MANAGEMENT AND INSEMINATION-RELATED FACTORS AFFECTING FERTILIZATION IN CATTLE

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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. When semen is purchased and transferred to the producer's liquid nitrogen tank, the maintenance of bull fertility is in the hands of the producer, farm and ranch employees, and AI technicians. Numerous management and insemination-related factors may affect fertilization in cattle including: a) semen storage, b) semen handling, site of semen deposition, and number of sperm deposited, c) semen quality, including "compensable" and "uncompensable" seminal traits, d) fertility associated antigen, e) accessory sperm number, f) time of AI, and g) timed AI.

Semen Storage

In order to realize the maximal potential fertility within straws of frozen semen, the liquid nitrogen tank must be managed properly. The liquid nitrogen tank consists of a "tank within a tank," with insulation under vacuum between the inner and outer tanks. Liquid nitrogen tanks should be stored 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 liquid nitrogen tank during transportation to avoid: a) tipping the tank over, and b) damaging the tank, both of which usually result in the premature loss of liquid nitrogen.

Regardless of whether the liquid nitrogen tank is stored 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 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°F. As would be expected, the temperature in the neck of the tank becomes warmer as the liquid nitrogen level in the tank decreases. Therefore, another best management practice is to monitor the liquid nitrogen level in your tank regularly, and fill the tank when appropriate.

Recrystallization, the transformation of small ice crystals in the extracellular fluid into larger ice crystals, occurs when the temperature changes from below -202°F to above -202°F to below -202 °F (Stroud, 2012). The larger ice crystals damage sperm cell membranes and organelles, the severity of which is dependent upon how high the temperature rises, and the duration of exposure, above -202°F (Stroud, 2012). Rapatz (1966) suggested that although

cell damage may occur at -202°F, the critical temperature may be closer to -148 to - 112°F. Indeed, 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). Furthermore, injury to sperm cannot be corrected by returning semen to liquid nitrogen (Berndtson et al., 1976; Saacke et al., 1978); therefore, damage from improper semen storage and removal from the tank is cumulative (Saacke et al., 1978).

Semen Handling

Labor efficient management strategies such as synchronization of estrus or ovulation and timed AI 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.

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 semen and sequence of insemination (1^{st}, 2^{nd}, 3^{rd} or 4^{th}) on conception rates, 2) whether conception rates achieved following AI by professional AI technicians (PAI) and herdsman-inseminators (HI) differed, and 3) the effect of elapsed time from initiation of thawing straws of semen to seminal deposition on conception rates. Although the average conception rate differed between PAI and HI (45% vs. 27%), simultaneous thawing and sequence of insemination (1^{st}, 2^{nd}, 3^{rd} or 4^{th}), and elapsed time from initial thaw to completion of fourth AI had no effect on conception rate within inseminator group (Dalton et al., 2004).

Although the elapsed time from initial thaw to completion of fourth AI was shorter for PAI than for HI (7.6 ± 0.22 vs. 10.9 ± 0.38 min; Dalton et al., 2004), the lower conception rate observed following AI by HI was not likely due to an extended time factor. When 0.5-mL semen straws were held at a constant temperature after thawing, Kaproth et al. (2002) reported no difference in mean progressive spermatozoal motility at 5 and 20 min post-thaw. In contrast, Kaproth et al. (2002) reported a decrease in mean progressive spermatozoal motility from 5 to 20 min post- thaw when 0.5-mL 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 conception rate achieved by HI.

Oliveira et al. (2012) investigated the effect of sequence of insemination after simultaneous thawing of 10 straws of semen on conception rate to timed AI 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 semen differentially affected conception rates following timed AI, depending on sire.

Critical to any discussion of semen handling is appropriate hygienic procedures. Bas et al. (2011) reported increased fertility (as measured by conception rate) in lactating dairy cows inseminated using a protective sheath covering the AI gun, compared to animals inseminated without a protective sheath (42.7% vs 36.1%, respectively).

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. Conception rates are most 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) utilize 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 of the cow within approximately 15 minutes after thawing.

Site of Semen Deposition

Many studies have compared semen deposition near the greater curvature of the uterine horns with conventional deposition into the uterine body. Although Senger et al. (1988), López-Gatius (1996) and Pursley (2004) reported increased conception rates when 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 semen deposition effect (interaction), with evidence of either an increase, decrease, or no effect of uterine horn deposition on conception rate for individual inseminators.

In a competitive insemination study, Dalton et al. (1999) reported a slight advantage in accessory sperm number attributed to semen deposition near the uterotubal junction compared with conventional deposition into the uterine body. In Nelore cows, Meirelles et al. (2012) reported increased fertility following deep intrauterine AI in the horn ipsilateral to the dominant follicle, as compared to conventional seminal deposition in the uterine body. Lastly, Carvalho et al. (2012) reported that deposition of semen in the uterine horns failed to improve fertilization rates in superovulated Holstein cows. Unfortunately, it is not clear why a few studies have shown a fertility advantage following uterine horn insemination while others have not.

A possible explanation for the positive effect of uterine horn inseminations 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).

Number of Sperm Deposited

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 numbers of sperm do not result in further increases in fertility. 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 that bulls differed in their maximal nonreturn rate, and in the rate at which they approached this maximum as sperm numbers per dose were increased (Figure 1). (Nonreturn rate, historically used by the dairy industry, is an indirect measure of fertility, specifically defined by Rycroft in 1992 "as the percentage of cows that are not rebred within a specified period of time after an insemination, typically 60 to 90 days.") Regarding semen quality, Pace et al. (1981) reported that fertility increases with increasing numbers of structurally intact and motile sperm.

