

INSEMINATION RELATED FACTORS AFFECTING FERTILIZATION IN ESTROUS-SYNCHRONIZED CATTLE

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Introduction

Estrous synchronization programs provide an organized and efficient approach to administering artificial insemination (AI) and the improvement of reproductive performance. Synchronization programs facilitate AI by altering the length of the estrous cycle and (or) through manipulation of follicular growth, thereby making the occurrence of estrus more predictable or allowing for appointment breeding (timed AI) without detection of estrus. Although protocol compliance (accurate cow identification, appropriate drug dosages, correct time and day of treatment, and route of administration) is very important to the success of an estrous or ovulation synchronization program, many other factors are also important. These factors include: a) semen handling, number of sperm deposited, and the site of insemination, b) semen quality, i.e., “compensable” and “uncompensable” seminal traits, c) fertilization status and embryo quality, and d) the bull effect and time of AI.

Factors contributing to the success of an estrous synchronization program

Semen handling

Labor efficient management strategies such as synchronization of estrus or ovulation and fixed-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 to determine: 1) the effect of simultaneous thawing of multiple 0.5-mL straws of semen and sequence of insemination (1st, 2nd, 3rd or 4th) 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 (1st, 2nd, 3rd or 4th), and elapsed time from initial thaw to completion of fourth AI had no effect on conception rate within inseminator group (Dalton et al., 2004). Nevertheless, 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.

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 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).

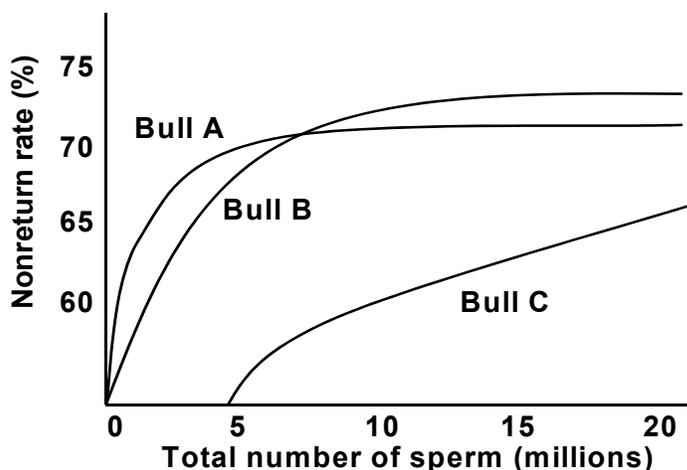


Figure 1. Relationship between nonreturn rate and the number of sperm inseminated. The semen of different bulls varies in the maximum nonreturn rate and in the rate at which the maximum fertility is achieved with increasing sperm dosage (Adapted from den Daas et al, 1998).

Compensable and uncompensable seminal traits

Collectively considering the previously mentioned literature, seminal deficiencies can be placed into two categories, “compensable” and “uncompensable.” 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 the 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. Consequently, bulls with high levels of abnormal sperm should not have semen collected, preserved and used for AI.

Nuclear vacuoles (craters or diadem) on otherwise normally shaped sperm heads appear as accessory sperm *in vivo* at the same frequencies as they occur in the inseminate (Saacke et al., 1998). The use of semen with these traits has been shown to depress embryo quality and fertilization rates compared with control semen where these traits were not evident or were minimized (Miller et al., 1982; DeJarnette et al., 1992; Saacke et al., 1992). Therefore, nuclear vacuoles (craters or diadem) on otherwise normally shaped sperm heads were originally considered uncompensable seminal traits. However, Acevedo et al. (2002) reported that normal shaped sperm with nuclear vacuoles (craters or diadem) lacked chromatin vulnerability to acid denaturation, a result that is contrary to the concept that normal shaped sperm with nuclear vacuoles (craters and diadem) are capable of fertilization, but not maintenance of the embryo. As suggested by Walters et al. (2005), decreased embryonic development after using morphologically abnormal sperm appears to be multifaceted and related to changes in head morphology.

