

Male Fertility Factors

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Introduction:

Since the early 60s, in our lab we sought useful correlations of seminal traits and fertility in order to ultimately predict the reproductive outcome of bovine semen used in artificial breeding or the fertility of a specific male under natural service. Despite these efforts, to this day we can only account for approximately 50-60% of the variation in fertility among males by measuring seminal traits, leaving the goal of predictability quite unachieved. In our frustration and need to progress in this endeavor, shared by many other reproduction laboratories throughout the world, the fate of sperm in the female reproductive tract, both quantitatively and qualitatively, has become an important consideration needing to be addressed. Quantitatively, the question asked centers on how few sperm from a male are necessary for optimal fertility? This question has been important toward maximizing influence of genetically sound males and has gained considerable importance with recent advancements in the x-y sorting of sperm, still a rather inefficient process requiring low sperm dosages for acceptable economic returns. Seminal traits addressing sperm number per dose are considered compensable. Qualitatively, we also know that seminal deficiencies exist that result in subfertility at any sperm dosage, suggesting incompetence in the fertilizing sperm. Such deficiencies are considered uncompensable. In this review, I would like to address some of the insights that have been derived from our growing understanding of the fate of sperm in the female tract and the interaction of seminal traits with that fate. Hopefully, they may at least explain some of the frustrations encountered or perhaps offer solutions to the critical eye as new tools become available in control of semen production as well as in the control of ovulation in the female. To do this, I would like to consider data from a variety of species.

Selective Sperm Transport and Retention in the Female:

Since the late 70s a rich literature has developed addressing the fate of sperm in the female as well as the influence of the female tract on the quality and quantity of sperm reaching the site of fertilization. I would like to encapsulate the portions of this work most meaningful to our current understanding of the female-male interaction, particularly as it relates to semen quality and the success or failure of an insemination. However, for those interested in the area of sperm transport in the female per se, other reviews should be consulted (Hawk, 1983; Hawk, 1987; Hunter and Wilmut, 1984 and Suarez et al. 1990, and for seminal quality aspects of sperm transport, Saacke, 1982).

In cattle, Van Demark and Hays (1954) first reported the very rapid transport of sperm from the site of deposition, naturally in the vagina or artificially in the uterus, to the oviductal ampulla as between 2.5 and 3.3 minutes. Years later, critical studies on the kinetics of sperm transport in the rabbit by Overstreet et al. (1978) showed that sperm transport occurred in two phases, the rapid and sustained. Indeed the first, rapid transport from the site of deposition to the site of fertilization, did occur within 1-15 minutes post-coitus or insemination; however, these were primarily dead or moribund sperm that were passively ushered through the tract by muscle contractions associated with coitus or insemination. However, there was an apparent

build-up of sperm in the lower isthmus of the oviduct which did not begin until approximately 4 hours post coitus, reaching highest sperm numbers 10 to 16 hours post coitus (sustained sperm transport). Drawn from this apparent oviductal sperm reservoir in the lower isthmus, nearly 100% viable spermatozoa were found in the vicinity of the upper isthmus and ampulla in proximity to ovulation (8-12 hours post-coitus), albeit in relatively low numbers.

Thus, the colonization of the lower oviduct by a population of predominantly viable spermatozoa during this sustained phase of transport is thought to provide the potential fertilizing sperm that reach the site of fertilization at the ampullary-isthmic junction of the oviduct; timing, however, may vary among species. For example, in the bovine, colonization of the lower isthmus occurred over a period of 6 to 12 hours post mating (Hunter and Wilmut (1984).

In the pig, Hunter (1980) provided evidence that the oviductal isthmus reservoir most likely served to maintain spermatozoal function until ovulation. Using mouse oviduct, which is translucent, Suarez (1987) observed the behavior of mouse sperm in situ and in proximity to ovulation. Sperm were retained in the isthmus by adherence of their heads to the mucosa as well as an apparent flagellar immobilization in this region. The adherence is mediated by sugar residues in the cell membrane overlying the sperm head region, fucose in the case of bovine (Lefebvre et al., 1997). One line of thought is that sperm in the isthmus reservoir are released by cue(s) to continue progress through the site of fertilization and the ampulla by events associated with ovulation (for review, Hunter 1998) permitting timely union of sperm and egg. For a number of species including mouse, rat, hamster, rabbit, sheep and cattle, Suarez et al. (1990) reviewed the numbers of sperm in the oviductal isthmus and the oviductal ampulla (near fertilization site) at the time of the impending ovulation. She also included the variation among species in numbers of sperm deposited at mating. Quite strong similarities were noted. Across species, the number of sperm inseminated ranged from 50 million for the mouse to 3 billion for the bovine, while numbers of sperm in the oviductal isthmus at the time of ovulation ranged from 4.4 thousand for sheep to 21.2 thousand for cattle, relatively small numbers when compared to the inseminates. Found in the oviductal ampulla at ovulation were only 2 sperm for the hamster and rat and 5, 10, 26 and 118 sperm for mice, cattle, sheep and rabbits, respectively. The very small number of sperm passing through the ampulla (near site of fertilization) at any one time has been thought to be an important natural safeguard against polyspermy (Hunter, 1988), known to result in early embryonic death.