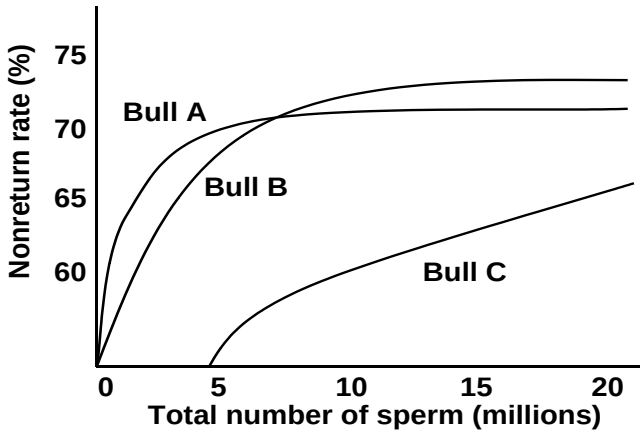

Figure 1. Relationship between nonreturn rate and the number of sperm inseminated. The semen of different bulls varies in the maximum nonreturn rate and in the rate at which the maximum fertility is achieved with increasing sperm dosage (Adapted from den Daas et al., 1998).

Sullivan and Elliot (1968) observed that low fertility bulls required more sperm in the inseminate than high fertility bulls in order to reach maximum fertility. Sullivan and Elliot (1968) postulated that more sperm were necessary due to the presence of abnormal sperm unable to gain access to the site of fertilization. As measured by accessory sperm trapped in the zona pellucida of embryos recovered 6 d after AI, the apparent inability of some abnormal sperm to gain access to the site of insemination was later shown to be true by Saacke 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 that 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.

DeJarnette et al. (1992) studied the effect of semen from bulls characterized as "average" or "below average" (as evaluated by the AI organization) based on percentage abnormal sperm. As shown in Figure 2, below average semen produced fewer excellent and good embryos and an increased number of degenerate embryos and unfertilized eggs when compared to semen of average quality.

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 40 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).

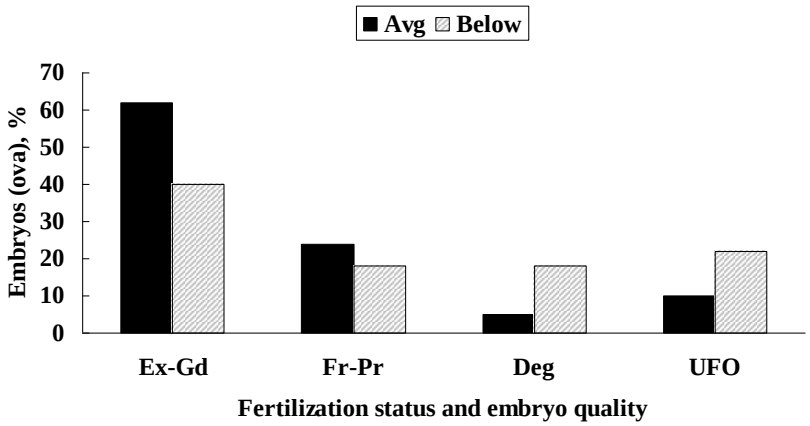

Figure 2. Effect of average and below average semen (based on percentage of abnormal sperm) on fertilization status and embryo quality in single-ovulating cattle. The shift in viable embryos (classified excellent to good and fair to poor) to degenerate and unfertilized caused by use of below average semen was significant.

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 sperm smears stained with toluidine blue and reported that 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 misshapened 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 misshapened 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 misshapened 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; Kolle et al., 2009). 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) by 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).

Fertility Associated Antigen

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 that 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 detectable fertility-associated antigen (FAA), a 31-kDa molecular weight protein on sperm, 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 to be used for AI. Bulls with sperm that were FAA-positive were 7 to 9 percentage points more fertile following first service AI than bulls producing sperm lacking FAA (Sprott et al., 2007).

To investigate whether presence of FAA could be used to evaluate the potential fertility of bulls to be used in 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, conducted at a commercial beef cattle ranch (Fazenda Anita, Mato Grosso do Sul, Brazil), suckled multiparous Nelore cows received TAI with frozen-thawed semen from one of the six bulls. Fertility (as measured by pregnancy per TAI) was not different between FAA-positive and FAA-negative bulls (41.5% vs. 39.3%, respectively). In Experiment 2, conducted at another commercial beef cattle ranch (Agropecuária Fazenda Brazil, Barra do Garças, Mato Grosso, Brazil), nulliparous Nelore heifers received TAI with frozen-thawed semen from one of the six bulls. Although fertility (pregnancy per 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 to be used in TAI.

Accessory Sperm Number

Accessory sperm quantification has been used to identify factors important to increasing the reproductive efficiency of cattle. In this procedure, embryos (ova) are recovered by uterine flush 6 d after AI. The fertilization rate is calculated, the morphological embryo quality grade is judged (Lindner and Wright, 1983) for morula-stage embryos, and the number of sperm trapped in the zona pellucida of each embryo (ova) is quantified following the procedure of DeJarnette et al. (1992). The number of accessory sperm in the zona pellucida has been positively associated with fertility in cattle (Hunter and Wilmut, 1984; Hawk and Tanabe, 1986; DeJarnette et al., 1992; Nadir et al., 1993; Cerri et al., 2009). Although accessory sperm are not directly involved in fertilization, they represent sperm able to access the oviduct, undergo capacitation, recognition, binding and the true acrosome reaction, and partially penetrate the zona pellucida. Accessory sperm are trapped in the zona pellucida by the "zona reaction," a functional block to polyspermy that occurs immediately following fertilization by the fertilizing sperm. Thus, accessory sperm are thought to be an indirect measure of sperm transport, and a quantitative measure of sperm available and competing for fertilization (DeJarnette et al., 1992).