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 ova recognition, binding, and penetration. For example, 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 in sperm membranes and seminal fluid were positively related to fertility in bulls.

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. 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 conception rates, 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.

Accessory sperm, fertilization status and embryo quality

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). 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

¹Embryo 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). In this paper, we will focus on two factors important to estrous synchronization and timed AI, the effect of the bull and 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)

The bull effect and time of AI

In 1993, Nadir et al. reported that accessory sperm numbers were improved by the use of a specific male (Table 3). Clearly, many sperm from Bull A gain access to the egg, as evidenced by the high median accessory sperm number compared to the other bulls. Bull A might be expected to be less vulnerable to semen handling and inseminator errors than the other bulls. Although bulls B and C might be expected to match the fertility and embryo quality of Bull A (based on median accessory sperm numbers of 8 and 13, respectively) the ability of Bulls B and C to produce an embryo might be expected to depend more heavily on inseminator competence and timing of insemination. Lastly, Bull D (with a median accessory sperm number of 2) might be marginal in fertilization rate and embryo quality under current use in AI. The seminal differences of these bulls are “compensable,” i.e., when the semen traits involved in these differences are known, AI organizations adjust the sperm dosage rate accordingly. Many compensable differences of bulls have not yet been elucidated. Consequently, the adjustment of sperm dosage rate can only be determined by fertility data resulting from the AI of a large number of cattle.

Table 3. Accessory sperm number per embryo (ovum) across bulls used at the same insemination dosage¹.

Bull	n	Median	Mean ± SD
A	25	40	53 ± 61
B	37	8	15 ± 23
C	16	13	36 ± 65
D	20	2	11 ± 16

¹Adapted from Nadir et al. (1993).

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. Currently, the best marker for uncompensable seminal deficiencies is the occurrence of abnormal sperm in semen. Abnormal sperm reflect both the health of spermatogenesis and the DNA contributed to the embryo.

Prior to acceptance into a young sire program, bulls are screened for the presence of significant numbers of abnormal sperm. In addition, reputable AI organizations routinely evaluate semen to monitor changes in a bull’s production of sperm. Although natural service is generally not recommended for estrous-synchronized cows, a breeding soundness evaluation is recommended for all natural service bulls. Detailed guidelines for breeding soundness evaluations were approved by the Society for Theriogenology in 1992, and have been reviewed elsewhere (Hopkins and Spitzer, 1997). Briefly, the breeding soundness evaluation consists of three sections: 1) physical examination, 2) scrotal circumference, and 3) semen collection and evaluation. Beef producers can minimize the risk associated with uncompensable seminal deficiencies by screening all natural service bulls with a breeding soundness evaluation.

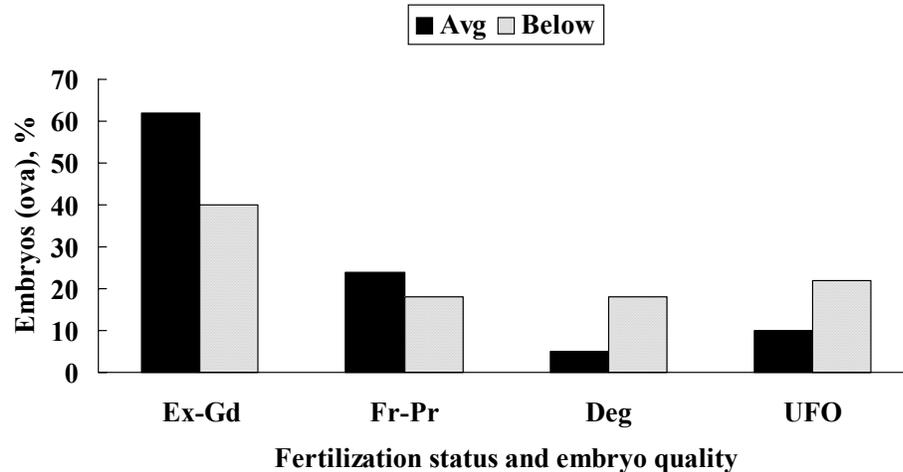


Figure 2. Effect of average and below average semen (based on percentage of abnormal sperm) on fertilization status and embryo quality in single-ovulating cattle. The shift in viable embryos (classified excellent to good and fair to poor) to degenerate and unfertilized caused by use of below average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

