

Sperm morphology: Its relevance to compensable and uncompensable traits in semen

R.G. Saacke*

*Virginia Polytechnic Institute and State University, Department of Dairy Science,
Blacksburg, VA 24061 0315, United States*

Abstract

The nature of subfertility due to the male or inseminate is as complex as that of the female. Fertilization failure, and failure in embryogenesis, are both of seminal origin. Males also differ in the number of sperms required to reach their maximum fertilization rate. Males requiring more sperm are considered to have compensable seminal deficiencies. These include a number of known viability and morphology traits (including both abnormal heads and tails) and unknown factors (functional or molecular traits) precluding sperm access to the ovum or ability of the sperm to engage the ovum sufficiently to initiate fertilization and the block to polyspermy. Differences in fertility among males or inseminates independent of sperm dosage are considered uncompensable. These seminal deficiencies are associated with fertilizing sperm that are incompetent to maintain the fertilization process or subsequent embryogenesis (once initiated), with most failures occurring prior to maternal recognition of pregnancy; these sperm would pre-empt fertilization by competent sperm. Evidence now exists supporting the concept that the uncompensable effect is due to chromatin aberrations in morphologically normal or near-normal fertilizing sperm present in abnormal ejaculates (elevated content of abnormal sperm). Thus, sperm morphology may be our best indication for the presence of an uncompensable deficiency, although we have yet to identify the incompetent fertilizing sperm clinically.

© 2008 Elsevier Inc. All rights reserved.

Keywords: Sperm morphology; Semen quality; Spermogram; Breeding soundness evaluation; Cattle

1. Introduction

Clearly, pregnancy success to AI is due to the health of the female population, as well as the quality, placement and handling of the inseminate. The focus of this paper is on semen quality. Currently, we can only account for approximately 50–60% of the variation in fertility among males by measuring seminal traits, leaving the goal of predicting fertility from laboratory evaluation of semen quite unachieved. Therefore, the

nature of subfertility due to the male or inseminate is as complex as that due to the female. Based on accessory sperm numbers in the egg vestments of 6-d-old non-surgically recovered embryos and the health of the embryo following AI, it was clear that failure of an inseminate to result in a pregnancy could be due to either fertilization failure (inadequate sperm access to the egg) or failure in development of the fertilized egg (competence of the fertilizing sperm). Both conditions were found to be of seminal origin [1]. How these sources of reproductive failure relate to our current evaluation of seminal traits in AI and the breeding soundness evaluation, is the topic of this review, with an emphasis on sperm morphology.

* Tel.: +1 540 953 0179; fax: +1 540 231 5014.

E-mail address: saacke@vt.edu.

2. Compensable and uncompensable seminal deficiencies

The complexity of subfertility due to the male has been apparent for many years, based upon field investigations of bull fertility at increasing doses of sperm inseminated [2,3]. Sullivan and Elliott [2] also showed the minimum number of motile sperm required for maximum fertility (threshold) differed among bulls, and that bulls differed in the maximum fertility at any dosage (Fig. 1). They also observed that low-fertility bulls required more sperm than high-fertility bulls to reach their maximum fertility. They postulated the requirement of more sperm by the subfertile bulls was due to the presence of morphologically abnormal sperm unable to negotiate barriers in the female tract, precluding their access to the site of fertilization. This was confirmed in a later study [4] where sperm with classically misshapen heads did not access the egg, i.e., appear as accessory sperm in eggs following AI. From AI data in the Netherlands [3], the minimum number of sperm required to reach maximum fertility for a given bull (threshold) was independent of the maximum fertility achievable by that bull. These studies have collectively provided the impetus to view seminal deficiencies as falling into two major categories (compensable and uncompensable). From these investigations, coupled with the accessory sperm/embryo quality research (use of the 6-d-old embryo as a biomonitor of the inseminate), it was clear that bulls differed in numbers of sperm accessing the egg, as well as the quality of the embryo generated, and that these two traits were independent of one another. Thus, in an effort to explain the nature of subfertility, bulls requiring more sperm to reach their maximum