Across several years of studies (using semen from nearly 30 bulls and 927 embryos (ova) recovered 6 d after AI), the relationship between median accessory sperm number, fertilization status, and embryo quality is clear (Table 1). Excellent and good embryos have more accessory sperm, as compared to fair and poor, degenerate, and unfertilized ova. The association of increased embryo quality and increased accessory sperm numbers is likely due to greater competition among potential fertilizing sperm at the time of fertilization. Howard et al. (1993)

described sperm selection by the zona pellucida, providing evidence that competition favors a more competent sperm. It should be clear from Table 1 that there is large variation in accessory sperm numbers within and across fertilization status and embryo quality categories. Consequently, this variation precludes the use of accessory sperm numbers as predictors of bull fertility. Nevertheless, the quest to increase accessory sperm numbers may help to develop future reproductive strategies to increase fertility.

Table 1. Relationship of accessory sperm per embryo (ovum) to fertilization status and embryo quality.

Fertilization status and embryo quality[1]	n	Mean ± SD	Median
Excellent and good	449	24.5 ± 44.1	7
Fair and poor	213	17.2 ± 32.2	5
Degenerate	80	13.5 ± 38.1	1
Degenerate/UFO	12	2.7 ± 5.7	0.5
Unfertilized	173	1.6 ± 16.5	0

[1]Embryo quality based on Linder and Wright (1983) as modified for degenerate embryos by DeJarnette et al. (1992).

Numerous studies seeking to increase accessory sperm numbers have been conducted (Table 2; see Saacke et al., 2000, for a review). In this paper we will focus on time of AI relative to ovulation.

Table 2. Summary of efforts to raise accessory sperm.

Effort	Outcome	Reference
Block retrograde sperm loss	No effect	DeJarnette et al. (1992)
Microencapsulation	Negative	Munkittrick et al. (1992)
Frozen vs. Fresh semen	No effect	Nadir et al. (1993)
Semen dosage	Positive	Nadir et al. (1993)
Select male	Positive	Nadir et al. (1993)
Extender (milk vs. egg yolk-citrate)	No effect	Dalton et al. (1994)
Seminal plasma	No effect	Nadir et al. (1995)
Site of insemination	Positive	Dalton et al. (1999)
Time of insemination	Positive	Dalton et al. (2001)

Time of AI

Dalton et al. (2001) reported on an experiment to determine the effect of insemination time on accessory sperm number per embryo (ovum), fertilization status, and embryo quality in single-ovulating dairy cows. All cows were continuously monitored for behavioral estrus by HeatWatch (CowChips, LLC, Denver, CO). HeatWatch uses radio frequency data communications, as previously described by Dransfield et al. (1998).

Previous work in dairy cattle revealed that ovulation occurs 27.6 ± 5.4 h after the first standing event for both natural estrus and prostaglandin-induced estrus (Walker et al., 1996), and between 24 to 32 h after the second GnRH injection in the Ovsynch protocol (Pursley et al., 1995). In our experiment, all cows received AI with one 0.5-mL straw (25×10^6 sperm) with semen from one of three bulls at 0, 12, or 24 h after the onset of estrus. Due to the logistics of monitoring the computer every 3 h and cow retrieval from pasture, actual times of insemination (mean ± SD) after the onset of estrus were 2.0 ± 0.9 h, 12.1 ± 0.6 h, and 24.2 ± 0.7 h for the 0, 12 and 24 h AI treatments respectively. Median accessory sperm values were greatest in embryos recovered following the 24-h AI treatment (Table 3). The fertilization rate was also greatest following the 24-h AI treatment (Table 3). Embryo quality declined with increasing intervals after the onset of estrus, from high quality embryos (0-h AI) to low quality embryos (24-h AI) (Figure 3).

Table 3. Effect of insemination time on accessory sperm per embryo (ovum)

and fertilization rate of recovered embryos (ova)[1].

		Accessory sperm per embryo (ovum)		
Treatment	n[2]	Mean ± SD	Median	Fertilization rate, %
0-h AI	39	9.5 ± 23.1	1	66
12-h AI	39	21.2 ± 46.2	2	74
24-h AI	39	33.0 ± 52.7	4	82

[1]Adapted from Dalton et al. (2001).
[2]Number of embryos (ova) recovered.

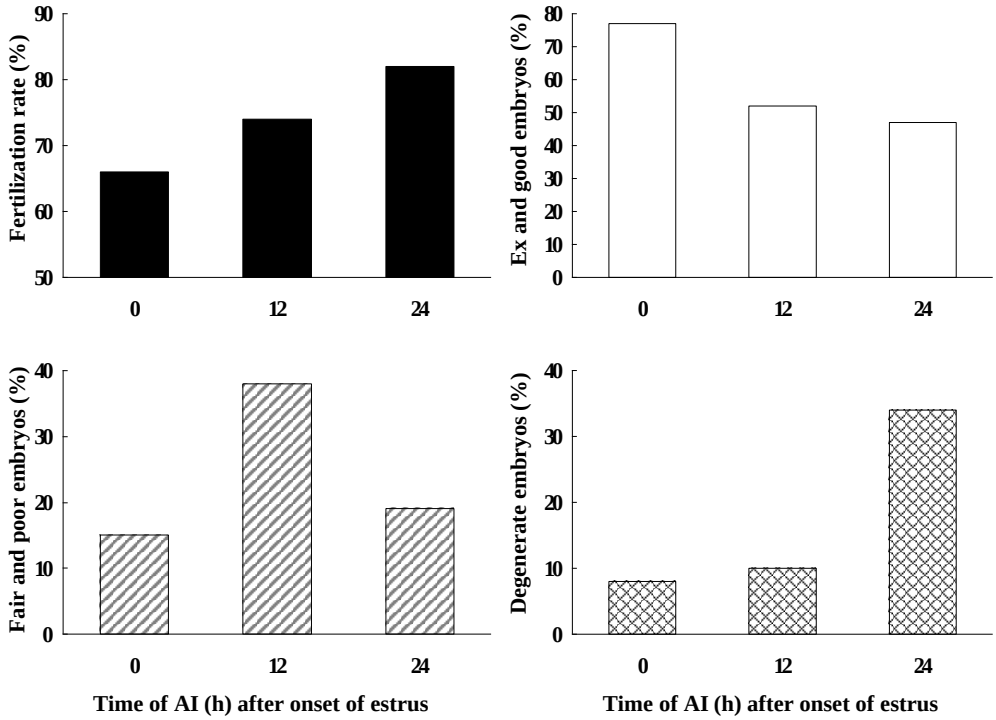

Figure 3. Effect of time of AI after onset of estrus (as determined by the first standing event in cattle continuously monitored by HeatWatch®) on fertilization status and embryo quality. (Adapted from Dalton et al., 2001).

