MANAGEMENT FACTORS ASSOCIATED WITH MALE FERTILITY

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Introduction

Artificial insemination (AI) is an efficient and cost-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 refrigerator, the maintenance of male fertility is in the hands of the producer, farm and ranch employees, and AI technicians. Management factors associated with male fertility include: a) semen storage and handling, b) site of semen deposition, c) the bull effect, d) time of AI, and e) timed AI.

Semen storage and handling

In order to realize the maximal potential fertility within straws of frozen semen, the liquid nitrogen refrigerator must be managed properly. The liquid nitrogen refrigerator consists of a "tank within a tank," with insulation under vacuum between the inner and outer tanks. Liquid nitrogen refrigerators 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). 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 refrigerator, 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. Previous reports have shown that sperm injury (as judged by sperm motility) occurs at temperatures as low as -79 degrees C (Etgen et al., 1957; Bean et al., 1963; DeJarnette, 1999). Furthermore, injury to sperm cannot be corrected by returning semen to the liquid nitrogen (Berndtson et al., 1976; Saacke et al., 1978).

When numerous cows must be inseminated on a given day, AI technicians routinely thaw multiple straws of semen simultaneously to facilitate AI in a timely manner. Dalton et al. (2004) conducted a field trial to determine: a) 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, b) whether conception rates achieved following AI by professional AI technicians (PAI) and herdsman-inseminators (HI) differed, and c) 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%, respectively), 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). 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) use appropriate hygienic procedures, f) maintain thermal protection of straws during AI gun assembly and transport to the cow, and g) deposit semen in the uterus 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.

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

Before continuing the discussion of management factors associated with male fertility, it is important to understand the relationship between accessory sperm, fertilization status, and embryo quality. In the accessory sperm quantification 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 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 and ova, 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.

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).

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.

Numerous studies seeking to increase accessory sperm numbers have been conducted (for a review please see Saacke et al., 2000). In the following two sections of this paper, we will focus on two factors associated with male fertility: a) the bull effect, and b) time of AI relative to ovulation.

The bull effect

In 1993, Nadir and coworkers reported that accessory sperm numbers were improved by the use of a specific male (Table 2). 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. Consequently, 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. When the semen traits involved in these differences are known, AI organizations adjust the sperm dosage rate accordingly. Briefly, deficiencies in semen quality can be placed into two categories, “compensable” and “uncompensable.” Seminal deficiencies, which can be overcome or minimized by increasing the sperm dosage are considered “compensable.” Seminal deficiencies

resulting in suppressed fertility regardless of sperm dosage are considered "uncompensable." Unfortunately, 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 2. Accessory sperm number per embryo (ovum) across bulls used at the same insemination dosage[1].

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

[1]Adapted 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 1, 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.

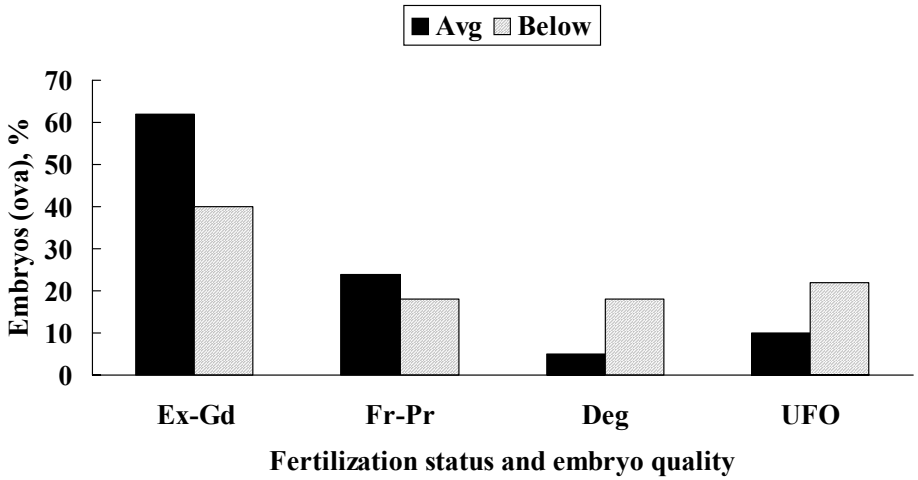

Figure 1. Effect of average and below average semen (based on percentage of abnormal sperm) on fertilization status and embryo quality in single-ovulating cattle. The shift in viable embryos (classified excellent to good and fair to poor) to degenerate and unfertilized caused by use of below average semen was significant.

