

SEMEN QUALITY FACTORS ASSOCIATED WITH FERTILITY

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Introduction

Throughout the world, cattle producers are interested in identifying the most fertile bulls, whether for use in AI or natural service. Unfortunately, although researchers and clinicians have tried for “nearly a century to develop techniques to accurately *predict* the fertility of a semen sample from an individual male, the goal has not been achieved” (Amann and Hammerstedt 1993). Why? This is due, at least in part, to the reality that studies investigating semen quality and fertility have produced inconsistent results. There are likely many reasons for the inconsistencies, a few of which are outlined below.

The accuracy of fertility data. Numerous factors may compromise fertility data (Table 1).

Table 1. Potential factors affecting fertility data.

Factor	Reference
Definition of fertility used (nonreturn rate or pregnancy diagnosis)	Saacke et al., 1980 Mocé and Graham, 2008
Non-sperm effects (management and environment on fertility of the cow)	Saacke et al., 1980 Mocé and Graham, 2008
AI of too few females to accurately estimate fertility	Mocé and Graham, 2008
Confounding due to the use of high sperm numbers	Saacke et al., 1980 DeJarnette, 2005 Mocé and Graham, 2008
Sample of bulls used in one study may not be representative of the population of bulls in other studies	Saacke et al., 1980

The multifaceted nature of sperm function. Currently, *some* attributes of sperm necessary for fertilization are known (Table 2; Amann and Hammerstedt, 1993), while others remain unknown. The multifaceted nature of sperm function is the foundation for the realization that “the malfunction of any one of many essential and independent sperm attributes (known and unknown) can render a given sperm incapable of fertilizing an oocyte” (Amann and Katz, 2004). Furthermore, attributes necessary for fertilization will certainly depend on whether AI or natural service is used (including, but not limited to, the effect of the site of semen deposition), whether ovulation synchronization and TAI is used, and female factors.

Table 2. Sperm attributes necessary for fertilization.¹

“Acceptable” morphology
Metabolism for production of energy
Progressive motility
Capacity for hyperactive motility
Stabilization of plasma and acrosomal membrane lipids
Acrosomal enzymes
Chromatin integrity

¹Partial list adapted from Amann and Hammerstedt (1993).

Variability in seminal quality measurements. Human bias, variation in personnel training, and use of different methods to evaluate seminal quality are likely causes of variability in seminal quality measurements within and between laboratories (Graham et al., 1980; Mocé and Graham, 2008; Brito, 2010).

This discussion does not imply, however, that we should stop investigating the relationship of semen quality and fertility. In fact, the development and refinement of techniques to evaluate seminal quality have actually led to enhanced “accuracy in positive fertility diagnosis by default, through identification and measurement of greater numbers of semen quality attributes that are associated with sub-fertile semen” (DeJarnette, 2005). Nevertheless, investigation of seminal quality factors and the relationship to fertility remains a daunting task, as DeJarnette (2005) states that “semen samples that possess sufficient levels of all known traits must still (at least initially) be considered of questionable fertility, because the sample could be deficient in other traits unknown or unmeasured.” Consequently, DeJarnette (2005) argues that “the accuracy of below average fertility diagnosis will likely always be more accurate than the acceptable diagnosis.”

Quantity and Quality of Sperm in the Inseminate

Historically, the assessment of male fertility has focused on the quantity and quality of sperm delivered to the female. 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 (Figure 1). 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. Nonreturn rate, defined by Rycroft (1992) “as the percentage of cows that are not rebred within a specified period of time after an insemination, typically 60 to 90 days,” has been historically used by the dairy industry as an indirect measure of fertility. Regarding semen quality, Pace et al. (1981) reported that fertility increases with increasing numbers of structurally intact and motile sperm.

