PREDICTING AND PROMOTING FERTILITY IN BULLS

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Introduction

The "predicting" portion of the title of this paper implies researchers and veterinarians understand *all* sperm attributes necessary for fertilization. Currently, *some* sperm attributes are known (Table 1), while others remain unknown.

Table 1. Sperm attributes necessary for fertilization.[1]
"Acceptable" morphology
Metabolism for production of energy
Progressive motility
Capacity for hyperactive motility
Stabilization of plasma and acrosomal membrane lipids
Acrosomal enzymes
Chromatin integrity

[1]Partial list adapted from Amann and Hammerstedt (1993).

Throughout the world, cattle producers are interested in identifying the most fertile bulls for natural service and AI. Unfortunately, although researchers and veterinarians have tried "to develop techniques to accurately predict the fertility of a semen sample from an individual male, the goal has not been achieved" according to Amann and Hammerstedt (1993).

This does not imply, however, that we should stop investigating the development of techniques to accurately predict fertility. In fact, the development and refinement of techniques to evaluate seminal quality have led to enhanced "accuracy in positive fertility diagnosis by default, through identification and measurement of greater numbers of semen quality attributes that are associated with sub-fertile semen" (DeJarnette, 2005).

The purpose of this paper is to review *a few selected practical topics and research results* focused on predicting and promoting fertility in bulls. Topics not included in the paper, but nonetheless important, include a discussion of fertility prediction vs. estimation (Utt, 2016), bull nutrition and development (Barth, 2012), and social dominance (Chenoweth, 1981).

Quantity and Quality of Sperm in the Inseminate

Historically, the assessment of male fertility has focused on the quantity and quality of sperm delivered to the female. Salisbury and VanDemark (1961) first suggested the relationship between sperm quantity and quality, when they proposed that fertility increases with increasing numbers of viable sperm inseminated up to a threshold level. After this threshold level has been attained, the female population becomes the limiting factor and increases in sperm numbers do not result in further increases in fertility (Figure 1). Sullivan and Elliot (1968) reported the minimum number of motile sperm required for maximum fertility differed among bulls, while den Daas et al. (1998) reported bulls differed in their maximal nonreturn rate, and in the rate at which they approached

this maximum as sperm numbers per dose were increased. Nonreturn rate, defined by Rycroft (1992) "as the percentage of cows that are not rebred within a specified period of time after an insemination, typically 60 to 90 days," has been historically used by the dairy industry as an indirect measure of fertility. Regarding semen quality, Pace et al. (1981) reported fertility increases with increasing numbers of structurally intact and motile sperm.
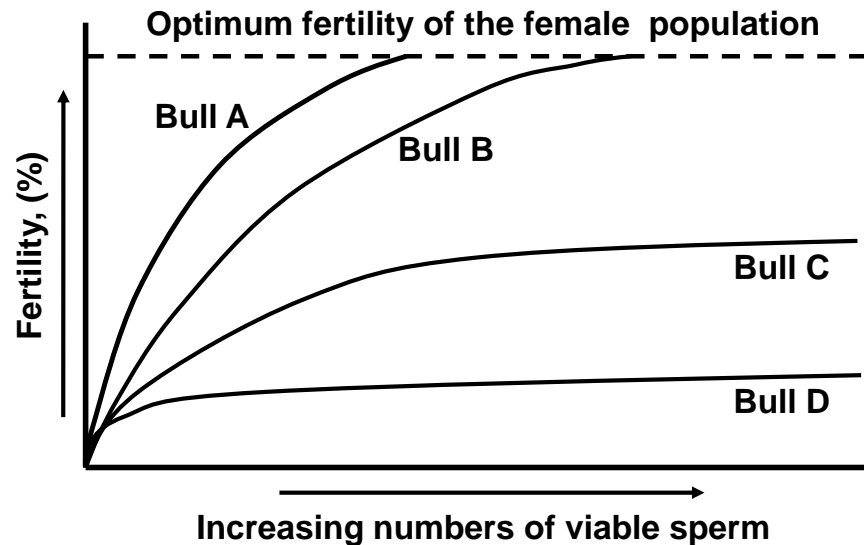

Figure 1. Relationship between number of viable sperm inseminated and fertility. The minimum number of viable sperm required for maximum fertility differs among bulls, as does the rate at which maximum fertility is achieved with increasing sperm dosage (Adapted from Salisbury and VanDemark, 1961, Sullivan and Elliot, 1968, and den Daas et al., 1998).