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 cows. All cows were continuously monitored for behavioral estrus by HeatWatch[®], which utilizes radio frequency data communications. This system included battery-powered, reusable pressure-sensing transmitters with a 0.4-km range, a repeater with a 0.8-km range, a signal receiver, a buffer that received and stored mounting activity data, and software that sorted information by cow, date, and time using a personal computer. Transmitters were contained in a nylon pouch on a 35- x 20-cm nylon mesh patch, which was glued with contact-type adhesive to the hair of the sacral region just anterior to the tailhead. At all times, the cows were housed in a pasture within working range of the transmitters and repeater. Transmitters were activated by continuous pressure (minimum of 2 s) from a mounting herdmate. Transmitted data included transmitter number, date, time and duration of standing events.

Previous work 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 \pm 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 4). The fertilization rate was also greatest following the 24-h AI treatment (Table 4). 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).

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 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.

Table 4. Effect of insemination time on accessory sperm per embryo (ovum) and fertilization rate of recovered embryos (ova)¹.

Treatment	n ²	Accessory sperm per embryo (ovum)		
		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

¹Adapted from Dalton et al. (2001)

²Number of embryos (ova) recovered.

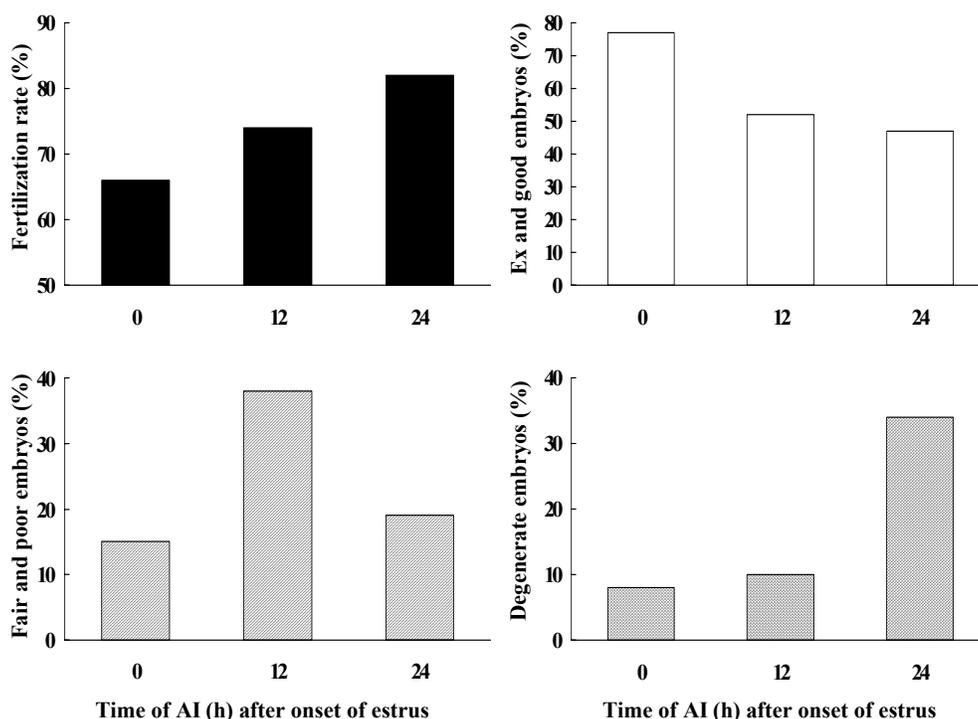


Figure 3. Effect of time of AI after onset of estrus (as determined by the first standing event in cattle continuously monitored by HeatWatch[®]) on fertilization status and embryo quality. (Adapted from Dalton et al., 2001).

