Sperm Morphology

R. G. Saacke, *PhD*
Dept of Dairy Science, Virginia Tech, Blacksburg, VA 24060

Abstract

Abnormal spermatozoa have been historically associated with male subfertility and sterility. As early as 1927, an insightful report by Williams and Savage in the *Cornell Vet* stated that "the dimensions of sperm heads from good fertility bulls are remarkably uniform". This is still true. Presence of abnormal spermatozoa in semen is one of our most sensitive indications that spermatogenesis of a male has been impaired. In addition, occurrence of certain types of abnormal sperm are more dangerous, and can indicate that normal-appearing sperm in the same sample may also be abnormal or incompetent in fertilization or sustaining embryogenesis once initiated. In this paper, we will summarize current concepts of how sperm morphology impacts fertilization rate, as well as early embryogenesis, by examining their transport in the female and engagement of the ovum. Also discussed is the relationship of sperm morphology as we measure it in the breeding soundness exam to what we recognize as the two main components comprising seminal deficiencies in artificial insemination: 1) those deficiencies which can be overcome by raising the sperm dosage to the female (compensable) 2) and those deficiencies where subfertility exists regardless of sperm dosage (uncompensable).

Introduction

Examination of semen for any trait is expected to provide an indication of fertility of the male or semen used in artificial insemination. We aspire to predict fertility from this endeavor. However, summarization of many experiments indicates the best tests of semen quality and or quantity only account for 25-50% of the variance in field pregnancy rates due to use of the inseminate or male. Combinations of tests may raise the accountability to as high as 60% of the variance in fertility, but rarely above. Thus, we are reminded that predictability has not been achieved and there is still much to be learned about the nature of subfertility due to the male, whose problems appear equally as complex as those associated with reproduction in the female. It is in this context that we discuss sperm morphology, one aspect of semen quality.

What is abnormal?

First we may have some difficulty in physically describing the abnormal sperm to everyone's satisfaction. Historically, problems associated with repeatability in measuring morphologically abnormal sperm were due to the fact that the same techniques were not always applied.[18] There were notable differences to be seen by varying staining, preservation or fixation methods as well as microscope optics and magnifications. Many of these exacerbated the natural human error of observation when slight deviations in cell shape or inclusions were present, or where multiple abnormalities occurred in the same cell. Efforts to make morphometric measurements more objective and precise show great promise.[36,37] In addition, advances in spermatozoal DNA evaluation add another dimension to recognition of abnormal cells. On this premise, I would like to think that we could agree on the behavior of abnormal sperm as: 1) those unable to participate in fertilization due to their morphology, or 2) those able to initiate fertilization or embryogenesis, but not competent to sustain either or both events. Sperm morphology associated with these two criteria is the focus of this presentation.

Impact on fertilization rate of sperm not capable of participating in fertilization would be dependent upon the total numbers of sperm in the dosage/inseminate and level of this seminal deficiency in the sample. Theoretically, such a deficiency can be overcome by increasing the sperm dosage to the female. Such an abnormal trait might therefore be considered compensable so long as the minimum number of sperm required by the female can be met by the "normal" sperm population in the inseminate. On the contrary, sperm capable of reaching the site of fertilization, penetrating the ovum vestments and initiating fertilization and/or embryogenesis, but not capable of sustaining either or both events would be uncompensable. In such a case, spermatozoal incompetence would preempt fertilization by a competent sperm in proportion to the frequency of occurrence of the incompetent sperm population in the inseminate. This would result in subfertility to sterility at any sperm dosage.