Compensable and Uncompensable Seminal Traits

Collectively, the work of Salisbury and VanDemark (1961), Sullivan and Elliot (1968), and den Dass et al. (1998) provides evidence there are seminal parameters which are "compensable" and others which are "uncompensable," as originally described by Saacke et al. (1994). Specifically, compensable traits of semen quality relate to the ability of inseminated sperm to not only reach the ovum, but also bind to and penetrate the zona pellucida, and initiate the block to polyspermy. Uncompensable traits of semen quality relate to the competence of fertilizing sperm to complete the fertilization process and sustain early embryonic development.

Therefore, seminal deficiencies, seen as reduced fertility when numbers of sperm are below threshold, which can be overcome or minimized by increasing sperm dosage, would be considered compensable. Reputable AI organizations routinely adjust the AI dose when compensable deficiencies are known. Seminal deficiencies resulting in suppressed fertility regardless of sperm dosage would be considered uncompensable. Bulls with semen exhibiting unacceptable levels of abnormal sperm are usually the main source of uncompensable traits. Reputable AI organizations, however, will not process semen with unacceptable levels of abnormal sperm. In practice, the impact of uncompensable seminal traits may be high when using natural service bulls which have not received a breeding soundness evaluation, and when using semen from non-accredited custom collection and freezing facilities.

Although normal sperm morphology is known to be related to embryo quality (DeJarnette et al., 1992) and fertility (Saacke et al., 1980), unfortunately, normal sperm morphology does not necessarily guarantee a decreased incidence of uncompensable seminal traits. Sperm with microscopically normal morphology, but with defective chromatin, have been implicated in cases of male subfertility for nearly 50 years (Gledhill, 1970), and are likely an uncompensable seminal trait (Ostermeier et al., 2001). The chromatin structure assay developed by Evenson et al. (1980) revealed a strong negative correlation between DNA fragmentation (as a result of vulnerability of sperm DNA to acid denaturation) and heterospermic fertility in bulls (in which parentage of calves was determined by blood typing and visual appraisal of phenotypic characteristics at birth) (Ballachey et al., 1988; Kasimanickam et al., 2006). Acevedo et al. (2002) reported that vulnerability of sperm DNA to acid denaturation was: a) positively associated with abnormal shaped sperm, and b) also extended to normal shaped sperm in abnormal samples. Beletti et al. (2005) used computational image analysis of stained sperm smears and reported sperm with chromatin abnormalities did not necessarily have abnormal sperm head morphology. Collectively, these results provide evidence that damage to chromatin integrity extends beyond morphologically abnormal sperm to apparently morphologically normal sperm.

Severely misshapen sperm do not appear as accessory sperm (Saacke et al., 1998), and thus are not thought to be able to traverse the barriers of the female reproductive tract. Consequently, severely misshapen sperm within an otherwise normal semen sample are considered a compensable seminal trait. Impaired progressive sperm motility may be one of the reasons for the exclusion of these sperm, as Dresdner and Katz (1981) reported that even small geometrical differences in sperm head morphology can cause large differences in sperm motility. In another possible scenario, the effects of abnormal spermatogenesis represented by morphologically abnormal sperm may extend to apparently morphologically normal sperm in the same semen samples. These apparently normal sperm and slightly misshapen sperm in an otherwise abnormal semen sample would be considered an uncompensable trait, and would be expected to depress fertility.

Compensable seminal traits cannot be explained completely by morphology and present-day in vitro viability measurements. Bulls whose sperm are able to access the ovum in vivo at low insemination dose based on fertility data (den Daas et al., 1998) or accessory sperm numbers per embryo (ova) (Nadir et al., 1993) may differ from sperm of other bulls in motility patterns or sperm surface modifications important to release from oviductal epithelium, ova recognition, binding, and penetration. For example, hyperactivated motility, which occurs after capacitation is thought to assist sperm release from the oviductal epithelium (Demott and Suarez, 1992). Furthermore, hyperactivated motility, instead of progressive motility, is thought to be more important for penetration of the zona pellucida in mice (Suarez and Dai, 1992). Additionally, Killian et al. (1993) reported that sperm surface modifications may involve seminal plasma proteins, while Bellin et al. (1994) determined that heparin-binding proteins (HBP) in sperm membranes and seminal fluid were positively related to fertility in bulls.

Although the recognition of compensable and uncompensable seminal traits is equally important, the focus should be on uncompensable traits, as these result in depressed fertility regardless of sperm numbers in the inseminate. Producers can minimize risk associated with uncompensable seminal deficiencies by: a) using semen from AI studs where sperm morphology is a routine part of the evaluation process, and b) screening all natural service bulls with a complete breeding

soundness evaluation, including sperm morphology. Detailed guidelines for breeding soundness evaluations have been reviewed elsewhere (Hopkins and Spitzer, 1997).