Consequently, AI at 12 h after onset of estrus provides a compromise between the potentially lower fertilization rate of 0-h AI and the lowered embryo quality (due to increased degenerate embryos) of 24-h AI (Figure 4). From these data, conception rates would be expected to be optimized following the 12-h AI (Figure 4). This agrees with Dransfield et al. (1998), in which the optimal time of AI for dairy cows identified in estrus by HeatWatch® was 4 to 16 h after the onset of estrus, based on conception rates determined by palpation between 35 and 75 d after AI. In our study, embryo quality at the late insemination may be impaired due to an aging ovum at the time of fertilization. In this scenario, 24-h AI would result in sperm reaching the site of fertilization at 30 + h after the onset of estrus, accounting for the time required for sustained sperm transport (6 to 12 h; Hawk, 1987; Hunter and Wilmut, 1983; Wilmut and Hunter, 1984). Consequently, fertilization of an aging ovum would occur, likely leading to lower embryo quality. The improved embryo quality associated with 0-h AI (Figure 3) suggests that the duration of sperm residence in the female reproductive tract may allow further selection pressure favoring competent sperm, thus optimizing embryo quality at early insemination. The high proportion of excellent and good embryos resulting from 0-h AI would be expected to establish pregnancies.

In an effort to determine the optimum time of AI in beef heifers, Dorsey et al. (2011) conducted a retrospective study of estrous synchronized beef heifers monitored for estrus by HeatWatch. Insemination criteria for the four experiments that comprised the retrospective study included once daily AI based on estrus, am/pm rule based on estrus, and fixed time AI. To analyze the effect of time of AI on fertility, Dorsey et al. (2011) assigned heifers to a 4-h time block based on time from onset of estrus (0 h) to insemination: 0–4, 4–8, 8–12, 12–16, 16–20, 20–24, and >24 h. Pregnancies generated in the 4–24 h onset of estrus to AI interval group (63.7%) were greater than the 0–4 h group (48.1%) and >24 h group (55.9%).

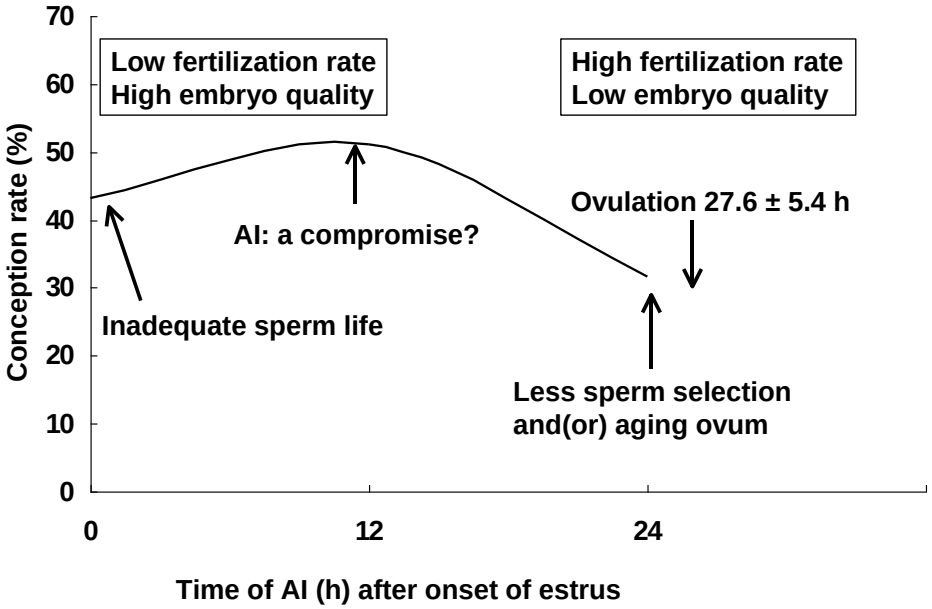

Figure 4. Artificial insemination at 12 h after onset of estrus in dairy cows appears to be a compromise between the low fertilization rate and high embryo quality of early inseminations and the high fertilization rate and low embryo quality of late inseminations. (Adaptation of data from Dransfield et al., 1998, and Dalton et al., 2001, originally published by Saacke et al., 2000).

The greater fertility to later insemination in beef heifers as reported by Dorsey et al. (2011), compared to dairy cows, may be related to a difference in time of ovulation in dairy and beef cattle (31.1 ± 0.6 h for beef heifers or cows; Stevenson et al., 1996; White et al., 2002; 27.6 ± 5.4 h in dairy cows; Walker et al., 1996).

Timed AI

In the past 15 years, numerous systematic breeding protocols have become available to the cattle producer, many of which incorporate timed AI (TAI). A common question asked by cattle producers is: Are there bull fertility differences following TAI? The simple answer is "*perhaps,"* depending on the bull and the protocol used. Critical to the discussion of potential sire fertility differences following TAI is the classic work of Macmillan and Watson (1975) who investigated the effect of the stage of estrus at the time of AI on non-return rates of above average, average, and below average fertility bulls. As shown in Figure 5, the high non-return rate following early AI among above average fertility bulls (as compared to average and below average fertility bulls) gives evidence that fertility may be associated with sperm longevity in the female reproductive tract. Consequently, TAI may magnify differences in fertility as the time interval from AI to ovulation increases. Alternatively, the magnitude of difference in fertility among bulls might be expected to be minimized when the synchronization protocol precisely controls ovulation and TAI within a distinct, although as yet unknown, "optimal interval."