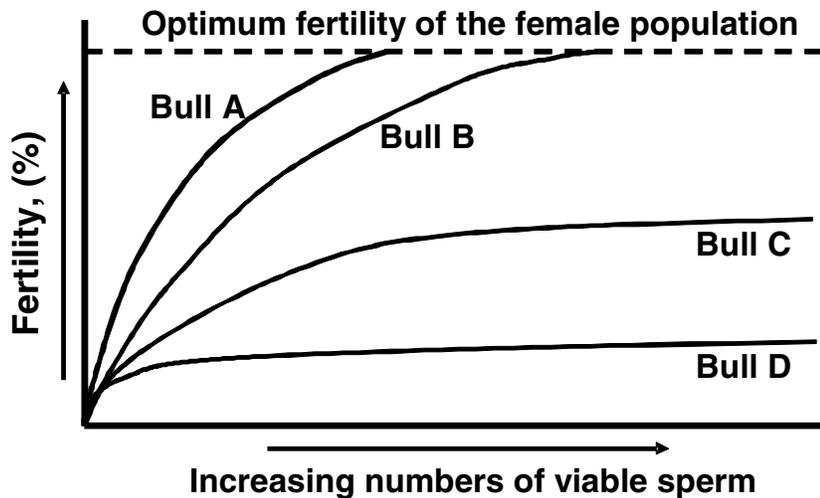


Figure 1. Relationship between number of viable sperm inseminated and fertility. The minimum number of viable sperm required for maximum fertility differs among bulls, as does the rate at which the maximum fertility is achieved with increasing sperm dosage (Adapted from Salisbury and VanDemark, 1961, Sullivan and Elliot, 1968, and den Daas et al. 1998).

Sullivan and Elliot (1968) also 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. (1998a).

Collectively, the work of Salisbury and VanDemark (1961), Sullivan and Elliot (1968), and den Daas et al. (1998) provides evidence that there are seminal factors which are “compensable” and others which are “uncompensable,” as originally described by Saacke et al. (1994). 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.” In contrast, seminal deficiencies resulting in suppressed fertility regardless of sperm dosage would be considered “uncompensable.” The independence of these traits indicates that reproductive efficiency of a particular male should be explainable by either compensable or uncompensable seminal traits, or by a combination of the two traits. Before continuing the discussion on compensable and uncompensable traits of semen, it is important to understand: a) sperm transport, b) retrograde loss of sperm, and c) semen quality and barriers to sperm transport.

Sperm Transport

In cattle, VanDemark and Hays (1954) first reported on the rapid transport of sperm from the site of deposition. Whether seminal deposition occurred by natural service in the vagina, or via AI

into the uterus, sperm were present in the oviductal ampulla as quickly as 2.5 to 3.3 minutes after deposition (VanDemark and Hays, 1954). Overstreet and Cooper (1978) and Overstreet et al. (1978) reported that sperm transport occurs in two distinct phases. The first, or rapid transport phase, has been shown to occur within a few minutes of insemination in rabbits (Overstreet and Cooper, 1978) and cattle (Hawk, 1987). During this phase, muscle contractions of the female reproductive tract transfer sperm from the site of deposition to the oviducts. In rabbits, many of these sperm are dead and subsequently cleared to the peritoneal cavity (Overstreet and Cooper, 1978). Thus, sperm in the rapid transport phase are not thought to be involved in fertilization.

The second, or sustained phase of sperm transport, brings sperm capable of fertilizing ova to the isthmus of the oviduct between 6 to 12 hours after insemination (Hunter and Wilmut, 1983; Wilmut and Hunter, 1984; Hawk, 1987). The number of predominantly viable sperm colonizing the isthmus progressively increases between 8 to 18 h after insemination (Hawk, 1987; Wilmut and Hunter, 1984).

There is evidence that the oviductal isthmus serves to maintain spermatozoal function until ovulation in the pig (Hunter, 1980). Suarez (1987) observed the behavior of mouse sperm in the oviduct *in situ* (by virtue of the translucence of the mouse oviduct) and in proximity to ovulation. In the isthmus, sperm were retained by: a) adherence of their heads to the mucosa, and b) flagellar immobilization in this region (Suarez, 1987). The adherence of sperm to the oviductal mucosa is mediated by sugar residues in the cell membrane overlying the sperm head region (fucose in the case of bovine; Lefebvre et al., 1997). In a review, Hunter (1998) postulated that sperm in the oviductal isthmus are released by cue(s) to continue to the site of fertilization by events associated with ovulation, thereby permitting the timely union of sperm and ovum.

