The Interaction of Male and Female in Success of Mating

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Introduction: To most in this audience, having a veterinary practice dealing with problems associated with reproduction is undoubtedly fun, profitable and rewarding. However, reproductive problems can also be most frustrating, often testing the limits of our knowledge and our patience. From personal experience, I can also say the same for research in reproductive physiology, particularly where we are interested in understanding factors important to reproductive success or identifying causes and remedies for reproductive failure. 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% 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 (quantitatively and qualitatively) has become an important question needing to be addressed. Answers in response to this question have been enlightening and although they are not "silver bullets" to predicting fertility, they have provided insight to the nature of reproductive success and failure and have exposed the roots of some of our frustrations. I would like to share some of these insights with you today. Hopefully, they may explain some of the frustrations encountered in a therio practice 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.

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 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. 29 (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, but these were primarily dead or moribund sperm that were passively ushered through the tract by muscle contractions associated with coitus or insemination. More importantly, 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 ampullaryisthmic junction of the oviduct. Timing of transport events would be expected to vary among species. For example, in the bovine, colonization of the lower isthmus by potential fertilizing sperm was found to occur over a period of 6 to 12 hours post mating (Hunter and Wilmut, 1984).

In the pig, Hunter (1984) 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 by an apparent flagellar immobilization in this region. The adherence is mediated by sugar residues in the cell membrane overlying the head region, fucose in the case of bovine (Lefebvre et al., 1997). One line of evidence suggests that sperm in the isthmic reservoir are released by cue(s), to continue progress through the site of fertilization and the ampulla, from 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 (site of fertilization) at any one time has been thought to be an important natural safeguard against polyspermy (Hunter, 1988). 30

Where do the millions to billions of sperm inseminated go? There is evidence that sperm are lost by simply passing through the reproductive tract into the body cavity, through ingestion by phagocytes 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 were 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 sperminjurious events such as 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) and ova in cattle (Nadir et al., 1993), making losses from cryo-cell injury compensable by cell dosage. I believe that we can say, that numbers of viable healthy 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. It appears that, in vivo, protection against polyspermy appears to be a strong priority in mammals.

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 privedged paths offered by the cervix and mucus of 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, removing cells with abnormal tails or protoplasmic droplets. Similarly, Dresdner and Katz (1981) have shown that small geometrical 31differences 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 in proximity to the ovum 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 or such 32 events as ability 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.
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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 abnormal cells. As stated earlier, this explains the higher sperm dosage threshold or compensable component of the lower fertility male. 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). Males having disturbances in spermatogenesis resulting in the ejaculation of abnormal sperm usually provide a broad spectrum in severity of morphological forms dependent upon the stage of spermatogenesis affected by the disturbance (Vogler et al., 1991, 1993). Saacke et al. 1998 showed that while classically abnormal sperm are indeed excluded from the ovum (accessory sperm), sperm with normal or subtly misshapen heads in otherwise abnormal ejaculates do gain access to the ovum. Which sperm are competent and which are not is unclear; however, it is accepted that normal appearing sperm in abnormal ejaculates are most likely the cause of the early embryonic death associated with the male. Thus, abnormal sperm in ejaculates appear to signify the "tip of the iceberg" with respect to uncompensable reproductive wastage experienced by the use of such semen. It should be recognized here that sperm with microscopically normal morphology, but with 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 and stability of sperm DNA to acid denaturation in bulls (Ballachey et al., 1988). Acevedo et al., (2001) recently reported that spermatogenic disturbances 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 abnormal 33 sperm in the same semen samples. 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.

Natural Service vs Artificial Insemination and Timing of Mating in Relation to Ovulation: Finally, a word or two should be said about the time of mating and the nature of the mating, i.e., artificial vs natural service since both should subscribe to our discussions above. In many species we know there is an optimum time of mating, i.e., breeding too early or too late will result in depressed pregnancy rates. In a recent study, using the 6 day-old bovine embryo as a bio-monitor for optimum time of insemination, Dalton et al., (2001) examined insemination times of: heat onset, 12 and 24 hours post onset. These times approximated 27 hours, 15 hours and 3 hours prior to the average ovulation time, respectively, which in this case was approximately 27 ± 5 hours post onset of heat. Of interest from this study was the nature of the loss due to early vs late insemination. Accessory sperm recovered from the embryos/ova showed sperm accessibility to the egg lowest when insemination was at heat onset and highest at insemination 24 hours post onset. As expected, fertilization rate followed in the same fashion low at heat onset, highest at 24 hours. However, embryo quality was reversed, being maximum at heat onset (greatest number of excellent embryos) and lowest (highest number of degenerate embryos) at 24 hours post onset. Thus, the insemination at 12 hours post onset would have provided the best pregnancy rate; however, it was clearly a compromise between fertilization failure and embryo failure. The early

insemination provided sufficient time for sperm transport and colonization of the oviductal isthmus, but lowered fertilization rates were undoubtedly an outcome of the limitation of sperm life or retention in the oviductal reservoir . On the contrary, the late insemination (24 hours post onset of heat) had high fertilization rates from the expected high sperm accessibility (accessory sperm number) but the embryo quality from this insemination was impaired. Embryo quality may have suffered due to inadequate time for sperm selection or the aging of ova in the oviduct awaiting sperm, since ovulation was occurring at 22 to 32 hours post heat onset. With approximately a 6-hour requirement for sustained sperm transport in the bovine, insemination at 24 hours post heat onset would not provide fertilizing sperm in the oviductal isthmus until 30 hours or more post estrus resulting in the aging of ova as they await arrival of sperm. These results were based upon the intrauterine artificial insemination of a normal dose (25 million cells) of cryopreserved semen. Dalton et al., 2001 also compared a single natural service at heat onset with artificial insemination at heat onset and 12 hours post onset utilizing the same bulls. For natural service an estimated 3 to 8 billion cells were deposited in the vagina (cranial to the cervix) and for artificial insemination 25 million cryopreserved sperm were deposited intrauterine (caudal to the cervix). The natural service resulted in higher numbers of sperm accessing the egg, higher fertility and higher embryo quality than did artificial insemination at either time. The greater numbers of sperm from natural service and the lack of injury from 34 cryopreservation explain some of the differences; however, one is lead to wonder what role the cervix might play in sperm selection in the bovine based upon the improved embryo quality achieved by semen deposition caudal to the cervix.

Conclusions: Although we could not touch all the critical literature on this interesting 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. 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. How far we can breed an animal prior to ovulation or how close undoubtedly matters depending upon the male, the uncompensable and compensable deficiencies in his semen, semen preservation techniques and associated injury, how many sperm are inseminated and what female barriers exist between sperm and egg, i.e., site of deposition. Artificial insemination in many species, exotic, endangered, recreational and food producing, continues to offer animal breeders great potential from both genetic and health standpoints. However, we still have much to learn from natural service with respect to sperm quality and quantity available for fertilization as well as in the form of a basis for judging semen preservation methods and other techniques. To close I would simply say, if I were to remain active in my research career designed to improve artificial insemination, gaining a better understanding of the events and concepts associated with natural service would get most of my attention along with continued efforts to understand the fate of sperm in the female tract.

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