

# **INSEMINATION RELATED FACTORS AFFECTING FERTILIZATION IN ESTROUS-SYNCHRONIZED CATTLE**

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## **Introduction**

Numerous insemination related factors may affect fertilization in estrous-synchronized cattle including: a) semen handling, site of semen deposition, and number of sperm deposited, b) semen quality, including “compensable” and “uncompensable” seminal traits, c) fertility associated antigen, d) accessory sperm number, e) time of AI, and f) timed AI.

## **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<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> or 4<sup>th</sup>) 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<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> or 4<sup>th</sup>), and elapsed time from initial thaw to completion of fourth AI had no effect on conception rate within inseminator group (Dalton et al., 2004).

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 to synchronize estrus or ovulation, b) accurately identify cows 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 retraining, the accuracy of technicians increased to 67%.

### **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.

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).

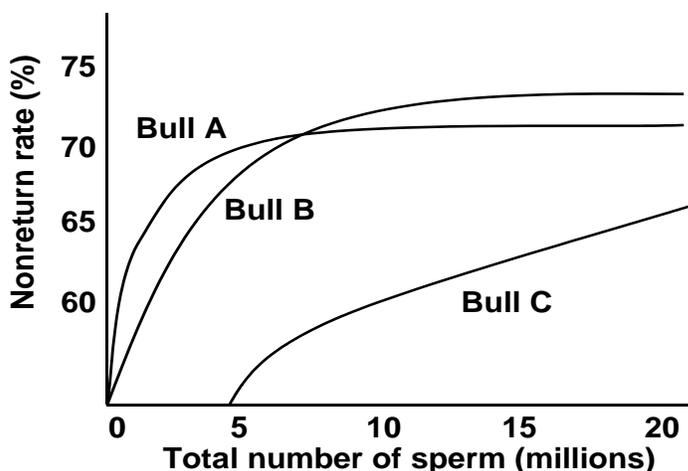


Figure 1. Relationship between nonreturn rate and the number of sperm inseminated. The semen of different bulls varies in the maximum nonreturn rate and in the rate at which the maximum fertility is achieved with increasing sperm dosage (Adapted from den Daas et al., 1998).

### 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.

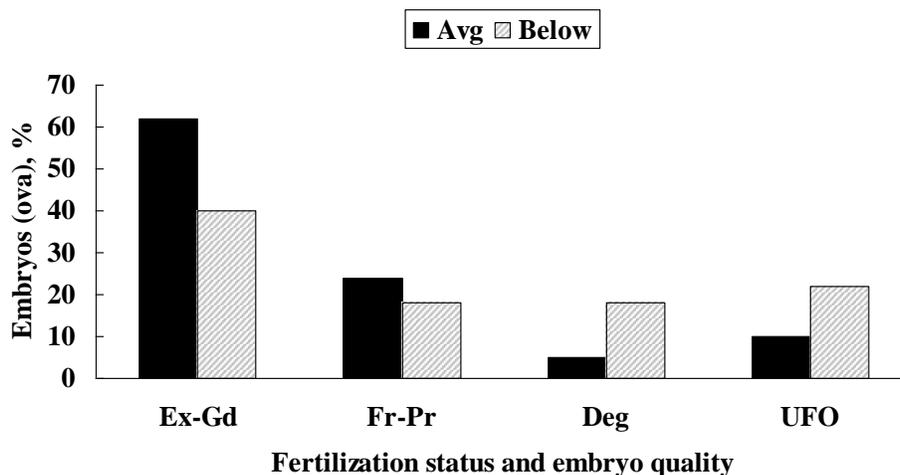


Figure 2. Effect of average and below average semen (based on percentage of abnormal sperm) on fertilization status and embryo quality in single-ovulating cattle. The shift in viable embryos (classified excellent to good and fair to poor) to degenerate and unfertilized caused by use of below average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

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).

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, 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) 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 <sup>1</sup>	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

<sup>1</sup>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

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 (CowChips, LLC, Denver, CO). HeatWatch uses radio frequency data communications, as previously described by Dransfield et al. (1998). 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 insemination on fertility, Dorsey et al. (2011) assigned heifers to a 4-h time block based on time from onset of estrus (h 0) 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%).

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.