Sperm Dosage and Fertility

The primary objective of a recent study (Menegatti Zoca et al., 2017) was to determine if beef bull fertility varied by number of sperm inseminated. A secondary objective was to characterize the potential impact of random variation through the use of two identical sperm per dose treatments, which differed only by straw color.

Ejaculates from five Angus bulls were collected, extended, and frozen at 10, 20, 20 or 40 x 10^6 sperm per dose in color-coded 0.5-mL straws. Multiparous cows (n = 4,866) in 10 Brazilian farms were synchronized for first service timed AI (TAI), and bull identification and straw color was recorded at TAI. Pregnancy was diagnosed by transrectal ultrasonography 30 to 90 d after TAI.

Pregnancy per TAI (P/TAI) was not different between dose (43.8, 45.3, 43.8 and 47.1% for 10, 20, 20 or 40 x 10^6 sperm respectively) (Menegatti Zoca et al., 2017). The P/TAI was different between bulls (Table 2), as bulls A and B exhibited higher P/TAI as compared to bull C (48.1 and 47.7 vs. 40.7 % respectively), while bulls D and E were intermediary (45.5 and 43.1% respectively).

Table 2. Proportion of cows pregnant per timed AI (P/TAI; %) across bulls in Brazilian beef cattle[1].

Bull	P/TAI, mean	n
A	48.1[a]	1,050
B	47.7[a]	1,058
C	40.7[c]	1,206
D	45.5[ab]	747
E	43.1[bc]	805

[1]Adapted from Menegatti Zoca et al. (2017).
[a,b,c] Different letters in the same column denotes statistical difference (P < 0.05).

Although the overall P/TAI between the two control groups (20-a: 45.3%; 20-b: 43.8%) were not different, the numerical variation within bull ranged from .5 (bull E) to 4.9 percentage points (bull D), providing evidence that random variation in reproductive field trials should not be ignored. In conclusion, although fertility differences between bulls were detected, fertility following TAI with 10, 20, 20 or 40 x 10^6 sperm per dose resulted in similar P/TAI at first service in synchronized beef cattle (Menegatti Zoca et al., 2017).

Next, Menegatti Zoca et al. (2018) investigated whether computer-assisted sperm analysis (CASA) and flow cytometry (FC) could explain differences in field fertility of the five Angus bulls described previously. The hypothesis was that high fertility bulls would exhibit the highest values for total motility, progressive motility, intact plasma membranes, intact acrosomes, and normal calcium influx, and lowest values for DNA fragmentation index.

As expected, CASA and FC characteristics were different between bulls (Menegatti Zoca et al., 2018). However, bulls with the highest fertility (A and B) did not display the highest values for total motility, progressive motility, intact plasma membranes, intact acrosomes, and normal calcium influx, nor the lowest value for DNA fragmentation index. Bull D showed the highest values for total motility, progressive motility, intact plasma membranes, intact acrosomes, and normal calcium influx, and the lowest value for DNA fragmentation index. Bull C, which had the lowest field fertility, did not present the lowest values in sperm analyses, whereas bull E showed the poorest in vitro values with an intermediate field fertility. In conclusion, CASA and FC were not able to explain the difference in field fertility between bulls (Menegatti Zoca et al., 2018).

Biomarkers: Fertility Associated Antigen, proAKAP4, AKAP4

During ejaculation, the seminal vesicles, prostate, and Cowper's glands secrete heparin-binding proteins (HBP) which coat the sperm (Miller et al., 1990; Nass et al., 1990). In 1994, Bellin and co-workers reported the distribution of specific forms of HBP on sperm corresponded to fertility potential of bulls used for natural service. Different patterns of HBP on bovine sperm have since been described (Bellin et al., 1996; Bellin et al., 1998).

Bulls with a 31-kDa molecular weight HBP on sperm (named fertility-associated antigen or FAA) were 9 to 40 percentage points more fertile (following natural service) than bulls producing sperm lacking FAA (Bellin et al., 1996; Bellin et al., 1998). Sprott et al. (2000) used 25 bulls of mixed breeds, including *Bos taurus, Bos taurus × Bos indicus,* and *Bos indicus* (Brahman) to investigate whether FAA could be used to assess the potential fertility of sperm for AI. Bulls with FAA-positive sperm were 7 to 9 percentage points more fertile following first service AI than bulls producing sperm lacking FAA (Sprott et al., 2000).