Where do the millions to billions of sperm inseminated go? There is evidence that sperm are lost through ingestion by phagocytes, passing through the reproductive tract and into the body cavity, or by retrograde loss to the exterior (Hafez, 1974). In a precise study of sperm loss in cattle following artificial insemination directly into the uterus, nearly 90% of the sperm inseminated was retrograded to the exterior or found in the vagina within 12 hours of insemination (Mitchell et al. 1985). In sheep (Mattner, 1969), pigs (Pursel et al., 1978) and cattle (Lineweaver et al., 1970) it has been shown that number of sperm reaching the oviducts and retained in these organs is influenced downward by the sperm-injurious events associated with cryopreservation. Presumably, injured sperm are more easily retrograded by the continuous caudal flow of female fluids or they are removed by phagocytes. Nevertheless, sperm dosage in the inseminate has been shown to be positively related to numbers of sperm accessing the oviduct in rabbits (Morton and Glover, 1974), making losses from cryo-cell

injury compensable by cell dosage. I believe that we can say, that numbers of viable (healthy, motile) sperm in the inseminate is important to the number of sperm colonizing the oviductal isthmus reservoir under the sustained transport system and is probably essential to the numbers or duration of sperm progressing from this reservoir to the ampulla or engaging the ovum at the ampullary-isthmic junction. There is not sufficient data available to speculate on the nature of this relationship between inseminate numbers of sperm and reservoir numbers of sperm. However, it would not be surprising if numbers of viable sperm inseminated were positively associated with those available for fertilization, but not in a linear fashion, rather a quadratic one with an asymptote. In vivo protection against polyspermy appears to be a strong priority in mammals (Suarez et al. 1990).

Barriers in the Female Tract to Sperm Transport.

Morphologically abnormal sperm in semen of males has been associated with subfertility and sterility for many years (Williams and Savage, 1925, 1927; Lagerlof, 1934). We now recognize that sperm with classically misshapened heads, described by these early workers using simple microscopes, do not traverse the female reproductive tract or participate in fertilization based upon recovery and evaluation of accessory sperm from ova and embryos (Saacke et al., 1998). 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 the bovine (Koeford-Johnson, 1972), rabbit (Mortimer, 1977) and human (Barros et al., 1984); the UTJ and lower isthmus impair traverse by sperm with abnormal heads in the mouse (Krzanowska, 1974, Nestor and Handel, 1984) and rabbit (Mortimer, 1977) and tails with droplets in the mouse (Nestor and Handel, 1984). Considering the very small, intricate privileged paths offered by the cervix and mucus for species having vaginal semen deposition (Mullins and Saacke, 1989) as well as the intricacies of the UTJ in species having uterine semen deposition (Hunter (1980), 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. In this regard, Dresdner and Katz (1981) have shown that even 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 proximal droplets (Amann et al., 2000) and abnormal heads were unable to attach to (Kot and Handel, 1987) or penetrate (Howard et al., 1993) the vestments of the ovum. In felids, Howard et al., (1993) reported that the zona pellucida itself provides a formidable barrier to abnormal heads with the most abnormal being on the outer most portions of the zona and those with improved morphology closest to the vitelline membrane. Spermatozoa with abnormal acrosomes were found to be impaired in their 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 (Thundathil et al. 2000). 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.

From the Females point of View: When are Seminal Deficiencies Compensable?:

Our current concepts are most clear from bovine data since artificial insemination records are the most extensive for this specie. In this concept, the interaction of the male and female was first depicted by Salisbury and VanDemark (1961) showing that fertility increases with increasing numbers of quality sperm delivered up to a threshold, after which, limiting factors in the reproductive capacity of the female population become important. Pace et al. (1981) showed this relationship to hold true for sperm viability traits of progressive motility, acrosomal integrity, and cell membrane integrity. For each of these measurable parameters, the number of sperm inseminated with the trait, not the percentage having the trait, was related to fertility and in an asymptotic fashion. Thus, seminal deficiencies resulting in subfertility due to below threshold numbers of viable sperm delivered to the female would be considered compensable in that adding sperm to the inseminate to above threshold numbers would eliminate the subfertility. Sullivan and Elliott (1968) were the first to show that bulls differed in the number of viable sperm necessary to reach the female threshold, thus complicating the matter. They postulated that one cause of the difference in threshold numbers among bulls was the abnormal sperm content of the semen. This has been since validated by the fact that most abnormal sperm do not access the ovum. However, in the bovine, males in artificial insemination can differ 10 fold or more in ability to access the ovum in vivo based upon fertility differences among bulls at low insemination dose (den Daas et al., 1998) or based upon accessory sperm number, differences among bulls measured at the same insemination dose (Nadir et al., 1993). 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 lower oviductal isthmus under sustained sperm transport. In addition such differences could easily be associated with the ability of sperm to undergo capacitation, sperm/egg recognition or even the acrosome reaction, all of which are still to be accurately quantified under laboratory conditions. On that basis, we can say that we still have much to learn before threshold sperm numbers in an inseminate or ejaculate can be calculated or predicted for a given male. It then follows that maximum dilution rates in artificial insemination or minimum inseminate dosages for our species of interest are still not in hand except for obtaining adequate numbers of breedings that can provide reliable fertility data to pinpoint the threshold for a given male.