Compensable vs. uncompensable sperm abnormalities and barriers in the female tract

Morphologically abnormal sperm in semen have been strongly associated with male subfertility and sterility for many years.[28,52,53] From the compensable standpoint, we now recognize that sperm with classically abnormal heads or tails, described by these early workers using simple microscopes, do not traverse the female reproductive tract or participate in fertilization. Barriers precluding their progression to the oviduct have been identified in a variety of species, all relevant to our discussion of the bovine. Barriers to abnormal tails and heads include the cervix and cervical mucus in the bovine,[24] rabbit,[30] and human;[4] the UTJ and lower isthmus impair traverse by sperm with abnormal heads in the mouse[27,34] and rabbit[30] and tails with droplets in the mouse.[34] Considering the very small, intricate privileged paths offered by the cervix and mucus of species having vaginal semen deposition[32] as well as the intricacies of the UTJ in species having uterine semen deposition,[23] it may be that flagellar pattern is important to sustained transport of sperm across these barriers, removing cells with abnormal tails or protoplasmic droplets. Similarly, Dresdner and Katz[14] have shown that small, geometrical differences in head morphology can cause large differences in sperm hydrodynamics. Thus, impaired or abnormal sperm motility may be the underlying basis for sperm exclusion based upon head morphology as well. It has also been observed that *in vitro*, sperm with abnormal heads[26] or acrosomes[48] in proximity to the ovum were unable to attach to or penetrate. In felids, Howard *et al*[22] reported that the zona pellucida itself provided a formidable barrier to abnormal heads, with the most abnormal being on the outermost portions of the zona and those with improved morphology closest to the vitelline membrane. On this basis, we can say that as long as there are sufficient numbers of normal sperm in the dosage of semen to satisfy the numbers needed for fertilization, we could consider abnormal sperm as a compensable deficiency.

Unfortunately, abnormal sperm content of a semen sample does not give us the option of simply compensating with additional sperm in the dosage. Sullivan and Elliott[47] were the first to demonstrate that low fertility males (at any sperm dosage) required more sperm to reach their maximum conception than did highly fertile males. They postulated this was due to the elevated abnormal sperm content in semen characteristic of low fertility males. For spermatozoal head abnormalities, this postulate has since been validated by the fact that sperm with classical abnormal heads do not access the ovum *in vivo* as determined by evaluation of accessory sperm.[40] Thus, the question is raised, can normal or near-normal appearing sperm in the abnormal inseminate be the uncompensable component of the low fertility male, i.e. account for subfertility at any dosage?

There is now good evidence that use of inseminates with lowered sperm quality, measured by both viability and morphology, can result in both lowered fertilization rate and very early embryonic failure prior to maternal recognition of pregnancy.[5,12,13,35,43] Differences among bulls in embryonic quality (6-7 day-old embryos) have been reported at the time of routine recovery for embryo transfer[30] and after observation of embryo survival in recipients.[11] Bulls were also shown to differ in the *in vitro* development of their embryos following *in vitro* fertilization.[15,17,21,44,51] Thus, evidence is strong for existence of incompetent sperm, capable of initiating fertilization and/or embryogenesis, but incapable of sustaining either event. Males having disturbances in spermatogenesis resulting in depressed viability and increased abnormalities usually provide a broad spectrum in severity of morphological forms, dependent upon the stage of spermatogenesis affected by the disturbance.[19,49,50] However, can the incompetent sperm be detected? Saacke *et al*[40] showed that while classically abnormal sperm are indeed excluded from the ovum (accessory sperm studies), sperm with normal or subtly misshapen heads (with or without vacuoles) in otherwise abnormal ejaculates do gain full access to the ovum based upon occurrence in the inseminate. *In vitro*, sperm with abnormal acrosomes signify incompetence in normal sperm in the same ejaculate[48] as do sperm with proximal protoplasmic droplets when levels exceed 30%.[2] Which fertilizing sperm are competent and which are not is still unclear; however, it is now accepted that normal or near-normal appearing sperm in abnormal ejaculates are the most likely cause of the very early embryonic death. Thus, the classical morphologically abnormal sperm in ejaculates (theoretically compensable) appear to represent the "tip of an iceberg", signifying the presence of the uncompensable component in these same ejaculates.

Morphologically normal, but incompetent sperm?