How many sperm are present in the oviducts near the time of ovulation? Suarez et al. (1990) reviewed the numbers of sperm in the isthmus and the ampulla (near the site of fertilization) near the time of ovulation in the mouse, rat, hamster, rabbit, sheep and cow. Although the number of sperm inseminated ranged from 50 million for the mouse to 3 billion for the cow, there were distinct similarities noted between animals in numbers of sperm in the oviduct. In sheep, there were approximately 4.4 thousand sperm present in the oviductal isthmus compared to 21.2 thousand for cows near the time of ovulation. In the oviductal ampulla there were only 2 sperm for the hamster and rat, and 5, 10, 26 and 118 sperm for mice, cows, sheep and rabbits, respectively, near the time of ovulation. The very small number of sperm passing through the ampulla (near the site of fertilization) at any one time has been thought to be an important natural safeguard against polyspermy (Hunter, 1988), which is known to result in early embryonic death.

Retrograde Loss of Sperm

The previously mentioned literature provides evidence that relatively small numbers of sperm, as compared to the total number in the inseminate, colonize the oviductal isthmus. So where do the sperm go? Following natural service, the majority of sperm are lost from the reproductive tract shortly after deposition. Those sperm which remain in the tract enter the uterus against the uterine mucosa after following privileged paths within grooves originating in the fornix vagina (Mullins and Saacke, 1989). Intrauterine AI bypasses the cervix, the major barrier to sperm transport, and allows for the use of low numbers of sperm relative to those required in natural

service. Nevertheless, within 12 h after insemination approximately 90% of sperm artificially inseminated are lost by retrograde flow (Mitchell et al., 1985).

Semen Quality and Barriers to Sperm Transport

The population of sperm reaching the site of fertilization (oviductal ampullary-isthmus junction) is enriched in both viability and normal morphology over that inseminated (Overstreet et al., 1978). Although morphologically abnormal sperm have been associated with subfertility and sterility for many years (Williams and Savage, 1925, 1927; Lagerlof, 1934), it is now known that sperm with classically misshapen heads do not traverse the barriers of the female reproductive tract or participate in fertilization based upon accessory sperm data from ova and embryos (Saacke et al., 1998a). 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.

Known barriers to sperm transport in the cow include the cervix-mucus complex (Mullins and Saacke, 1989) and the uterus (Mitchell et al., 1985), while the utero-tubal junction and lower isthmus of the oviduct (Krzanowska, 1974) are a formidable barrier in the mouse. The relationship between barriers to sperm transport and specific morphological abnormalities has been elucidated by numerous investigators. In the cow, the cervix-mucus complex is a barrier to abnormal tails and heads (Koeford-Johnsen, 1972), while the utero-tubal junction and lower isthmus impair traverse by sperm with abnormal head morphology (Krzanowska, 1974; Nestor and Handel, 1984) and tails with droplets (Nestor and Handel, 1984) in the mouse. The zona pellucida of the ovum, however, may be the most formidable barrier to participation in fertilization of morphologically abnormal, viable sperm (Howard et al., 1993). Further, sperm with abnormal acrosomes have been reported to: a) be impaired in their ability to attach to the ovum in vitro, and b) fail to penetrate the zona pellucida (Thundathil et al., 2000). Lastly, apparently normal sperm in the inseminate of bulls with a high percentage of abnormal acrosomes were also found to be deficient, as embryonic development following fertilization with apparently normal sperm was also impaired (Thundathil et al., 2000).