fertilization rate were considered to have compensable seminal deficiencies (Male B vs. Male A, Fig. 1). In contrast, males having lowered fertility independent of sperm dosage were considered to have uncompensable seminal deficiencies (Male C vs. Males A and B, Fig. 1). It has often been said that it is easier to identify the lower fertility bulls than it is to recognize the high-fertility bulls by virtue of semen evaluation. Although not universally true, low-fertility bulls are those with uncompensable traits, with the possible exception of a male having such high levels of compensable deficiencies that threshold numbers of sperm to the female cannot be met in the AI dose (Male D, Fig. 1). This would be rare in today's AI industry, but not in unselected natural service or the population of bulls subjected to a breeding soundness evaluation. Barriers in the female tract between the site of semen deposition and the site of fertilization lay claim to much of the seminal differences involved in the compensable component.

3. Barriers in the female tract to sperm transport: compensable deficiencies

Morphologically abnormal sperm in semen of males have been associated with subfertility and sterility for many years [5–7]. We now recognize that sperm with classically misshapen heads, identified by early workers using simple microscopes, do not traverse the female reproductive tract and/or participate in fertilization, based upon recovery and evaluation of accessory sperm from ova and embryos [4]. Barriers precluding their progression to the oviduct have been identified in a variety of species. Barriers to abnormal tails and heads include the cervix and cervical mucus, in cattle [8], rabbit [9] and human [10], the uterotubal junction and lower isthmus impair traverse by sperm with abnormal heads in the mouse [11,12] and rabbit [9], and tails with protoplasmic droplets in the mouse [12]. Considering the very small, intricate privileged paths offered by the cervix and mucus for species having intravaginal semen deposition, e.g., cattle and sheep [13], as well as the similar intricacies presented at the uterotubal junction in species having intrauterine semen deposition, e.g., pig [14], it may be that flagellar pattern is important to sustained transport of sperm, resulting in removal or retrograde of cells with abnormal tails and protoplasmic droplets. Similarly, even small geometrical differences in head morphology can cause large differences in sperm hydrodynamics [15]. Thus, impaired or abnormal sperm motility may be the underlying basis for sperm exclusion, based upon head morphology as well. It has

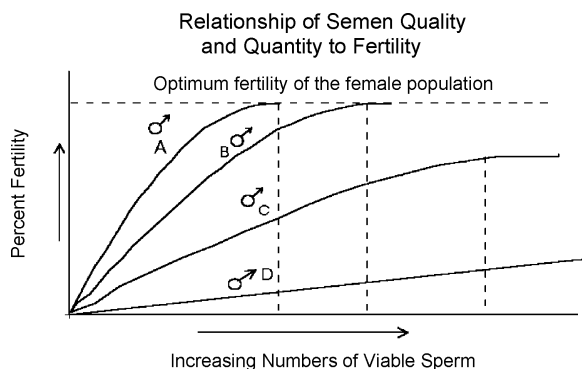


Fig. 1. Relationship between pregnancy rate and the number of spermatozoa inseminated. The semen of individual bulls varies in the maximum non-return rate and in the rate at which the maximum fertility is achieved with increasing sperm dosage (modified from Sullivan and Elliott [2]).

also been observed that in vitro, sperm with proximal droplets [16] and abnormal heads [17] were unable to attach to or penetrate [18] the vestments of the ovum. In felids, Howard et al. [18] reported that the zona pellucida provided a formidable barrier to abnormal heads, with the most abnormal being on the outer most portions of the zona, and those with better morphology closest to the vitelline membrane. Spermatozoa with abnormal acrosomes had impaired ability to attach to the ovum in vitro and thus would not be thought to participate in fertilization; however, their presence in an ejaculate apparently signifies incompetence in the accompanying sperm of the ejaculate where embryonic development from fertilized eggs is impaired [19]. This apparently holds for other sperm abnormalities as well, to the point that we are beginning to view sperm abnormalities as the tip of an iceberg impairing reproductive efficiency. Clearly, these morphological differences in sperm among bulls account for part of the compensable deficiency differences among bulls, as postulated by Sullivan and Elliott [2]. However, Pace et al. [20] reported that differences in numbers (not percent) of sperm having the viability traits of progressive motility, acrosomal and cell membrane integrity, were all negatively related to minimum numbers of sperm required for a male to reach maximum fertility. Thus, these viability traits along with the morphological distortions would all be considered compensable.

