

THE ROLE OF THE SIRE ON PREGNANCY SUCCESS OF A SYNCHRONIZED BREEDING PROGRAM

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Introduction

Artificial insemination (AI) is an effective strategy to improve the genetics and reproductive performance of a herd. Reputable commercial AI stud and custom semen collection businesses, through stringent collection, processing and quality control, provide a highly fertile product to their customers. Once transferred to the beef producer's liquid nitrogen semen storage tank, the maintenance of sire fertility is the responsibility of the owner, employees, and AI technicians. Overall, however, the role of the sire on pregnancy success of a synchronized breeding program is multi-faceted.

Semen Storage

In order to realize the maximal potential fertility within straws of frozen semen, the liquid nitrogen semen storage tank must be managed properly. The tank consists of a "tank within a tank," with insulation under vacuum between the inner and outer tanks. Liquid nitrogen semen storage tanks should be housed in a clean, dry area, and preferably on a wood stand to avoid possible corrosion (due to contact with wet or damp concrete). Be sure to securely fasten the tank during transportation to avoid tipping the tank over, and damaging the tank, both of which usually result in the premature loss of liquid nitrogen. Regardless of whether the liquid nitrogen semen storage tank is housed in an office or transported in a vehicle to a location closer to the cows to be serviced, a detailed inventory of semen should be easily accessible, so that straws may be located and removed from the tank quickly to avoid exposure of semen to ambient temperature.

When removing a straw from a liquid nitrogen semen storage tank, it is imperative that the technician keep the canister, cane and unused semen straws as low as possible in the neck of the tank. A best management practice is to keep all unused straws below the frost-line in the neck of the tank. Keep in mind that although the temperature of liquid nitrogen is -320°F , there is a temperature gradient in the neck of the tank. For example, a tank with a neck tube that measures 6 inches long may have a temperature of -103°F in the middle of the neck (3 inches below the top), while the temperature at 1 inch below the top may be $+5^{\circ}\text{F}$.

A recent study (Ahmadzadeh et al., 2022) provides evidence the temperature in the neck of a semen storage tank, especially in the area below the frost-line exhibits wide variation and is influenced by liquid nitrogen level. Berndtson et al. (1976) reported the liquid nitrogen level in a tank can dramatically affect the temperature of straws repeatedly raised and lowered in the tank. When a tank was full of liquid nitrogen, elevation of a cane (containing a goblet with 5 semen straws) into the neck of the tank for 1 minute resulted in a straw temperature increase of 28°F (from -320 to -292 ; Berndtson et al., 1976). It is important to recognize, however, that the increase in straw temperature was minimized as the goblet contained liquid nitrogen at the start of the exposure. Furthermore, the straw temperature reached -320°F within 1 minute, even after five repetitions (Berndtson et al., 1976).

When the liquid nitrogen level in the tank was low (~ 7 inches), Berndtson et al. (1976) reported the temperature of straws increased 131°F (from -320 to -189) during the first minute, and straw temperatures cooled only 86 to 104°F two minutes after returning to the tank. Consequently, higher temperatures were reached during the second, third, fourth, and fifth repetitions. Berndtson et al. (1976) reported greater than 10 minutes were required for the straw temperature to reach -320°F upon return after the fifth repetition. Consequently, another best management practice is to monitor the liquid nitrogen level in your tank regularly, and never let the level of liquid nitrogen go below 10 inches.

The ice pattern within bovine semen extender may change considerably (termed recrystallization) as it is warmed (Rapatz, 1966). Larger, extracellular ice crystals are associated with damage to sperm cell membranes and organelles (Stroud, 2016), and previous reports have shown that sperm injury (as judged by sperm motility) occurs at temperatures as low as -110°F (Etgen et al., 1957; Bean et al., 1963; DeJarnette, 1999).

When Berndtson et al. (1976) raised a 0.5-mL straw of semen into the neck of a tank using tweezers, the time necessary for the temperature to change from -320 to -148°F was 10 to 15 seconds. Therefore, to provide a margin of safety and avoid recrystallization injury to sperm in straws not removed from the same goblet or canister, the recommendation is for a cane (or canister containing canes) of frozen semen to be limited to eight seconds in the neck of a tank (below the frost-line) when retrieving straws (Berndtson et al., 1976; Saacke, 1978; Stroud, 2016).

Depending on the time required to remove a straw, other straws on the cane may be exposed to injurious high temperatures (Saacke, 1974). Carelessly working above the frost line will likely be particularly damaging to sperm even when applying the “eight second rule,” as straw temperatures will rapidly enter the danger zone (Saacke, 1978; Berndtson et al., 1976; Stroud, 2016). Injury to sperm cannot be corrected by returning semen to the liquid nitrogen (-320°F; Berndtson et al., 1976; Saacke, 1978); therefore, damage to sperm in straws not removed during improper retrieval of other straws is additive (Saacke, 1974; Berndtson et al., 1976; Stroud, 2016).