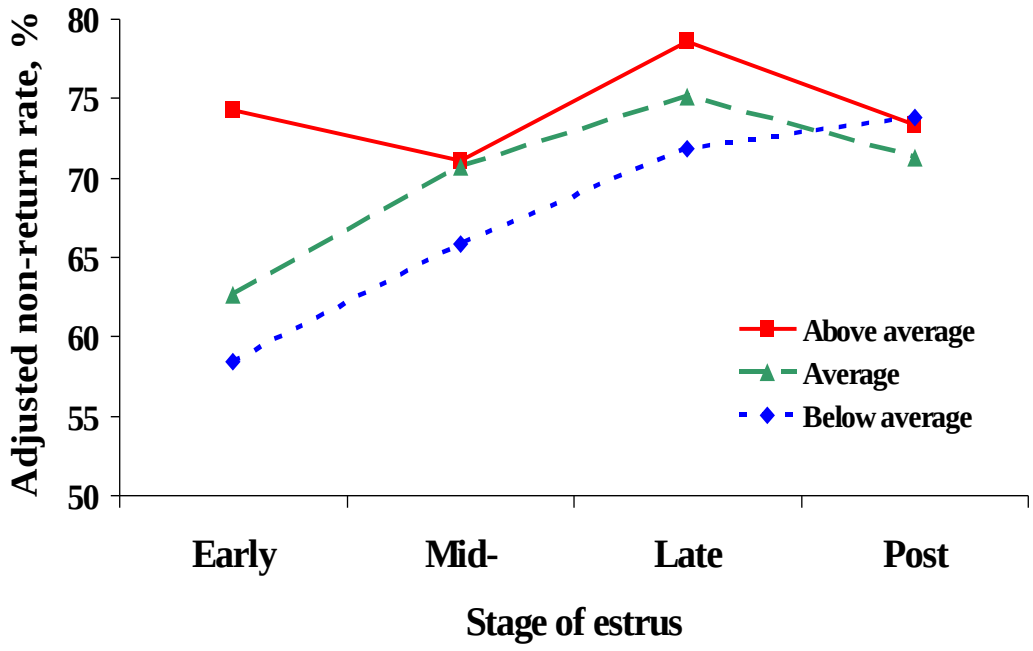

Figure 5. The effect of bull fertility group and stage of estrus at AI on adjusted non-return rates. (Adapted from MacMillan and Watson, 1975).

Practically speaking, what can a cattle producer do to manage potential sire fertility differences following TAI? First, all producers should acquire semen from reputable AI studs and custom collection businesses, as it is widely known that processing semen for cryopreservation can influence fertility, as judged by percentage motility and intact

acrosomes post-thaw (Ennen et al., 1976; Robbins et al., 1976). Furthermore, as mentioned previously, semen storage, semen handling, and site of semen deposition are critical factors that can be easily managed on the farm or ranch. Lastly, choice of a estrous synchronization and TAI protocol, and compliance (the correct drug and dosage, at the correct time and day, to the correct animal), may play a role in sire fertility, especially in bulls requiring the precise control of follicular development and ovulation to minimize the effect of a short duration of sperm longevity.

Recent research provides evidence that delayed insemination of non-estrous beef heifers in a TAI protocol yields a higher pregnancy per TAI than heifers inseminated at fixed time regardless of expression of estrous (Thomas et al., 2014). Sixteen days after a 14-d CIDR regimen (on d 30) all heifers received prostaglandin and an estrous detection aid to facilitate detection of estrus. On d 33, GnRH was administered and estrous expression was recorded. Heifers received either: 1) TAI (concurrent with GnRH administration 66 h after prostaglandin) regardless of estrous expression, or 2a) TAI (concurrent with GnRH administration 66 h after prostaglandin) for heifers expressing estrus, or 2b) delayed AI 20 h after GnRH for heifers failing to express estrus.

Overall, the pregnancy per TAI was higher for heifers in the delayed insemination or "split-time" treatment compared with those receiving TAI regardless of estrous expression (54 vs 46%, respectively; Thomas et al., 2014). The increase in pregnancy per TAI is thought to be due to the delayed AI of non-estrous heifers in the second treatment, as pregnancy per TAI for non-estrous heifers was higher than non-estrous heifers in the first treatment (49 vs 34%, respectively; Thomas et al., 2014).

The mechanism by which delaying inseminations of non-estrous beef heifers leads to increased fertility is not known; however, Thomas et al. (2014) theorize that waiting 20 h after GnRH administration to AI may: 1) align the lifespan of capacitated sperm with the timing of ovulation, and (or) 2) allow further time for initiation of estrus among a larger percentage of heifers, resulting in a more favorable uterine environment in estrual heifers. In a study in which beef cows were synchronized with a 7d CO-Synch + CIDR protocol, however, no increase in fertility was observed using a similar delayed insemination strategy (Thomas et al., 2014).

Conclusions

For optimal reproductive efficiency, beef cows should produce a live calf each year. Consequently, to maximize fertility, beef producers should consider the following points:

- Proper semen 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.
- Ovulation occurs approximately 28 h after the first standing event in dairy cows and 31 h after the first standing event in beef cows and heifers.
- Sustained sperm transport requires 6 to 12 h; therefore, time of AI should occur close enough to ovulation to maximize sperm access to the ovum, but not too late to have an aging ovum awaiting sperm arrival at the site of fertilization in the oviduct.

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References

Acevedo, N., J. Bame, L.A. Kuehn, W.D. Hohenboken, D.P. Evenson, and R.G. Saacke. 2002. In: Proc. Nat'l. Assoc. Anim. Breeders 19th Tech. Conf. Artif. Insem. and Reprod., Columbia, MO, pp.84-90.