To investigate whether presence of FAA could be used to evaluate the potential fertility of bulls for TAI, Dalton et al. (2012) collected semen from Nelore (*Bos indicus)* bulls and identified six bulls (FAA-negative: n = 3; FAA-positive: n = 3) to be used in two field trials. In Experiment 1, suckled multiparous Nelore cows (n = 835) received TAI with frozen-thawed semen from one of the six bulls. Fertility (as measured by P/TAI) was not different between FAA-positive and FAA-negative bulls (41.5% vs. 39.3%, respectively). In Experiment 2, nulliparous Nelore heifers (n = 617) received TAI with frozen-thawed semen from one of the six bulls. Although fertility (P/ TAI) was different between FAA-positive and FAA-negative bulls (33.7% vs. 40.7%, respectively), our results did not support previous results. In fact, results from these two experiments provide evidence that FAA-negative status was not a limiting fertility factor. The identification of FAA-positive and FAA-negative status was unsuccessful as a method to evaluate the potential fertility of bulls for TAI.

Peddinti et al. (2008) compared sperm protein expression profiles and reported sperm from high fertility bulls have higher expression of proteins involved in energy metabolism, cell communication, spermatogenesis, and motility, as compared to sperm from low fertility bulls. Peddinti et al. (2008) further described differential expression of 125 proteins between high and low fertility bull sperm and theorized these proteins may be potential biomarkers of bull fertility.

A-kinase anchor protein-4 (AKAP4) is a major fibrous sheath protein of the principal piece of the sperm flagellum and is thought to be important to flagellar function in the mouse (Carrera et al. 1994), bovine (Moss et al., 1999), and human (Turner et al., 1998). Peddinti et al. (2008) described greater expression of AKAP4 in sperm from high fertility bulls and theorized the increased presence of AKAP4 may result in greater motility. A commercial kit (https://4biodx-breeding.com/index.php/en/portfolio-item/elisa-kit-bull/) that claims to quantify expression of proAKAP4 (the precursor which is processed into the AKAP4 functional protein; Sergeant et al., 2019) in the ejaculate is currently available. Sergeant et al. (2019) argues the fertilizing ability of ejaculated sperm may be evaluated using the kit. While the development of a test to predict bull fertility has long been investigated, the words of Amann and Hammerstedt (1993) that "some but not all" sperm attributes necessary for fertilization are known still ring true today. While proAKAP4 and AKAP4 are undoubtedly important to fertility, we must remember the plethora of factors, known and unknown, that affect bull fertility.

Bull Breeding Soundness Evaluation

A breeding soundness evaluation (BSE) is a critical management strategy to ensure optimal health and promotion of fertility. A BSE should be performed on all natural service bulls: a) prior to purchase, b) annually, and c) whenever there is concern relative to the potential fertility of a bull. Comprehensive guidelines for the BSE were approved by the Society for Theriogenology in 1992. A new, computer generated BSE form (Myers, 2014) is available from the Society for Theriogenology (www.therio.org). The new form is easy and quick to fill out, and offers the ability to upload data to a centralized location (Myers, 2014).

In 2009 NAHMS reported nearly 27% of beef operations surveyed "semen tested" bulls, while 16% measured scrotal circumference. No data, however, was provided regarding the use of a complete BSE. As shown below, the BSE is a comprehensive evaluation and consists of three sections:

1) Physical examination. For natural service to be successful, bulls must be able to identify cows in heat and copulate. Consequently, the BSE begins with a physical examination of the bull, with particular importance paid to the soundness of eyes, feet and legs. Vision plays a key role in identifying potentially receptive females (Geary and Reeves, 1992). In order for copulation to occur, the bull must be able to physically support a large portion of his weight on his rear legs, fully extend his penis, and gain adequate intromission to deliver the ejaculate. Bulls with sore feet, legs or back, or with traumatic injuries, may not adequately or efficiently service cows. In addition, bulls with poor conformation (post-legged or sickle-hocked) have an increased risk of lameness compared with bulls of proper conformation. The BSE also includes examining the testes, epididymides, penis, and prepuce, as well as internal organs (seminal vesicles, prostate, and ampullae).

2) Scrotal circumference. Scrotal circumference is highly correlated to sperm production, especially in young bulls (Willet and Ohms, 1957; Hahn et al., 1969). Consequently, bulls with insufficient scrotal circumference (relative to age) may not efficiently generate pregnancies. Increased scrotal circumference is associated with decreased age at puberty in related females (Brinks, 1978). An earlier age at puberty for beef heifers is associated with increased lifetime fertility and pounds of calf produced (Brinks, 1994). Veterinarians and producers must also realize, however, that scrotal circumference is of less value as an indicator

of sperm production in bulls greater than 5 years of age, as there is a reduction in sperm output per gram of testis in old bulls (Hahn et al., 1969).

