1882.

Elliott, F. I. 1974. Heterospermic trials at ABS. Proc. V Tech. Conf. Artif. Insem. Reprod. National Asso. Anim. Breeders. pp 65-66.

Filseth, O., K. Komisrud, and T. Graffer. 1992. Effect of dilution rate on fertility of frozen bovine semen. Proc. XII Intl. Cong. Reprod. and Artif. Insem. (Hague) Vol III:1409-1411.

Foote, R. H. 2003. Fertility estimation: a review of past experience and future prospects. Anim. Reprod. Sci. 75:119-139.

Gallagher, G. R. and P. L. Senger. 1989. Concentrations of spermatozoa in the vagina of heifers after deposition of semen in the uterine horns, uterine body or cervix. J. Reprod. Fert. 86:19-25.

Garner, D. L. 1997. Ancillary test of bull semen quality. Food Anim. Practice 13:313-330.

Geary, T. W., and J. C. Whittier. 1998. Effects of a timed insemination following synchronization of ovulation using the Ovsynch or CO-Synch protocol in beef cows. Prof. Anim. Sci. 14:217-220.

Hendriksen, P. J. M. 1999. Do X and Y spermatozoa differ in proteins? Theriogenology 52:1295-1307.

Hiers, E. A., C. R. Barthle, MK. V. Dahms, G. E. Portillo, G. A. Bridges, D. O. Rae, W. W. Thatcher, and J. V. Yelich. 2003. Synchronization of Bos indicus x Bas Taurus cows for timed artificial insemination using gonadotropin-releasing hormone plus prostaglandin $F_{2\alpha}$ in combination with melengestrol acetate. J. Anim. Sci. 81:830-835.

Kastelic, J.P., R. B. Cook, R. A. Pierson, and G. H. Coulter. 2001. Relationships among scrotal and testicular characteristics, sperm production and seminal quality in 129 beef bulls. Can. J. Vet. Res. 65:111-115.

Kastelic, J.P., G. J. Mears, and G Wallins. 1995. Neonatal hypothyroidism induced with 6-propyl-2-thiouracil does not enhance gonadal development in bulls and heifers. Proc. Amer.Soc. Anim. Sci., Western Section, 46:223-226, 1995.

Kidder, H. E., W. G. Black, J. N. Wiltbank, L. C. Ulberg, and L. E. Casida. 1954. Fertilization rates and embryonic death rates in cows bred to bulls of different levels of fertility. J. Dairy Sci. 37:691-697.

Killian, G. J., D. A. Chapman, and L. A. Rogowski. 1993. Fertility-associated proteins in Holstein bull seminal plasma. 49:1202-1207.
Linford, E., F. A. Glover, C. Bishop, and D. L. Stewart. 1976. The relationship between semen evaluation methods and fertility in the bull. J. Reprod. Fert. 47:283-291.
Macmillan, K. L. and J. D. Watson. 1975. Fertility differences between groups of sires relative to the stage of oestrus at the time of insemination. Anim. Prod. 21:243-249.
Martin, T. L., G. L. Williams, D. D. Lunstra, and J. J. Ireland. 1991. Immunoneutralization of inhibin modifies hormone secretion and sperm production in bulls. Biol. Reprod. 45:73-77.
Maxwell, W. M. C., and P. F. Watson. 1996. Recent progress in the preservation of ram semen. Anim. Reprod. sci. 42:55-65.
McCauley, T. C., G. R. Dawson, J. N. Oyarzo, J. S. McVicker, H. F. Marks, and R. L. Ax. 2004. Development and validation of a lateral-flow cassette for fertility diagnostics in bulls. In Vitro Diagnostic Technology: *In press.*
Meyers, S. A., J. W. Overstreet, I. K. M. Liu, and E. Z. Drobnis. 1995. Capacitation in vitro of stallion spermatozoa: comparison of progesterone-induced acrosome reactions in fertile and subfertilte males. J. Andrology 16:47-54.
Mitchell, J. R., P. L. Senger, and J. L. Rosenberger. 1985. Distribution and retention of spermatozoa with acrosomal and nuclear abnormalities in the cow genital tract. J. Anim. Sci. 61:956-967.
Nelson, V. E., E. P. Aalseth, C. H. Hawman, G. D. Adams, L. J. Dawson, and R. W. McNew 1987. Sperm discharge and distribution within the cow's reproductive tract after AI. J. Anim. Sci. 65(Suppl. 1):401 (Abstr.).
Parrish, J. J., G. C. Ostermeier, and M. M. Pace. 1998. Fourier harmonic analysis of sperm morphology. Proc. 17th Tech. Conf. Artif. Insem. Reprod., Natl. Assoc. Anim. Breeders, Columbia MO. pp 25-31.
Pursley, J. R., M. R. Kosorok, M. C., Wiltbank. 1997. Reproductive management of lactating dairy cows using synchronization of ovulation. J. Dairy Sci. 80:301-306.
Saacke, R. G. 1998. AI fertility: Are we getting the job done? Proc. 17th Tech. Conf. Artif. Insem. and Reprod., Natl. Assoc. Animal Breeders, Columbia, MO. pp 6-13.
Saacke, R. G., W. E. Vinson, M. L. O'Connor, J. E. Chandler, J. K. Mullins, R. P. Amann, C. E.Marshall, R. A. Wallace, W. N. Vincel, and H. C. Kellgren. 1980. The relationship of semen quality and fertility. Proc. 8th Tech. Conf. Artif. Insem. Reprod., Natl. Assoc.Anim. Breeders, Columbia MO. pp 71-78.
Saacke, R. G. and J. M. White. 1972. Semen quality tests and their relationship to fertility. Proc.4th Tech. Conf. Artif. Insem. Reprod., Natl. Assoc. Anim. Breeders, Columbia MO. pp 22-27.
Salisbury, G. W. and N. L. VanDemark. 1961. Significance of semen quality. Pages 359-379 in Physiology of reproduction and artificial insemination in cattle. 1st ed. W. H. Freeman and Co. San Francisco.
Seidel, G. E., Jr., J. L. Schenk, L. A. Herickhoff, S. P. Doyle, Z. Brink, R. D. Green, and D. G. Cran. 1999. Insemination of heifers with sexed sperm. Theriogenology 52:1407-1420.
Senger, P. L. 1994. The estrus detection problem: new concepts, technologies, and possibilities. J Dairy Sci. 77:2745-2753.