Evidence of a relationship between reduced embryonic development and sperm with apparently normal morphology in an otherwise abnormal ejaculate was initially reported by Setchell et al. (1988) in mice. The impaired reproductive performance by apparently normal sperm in an abnormal ejaculate of bulls and mice (Thundathil et al., 2000; Setchell et al., 1988) provides alarming evidence that sperm abnormalities may only be the visible portion of an iceberg impairing reproduction.

Compensable and Uncompensable Seminal Traits

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. Seminal deficiencies, seen as reduced fertility when numbers of sperm are below threshold, which can be overcome or minimized by increasing sperm dosage, are considered compensable.

Compensable seminal traits are thought to be closely associated with measures of sperm viability, including motility, and acrosomal and cell membrane integrity (DeJarnette, 2005). Reputable AI organizations routinely adjust the AI dose when compensable deficiencies are known.

Uncompensable traits of semen quality relate to the competence of fertilizing sperm to complete fertilization and sustain early embryonic development. Seminal deficiencies resulting in suppressed fertility regardless of sperm dosage are considered uncompensable. Bulls with semen exhibiting unacceptable levels of abnormal sperm are usually the main source of uncompensable traits. Nevertheless, uncompensable traits have also been associated with errors in spermatozoal chromatin, and may actually be most important in the morphologically normal or near-normal spermatozoa that *accompany* abnormal sperm (mainly misshapen heads) in subfertile males (Saacke, 2008). Consequently, bulls with high levels of abnormal sperm should not have semen collected, cryopreserved, and used for AI.

Vogler et al. (1993) (Figure 2) studied the effect of a 48-h mild scrotal insulation on sperm viability (motility and acrosomal integrity) and morphology in *Bos taurus* bulls. Testicular surface temperatures ranged from 33.3°C to 36.4°C, with a mean of 34.8°C. Every three days during the study, two ejaculates were collected in succession by artificial vagina.

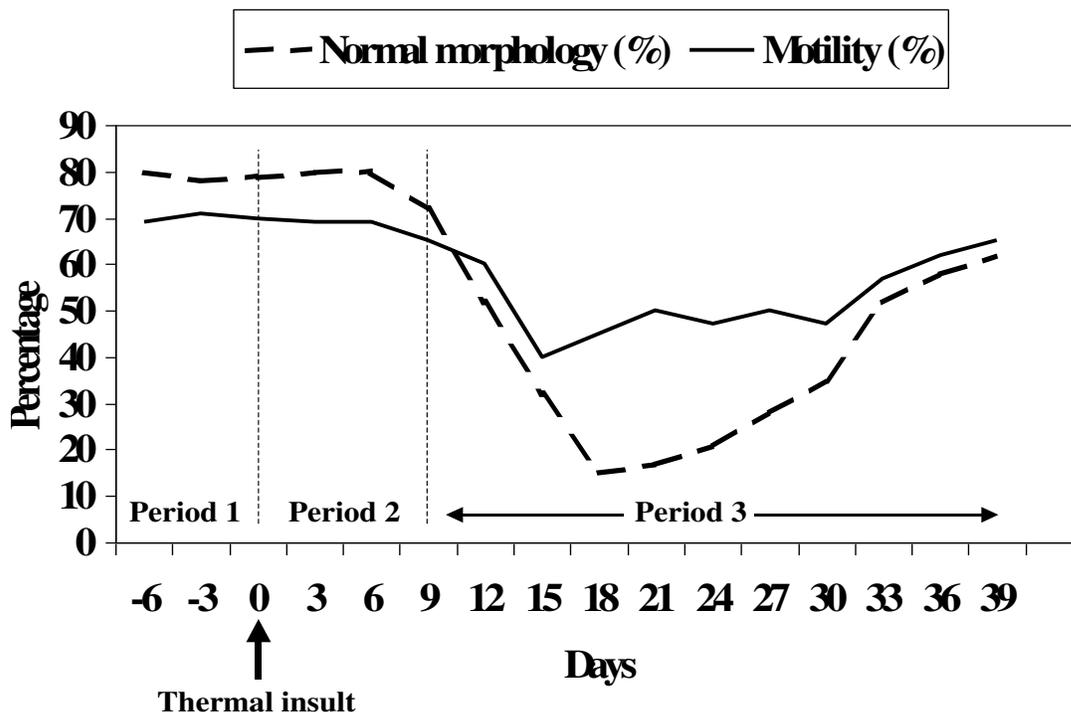


Figure 2. The effect of a 48-h scrotal insulation (thermal insult) on the percentage normal sperm morphology and motility. (Adapted from Vogler et al., 1993).