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. Cattle producers using natural service as

a component of the reproductive program should have a veterinarian perform a breeding soundness evaluation on all natural service bulls. Furthermore, all bulls used for custom semen collection should have a breeding soundness evaluation prior to collection. 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: a) physical examination, b) scrotal circumference, and c) semen collection and evaluation. Beef producers can minimize the risk associated with uncompensable seminal deficiencies by screening all natural service bulls (and bulls due to be custom-collected) with a breeding soundness evaluation.

Time of AI

Dalton and coworkers (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) of 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 2).

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.

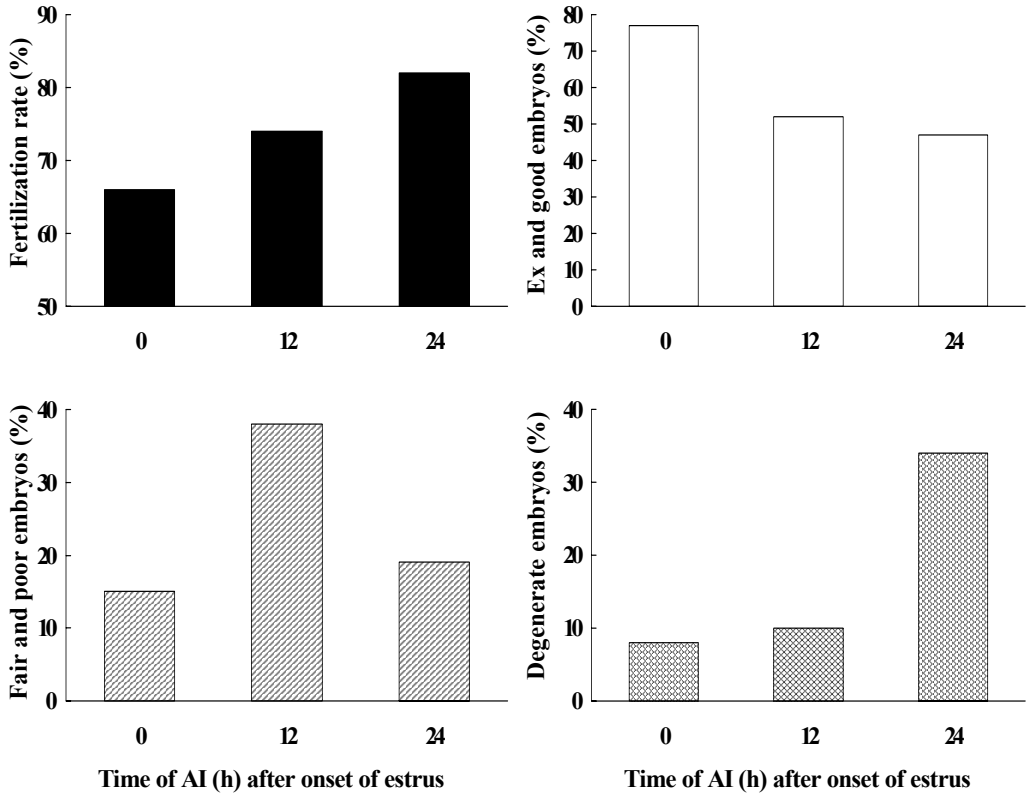

Figure 2. Effect of time of AI after onset of estrus (as determined by the first standing event in cattle continuously monitored by HeatWatch®) on fertilization status and embryo quality. (Adapted from Dalton et al., 2001).