In cattle, bulls used for AI can differ 10-fold or more in ability to access the ovum in vivo, based upon fertility differences among bulls at low insemination doses [3], or based upon accessory sperm number differences among bulls at the same insemination dose [21]. This strongly suggests that there are compensable seminal deficiencies that cannot be explained by conventional assessments of sperm viability or morphology. Most likely important to sperm accessing the egg would be differences among bulls in molecular events on the sperm surface, or functional changes of sperm associated with colonizing and storage in the major sperm reservoir under sustained sperm transport, i.e., the lower oviductal isthmus. Such changes could be imparted via the seminal fluids accompanying the sperm [22,23]. In addition, these differences could easily be associated with the ability of sperm to undergo capacitation, sperm/egg recognition, or even the true acrosome reaction, all of which are still to be accurately quantified under laboratory conditions for evaluating semen. On the basis of this discussion, although both sperm morphology and viability comprise part of the important compensable seminal traits, there clearly are others that remain to be characterized.

4. From the female perspective, when are seminal deficiencies uncompensable?

Sullivan and Elliott [2] also demonstrated that bulls could have depressed fertility regardless of semen dose. These low-fertility bulls generally had higher seminal content of morphologically abnormal cells. As stated earlier, this explains the higher sperm dosage threshold or compensable component of the lower fertility male, since such sperm could not negotiate the barriers in the female tract and access the site of fertilization. But, what about the uncompensable component of the low-fertility male, i.e., subfertility at any dosage? There is now good evidence that many sperm with normal motility and morphology that are present in abnormal ejaculates are able to access the egg, but are not competent to complete fertilization or sustain embryogenesis once these events are initiated [24–28]. Differences among bulls in embryonic development of their conceptuses have been reported at the time of routine recovery for embryo transfer [29], and after observation of embryo survival in recipients [30]. Bulls also differed in the development of their embryos following in vitro fertilization [31–34]. In low-fertility bulls, early cleavage rates were reduced and pronuclear formation was delayed [34] or impaired [35]. Thus, incompetence in morphologically normal or near-normal spermatozoa of abnormal ejaculates appeared to be the cause of the uncompensable component.

Sperm with microscopically normal morphology but defective chromatin have been implicated in cases of male subfertility for some time [36]. The chromatin structure assay developed by Evenson et al. [37] revealed a strong positive association between heterospermic fertility in bulls (based upon genetic markers at birth) and stability of the sperm DNA to acid denaturation [38]. Based on this same assay, sperm ejaculated before a mild thermal insult of the testis by scrotal insulation had more stable DNA than those ejaculated after scrotal insulation, where abnormal sperm were also evident [39,40]. Acevedo et al. [41] modified the chromatin structure assay, such that sperm DNA stability to acid denaturation could be evaluated on the same sperm as judged morphologically. Applying this modification to the semen of scrotally insulated bulls, they reported that spermatogenic disturbance caused by elevated testicular temperature resulted in the production of abnormal sperm and that vulnerability of sperm DNA to acid denaturation was positively associated with sperm having misshapen heads, but also extended to normal-shaped sperm in the abnormal samples. This provided more support for the

assertion that the occurrence of sperm with misshapen heads can signal chromatin abnormalities and potential incompetence among both morphologically normal and abnormal sperm in the same sample. Furthermore, although selection of sperm at barriers in the female tract appears amazingly strong (based upon sperm shape and motility), it is undoubtedly far from absolute in excluding incompetent sperm from accessing the egg.