Ballachey, B.E., D.P. Evenson, and R.G. Saacke. 1988. The sperm chromatin structure assay: relationship with alternate tests of semen quality and heterospermic performance of bulls. J. Androl. 9:109-115.

Bas, S., A. Hoet, P. Rajala-Schultz, D. Sanders, and G.M. Schuenemann. 2011. The use of plastic cover sheaths at the time of artificial insemination improved fertility of lactating dairy cows. J. Dairy Sci. 94:793–799.

Bean, B.H., B.W. Pickett, and R.C. Martig. 1963. Influence of freezing methods, extenders and storage temperatures on motility and pH of frozen bovine semen. J. Dairy Sci. 46:145.

Beletti, M.E., L.F. Costa, and M.M. Guardieiro. 2005. Morphometric features and chromatin condensation abnormalities evaluated by toluidine blue staining in bull spermatozoa. Braz. J. Morphol. Sci. 22(2):85-90.

Bellin, M.E., H.E. Hawkins, and R.L. Ax. 1994. Fertility of range beef bulls grouped according to presence or absence of heparin-binding proteins in sperm membranes and seminal fluid. J. Anim. Sci. 72:2441-2448.

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.

Bellin, M.E., J.N. Oyarzo, H.E. Hawkins, H. Zhang, R.G. Smith, D.W. Forrest, L.R. Sprott, and R.L. Ax. 1998. Fertility-associated antigen on bull sperm indicates fertility potential. J. Anim. Sci. 76:2032−2039.

Berndtson, W.E., B.W. Pickett, and C.D. Rugg. 1976. Procedures for field handling of bovine semen in plastic straws. In: Proc. Nat'l. Assoc. Anim. Breeders 6th Tech. Conf. on Artif. Insem. and Reprod., Columbia, MO, p. 51.

Brown, D.W. Jr., P.L. Senger, and W.C. Becker, 1991. Effect of group thawing on post-thaw viability of bovine spermatozoa packaged in .5-milliliter French straws. J. Anim. Sci. 69:2303-2309.

Carvalho, P.D., A.H. Souza, A.R. Dresch, L.M. Vieira, K.S. Hackbart, D. Luchini, S. Bertics, N. Betzold, M.C. Wiltbank, and R.D. Shaver. 2012. Placement of semen in uterine horns failed to improve fertilization rates in superovulated Holstein cows. J. Dairy Sci. 95: (Suppl. 2): 575 (Abstr.).

Cembrowics, H.J. 1964.Efficiency of inseminators. In: Proc.5th Int. Congr. Anim. Reprod. Artif. Insem. 4:624.

Cerri, R.L.A., H.M. Rutigliano, R.C. Chebel, and J.E.P. Santos. 2009. Period of dominance of the ovulatory follicle influences embryo quality in lactating dairy cows. Reproduction 137: 813-823.

Dalton, J.C., S. Nadir, S. Degelos, J. Bame, and R.G. Saacke. 1994. Effect of cream added to the inseminate on accessory sperm number in cattle. J. Anim. Sci. 72:(Suppl. 1):172 (Abstr.).

Dalton, J.C., S. Nadir, J.H. Bame, and R.G. Saacke. 1999. Effect of a deep uterine insemination on spermatozoal accessibility to the ovum in cattle. Theriogenology 51:5:883-890.

Dalton, J.C., S. Nadir, J.H., Bame, M. Noftsinger, R.L. Nebel, and R.G., Saacke. 2001. Effect of time of insemination on number of accessory sperm, fertilization rate, and embryo quality in nonlactating dairy cattle. J. Dairy Sci. 84:2413-2418.

Dalton, J.C., A. Ahmadzadeh, B. Shafii, W.J. Price, and J.M. DeJarnette. 2004. Effect of thawing multiple 0.5-mL semen straws and sequential insemination number on conception rates in dairy cattle. J. Dairy Sci. 87:972-975.

Dalton, J.C., L. Deragon, J.L.M. Vasconcelos, C.N. Lopes, R.F.G. Peres, and A. Ahmadzadeh. 2012. Fertility-associated antigen on Nelore bull sperm and reproductive outcomes following first-service fixed-time AI of Nelore cows and heifers. Theriogenology 77:389–394.

DeJarnette, J.M., R.G. Saacke, J. Bame, and C.J. Vogler. 1992. Accessory sperm: Their importance to fertility and embryo quality, and attempts to alter their numbers in artificially inseminated cattle. J. Anim. Sci. 70:484-491.

DeJarnette, J.M. 1999. Factors affecting the quality of frozen semen after thawing. In: Proc. Soc. for Therio. Ann. Conf., Nashville, TN, pp. 267-279.

Demott, R.P., and S.S. Suarez. 1992. Hyperactivated sperm progress in the mouse oviduct. Biol Reprod. 46(5):779–785.

den Daas, J.H.G., G. DeJong, 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.

Diskin, M.G., J.R. Pursley, D.A. Kenny, J.F. Mee, and J.M. Sreenan. 2004. The effect of deep intrauterine placement of semen on conception rate in dairy cows. J. Dairy Sci. 87:(Suppl. 1):257 (Abstr.).

Dorsey, B.R., R. Kasimanickam, W.D. Whittier, R.L. Nebel, M.L. Wahlberg, and J.B. Hall. 2011. Effect of time from estrus to AI on pregnancy rates in estrous synchronized beef heifers. Anim. Reprod. Sci. 127:1-6.

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.

Dresdner, R.D., and D.F. Katz. 1981. Relationship of mammalian sperm motility and morphology to hydrodynamic aspects of cell function. Biol. Reprod. 25:920-930.

Etgen, W.M., J.M. Ludwick, H.E. Rickard, E.A. Hess, and F. Ely. 1957. Use of mechanical refrigeration in preservation of bull semen. J. Dairy Sci. 40:774.