Vogler et al. (1993) reported that Period 1 (pre-insult) and 2 (post-insult, sperm present in the epididymis or rete testis at the time of scrotal insult) did not differ in sperm motility and morphology (Figure 2). However, total sperm abnormalities increased and sperm motility decreased in Period 3 (post-insult, sperm undergoing spermatogenesis at the time of scrotal insult) compared to Period 1 (Figure 2). Sperm motility was depressed between 10 to 20 percentage points, and was most apparent on days +15 to +18 after insult. Abnormal sperm content increased beginning on day +12, peaked at day +18, and persisted longer than the depressed motility. At the termination of the study on day +39, the ejaculate content of abnormal sperm was approaching pre-insult levels. Although bulls varied in the type of abnormal spermatozoa produced and in magnitude of response, specific abnormalities appeared in ejaculates in a predictable chronological sequence following scrotal insult on day 0. The sequence was: decapitated sperm, (days +12 to +15); diadem, (day +18); pyriform and nuclear vacuoles, (day +21); knobbed acrosome, (day +27); and dag defect (abnormal axonemal structure), (day +30).

In an effort to learn more about specific morphological abnormalities and compensable and uncompensable seminal traits, an experiment with semen collected from a bull in the scrotal insulation study (Vogler et al., 1993) was used to inseminate nonlactating, superovulated *Bos taurus* cows (Saacke et al., 1992). High levels of vacuolated sperm (19 to 38%, diadem and random, respectively) from day +21 was compared with pre-insult control semen (day -3 and -6) as well as semen from day +9 (after insulation, but before appearance of visible morphological abnormalities) (Table 3). It is clear that use of semen with high levels of nuclear vacuoles resulted in depressed embryo quality (Saacke et al., 1992).

Table 3. Effect of an inseminate containing a high proportion of random vacuoles and diadem defects on embryo quality in nonlactating, superovulated *Bos taurus* cows.¹

Cows, (n)	Embryos, (n)	Semen	Embryo Quality ² , (%)		
			Excellent-Good	Fair-Poor	Degenerate
7	90	Day -3	74.4	11.1	14.4
8	85	Day +21	38.3	21.2	35.3
7	87	Day -6	66.7	11.5	17.2
9	141	Day +9	56.0	22.5	19.9

¹Adapted from Saacke et al. (1992).

²Embryo quality based on compactness and homogeneity of a day 6 morula as previously described by Lindner and Wright (1983).

Because the use of semen with nuclear vacuoles has been shown to depress embryo quality and fertilization rates compared with control semen (Miller et al., 1982; DeJarnette et al., 1992; Saacke et al., 1992), nuclear vacuoles (random 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 (random or diadem) lacked chromatin vulnerability to acid denaturation, a result that is contrary to the concept that uncompensable traits affecting embryo quality are associated with errors in spermatozoal chromatin. The apparent incompatibility of the results of Saacke et al. (1992) and Acevedo et al. (2002) were disturbing and did not agree with the original assertion that nuclear vacuoles (random or diadem) on otherwise normally shaped sperm heads were uncompensable seminal traits. In fact, as briefly mentioned previously and described in further detail below, Acevedo et al. (2002) discovered errors in

spermatozoal chromatin appear in morphologically normal or near-normal spermatozoa that *accompany* abnormal sperm (mainly misshapen heads) in the same ejaculate.