From the Females point of View: When are Seminal Deficiencies Uncompensable?:

Sullivan and Elliott (1968) also demonstrated that low fertility males (at any dosage) generally required more sperm to reach their maximum conception than did highly fertile males. Such low fertility males 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 not competent to complete fertilization or sustain embryogenesis once these events are initiated (Barth, 1992; Courot and Colas, 1986; DeJarnette et al., 1992; Orgebin-Crist and Jahad, 1977; Setchell et al, 1988). Differences among bulls in embryonic development of their conceptuses have been reported at the time of

routine recovery for embryo transfer (Miller et al., 1982) and after observation of embryo survival in recipients (Coleman et al., 1987). Bulls were also shown to differ in the development of their embryos following in vitro fertilization (Eyestone and First, 1989; Hillery et al., 1990; Shi et al., 1990; Eid et al., 1994). In low fertility bulls, early cleavage rates were reduced and pronuclear formation was delayed (Eid et al., 1994). Thus, incompetence in morphologically normal or near-normal spermatozoa of abnormal ejaculates appear to be the cause of the uncompensable component.

It should be recognized that sperm with microscopically normal morphology but defective chromatin have been implicated in cases of male subfertility for some time (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 the sperm DNA to acid denaturation (Ballachey et al., 1988). Using this same assay, Karabinus et al., (1997) have shown that sperm ejaculated before a mild thermal insult of the testis by scrotal insulation have more stable DNA than those ejaculated after scrotal insulation where abnormal sperm are also evident (Vogler et al., 1993). Acevedo et al., (2001, 2002), 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 abnormal shaped sperm, but also extended to normal shaped sperm in the abnormal samples. This again, tends to confirm that occurrence of morphologically abnormal sperm can signal chromatin abnormalities and potential incompetence among both normal and abnormally appearing sperm in the same sample. It also underlines the fact that while female sperm selection appears amazingly strong based upon sperm shape and motility, it is 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., (1995, 1996) from which they speculated that flaws in packaging and condensation of sperm chromatin during spermiogenesis resulted in the instability of the DNA in subfertile men. The instability of the DNA is thought to be due to limitations in disulfide bonds essential for DNA condensation in the sperm nucleus which is, 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 for normal embryonic development and to signal maternal recognition of pregnancy. Recently, utilizing the scrotal insulation model in the bull, Walters et al., (2006) compared pronuclear development following IVF for semen obtained prior to and following development of denatured DNA by elevated testicular temperature. Clearly, they verified that the observations of Eid et al., (1994) were correct that 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.

Although it is important that we recognize both the compensable as well as the uncompensable seminal traits in our breeding male populations, it is clear that we must focus most seriously on the uncompensable traits since these result in subfertility regardless of sperm numbers in the inseminate, AI technique or reproductive strategy applied. Bulls having uncompensable deficiencies in their semen should be eliminated from use in our herds wherever possible. At our current state of knowledge, such bulls are best avoided by using AI bulls from reliable sources where semen morphology is a routine part of the evaluation process or in the case of natural bulls where semen morphology is strong part of the BSE exam.

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). 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 of which the zona pellucida may be the most formidable. Differences among males or semen samples exist with respect to accessibility of sperm to the ovum that cannot be explained by conventional sperm viability or morphological assessment. Spermatozoal 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 before we have a full appreciation of the 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 in subfertile males. Such sperm do access the ovum *in vivo*. The nature of the subfertility due to these incompetent sperm 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 to the point of failure to establish maternal recognition of pregnancy. Recognition of the existence of these uncompensable cells in the ejaculate is currently best indicated by abnormal levels of sperm with misshapen heads.

Although we could not touch all the critical literature on this topic, clearly that which we did touch supports the concept that understanding what happens to all those sperm and their fate in the female addresses many of the male/female interactions important to reproductive success or failure with emphasis on seminal quality. It has also provided answers to some of our frustrations in trying to predict the outcome of a mating or why our measures of reproductive ability do not often track with results. Clearly, the sperm that reach the site of fertilization are not the ones we evaluate in a BSE exam of a male or routine semen evaluations in the AI lab. However, the importance of knowing the viability and morphology

of the inseminate does give us critical insights upon which to, at the least, eliminate the majority of subfertile males and semen. It should also be clear that we still have much to learn with respect to sperm quality and quantity required for optimum fertilization as well as in judging cryopreservation and other techniques. There is no current method to accurately determine the minimum sperm dosage providing optimum fertility for a given male, or precisely indentify all subfertile males in the population.

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