Ennen, B.D., W.E. Berndtson, R.G. Mortimer, and B.W. Pickett. 1976. Effect of processing procedures on motility of bovine spermatozoa frozen in 0.25-mL straws. J. Anim. Sci. 43:651–656.

Evenson, D.P., Z. Darznikiewicz, and M.R. Melamed. 1980. Relation of mammalian sperm chromatin heterogeneity to fertility. Science 240:1131-1134.

Gledhill, B.L. 1970. Enigma of spermatozoal DNA and male infertility: A review. Am. J. Vet. Res. 31:539-549.

Hawk, H.W. 1987. Transport and fate of spermatozoa after insemination of cattle. J. Dairy Sci. 70:1487-1503.

Hawk, H.W., and T.Y. Tanabe. 1986. Effect of unilateral cornual insemination upon fertilization rate in superovulating and single-ovulating cattle. J. Anim. Sci. 63:551-560.

Hopkins, F.M., and J.C. Spitzer. 1997. The new Society for Theriogenology breeding soundness evaluation system. Vet. Clin. North Am.: Food Anim. Pract. 2:13:283-293.

Howard, J.G., A.M. Donoghue, L.A. Johnston, and D.E. Wildt. 1993. Zona pellucida filtration of structurally abnormal spermatozoa and reduced fertilization in teratospermic cats. Biol. Reprod. 49:131-139.

Hunter, R.H.F., and I. Wilmut. 1984. Sperm transport in the cow: peri-ovulatory redistribution of viable cells within the oviduct. Reprod. Nutr. Develop. 24:597-608.

Hunter, R.H.F., and I. Wilmut. 1983. The rate of functional sperm transport into the oviducts of mated cows. Anim. Reprod. Sci. 5:167-173.

Kaproth, M.T., J.E. Parks, G.C. Grambo, H.E. Rycroft, and J.A. Hertl. 2002. Effect of preparing and loading multiple insemination guns on conception rate in two large commercial dairy herds. Theriogenology 57:909–921.

Kasimanickam, R., R.L. Nebel, I.D. Peeler, W.L. Silvia, K.T. Wolfe, A.J. McAllister, and B.G. Cassell. 2006. Breed differences in competitive indices of Holstein and Jersey bulls and their association with sperm DNA fragmentation index and plasma membrane integrity. Theriogenology. 66:1307-1315.

Killian, G.J., D.A. Chapman, and L.A. Rogowski. 1993. Fertility-associated proteins in Holstein bulls. Biol. Reprod. 49:1202-1208.

King, J., and J.W. Macpherson. 1965. Observations on retraining of artificial insemination technicians and its importance in maintaining efficiency. Can. Vet. Jour. 6(4):83-85.

Kolle, S., S. Dubielzig, S. Reese, A. Wehrend, P. Konig, and W. Kummer. 2009. Ciliary transport, gamete interaction, and effects of the early embryo in the oviduct: Ex-vivo analyses using a new digital videomicroscopic system in the cow. Biol. Reprod. 81:2:267-274.

Lindner, G.M., and R.W. Wright. 1983. Bovine embryo morphology and evaluation. Theriogenology 20:407-416.

López-Gatius, F. 1996. Side of gestation in dairy heifers affects subsequent sperm transport and pregnancy rates after deep insemination into one uterine horn. Theriogenology 45:417-425.

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.

Macpherson, J.W. 1968. Semen placement effects on fertility in bovines. J. Dairy Sci. 51:807-808.

McKenna, T., R.W. Lenz, S.E. Fenton, and R.L. Ax. 1990. Nonreturn rates of dairy cattle following uterine body or cornual insemination. J. Dairy Sci. 73:1779-1783.

Meirelles, C., L.E. Kozicki, R.R. Weiss, M.S. Segui, A. Souza, I. W. dos Santos, and J.C. dos Santos Breda. 2012. Comparison between deep intracornual artificial insemination (DIAI) and conventional artificial insemination (AI) using low concentration of spermatozoa in beef cattle. Braz. Arch. Biol. Technol. 55(3):371-374.

Miller, D.J., M.A. Winer, and R.L. Ax. 1990. Heparin-binding proteins from seminal plasma bind to bovine spermatozoa and modulate capacitation by heparin. Biol. Reprod. 42:899−915.

Munkittrick, T.W., R.L. Nebel, and R.G. Saacke. 1992. Accessory sperm numbers for cattle inseminated with protamine sulfate microcapsules. J. Dairy Sci. 75:725-731.

Nadir, S., R.G. Saacke, J. Bame, J. Mullins, and S. Degelos. 1993. Effect of freezing semen and dosage of sperm on number of accessory sperm, fertility, and embryo quality in artificially inseminated cattle. J. Anim. Sci. 71:199-204.

Nadir, S., J.C. Dalton, S.D. Degelos, J. Bame, and R.G. Saacke. 1995. Accessory sperm number in artificially inseminated cattle using cauda epididymal sperm cryopreserved in the presence and absence of a normal complement of seminal plasma. J. Dairy Sci. 78:(Suppl. 1):303 (Abstr.).

Nass, S.J., D.J. Miller, M.A. Winer, and R.L. Ax. 1990. Male accessory sex glands produce heparin-binding proteins that bind to caudal epididymal spermatozoa and are testosterone dependent. Mol. Reprod. Dev. 25:237-246.

Oliviera, L.Z., R.P. Arruda, A.F.C. de Andrade, R.M. Santos, M.E. Beletti, R.F.G. Peres, J.P.N. Martins, and V.F.M. Hossepian de Lima. 2012. Effect of sequence of insemination after simultaneous thawing of multiple semen straws on conception rate to timed AI in suckled multiparous Nelore cows. Theriogenology 78:1800–1813.