The nature of the interference of chromatin incompetent sperm with pregnancy rate was addressed in studies by Sakkas et al. [42,43]; they speculated that flaws in packaging and condensation of sperm chromatin during spermiogenesis resulted in the instability of sperm DNA in subfertile men. The instability of the DNA was thought to be due to limitations in disulfide bonds essential for DNA condensation in the sperm nucleus which was, in turn, important to the ultimate sperm head shape for the specific species. This same DNA condensation and packaging that occurs in the male testis, must undergo a reversal in condensation (i.e., decondensation) in the egg following fertilization, where the male pronucleus is formed in preparation for union with the female pronucleus, resulting in the restoration of the 2N DNA of the newly formed embryo. This must occur in a timely fashion for the embryo to progress at a rate compatible with normal embryonic development and provide the signals necessary for maternal recognition of pregnancy. Recently, utilizing the scrotal insulation model in the bull, Vogler et al. [40], Walters et al. [35] compared pronuclear development following IVF with semen obtained prior to and following development of sperm having denaturable DNA caused by elevated testicular temperature. Clearly, they verified that the observations of Eid et al. [34] were correct; the retarded cleavage rate of male-related embryonic failure was due to delayed pronuclear formation, with stages of pronuclear formation involving decondensation of the sperm nucleus the apparent limiting factor. In the scrotal insulation model of Vogler et al. [40], detached sperm heads and nuclear vacuoles appeared prior to misshapen sperm heads. Sperm incompetent to form a pronucleus in a timely fashion accompanied the misshapen heads, but not the detached heads or nuclear vacuoles on otherwise normally shaped heads [35] supporting the observations of Acevedo et al. [41] that misshapen heads are at least one major signal that an ejaculate contains uncompensable deficiencies.

Although it is important that we recognize both the compensable as well as the uncompensable seminal traits in our breeding males, it is clear that we must focus most on the uncompensable traits, since these

result in subfertility regardless of sperm numbers in the inseminate, AI technique or reproductive strategy applied (including natural service). Bulls having substantial numbers of uncompensable deficiencies in their semen should be eliminated. At our current state of knowledge, such bulls are best avoided by using semen for AI from reliable sources, where semen morphology is a routine part of the evaluation process, or in the case of natural service, bulls where semen morphology is a strong part of the breeding soundness evaluation. Clearly, abnormal-shaped heads are of primary concern. In this respect, the most promising method of quantifying and following changes in sperm head shape is that utilizing the Fourier Harmonic Amplitude analysis [44,45]. Evolution of this approach should prove useful in the AI industry and to veterinarians providing breeding soundness evaluations.

5. Summary and conclusions

Seminal traits important to reproductive efficiency *in vivo* can be considered to fall into two major categories: (1) those important to sperm transport and function in the female reproductive tract, up to and including initiation of the fertilization process and the block to polyspermy (compensable traits); and (2) those important to the maintenance of the fertilization event and subsequent embryogenesis, once initiated (uncompensable traits). Both sperm viability and morphology are important to the compensable traits, because aberrations in either result in complete or partial exclusion at several barriers in the female tract (the zona pellucida may be the most formidable). There are differences among males or semen samples with respect to accessibility of sperm to the ovum that cannot be explained by conventional sperm viability or morphological assessment. Sperm traits at the functional or molecular level important to colonization of the oviduct and to binding and traversing the ovum vestments remain to be identified and quantified to provide a more complete appreciation of compensable factors.

Uncompensable traits affecting embryo quality have been associated with errors in spermatozoal chromatin. The errors appear to be most important in the morphologically normal or near-normal spermatozoa that accompany abnormal sperm (mainly misshapen heads) in subfertile males. Although these sperm access the ovum *in vivo*, the nature of their subfertility appears to center on the inability of their DNA to decondense in a timely or normal fashion following fertilization, with subsequent retardation in formation of the male pronucleus. This is currently thought to result in

delayed cleavage and development of the embryo, resulting in failure to establish maternal recognition of pregnancy. Recognition of the existence of these uncompensable cells in the ejaculate is currently best based on abnormal levels of sperm with misshapen heads.