It should be recognized that sperm with microscopically normal morphology, but defective chromatin, have been implicated in cases of male subfertility since 1970.[20] The chromatin structure assay developed by Evenson *et al*[16] revealed a strong positive association between heterospermic fertility and stability of sperm DNA to acid denaturation in bulls.[3] Most recently, Acevedo *et al*[1] reported that spermatogenic disturbances caused by elevated testicular temperature resulted in the production of abnormal sperm, and vulnerability of sperm chromatin to acid denaturation accompanied an increase in misshapen sperm heads. However, vulnerability of

sperm chromatin to denaturation was not restricted to abnormal cells; rather, it extended to morphologically normal cells in the same ejaculates as those having the misshapen sperm heads.[1] This tends to confirm that occurrence of morphologically abnormal sperm can signal chromatin abnormalities and potential incompetence among both normal and abnormal sperm in the same semen samples.

Flaws in packaging and condensation of sperm chromatin during spermiogenesis has been speculated to be involved in the instability of DNA of subfertile semen.[41,42] The instability of the DNA is thought, in turn, to be due to limitations in disulfide bonding within nuclear chromatin during the nuclear/chromatin condensation process,[25] which begins during early spermiogenesis (formation of the sperm head) and continues through epididymal maturation.[8] The association of DNA vulnerability to acid denaturation with onset of abnormal heads in males subjected to elevated testicular temperatures[1,19] may be due to the interference of elevated testicular temperature with substitution of sperm nuclear proteins, protamines (rich in sulfhydryl groups), for the somatic cell nuclear proteins, histones. The fertilizing sperm has a very condensed and compact chromatin. However, the process of nuclear condensation reverses itself after fertilization by the decondensation of the sperm nucleus within the egg, an oxidative process resulting in the production of thiol groups (SH) from the dissulfide (SS) linkages. Qui *et al*[38] have shown that flaws in sperm DNA affect the kinetics of rate of decondensation and subsequent pronuclear formation following fertilization. Regarding the embryological impact of an incompetent sperm, this timing agrees quite well with the uncompensable seminal component in bulls. This component affects kinetics of rate of pronuclear formation and early cleavage in the bovine,[15] leading to the concept that the uncompensable-or incompetent-fertilizing sperm cannot decondense the nucleus, forming a pronucleus in a timely fashion to permit normal rate of embryogenesis. This concept will need additional testing but is thought to be, at the least, a partial explanation for the incompetence of the morphologically normal fertilizing sperm in sustaining early embryogenesis.

Morphological deviations within a male are of importance to fertility

Finally, we should revisit two of the early conclusions of Williams and Savage:[52,53] 1) the dimensions of sperm heads from good fertility bulls are remarkably uniform and 2) permissible numbers of abnormal sperm in an ejaculate depend largely on the type of abnormalities present.

Did Williams and Savage[52,53] see slight differences in sperm morphology among males that, if consistent or uniform, were inconsequential to fertility? – apparently so. However, we might then ask the question, is "abnormal" animal-dependent? To this, we must say yes and no. First, which abnormality are we questioning, and what other abnormalities accompany it? The confusion comes from our past lack of understanding the degree to which specific deviations in morphology interfere with fertility or signify an underlying problem. This should now be in better focus. Heritable differences in the dimensions and shape of spermatozoal organelles from male to male within a species are known to occur without impact on fertility. These include subtle differences in head shape[7] and middle piece length.[29] For example, Sullivan[45] reported a normal fertile bull that repeatedly produced nearly absolute levels of slightly elongated sperm heads and another fertile bull having heads uniformly slightly short. Sperm from the bull giving longer sperm were shown to access (accessory sperm) and fertilize the egg normally.[33] On the contrary, specific abnormalities have been well documented as being heritable and resulting in degrees of subfertility to sterility (for reviews: Barth and Oko,[6] Blom,[9] Sullivan[46]). Examples include absolute levels of abnormal acrosomes in semen, resulting in sterility where sperm could not even attach to the egg *in vivo*,[10] versus moderate levels of similar defects resulting in subfertility among related bulls.[39]

Clearly, variation in sperm morphology among cells within ejaculates indicates a perturbation of spermatogenesis signifying the presence of both compensable and uncompensable components impacting fertility. Variance in sperm head morphology is of greatest concern, because it is associated with abnormal chromatin that even extends to normal cells in the same ejaculates. Slight deviations in head shape, if uniform, may be tolerated, particularly if characteristic of the male are not seasonally influenced. However, any specific trait known to preclude spermatozoal access to the ovum should be open for critical scrutiny regardless of uniformity.