Semen Handling

Synchronization of estrus or ovulation and timed AI (TAI) protocols are becoming more common. Consequently, numerous cows must be inseminated on a given day. To facilitate AI in a timely manner, AI technicians routinely thaw multiple straws of semen simultaneously.

Oliveira et al. (2012) investigated the effect of sequence of insemination after simultaneous thawing of 10 straws of conventional semen on pregnancy per AI (P/AI) to TAI in suckled multiparous Nelore cows. Semen from 1 of 3 bulls resulted in decreased fertility for straws 7, 8, 9, and 10; however, fertility of the other 2 bulls was not different across all 10 straws. The results of extensive laboratory analyses of the semen failed to explain the observed decrease in fertility. Oliveira et al. (2012) concluded that sequence of insemination after simultaneous thawing of 10 straws of conventional semen differentially affected P/AI following TAI, depending on sire.

Dalton et al. (2004) conducted a field trial with Holstein dairy cattle to determine: 1) the effect of simultaneous thawing of multiple 0.5-mL straws of conventional semen and sequence of insemination (first, second, third or fourth) on P/AI, 2) whether P/AI achieved following AI by

professional technicians and herdsman-inseminators differed, and 3) the effect of elapsed time from initiation of thawing straws of conventional semen to seminal deposition on P/AI. Although the average P/AI differed between professional technicians and herdsman-inseminators (45% vs. 27%), simultaneous thawing and sequence of insemination (first, second, third or fourth), and elapsed time from initial thaw to completion of fourth AI had no effect on P/AI within inseminator group (Dalton et al., 2004).

Although the elapsed time from initial thaw to completion of fourth AI was shorter for professional technicians than for herdsman-inseminators (7.6 ± 0.22 vs. 10.9 ± 0.38 min; Dalton et al., 2004), the lower P/AI observed following AI by herdsman-inseminators was not likely due to an extended time factor. When 0.5-mL conventional semen straws were held at a constant temperature after thawing, Kaproth et al. (2002) reported no difference in mean progressive sperm motility at 5 and 20 min post-thaw. In contrast, Kaproth et al. (2002) reported a decrease in mean progressive sperm motility from 5 to 20 min post-thaw when 0.5-mL conventional semen straws were thawed at 95°F but held at 72°F. It is possible that failure to maintain straws at a constant temperature during AI gun assembly and transport to the cow is one of many contributing factors to the decreased fertility achieved by herdsman-inseminators.

In relation to sexed semen packaged in 0.25-mL straws, ABS Global (2009) reported decreased progressive sperm motility at 10 and 15 min post-thaw, when straws were held at either at 108°F or 40°F, simulating heat shock and cold shock conditions, respectively. Similar to conventional semen (Kaproth et al., 2002), ABS Global (2009) reported no difference in progressive sperm motility when sexed semen was held at constant temperature (98.6°F). Taken together, a reasonable strategy to maintain progressive sperm motility (and ultimately, fertility) is to maintain thermal protection of sexed semen straws and deposit semen in the uterus within approximately 5 minutes after thawing.

A general recommendation as to the number of straws that may be thawed simultaneously detracts from the overall importance of proper semen handling for successful AI. Fertility is likely to be maximized when personnel: a) accurately identify and administer the appropriate treatments to all cows and heifers to synchronize estrus or ovulation, b) accurately identify cows and heifers in estrus, c) follow the AI stud's recommendations for thawing semen, d) prevent direct straw-to-straw contact during thawing to avoid decreased post-thaw sperm viability as a result of straws freezing together (Brown et al., 1991), e) use appropriate hygienic procedures, f) maintain thermal protection of straws during AI gun assembly and transport to the cow, and g) deposit semen in the uterus within approximately 5 minutes (sexed semen) or 10-15 minutes (conventional semen) after thawing.

Site of Semen Deposition

Many studies have compared conventional semen deposition near the greater curvature of the uterine horns with deposition into the uterine body. Although Senger et al. (1988), López-Gatius (1996) and Pursley (2004) reported increased P/AI when conventional semen was deposited in the uterine horns rather than the uterine body, Hawk and Tanabe (1986), Williams et al. (1988), and McKenna et al. (1990) found no difference in fertility when comparing uterine body and uterine horn inseminations. Furthermore, Diskin et al. (2004) reported an inseminator and site of

conventional semen deposition effect (interaction), with evidence of either an increase, decrease, or no effect of uterine horn deposition on P/AI for individual inseminators.

In a competitive insemination study, Dalton et al. (1999) reported a slight advantage in accessory sperm number attributed to conventional semen deposition near the uterotubal junction compared with deposition into the uterine body. In Nelore cows, Meirelles et al. (2012), using conventional semen, reported increased fertility following deep intrauterine AI in the horn ipsilateral to the dominant follicle, as compared to seminal deposition in the uterine body. In contrast, Carvalho et al. (2013) reported deposition of conventional semen in the uterine horns failed to improve fertilization rates in superovulated Holstein cows. Lastly, there is no evidence that sexed semen deposition into the uterine horns enhances fertility as compared to deposition into the uterine body (Seidel et al. 1999; Seidel and Schenk, 2008).