Sperm with microscopically normal morphology, but with defective chromatin, have been implicated in cases of male subfertility for at least 35 years (Gledhill, 1970). The chromatin structure assay developed by Evenson et al. (1980) revealed a strong positive association between heterospermic fertility in bulls (based upon genetic markers at birth) and stability of sperm DNA to acid denaturation (Ballachey et al., 1988; Kasimanickam et al., 2006). Using this same assay and cryopreserved semen from bulls subjected to a mild thermal insult of the testis by scrotal insulation (Vogler et al., 1993), Karabinus et al. (1997) reported that sperm ejaculated before scrotal insulation have more stable DNA than those ejaculated after scrotal insulation (where abnormal sperm are also evident).

Acevedo et al. (2002) also applied a 48-h scrotal insult to Holstein bulls and modified the chromatin structure assay so that sperm DNA stability to acid denaturation could be evaluated on the same sperm as judged morphologically. 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. Although female sperm selection appears strong based upon sperm shape and motility, it is apparent that total exclusion of incompetent sperm from accessing the ovum does not occur.

How might chromatin stability affect fertility? Sakkas et al. (1995, 1996) speculated that flaws in packaging and condensation of sperm chromatin during spermiogenesis resulted in the instability of the DNA of subfertile men. Specifically, limitations in disulfide bonds essential for DNA condensation in the sperm nucleus are thought to be the source of the instability. The same DNA condensation that occurs in the testis during spermiogenesis must be reversed (i.e., decondensation) in the ovum following fertilization, ultimately resulting in the restoration of the 2N DNA of the embryo. Decondensation of the sperm nucleus and development of the male pronucleus must occur in a timely manner for the embryo to develop and generate a signal for maternal recognition of pregnancy. Walters et al. (2006) compared pronuclear development following IVF for semen collected before and after scrotal insulation in *Bos taurus* bulls, and concluded that normal sperm maturation is disrupted during scrotal insulation, as evidenced by a lack of timely decondensation after penetration of the ovum. The results of Walters et al. (2006) confirm the previous results of Eid et al. (1994) in which early cleavage rates were reduced due to delayed pronuclear formation following the use of semen from low fertility bulls in vitro. Collectively, it appears the apparent limiting factor is decondensation of the sperm nucleus. Lastly, as described by Walters et al. (2006), the impact of morphologically abnormal sperm occurred prior to cleavage during the early stages of fertilization. Consequently, altered chromatin stability is considered an uncompensable seminal trait.

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.

Although the recognition of compensable and uncompensable seminal traits is equally important, the focus of future research should concentrate on uncompensable traits, as these result in depressed fertility regardless of sperm numbers in the inseminate. Producers can minimize the 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 (Hopkins and Spitzer, 1997).

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 (Saacke et al., 1998b; Table 4). 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 4 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.

Table 4. Relationship of accessory sperm per embryo (ovum) to fertilization status and embryo quality.¹

Fertilization status and embryo quality ²	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

¹Adapted from Saacke et al. (1998b).

²Embryo 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 (see Saacke et al., 2000, for a review). Relative to the discussion in this paper, accessory sperm numbers and (or) embryo quality have been improved by: a) raising sperm dosage in the inseminate (Nadir et al., 1993), and b) through the use of semen with “average” vs. “below average” semen quality (DeJarnette et al. 1992).

The effect of sperm dosage on accessory sperm values is presented in Table 5. In Experiment 1, median accessory sperm values were not different between treatments (20×10^6 vs. 40×10^6 ; DeJarnette et al., 1992). However, increasing the sperm dosage to 100×10^6 resulted in an increased median accessory sperm number in Experiment 2 (Nadir et al. 1993). Furthermore, insemination with the high dose improved the percentage of embryos (ova) with accessory sperm, fertilization rate, and embryo quality (Nadir et al., 1993; Figure 3).