3) Semen collection and evaluation. Following collection of a semen sample (usually by electroejaculation), sperm motility and morphology are evaluated. In general, fertile bulls have a greater percentage of progressively motile sperm and a lower percentage of morphologically abnormal sperm than sub-fertile or infertile bulls (Williams and Savage, 1925; Lagerlof, 1934; Saacke, 1982; Barth and Oko, 1989).

Based on data resulting from a BSE, bulls may be classified as: a) a satisfactory potential breeder, b) an unsatisfactory potential breeder, or c) classification deferred. A bull must meet minimum criteria for scrotal circumference, sperm motility and morphology to be classified as a satisfactory potential breeder. These bulls must also be free of other problems (feet, legs, eyes, penile, preputial, accessory sex glands) that may reduce fertility in natural mating situations.

The classification of a bull as a satisfactory potential breeder ***does not guarantee*** the bull is currently a satisfactory breeder, or that the bull will be a satisfactory breeder in the future. The BSE outlines minimum standards that must be achieved, in addition to passing a physical exam. A BSE does not evaluate a bull's libido, a highly desirable and measurable trait. Libido is most often evaluated through the use of serving capacity tests that evaluate the number of services attempted by bulls during a limited period of time using restrained, non-estrual cows at predetermined bull:cow ratios (Blockey, 1976).

Bulls with physical abnormalities, and (or) those bulls not meeting the required minimum standards should be classified as an unsatisfactory potential breeder. Classification may be deferred at the discretion of the veterinarian for bulls that cannot be classified as satisfactory but may improve with time. This category includes young bulls with immature ejaculates and any bull with unacceptable sperm motility and morphology, considered to be temporary, and capable of improving. Sperm production (spermatogenesis) is a continuous, dynamic process that takes 60 days to complete. Consequently, physiologically stressful events (e.g., illness or environmental heat stress) may affect sperm motility and morphology for nearly two months after the event. Therefore, bulls that receive a "classification deferred" status on a BSE (due to poor sperm motility and morphology) should be re-tested in 60 days.

The importance of using BSE's cannot be overemphasized. In 2018, Roberts reported 82% of beef bulls from 2,883 BSE's conducted over 10 years (2007-2017) were classified as satisfactory potential breeders, with 15% deferred and 3% unsatisfactory. Carson and Wenzel (1995) evaluated 1,276 beef and dairy bulls and reported 37.1% received unsatisfactory (28.9%) or deferred (8.2%) classification. The main reasons cited for unsatisfactory or deferred classification were unacceptable sperm morphology, insufficient scrotal circumference, and physical problems, including eye lesions, lameness, and internal and external sex organ lesions. This gives evidence of the superiority of the BSE as compared to semen testing for the evaluation of breeding soundness.

Menegassi et al. (2011) reported a benefit/cost ratio of $19.67 for each $1 invested in the BSE. Similarly, Chenoweth (2002) argued a benefit/cost ratio of $20.00 for each $1 invested in the BSE. Using these values as a starting point, coupled with the realization that greater than 80% of

beef operations surveyed did not "semen test" bulls, let alone have a BSE performed on bulls used for natural service (NAHMS, 2009), the BSE is clearly a prudent, yet underappreciated and underutilized economic tool.

Conclusions

For many years, researchers have tried to develop techniques to predict fertility. Unfortunately, this has not been accomplished. Nevertheless, the quest to predict fertility has led to enhanced accuracy of fertility estimation by default, through identification of attributes associated with sub-fertile semen. Beef producers should remember the following points when attempting to maximize bull fertility:

- "Compensable" traits of semen quality relate to the ability of inseminated sperm to reach the ovum, bind to and penetrate the zona pellucida, and initiate the block to polyspermy.
- "Compensable" seminal deficiencies can be overcome or minimized by increasing the sperm dosage. (Reputable AI organizations adjust the AI dose when compensable seminal deficiencies are known.)
- "Uncompensable" traits of semen quality relate to the competence of fertilizing sperm to complete fertilization and sustain early embryonic development.
- "Uncompensable" seminal deficiencies result in suppressed fertility regardless of sperm dosage.
- Fertility following TAI with 10, 20, 20 or 40 x 10^6 sperm per dose resulted in similar P/TAI at first service in synchronized beef cattle. Fertility differences between bulls were detected, however, CASA and FC were not able to explain the differences in field fertility.
- A complete BSE (physical examination, scrotal circumference, and semen collection and evaluation) has a benefit/cost ratio of $20 for each $1 invested in the BSE.

Acknowledgements

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