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 3). From these data, conception rates would be expected to be optimized following the 12-h AI (Figure 3). 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.

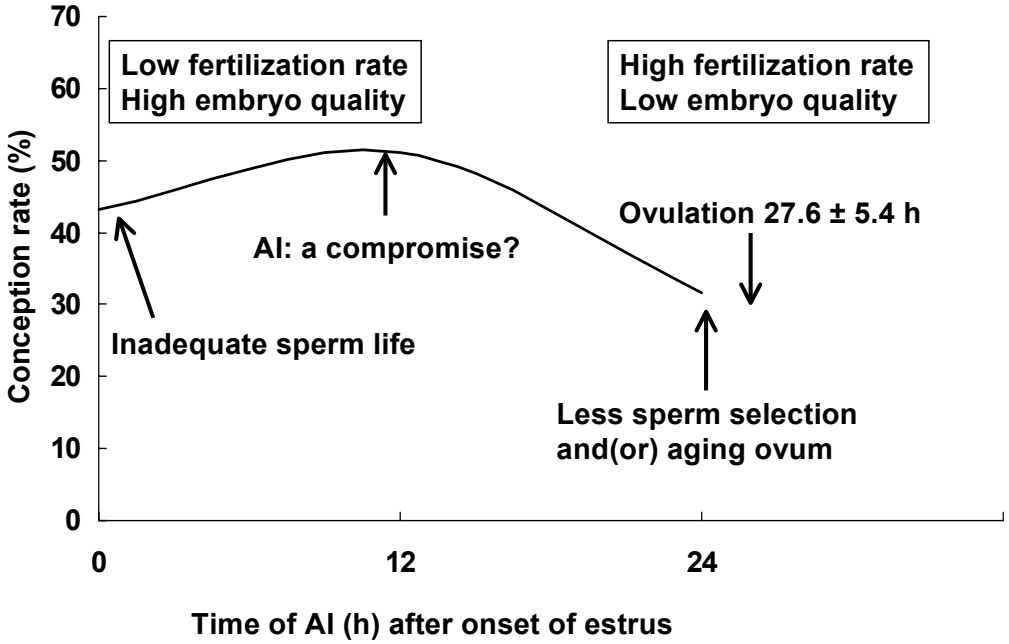

Figure 3. Artificial insemination at 12 h after onset of estrus appears to be a compromise between the low fertilization rate and high embryo quality of early inseminations and the high fertilization rate and low embryo quality of late inseminations. (Adaptation of data from Dransfield et al., 1998, and Dalton et al., 2001, originally published by Saacke et al., 2000).

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.

Timed AI

Research on the bull effect and time of AI was completed using either visual detection of estrus (Nadir et al., 1993) or HeatWatch® (Dalton et al., 2001). In the past decade, numerous systematic breeding protocols have become available to the cattle producer, many of which incorporate timed AI (TAI). So, is there evidence of bull fertility differences following TAI? The simple answer is yes *and* no, as there appear to be differences in some studies, while other studies report no differences. Before discussing the evidence, a quick review of the work of MacMillan and Watson (1975) is warranted. Macmillan and Watson (1975) investigated the effect of the interval from observed estrus to AI on non-return rates of above average, average, and below average fertility bulls. (Non-return 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.") As shown in Figure 4, 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 TAI and ovulation within a distinct, although as yet unknown, "optimal interval."