DeJarnette et al. (1992) studied the effect of semen quality on accessory sperm number, fertilization status, and embryo quality in a study using semen from bulls characterized as “average” or “below average” (as evaluated by the AI organization; Table 6). As shown in Figure 4, below average quality semen produced fewer excellent and good embryos and an increased number of degenerate embryos and unfertilized ova when compared to semen of average quality. Currently, the best markers for uncompensable seminal deficiencies is 1) the occurrence of abnormal sperm in semen, and 2) errors in sperm chromatin. As previously mentioned, producers can minimize the 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 (Hopkins and Spitzer, 1997).

Table 5. Effect of sperm dose on accessory sperm values and fertilization rate in single-ovulating *Bos taurus* cattle.

Sperm dose (x 10 ⁶)	n	Accessory sperm per embryo(ovum)		Embryos (ova) with accessory sperm (%)	Fertilization rate (%)
		Median	Mean ± SD		
20 ^a	42	0	8 ± 22	52	66
40 ^a	39	1	10 ± 17	59	69
20 ^b	38	3	29 ± 63	79	79
100 ^b	38	27**	38 ± 38	92	97

^aExperiment 1: DeJarnette et al. (1992)

^bExperiment 2: Nadir et al. (1993)

** (P<0.05)

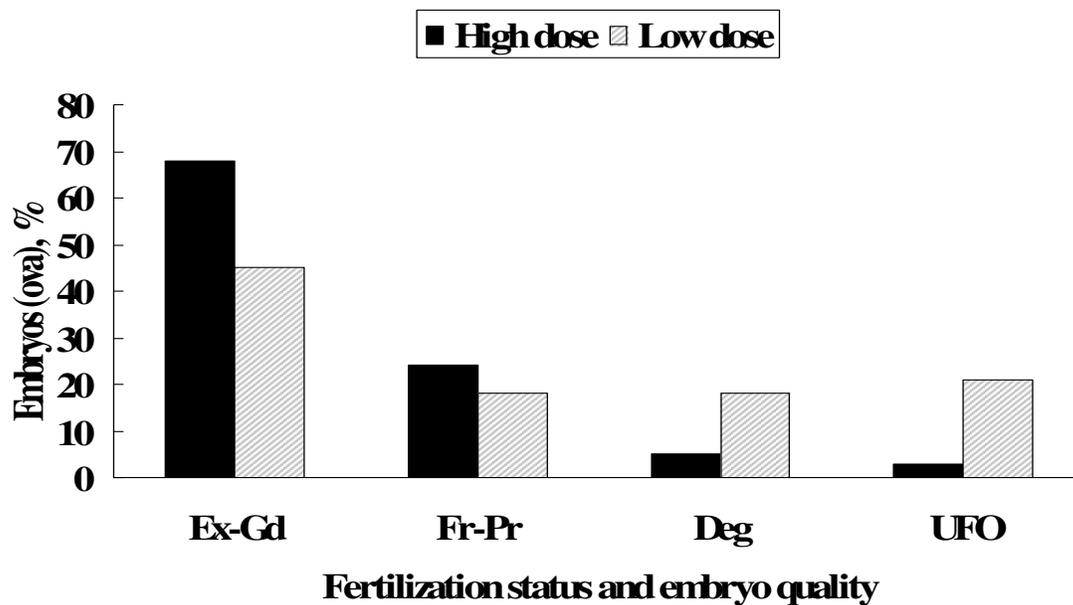


Figure 3. Effect of AI with 100 x 10⁶ sperm (High dose) or 20 x 10⁶ sperm (Low dose) on fertilization status and embryo quality in single-ovulating *Bos taurus* cattle. The shift in viable embryos classified excellent to good (Ex-Gd) and fair to poor (Fr-Pr) to degenerate (Deg) and unfertilized (UFO) due to the lower dose was significant (P<0.05; Nadir et al., 1993).

Table 6. Percentage post-thaw motility, acrosomal integrity, and morphology of average and below average semen used by DeJarnette et al. (1992).

Semen characteristic	Semen quality	
	Average	Below average
Motility		
0 h ^a	40 ^c	30
3 h ^b	30	20
Intact acrosomes		
0 h	89 ^d	69
3 h	82	58
Morphology		
Normal	81 ^e	61
Abnormally-shaped heads	8	17
Nuclear vacuoles	5	10
Protoplasmic droplets	4	8
Abnormal tails	2	4

^aEvaluation immediately after thawing.

