

## Factors affecting fertility of conventional and sexed semen

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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. Numerous factors, however, may affect the fertility of conventional and sexed semen including: a) semen handling, b) site of semen deposition, c) number of sperm deposited, d) semen quality, including “compensable” and “uncompensable” seminal traits, e) time of AI, and f) timed AI.

### 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 (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> or 4<sup>th</sup>) 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 (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 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 ( $7.6 \pm 0.22$  vs.  $10.9 \pm 0.38$  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 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 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 spermatozoal 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 spermatozoal motility when sexed

semen was held at constant temperature (98.6°F). Taken together, a reasonable strategy to maintain progressive spermatozoal motility (and ultimately, fertility) is to maintain thermal protection of sexed semen straws and deposit semen in the uterus within 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) 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 within approximately 5 minutes (sexed semen) or 10 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).

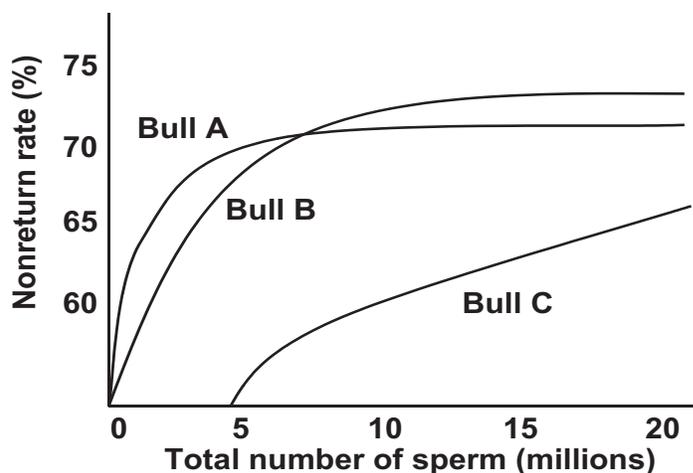
A possible explanation for the positive effect of uterine horn inseminations in a few of the aforementioned studies may be related to the minimization or elimination of cervical semen deposition. Cervical insemination errors account for approximately 20% of attempted uterine body depositions (Peters 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).

### **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).



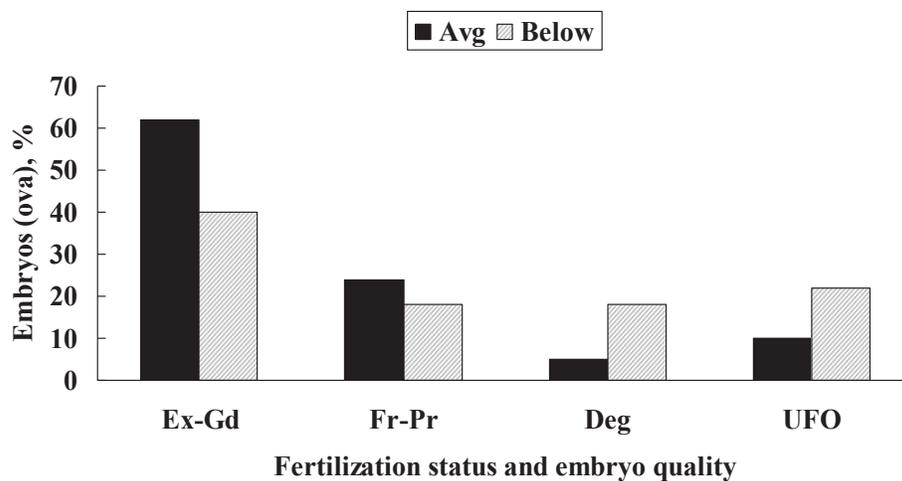
**Figure 1.** Relationship between nonreturn rate and the number of sperm inseminated. The semen of different bulls varies in the maximum nonreturn rate and in the rate at which the maximum fertility is achieved with increasing sperm dosage (Adapted from den Daas et al., 1998).

### 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 of conventional semen 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 45 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 (with conventional semen) 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 and reported that sperm with chromatin abnormalities did not necessarily have abnormal sperm head morphology. Gosálvez et al. (2011) investigated the rate of spermatozoal DNA fragmentation in frozen-thawed sexed semen incubated at 98.6°F for 72 hours, and reported an increased percentage DNA fragmentation appearing between 24 to

48 hours as compared to frozen-thawed conventional semen. Collectively, these results provide evidence that damage to chromatin integrity a) extends beyond morphologically abnormal sperm to apparently morphologically normal sperm, and b) occurs in sexed 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; Kollé 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).

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).