Conclusions

Abnormal sperm content of semen is the most sensitive seminal parameter related to lowered reproductive performance, and should be given serious attention by veterinarians and AI personnel.

Abnormal sperm content in semen may reduce reproductive efficiency, by either insufficient numbers of sperm capable of reaching the site of fertilization, or by their inability to complete fertilization or sustain the embryo following fertilization.

Variation in sperm head and tail morphology within a sample reflects a disturbance of spermatogenesis, with sperm head morphology signaling presence of abnormal spermatozoal chromatin. The abnormal

chromatin extends to cells of normal morphology in the same sample. Spermatozoa with normal morphology, but abnormal chromatin, are thought to be the cause of the uncompensable seminal traits leading to early embryonic death.

References

1. Acevedo N, Bame JH, Kuehn LA, Hohenboken WD, Evenson DP, Saacke RG: Effects of elevated testicular temperature on spermatozoal morphology and chromatin stability to acid denaturation in the bovine. *Biol Reprod* 64 (Suppl 1): 217-218, 2001.
2. Amann RP, Seidel GE, Mortimer RG: 2000. Fertilizing potential *in vitro* of semen from young beef bulls containing a high or low percentage of sperm with a proximal droplet. *Therio* 54:1499-1515.
3. Ballachey BE, Evenson DP, Saacke RG: The sperm chromatin structure assay: relationship with alternate tests of semen quality and heterospermic performance of bulls. *J Andrology* 9:109-115, 1988.
4. Barros C, Vigil P, Herrera E, Arguello B, Walker R: Selection of morphologically abnormal sperm by human cervical mucus. *Arch Androl* 12 (suppl): 95-100, 1984.
5. Barth AD: The relationship between sperm abnormalities and fertility in *Proc 14th Tech Conf on Artif Insemin And Reprod.*, Nat'l Assoc Animal Breeders, Columbia, MO, 1992, pp 47-63.
6. Barth AD, Oko RJ: *Abnormal morphology of bovine spermatozoa.* Iowa State University Press, Ames, Iowa, 1989.
7. Beatty RA: Genetics of the mammalian gamete. *Biol Rev* 45:73-104, 1970.
8. Bedford JM, Calvin HI: The occurrence and possible functional significance of s-s crosslinks in sperm heads with particular reference to eutherian mammals. *J Exp Zool* 188:137-156, 1974.
9. Blom E: The ultrastructure of some characteristic sperm defects and a proposal for a new classification of the bull spermiogram. *Nord Vet Med* 25:383-393, 1973.
10. Buttle HRL, Hancock JL: Sterile boars with "knobbed" spermatozoa. *J Agr Sci* 65:255-262, 1965.
11. Coleman DA, Dailey RE, Leffel RE, Baker RD: Estrous synchronization and establishment of pregnancy in bovine embryo transfer recipients. *J Dairy Sci* 70:858-866, 1987.
12. Courot M, Colas G: The role of the male in embryonic mortality (cattle and sheep), in Greenan JM, Diskin MG (eds): *Embryonic Mortality in Farm Animals.* Dordrecht, Martinus Nijhoff, 1986, pp 95-203.
13. 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* 70:484-491, 1992.
14. Dresdner RD, Katz DF: Relationship of mammalian sperm motility and morphology to hydrodynamic aspects of cell function. *Biol Reprod* 25:920-930, 1981
15. Eid LN, Lorton SP, Parrish JJ: Paternal influence on S-phase in the first cell cycle of the bovine embryo. *Biol Reprod* 51:1232-1237, 1994.
16. Evenson DP, Darznikiewicz Z, Melamed MR: Relation of mammalian sperm chromatin heterogeneity of fertility. *Science* 240:1131-1134, 1980.
17. Eyestone WH, First NL: Variation in bovine embryo development in vitro due to bulls. *Therio* 31:191-196, 1989.
18. Foote RH: Effect of processing and measuring procedures on estimated sizes of bull sperm heads. *Therio* 59:1765-1733, 2003.
19. Geiger LN, Enwall LE, Kaya A, Bergfelt RS, Parrish JJ: The effects of scrotal insulation on bovine sperm morphology as determined by Fourier harmonic analysis and its relation to *in vitro* fertilization. *Biol Reprod* 68 (Suppl. 1): 151, 2003.