Previous work in dairy cattle revealed that ovulation occurs  $27.6 \pm 5.4$  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 ( $25 \times 10^6$  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  $\pm$  SD) after the onset of estrus were  $2.0 \pm 0.9$  h,  $12.1 \pm 0.6$  h, and  $24.2 \pm 0.7$  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)<sup>1</sup>.

Treatment	n <sup>2</sup>	Accessory sperm per embryo (ovum)		Fertilization rate, %
		Mean $\pm$ SD	Median	
0-h AI	39	9.5 $\pm$ 23.1	1	66
12-h AI	39	21.2 $\pm$ 46.2	2	74
24-h AI	39	33.0 $\pm$ 52.7	4	82

<sup>1</sup>Adapted from Dalton et al. (2001).

<sup>2</sup>Number of embryos (ova) recovered.

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<sup>®</sup> 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.

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 ( $31.1 \pm 0.6$  h for beef heifers or cows; Stevenson et al., 1996; White et al., 2002;  $27.6 \pm 5.4$  h in dairy cows; Walker et al., 1996).

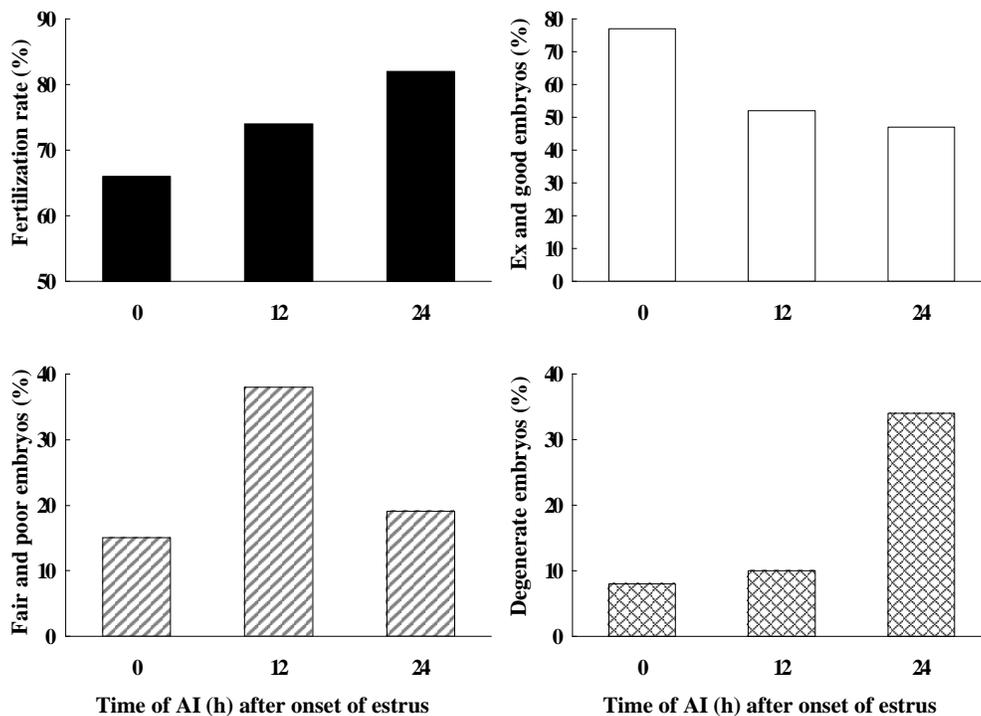


Figure 3. Effect of time of AI after onset of estrus (as determined by the first standing event in cattle continuously monitored by HeatWatch<sup>®</sup>) on fertilization status and embryo quality. (Adapted from Dalton et al., 2001).