^bEvaluation after 3 h of incubation at 37 degrees C after thawing.

^cEstimate of progressive motility to the nearest 10% after viewing two smears.

^dMeans of two direct counts of 100 cells each.

^eMeans of three differential counts of 100 cells each.

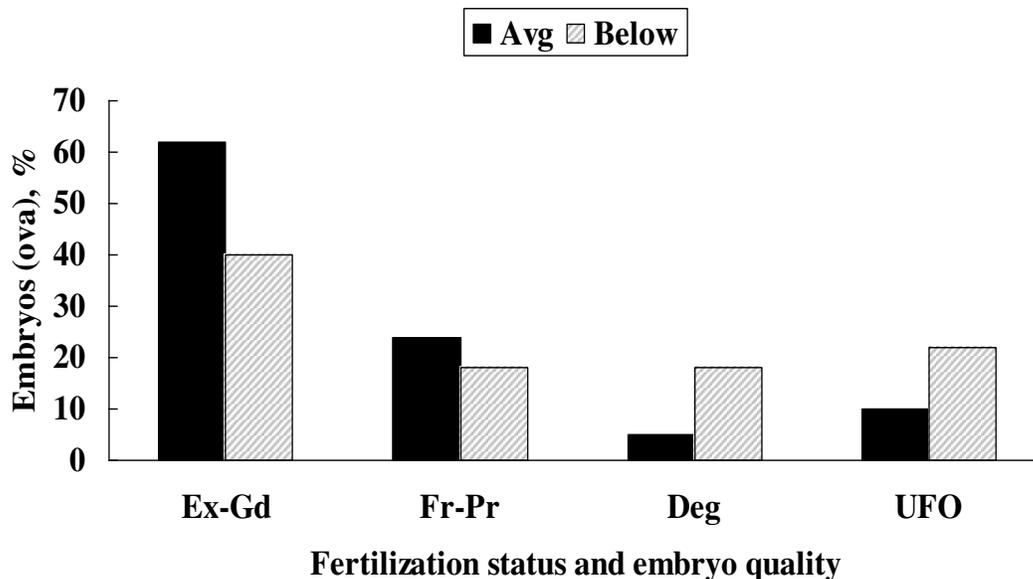


Figure 4. Effect of average and below average semen on fertilization status and embryo quality in single-ovulating *Bos taurus* cattle. The shift in viable embryos classified excellent to good (Ex-Gd) and fair to poor (Fr-Pr) to degenerate (Deg) and unfertilized (UFO) caused by use of below average quality semen was significant. ($P < 0.06$; DeJarnette et al., 1992).

Conclusions

Semen quality factors associated with fertility in cattle can be placed into two categories: compensable and uncompensable seminal traits.

- 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.
 - 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.
 - Unfortunately, compensable seminal traits cannot be explained completely by morphology and present-day in vitro viability measurements.
- Uncompensable traits of semen quality relate to the competence of fertilizing sperm to complete the fertilization process and sustain early embryonic development.
 - 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. Furthermore, altered chromatin stability is considered an uncompensable seminal trait.
 - There is evidence that damage to chromatin stability extends beyond morphologically abnormal sperm to apparently morphologically normal sperm.
 - Cattle producers can minimize the risk associated with uncompensable seminal deficiencies by:
 - using semen from AI studs where sperm morphology is a routine part of the evaluation process, and
 - screening all natural service bulls with a complete breeding soundness evaluation, including sperm morphology.

Although the recognition of compensable and uncompensable seminal traits is equally important, the focus of future research should concentrate on uncompensable traits, as these result in depressed fertility regardless of sperm numbers in the inseminate. Lastly, although progress continues to be made, there is currently no way to accurately determine the minimum sperm dosage to provide maximal fertility for a given male, or to identify all subfertile males.

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