A possible explanation for the positive effect of uterine horn inseminations in a few of the aforementioned studies may be related to the minimization or elimination of cervical semen deposition. Cervical insemination errors account for approximately 20% of attempted uterine body depositions (Peters et al., 1984). Macpherson (1968) reported that cervical insemination resulted in a 10% decrease in fertility when compared with deposition of semen in the uterine body.

Clearly, all AI technicians must develop sufficient skill to recognize when the tip of the AI gun remains in the cervix. To maximize fertility, AI technicians must continue to manipulate the reproductive tract until the tip of the AI gun is past the cervix and deposition into the uterus can be accomplished. The importance of retraining, however, cannot be minimized. King and Macpherson (1965) used excised reproductive tracts and AI guns with dye-filled straws and reported approximately 25% accuracy of technicians in placing the dye in the uterine body. After initial retraining, the accuracy of technicians increased to 67%. Retraining was continued every three months until 80 to 85% accuracy of dye placement in the uterine body was achieved (King and MacPherson, 1965). Lastly, a strong relationship between technician accuracy with dye placement and subsequent fertility has been reported (King and MacPherson, 1965; Cembrowics, 1964).

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.

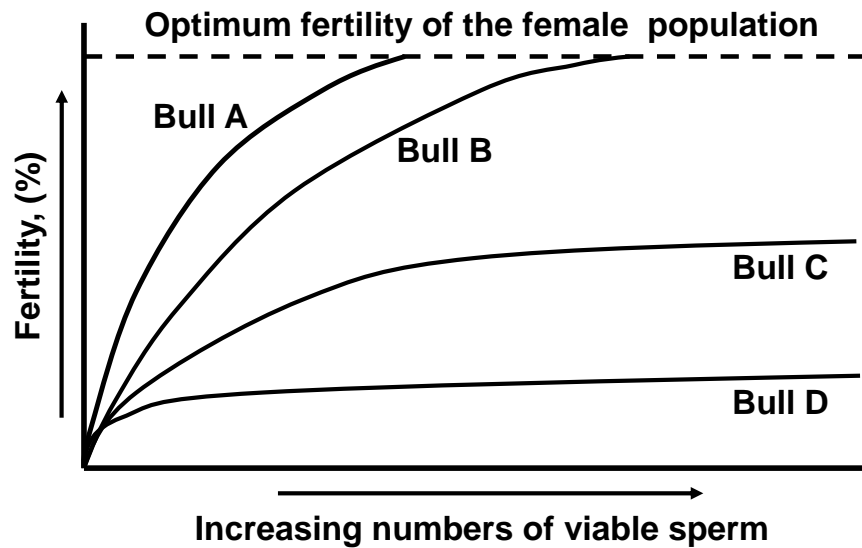


Figure 1: Relationship between number of viable sperm inseminated and fertility. The minimum number of viable sperm required for maximum fertility differs among bulls, as does the rate at which maximum fertility is achieved with increasing sperm dosage. Bulls A and B reach the threshold, whereas the maximal fertility of Bulls C and D is a function of uncompensable traits, and consequently, limited to below threshold (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 greater than 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 is thought to assist sperm release from the oviductal epithelium (Demott and Suarez, 1992; Suarez and Cho, 2003). Furthermore, hyperactivated motility, instead of progressive motility, is thought to be more important for penetration of the zona pellucida (Suarez and Dai, 1992; Suarez and Cho, 2003). 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 (Koziol and Armstrong, 2018).

Sperm Dosage and Fertility

The primary objective of a recent study (Menegatti Zoca et al., 2020) 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 10⁶ 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 10⁶ sperm respectively) (Menegatti Zoca et al., 2020). The P/TAI was different between bulls, 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). 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 10⁶ sperm per dose resulted in similar P/TAI at first service in synchronized beef cattle (Menegatti Zoca et al., 2020).

Next, Menegatti Zoca et al. (2020) 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, and 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., 2020). However, bulls with the highest fertility (A and B) did not display the highest values for total motility, progressive motility, and intact plasma membranes, intact acrosomes, and normal calcium influx, nor the lowest value for DNA fragmentation index. Bull D (intermediary fertility) showed the highest values for total motility, progressive motility, and 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 intermediary field fertility. In conclusion, CASA and FC were not able to explain the difference in field fertility between bulls (Menegatti Zoca et al., 2020).

Conclusions

For optimal reproductive efficiency, beef cows should produce a live calf each year. The role of the sire on pregnancy success of a synchronized breeding program is multi-faceted. To maximize fertility, beef producers should consider the following points:

- Proper semen storage and handling, including the delivery of sufficient numbers of viable sperm, is critical to a successful AI program.
- “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.
- Producers can minimize risk associated with uncompensable seminal deficiencies by
 - using semen from AI studs where sperm morphology is a routine part of the evaluation process
 - screening all natural service bulls with a complete breeding soundness evaluation, including sperm morphology.
- Fertility following TAI with 10, 20, 20 or 40 x 10⁶ 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.

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