Ostermeier, G.C., G.A. Sargeant, B.S. Yandell, D.P. Evenson, and J.J. Parrish. 2001. Relationship of bull fertility to sperm nuclear shape. J. Androl. 22:4:595-603.

Pace, M.M., J.J. Sullivan, F.I. Elliott, E.F. Graham, and G.H. Coulter. 1981. Effects of thawing temperature, number of spermatozoa and spermatozoal quality on fertility of bovine spermatozoa packaged in 0.5-mL french straws. J. Anim. Sci. 53:693-701.

Peters, J.L., P.L. Senger, J.L. Rosenberger, and M.L. O'Connor. 1984. Radiographic evaluation of bovine artificial inseminating technique among professional and herdsman-inseminators using .5- and .25-mL French straws. J. Anim. Sci. 59:1671-1683.

Pursley, J.R., M.O. Mee, and M.C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using $PGF_{2\alpha}$ and GnRH. Theriogenology 44:915–923.

Pursley, J.R. 2004. Deep uterine horn AI improves fertility of lactating dairy cows. J. Dairy Sci. 87: (Suppl. 1):372 (Abstr.).

Rapatz, G.L. 1966. What happens when semen is frozen? In: Proc. Nat'l. Assoc. Anim. Breeders 1st Tech. Conf. Artif. Insem. and Reprod., Columbia, MO, p.45.

Robbins, R.K., R.G. Saacke, and P.T. Chandler. 1976. Influence of freeze rate, thaw rate and glycerol level on acrosomal retention and survival of bovine spermatozoa frozen in French straws. J. Anim. Sci. 42:145-154.

Rycroft, H. 1992. Factors influencing non-return data. In: Proc. Nat'l. Assoc. Anim. Breeders 14th Tech. Conf. Artif. Insem. and Reprod., Columbia, MO, pp.43-46.

Saacke, R.G., J.A. Lineweaver, and E.P. Aalseth. 1978. Procedures for handling frozen semen. In: Proc. 12th Conf. AI of Beef Cattle, Nat'l Assoc Anim Breeders, Columbia. MO, pp. 46-61.

Saacke, R.G., W.E. Vinson, M.L. O'Connor, J.E. Chandler, J. Mullins, and R.P. Amann. 1980. The relationship of semen quality and fertility: A heterospermic study. In: Proc. Nat'l. Assoc. Anim. Breeders 8th Tech. Conf. Artif. Insem. and Reprod., Columbia, MO, pp. 71–78.

Saacke, R.G., S. Nadir, and R.L. Nebel. 1994. Relationship of semen quality to sperm transport, fertilization, and embryo quality in ruminants. Theriogenology 41:45-50.

Saacke R.G., J.M. DeJarnette, J.H. Bame, D.S. Karabinus, and S.S. Whitman. 1998. Can spermatozoa with abnormal heads gain access to the ovum in artificially inseminated super- and single-ovulating cattle? Theriogenology 50:117-128.

Saacke, R.G., J.C. Dalton, S. Nadir, R.L. Nebel, and J.H. Bame. 2000. Relationship of seminal traits and insemination time to fertilization rate and embryo quality. Anim. Reprod. Sci. 60-61:663-677.

Salisbury, G.W., and N.L. VanDemark. 1961. In: Physiology of Reproduction and Artificial Insemination of Cattle, p. 361. W.H. Freeman and Company, San Francisco, CA.

Senger, P.L., W.C. Becker, S.T. Davidge, J.K. Hillers, and J.J. Reeves. 1988. Influence of corneal insemination on conception in dairy cattle. J. Anim. Sci. 66:3010-3016.

Sprott, L.R., M.D. Harris, D.W. Forrest, J. Young, H.M. Zhang, J.N. Oyarzo, M.E. Bellin, and R.L. Ax. 2000. Artificial insemination outcomes in beef females using bovine sperm with a detectable fertility-associated antigen. J. Anim. Sci. 78:795-798.

Stevenson, J.S., M.W. Smith, J.R. Jaeger, L.R. Corah, and D.G. LeFever. 1996. Detection of estrus by visual observation and radiotelemetry in peripubertal, estrus synchronized beef heifers. J. Anim. Sci. 74:729–735.

Stroud, B. 2012. Consequences of mishandling frozen semen and embryos. In: Proc. Appl. Reprod. Strat. Beef Cattle, Sioux Falls, SD, pp. 191-203.

Suarez, S.S., and X. Dai. 1992. Hyperactivation enhances mouse sperm capacity for penetrating viscoelastic media. Biol. Reprod. 46:686-691.

Sullivan, J.J., and F.I. Elliott. 1968. Bull fertility as affected by an interaction between motile spermatozoa concentration and fertility level in artificial insemination. VI Int'l. Cong. Anim. Reprod. Artif. Insem. 2:1307.

Thomas, J.M., S.E. Poock, M.R. Ellersieck, M.F. Smith, and D.J. Patterson. 2014. Delayed insemination of non-estrous heifers and cows when using conventional semen in timed artificial insemination. J. Anim. Sci. 92:4189-4197.

Walker, W.L., R.L. Nebel, and M.L. McGilliard. 1996. Time of ovulation relative to mounting activity in dairy cattle. J. Dairy Sci. 79:1555-1561.

White, F.J., R.P. Wettemann, M.L. Looper, T.M. Prado, G.L. Morgan. 2002. Seasonal effects on estrous behavior and time of ovulation in nonlactating beef cows. J. Anim. Sci. 80:3053–3059.

Williams, B.L., F.C. Gwazdauskas, W.D. Whittier, R.E. Pearson, and R.L. Nebel. 1988. Impact of site of inseminate deposition and environmental factors that influence reproduction of dairy cattle. J. Dairy Sci. 71:2278-2283.

Wilmut, I. and R.H.F. Hunter. 1984. Sperm transport into the oviducts of heifers mated early in oestrus. Reprod. Nutr. Develop. 24:461-465.