References

- [1] Saacke RG, Dalton JC, Nadir S, Nebel RL, Bame JH. Relationship of seminal traits and insemination time to fertilization rate and embryo quality. *Anim Reprod Sci* 2000;60–61:663–77.
- [2] Sullivan JJ, Elliott FI. Bull fertility as affected by an interaction between motile spermatozoa concentration and fertility level in artificial insemination. *VI Int Cong Anim Reprod Artif Insem* 1968;2:1307.
- [3] den Daas JH, DeJong GG, Lansbergen L, Van Wagtendonk-De Leeuw AM. The relationship between the number of spermatozoa inseminated and the reproductive efficiency of individual bulls. *J Dairy Sci* 1998;81:1714–23.
- [4] Saacke RG, DeJarnette JM, Bame JH, Karabinus DS, Whitman S. Can spermatozoa with abnormal heads gain access to the ovum in artificially inseminated super- and single-ovulating cattle? *Theriogenology* 1998;51:117–28.
- [5] Williams WW, Savage A. Observations on the seminal micro-pathology of bulls. *Cornell Vet* 1925;15:353–75.
- [6] Williams WW, Savage A. Methods of determining the reproductive health and fertility of bulls. A review with additional notes. *Cornell Vet* 1927;17:374–6.
- [7] Lagerlof N. Morphological studies on the changes in the sperm structure and in the testes of bulls with decreased or abolished fertility. *Acta Path Microbiol Scand* 1934;19:254–67.
- [8] Koeford-Johnsen HH. Cervical secretions as a selective filter for abnormal types of spermatozoa. *Arsberetning Inst For Sterilitetsforskning Konelige Veterinaer-og Landbohojskole* 1972;15: 171–6.
- [9] Mortimer D. The survival and transport to the site of fertilization of diploid rabbit spermatozoa. *J Reprod Fertil* 1977;51:99–105.
- [10] Barros C, Vigil P, Herrera E, Arguello B, Walker R. Selection of morphologically abnormal sperm by human cervical mucus. *Arch Androl* 1984;12(Suppl):95–100.
- [11] Krzanowski H. The passage of abnormal spermatozoa through the uterotubal junction of the mouse. *J Reprod Fertil* 1974;38: 81–90.
- [12] Nestor A, Handel MA. The transport of morphologically abnormal sperm in the female reproductive tract. *Gamete Res* 1984;10:119–26.
- [13] Mullins J, Saacke RG. Study of the functional anatomy of the bovine cervical mucosa with special reference to mucus secretion and sperm transport. *Anat Rec* 1989;225:106–17.
- [14] Hunter RHF. Physiology and technology of reproduction in the female. London: Pub. Academic Press; 1980. p. 122–7.
- [15] Dresdner RD, Katz DF. Relationship of mammalian sperm motility and morphology to hydrodynamic aspects of cell function. *Biol Reprod* 1981;25:920–30.
- [16] Amann RP, Seidel GE, Mortimer RG. Fertilizing potential in vitro of semen from young beef bulls containing a high or low percentage of sperm with a proximal droplet. *Theriogenology* 2000;54:1499–515.
- [17] Kot MC, Handel MA. Binding of abnormal sperm to mouse egg zona pellucida in vitro. *Gamete Res* 1987;18:57–63.
- [18] Howard JG, Donoghue AM, Johnston LA, Wildt DE. Zona pellucida filtration of structurally abnormal spermatozoa and reduced fertilization in teratospermic cats. *Biol Reprod* 1993;49:131–9.
- [19] Thundathil JR, Meyer A, Palasz T, Barth AD, Mapletoft RJ. Effect of the knobbed acrosome defect in bovine sperm on IVF and embryo production. *Theriogenology* 2000;54:921–34.
- [20] Pace MM, Sullivan JJ, Elliott FI, Graham EF, Coulter GH. Effects of thawing temperature, number of spermatozoa and spermatozoal quality on fertility of bovine spermatozoa packaged in 0.5-ml French straws. *J Anim Sci* 1981;53:693–701.
- [21] Nadir S, Saacke RG, Bame JH, Mullins, Degelos JS. Effect of freezing semen and dosage of sperm on number of accessory sperm, fertility and embryo quality in artificially inseminated cattle. *J Anim Sci* 1993;71:199–204.
- [22] Bellin ME, Hawkins HE, Ax RL. Fertility of range beef bulls grouped according to presence or absence of heparin-binding proteins in sperm membranes and seminal fluid. *J Anim Sci* 1994;72:2441–8.
- [23] Killian GJ, Chapman DA, Rogowski LA. Fertility-associated proteins in Holstein bulls. *Biol Reprod* 1993;49:1202–8.
- [24] Barth AD. The relationship between sperm abnormalities and fertility. In: *Proc 14th Tech Conf on Artif Insem and Reprod*; 1992. p. 47–63.
- [25] Courot M, Colas G. The role of the male in embryonic mortality (cattle and sheep). In: Greenan JM, Diskin MG, editors. *Embryonic mortality in farm animals*. Dordrecht: Martinus Nijhoff; 1986. p. 95–203.
- [26] DeJarnette JM, Saacke RG, Bame JH, Vogler CJ. Accessory sperm: their importance to fertility and embryo quality, and attempts to alter their numbers in artificially inseminated cattle. *J Anim Sci* 1992;70:484–91.
- [27] Orgebin-Crist M, Jahad C. Delayed cleavage of rabbit ova after fertilization by young epididymal spermatozoa. *Biol Reprod* 1977;16:358–63.
- [28] Setchell BP, Occhio MJ, Hall MS, Lourie MS, Tucker MJ, Zupp JL. Is embryonic mortality increased in normal female rats mated to subfertile males? *J Reprod Fertil* 1988;83:567–74.
- [29] Miller D, Hrudka FM, Cates WF, Mapletoft R. Infertility in a bull with a nuclear sperm defect: a case report. *Theriogenology* 1982;17:611–21.
- [30] Coleman DA, Dailey RE, Leffell RE, Baker RD. Estrous synchronization and establishment of pregnancy in bovine embryo transfer recipients. *J Dairy Sci* 1987;70:858–66.
- [31] Eyestone WH, First NL. Variation in bovine embryo development in vitro due to bulls. *Theriogenology* 1989;31:191–6.
- [32] Hillery FL, Parrish JJ, First NL. Bull specific effect on fertilization and embryo development in vitro. *Theriogenology* 1990;33: 249–52.
- [33] Shi KS, Lu KH, Gordon I. Effect of bulls on fertilization of bovine oocytes and their subsequent development in vitro. *Theriogenology* 1990;33:324–33.
- [34] Eid LN, Lorton SP, Parrish JJ. Paternal influence on S-phase in the first cell cycle of the bovine embryo. *Biol Reprod* 1994;51:1232–7.
- [35] Walters AH, Saacke RG, Pearson RE, Gwazdauskas FC. Assessment of pronuclear formation following in vitro fertilization with bovine spermatozoa obtained after thermal insulation of the testis. *Theriogenology* 2006;65:1016–28.
- [36] Gledhill BL. Enigma of spermatozoal DNA and male infertility: a review. *Am J Vet Res* 1970;31:539–49.