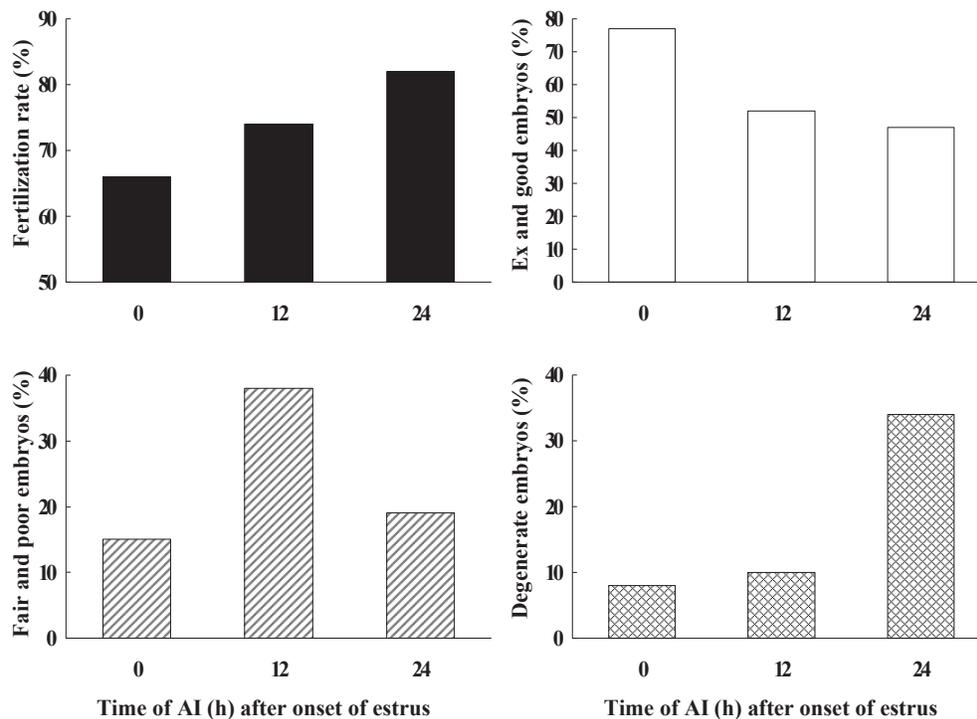
## Time of AI

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  $27.6 \pm 5.4$  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 conventional semen from one of three bulls at 0, 12, or 24 h after the onset of estrus. Median accessory sperm values were greatest in embryos recovered following the 24-h AI treatment (Table 1). The fertilization rate revealed an upward trend, with the highest numerical value associated with the 24-h AI treatment (Table 1). Embryo quality, however, 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).

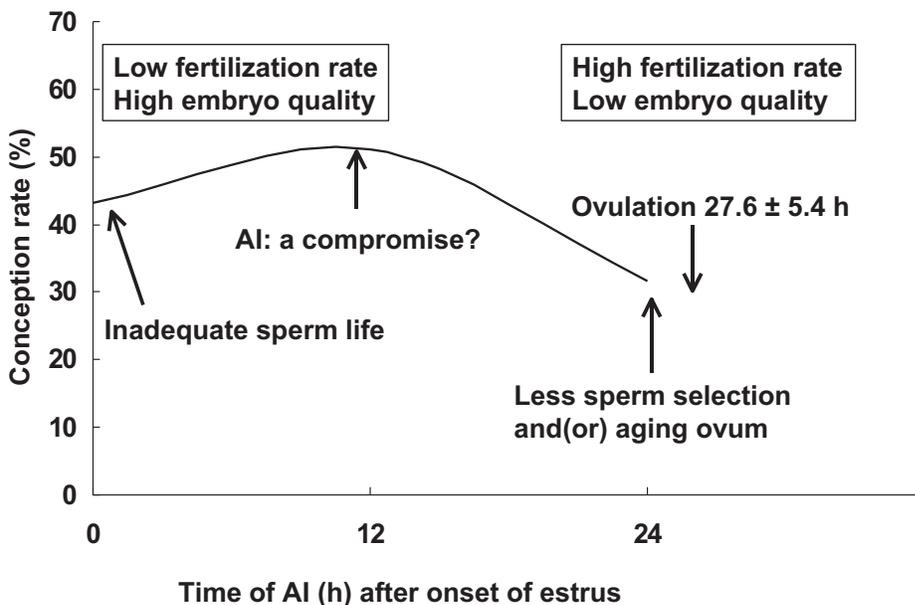
**Table 1.** Effect of insemination time on accessory sperm per embryo (ovum) and fertilization rate of recovered embryos (ova)<sup>1</sup>.

Treatment	n <sup>2</sup>	Accessory sperm per embryo (ovum)		Fertilization rate, %
		Mean ± SD	Median	
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

<sup>1</sup>Adapted from Dalton et al. (2001).<sup>2</sup>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, P/AI 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. 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.