Stahlberg, R., B. Harlizius, K. F. Weitze, and D. Waberski. 2000. Identification of embryo paternity using polymorphic DNA markers to assess fertilizing capacity of spermatozoa after heterospermic insemination in boars. Theriogenology 53:1365-1373.

Sullivan, J. J. 1970. Sperm numbers required for optimum breeding efficiency in cattle. Proc.III Tech. Conf. Artif. Insem. Reprod., Natl. Assoc. Anim. Breeders, Columbia MO. pp 36-43.

Trimberger, G. W. 1948. Breeding efficiency in dairy cattle from artificial insemination at various intervals before and after ovulation. Nebraska Agric. Exp. Stn. Res. Bull. 153:1-26.

Trimberger, G. W. and H. P. Davis. 1943. Conception rate in dairy cattle from artificial insemination at various stages of estrus. Nebraska Agric. Exp. Stn. Res. Bull. 129:1-14.

USDA. 1961. Milk. Cows on farms, production per cow, and total production. Statistical reporting service, Crop reporting board, Statistical bulletin no. 289.

USDA. 1999. Milk cows and production, final estimates 1993-97. Natl. Agric. Statistics Service Publ. 952.

van Giessen, R. C., C. A. Zuidberg, W. Wilmink, W. v/d Veene, and N. den Daas. 1992. Optimum use of a bull with high genetics. Proc. XII Intl. Cong. Reprod. and Artif. Insem. (Hague) Vol III:1493-1495.

Vicente, J., M. V. de Castro, R. Lavara, and E. Mocé. 2004. Study of fertilizing capacity of spermatozoa after heterospemic insemination in rabbit using DVA markers. Theriogenology 61:1357-1365.

Vishwanath, R., 2003. Artificial insemination: the state of the art. Theriogenology 59:571-584.

Vishwananth, R., R. L. Nebel, W. H. McMillan, C. J. Pitt, and K. L. Macmillan. 1997. Selected times of insemination with microencapsulated bovine spermatozoa affect pregnancy rates of synchronized heifers. Theriogenology 48:369-376.

Vogler, C.J., J.H. Bame, J.M. DeJarnette, M.L. McGilliard and R.G. Saacke. 1993. Effects of elevated testicular temperature on morphology characteristics of ejaculated spermatozoa in the bovine. Theriogenology 40:1207-1219.

Watson, P. F. 1995. Recent developments and concepts in the cryopreservation of spermatozoa and the assessment of their post-thawing function. Reprod. Fertil. Dev. 7:213-233.

NOTES