20. Gleldhill BL: Enigma of spermatozoal DNA and male infertility: a review. *Am J Vet Res* 31:539-549, 1970.
21. Hillery FL, Parrish JJ, First NL: Bull specific effect on fertilization and embryo development *in vitro*. *Therio* 33:249, 1990.
22. Howard JG, Donoghue AM, Johnston LA, Wildt DE: Zona pellucida filtration of structurally abnormal spermatozoa and reduced fertilization in teratospermic cats. *Biol Reprod* 49:131-139, 1993.
23. Hunter RHF: *Physiology and Technology of Reproduction.* London UK, Academic Press, pp 104-144, 1980.
24. Koeford-Johnsen HH: Cervical secretions as a selective filter for abnormal types of spermatozoa. Arsberetnig Inst. For Sterilitetsforskning, Konelige Veterinaer-og Landbohojskole 15:171-176, 1972.
25. Kosower NS, Katayose H, Yanagamachi R: Thiol-disulfide status and acridine orange fluorescence of mammalian sperm nuclei. *J Andrology* 13:342-348, 1992.
26. Kot MC, Handel MA: Binding of abnormal sperm to mouse egg zonae pellucidae *in vitro*. *Gamete Res* 18: 57-63, 1987.
27. Krzanowski H: The passage of abnormal spermatozoa through the uterotubal junction of the mouse. *J Reprod Fertil* 38:81-90, 1974.
28. 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* 19:254-267,1934.
29. Lukefahr SD, Hohenboken W: Characteristics of spermatozoan midpiece length and its relationship with economically important traits in cattle. *J Dairy Sci* 64:508-512, 1981.
30. Miller D, Hrudka M , Cates WF, Mapletoft R: Infertility in a bull with a nuclear sperm defect: a case report. *Theriog* 17:611-621, 1982.
31. Mortimer D: The survival and transport to the site of fertilization of diploid rabbit spermatozoa. *J Reprod Fertil* 51: 99-105, 1977.
32. Mullins J, Saacke RG: Study of the functional anatomy of the bovine cervical mucosa with special reference to mucus secretion and sperm transport. *The Anat Record* 225:106-117, 1989.
33. Munkittrick TW, Nebel RL, Saacke RG: Effect of microencapsulation on accessory sperm in the zona pellucida. *J Dairy Sci* 75:725-731, 1992.
34. Nestor A, Handel MA: The transport of morphologically abnormal sperm in the female reproductive tract. *Gamete Res* 10:119-126, 1984.
35. Orgebin-Crist M, Jahad C: Delayed cleavage of rabbit ova after fertilization by young epididymal spermatozoa. *Biol Reprod* 16:358:363, 1977.
36. Ostermeier GC, Sargeant GA, Yandell BS, Parrish JJ: Measurement of bovine sperm nuclear shape using Fourier harmonic amplitudes. *J Androl* 22:584-594, 2001.
37. Ostermeier GC, Sargeant GA, Yandell BS, Evenson DP, Parrish JJ: Relationship of bull fertility to sperm nuclear shape. *J Androl* 22: 595-603, 2001.
38. Qiu J, Hales BF, Robaire B: Effects of chronic low-dose cyclophosphamide exposure on the nuclei of rat spermatozoa. *Biol Reprod* 52:33-40, 1995.
39. Saacke RG, Amann RP, Marshall CE: Acrosomal cap abnormalities of sperm from subfertile bulls. *J Anim Sci* 27:1391-1398, 1968.
40. Saacke RG, DeJarnette JM, Bame JH, Karabinus D S, Whitman S: Can spermatozoa with abnormal heads gain access to the ovum in artificially inseminated super- and single-ovulating cattle? *Therio* 51:117-128, 1998.
41. Sakkas D, Manicardi G, Bianchi P G, Bizzaro D, Bianchi U: Relationship between the presence of endogenous nicks and sperm chromatin packaging in maturing and fertilizing mouse spermatozoa. *Biol Reprod* 52:1140-1155, 1995..
42. Sakkas D, Urner F, Bianchi PG, Bizzaro D, Wagner I, Jaquenoud N, Manicardi G, Campana A: Sperm Chromatin anomalies can influence decondensation after intracytoplasmic sperm injection. *Human Reprod* 11: 837-843, 1996.
43. 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* 83:567-574, 1988.
44. Shi KS, Lu KH, Gordon I: Effect of bulls on fertilization of bovine oocytes and their subsequent development *in vitro*. *Therio* 33:324, 1990.