- [37] Evenson DP, Darznikiewicz Z, Melamed MR. Relation of mammalian sperm chromatin heterogeneity of fertility. *Science* 1980;240:1131–4.
- [38] Ballachey BE, Evenson DP, Saacke RG. The sperm chromatin structure assay: relationship with alternate tests of semen quality and heterospermic performance of bulls. *J Androl* 1988;9: 109–15.
- [39] Karabinus D, Vogler CJ, Saacke RG, Evenson DP. Chromatin structural changes in bovine sperm after scrotal insulation of Holstein bulls. *J Androl* 1997;18:549–55.
- [40] Vogler CJ, Bame J, DeJarnette JM, McGilliard ML, Saacke RG. Effects of elevated testicular temperature on morphology characteristics of ejaculated spermatozoa in the bovine. *Theriogenology* 1993;40:1207–19.
- [41] Acevedo NJ, Bame H, Kuehn LA, Hohenboken WD, Evenson, DP, Saacke RG. Sperm chromatin structure assay (SCSA) and sperm morphology. In: *Proc 19th Tech Conf Artif Insem and Reprod*; 2002.p. 84–90.
- [42] Sakkas D, Manicardi G, Bianchi PG, Bizzaro D, Bianchi U. Relationship between the presence of endogenous nicks and sperm chromatin packaging in maturing and fertilizing mouse spermatozoa. *Biol Reprod* 1995;52:1140–55.
- [43] Sakkas D, Urner F, Bianchi PG, Bizzaro D, Wagner I, Jaquenoud N, et al. Sperm chromatin anomalies can influence decondensation after intracytoplasmic sperm injection. *Human Reprod* 1996;11:837–43.
- [44] Ostermeier GC, Sargeant GA, Yandell BS, Parrish JJ. The measurement of bovine sperm nuclear shape using the Fourier harmonic amplitudes. *J Androl* 2001;22:584–94.
- [45] Parrish JJ, Kaya A, Pawshe C, Diddiqui M, Shamsuddin M. Sperm shape research: an update. In: *Proc 21st Tech Conf Artif Insem and Reprod*; 2006.p. 19–26.