**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).

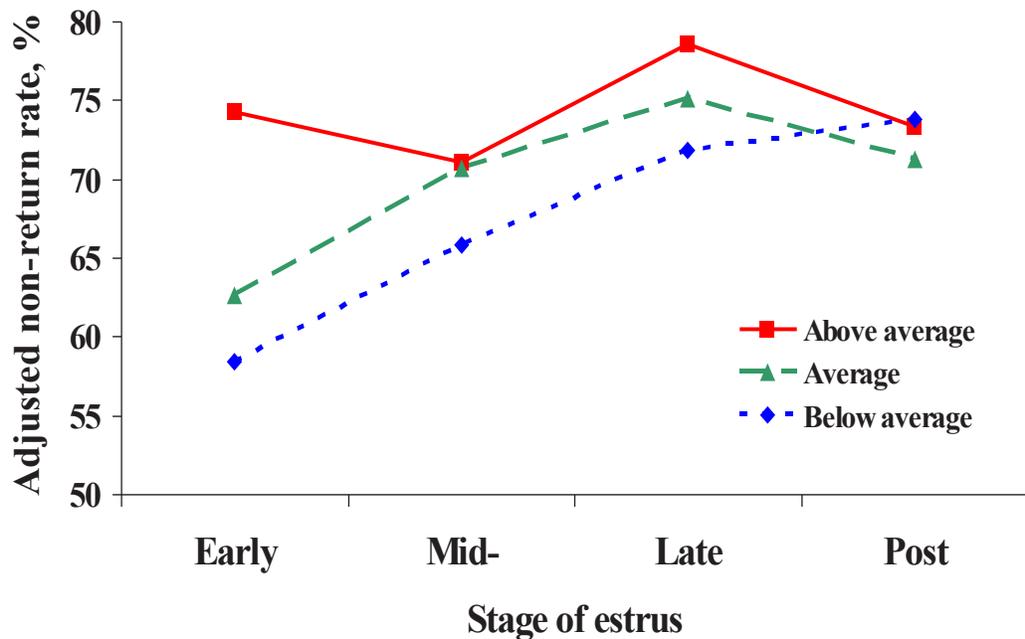
In an effort to determine the optimum time of AI in beef heifers, Dorsey et al. (2011) conducted a retrospective study of estrous-synchronized beef heifers monitored for estrus by HeatWatch. Insemination criteria for the four experiments that comprised the retrospective study included once daily AI based on estrus, am/pm rule based on estrus, and TAI. 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%).

The greater fertility to later insemination in beef heifers (Dorsey et al., 2011) compared to dairy cows (Dransfield et al., 1998; Dalton et al., 2001), 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).

## Timed AI (TAI)

In the past 15 years, numerous systematic breeding protocols have become available to the cattle producer, many of which incorporate 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 an 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 using conventional semen provides evidence that delayed insemination of non-estrous beef heifers in a TAI protocol yields a higher P/AI than heifers inseminated at fixed time regardless of expression of estrous (Thomas et al., 2014a). 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 P/AI 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., 2014a). The increase in P/AI is thought to be due to the delayed AI

of non-estrous heifers in the second treatment, as P/AI for non-estrous heifers was higher than non-estrous heifers in the first treatment (49 vs 34%, respectively; Thomas et al., 2014a).

Thomas et al. (2014b) also conducted a delayed insemination study using sexed semen in suckled beef cows. Estrus was synchronized with the 7-d CO-Synch + CIDR protocol (Beef Reproduction Task Force, 2015). Estrous detection aids were applied at CIDR removal on d 7, and estrous expression was recorded at GnRH administration on d 10. Three treatments were used: 1) TAI with conventional semen regardless of estrous expression (concurrent with GnRH, 66 h after CIDR removal), 2) TAI with sexed semen regardless of estrous expression (concurrent with GnRH, 66 h after CIDR removal), or 3) TAI with sexed semen (concurrent with GnRH, 66 h after CIDR removal) for cows having expressed estrus, and delayed AI 20 h after final GnRH for cows failing to express estrus. Not surprisingly, higher P/AI was reported with conventional semen (77%) as compared to sexed semen (51 and 42% for treatments 2 and 3, respectively) among cows that expressed estrus. In contrast, however, among cows that failed to express estrus, Thomas et al. (2014b) reported delayed insemination with sexed semen resulted in higher P/AI than with sexed semen at the standard time (36 vs. 3%, respectively). Lastly, among cows that failed to express estrus, P/AI (36%) from sexed semen at the delayed time was similar to P/AI achieved using conventional semen at the standard time (37%). Thomas et al. (2014b) concluded that delayed insemination (of cows that failed to express estrus) by 20 h from the standard TAI improved P/AI in suckled beef cows inseminated with sexed semen.

The mechanism by which delaying inseminations of non-estrous beef heifers (conventional semen; Thomas et al., 2014a) and beef cows (sexed semen; Thomas et al., 2014b) leads to increased fertility is not known; however, Thomas et al. (2014a and b) theorize that waiting 20 h after GnRH administration to AI may: a) align the lifespan of capacitated sperm with the timing of ovulation, and (or) b) allow further time for initiation of estrus among a larger percentage of animals, resulting in a more favorable uterine environment in estrual heifers and cows.

## 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.
- 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

Research investigating compensable and uncompensable traits of semen, accessory sperm, and time of AI 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.

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