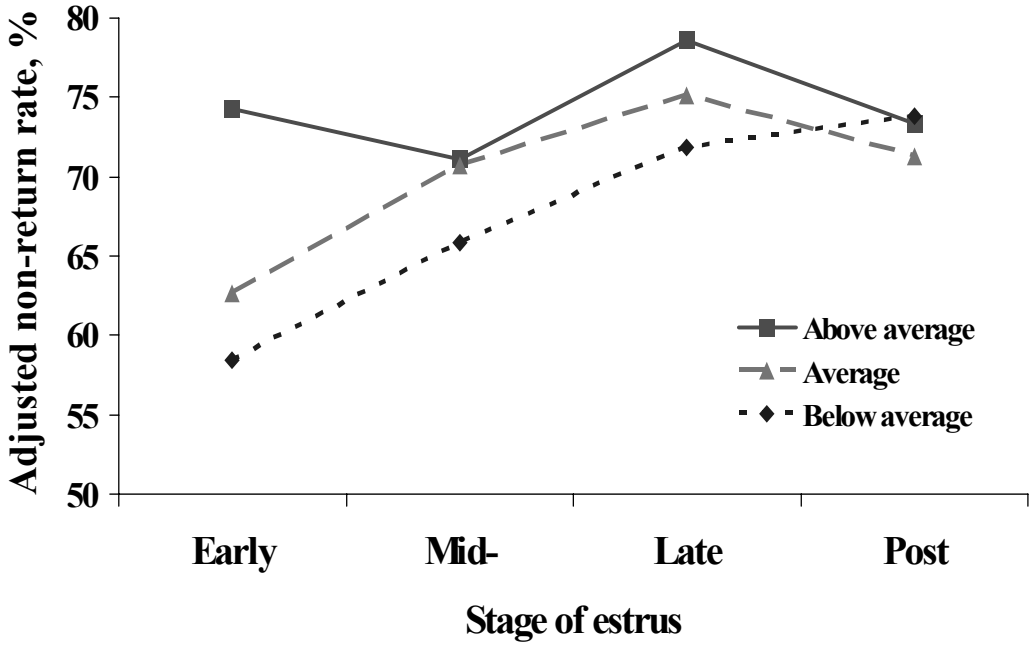

Figure 4. The effect of bull fertility group and stage of estrus at AI on adjusted non-return rates. (Adapted from MacMillan and Watson, 1975).

Hiers et al. (2003) reported an effect of AI sire on TAI pregnancy rates of nonlactating *bos indicus* × *bos taurus* cows for one of three herds studied. Macfarlane (2003) synchronized first service lactating cows with Presynch + Ovsynch and compared pregnancy rate per AI following TAI 8 h before or 16 h after the final GnRH injection, which corresponded to AI ~36 h or 12 h prior to ovulation. At 56 d after AI, there was a 9% difference in pregnancy rate per AI (23.4% vs. 32.3%, for the -8 h and +16 h groups, respectively). In one of the farms, Macfarlane (2003) noted that the highest fertility bull performed equally well at -8 and +16 h, whereas the lowest fertility bull had decreased fertility at -8 h, but improved when used at +16 h. This agrees with the data from MacMillan and Watson (1975), as shown in Figure 4. In contrast, Cornwell et al. (2006) investigated TAI after the final GnRH in a Presynch + Ovsynch protocol and reported that neither TAI at 0 and 24 h after GnRH affected pregnancy rate per AI (26.7% vs. 25.7%, respectively), nor was pregnancy rate per AI statistically different between the average and high fertility bulls used (23.2% vs. 29.4%, respectively).

Given the aforementioned evidence in which limited numbers were used, there may be a difference in sire fertility following TAI. For a meaningful conclusion to be drawn, however, further research with sufficient numbers of observations must be conducted.

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 and handling, and site of semen deposition are critical factors that can be easily managed on the farm or ranch. Lastly, choice of a 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

Reputable commercial AI stud and custom semen collection businesses provide a highly fertile product to their customers. When semen is purchased and transferred to the producer's liquid nitrogen refrigerator, the maintenance of male fertility is in the hands of the producer, farm and ranch employees, and AI technicians. To achieve the maximal fertility associated with frozen semen for AI use, the following points are important for all beef producers to remember:

- Proper semen storage and handling, including the delivery of sufficient numbers of viable sperm, is critical to a successful AI program.
- 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.
- Protocol compliance (accurate cow identification, appropriate drug dosages, correct time and day of treatment, and route of administration) is very important.
- Choice of a TAI protocol 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.

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