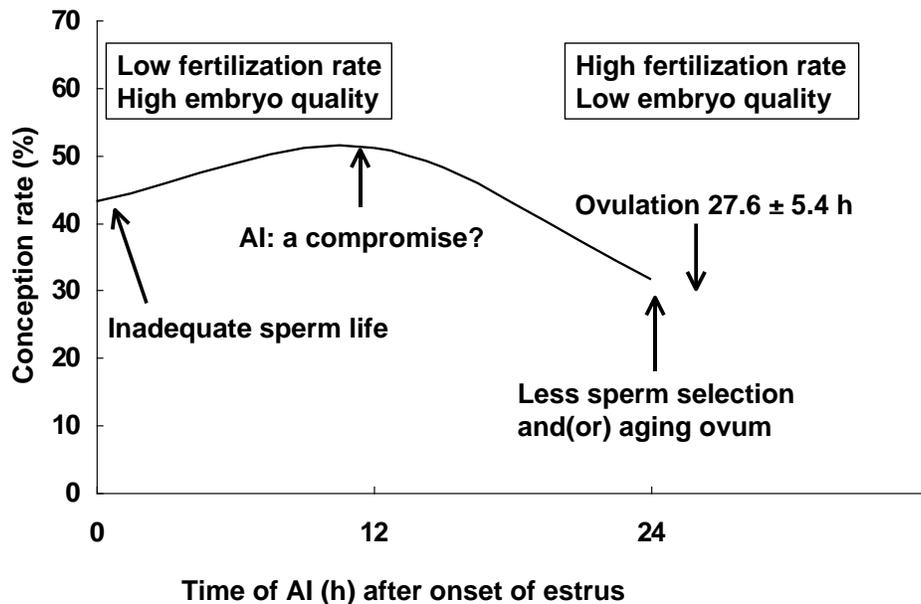


Figure 4. Artificial insemination at 12 h after onset of estrus in dairy cows appears to be a compromise between the low fertilization rate and high embryo quality of early inseminations and the high fertilization rate and low embryo quality of late inseminations. (Adaptation of data from Dransfield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

### 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.”

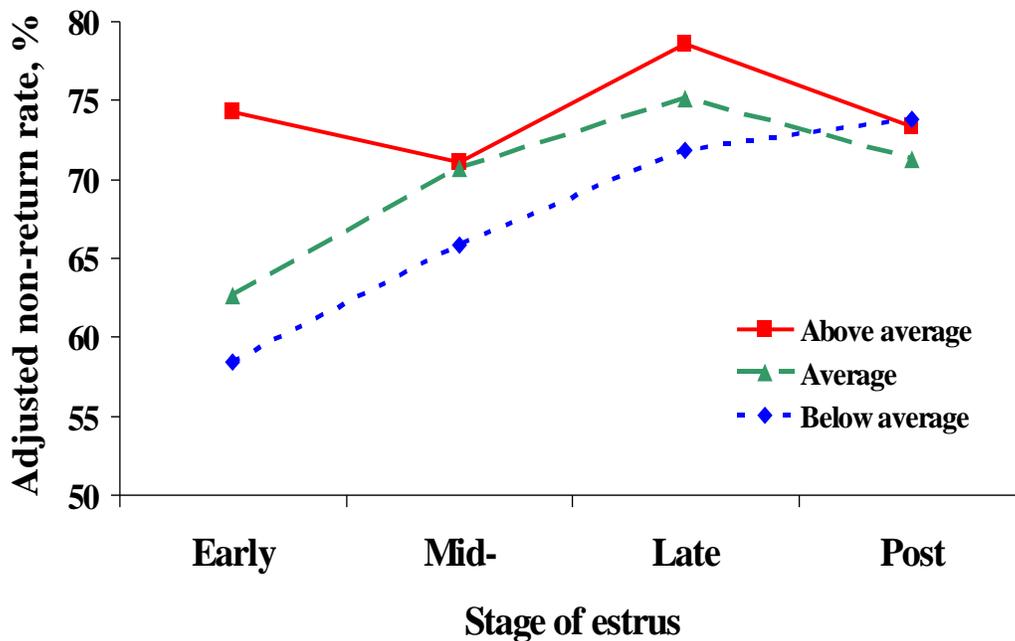


Figure 5. The effect of bull fertility group and stage of estrus at AI on adjusted non-return rates. (Adapted from MacMillan and Watson, 1975).

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 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.

### 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.

### **Acknowledgements**

This research was supported by grants to R.G. Saacke from Select Sires Inc., Plain City, OH, the National Association of Animal Breeders, Columbia, MO, and the Virginia Agricultural Council. Select Sires, Inc., also provided funding to J.C. Dalton for research investigating multiple straw thawing and sequential insemination number. Funding for investigation of Fertility Associated Antigen was provided to J.C. Dalton by Alta Genetics Brazil, Uberaba, São Paulo, Brazil, and UNESP Botucatu, São Paulo, Brazil.

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