The improved embryo quality associated with 0-h AI 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. This agrees with Pursley et al. (1998) regarding pregnancy loss after timed insemination of lactating cows following Ovsynch. According to Pursley et al. (1998), cows inseminated at the time of the second injection of GnRH (0 h) had the lowest pregnancy loss when compared with cows inseminated 8, 16, 24, or 32 h after the second injection of GnRH. Furthermore, Pursley et al. (1998) reported more female calves than male calves were born after AI at 0 and 32 h than would be expected. Sex ratio of calves born from AI at 8, 16, and 24 h were not different from expected values.

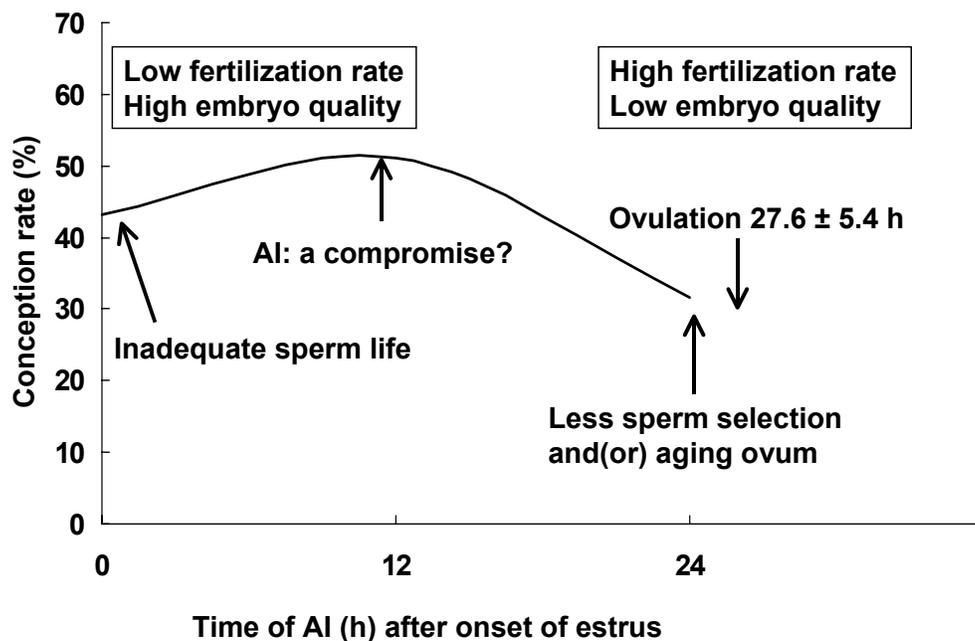


Figure 4. Artificial insemination at 12 h after onset of estrus appears to be a compromise between the low fertilization rate and high embryo quality of early inseminations and the high fertilization rate and low embryo quality of late inseminations. (Adaptation of data from Dransfield et al., 1998,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

Conclusions

For optimal reproductive efficiency, beef cows should produce a live calf each year. Estrous synchronization programs provide an organized and efficient approach to administering AI and the improvement of reproductive efficiency. Although administration of estrous synchronization programs may differ between herds, the following points are important for all beef producers to remember:

- Protocol compliance (accurate cow identification, appropriate drug dosages, correct time and day of treatment, and route of administration) is very important
- 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 (Bulls with unacceptably high levels of abnormal sperm should not have semen collected, preserved and used for AI)
- Ovulation occurs 27.6 ± 5.4 h after the first standing event for both natural estrus and prostaglandin-induced estrus, and between 24 to 32 h after the second GnRH of Ovsynch
- 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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