45. Sullivan JJ: Personal communication 1974.
46. Sullivan JJ: Morphology and motility of spermatozoa, in: Salisbury GW, VanDemark NL and Lodge JR (eds): ***Physiology of Reproduction and Artificial Insemination of Cattle,*** ed 2, W. H. Freeman and Co. San Francisco, CA, 1978, pp 286-328.
47. Sullivan JJ, Elliott FI:1968. Bull fertility as affected by an interaction between motile spermatozoa concentration and fertility level in artificial insemination. ***VI Int'l Cong Anim Reprod Artif Insem 2:1307, 1968.***
48. Thundathil J, Meyer JR, Palasz AT, Barth AD, Mapletoft RJ: Effect of the knobbed acrosome defect in bovine sperm on IVF and embryo production. ***Therio*** 54: 921-934, 2000.
49. Vogler CJ, Saacke RG, Bame JH, DeJarnette JM, McGilliard ML: Effects of scrotal insulation on viability characteristics of cryopreserved bovine semen. ***J Dairy Sci*** 74:3827-3835, 1991.
50. Vogler CJ, Bame JH, DeJarnette JM, McGilliard ML, Saacke RG: Effects of elevated testicular temperature on morphology characteristics of ejaculated spermatozoa in the bovine. ***Therio*** 40:1207-1219, 1993.
51. Walters A, Eyestone W, Saacke RG, Gwazdauskas FC: Does sperm preparation methods affect bovine embryonic development after IVF with semen after thermal insult. ***Biol Reprod*** 68 (Suppl 1):338, 2003.
52. Williams WW, Savage A: Observations on the seminal micropathology of bulls. ***Cornell Vet*** 15:353-375, 1925.
53. Williams WW, Savage A: Methods of determining the reproductive health and fertility of bulls. A review with additional notes. ***Cornell Vet*** 17:374-376, 1927.

Longer-acting metaphylaxis –TETRADURE™ 300 (oxytetracycline) Injection is the only injectable antibiotic that protects high-risk cattle from BRD[1] for 7 to 8 days.[2] This gives TETRADURE 300 the power to add an entirely new dimension to metaphylactic therapy. And it delivers results comparable to MICOTIL® (tilmicosin) or NUFLOR® (florfenicol).[3] To discover the benefits of longer-acting BRD control for your clients, contact your Merial Representative today.

Not for use in lactating dairy animals. Adverse reactions, including injection site swelling, restlessness, ataxia, inflammation and respiratory abnormalities, have been reported.

TETRADURE™ 300
(oxytetracycline) Injection

1. Associated with *Mannheimia (Pasteurella) haemolytica*.
2. Based on label claims and FOI Summary.
3. Trials available upon request.

MERIAL

www.tetradure.com
1-888-637-4251

Better products mean better results™

RAGEVTD401A (01/04)