Asian Journal of Andrology (2015) 17, 522–528

www.asiaandro.com; www.ajandrology.com

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INVITED OPINION

Sperm Biology

Odyssey of the spermatozoon

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Asian Journal of Andrology (2015) **17**, 522–528; doi: 10.4103/1008-682X.153544; published online: 28 April 2015

This Opinion piece is offered as a cursory overview of sperm development, function, and transport through the eyes of an equine veterinarian. My professional background is predominantly clinical in nature, but my fascination with sperm function and preservation has led to a fairly sizeable review of the scientific literature over the years in hopes of extracting laboratory findings that have application to my daily activities in the clinical arena. Spermatozoa are quite unique among cellular types with regard to both form and function, and represent the only endogenously derived cell type that exerts its action in a separate being. This paper takes the reader on a voyage with a mammalian spermatozoon, from its formative stages through its transport in the male and female reproductive tracts, and culminating with its interaction with an ovulated oocyte at the time of fertilization. Specific emphasis is placed on equine spermatozoa when notable research findings have been unveiled.

With an odyssey defined as a long journey during which many things happen, it would seem that the spermatozoon epitomizes this term. For spermatologists and clinicians, however, the voyage of a spermatozoon might be best-described as a mystery expedition. Our understanding of sperm physiology remains fairly superficial despite an exhaustive number of manuscripts relating to the spermatozoon (over 63 000 entries on PubMed alone when sorting by the terms "spermatozoon" or "spermatozoa"). The uninformed individual might describe a spermatozoon as a highly specialized, but simple, cell with only one role to fulfill… that of fertilization. While fertilization might be considered the endpoint of sperm function, this cell must be extremely sophisticated and adaptable to achieve this task and the process involves a series of highly coordinated cellular and molecular events. Following is a list of some requirements ascribed to a mammalian spermatozoon:

1. Loss of most organelles and cytoplasm during formation in the testis and maturation in the epididymis (requires a host of intracellular and intercellular signaling events).
2. Remodeling of sperm chromatin within the epididymis as a protective mechanism against environmental injury (requires repackaging of nuclear DNA into a highly condensed form through the aid of specialized proteins termed protamines).
3. Plasma membrane alterations within the epididymis to yield proteins important to fertilization (requires various enzymatic-linked alterations of existing proteins, as well as uptake of proteins from epididymal fluid or from the epididymal epithelium).
4. Passage through the uterus and uterotubal junction of the female at the time of insemination (requires activated flagellar movements, protection against immunologic attack, and posttranslational modification of sperm-derived proteins).
5. Binding to oviductal epithelial cells to form a sperm reservoir (requires specific cell-cell attachment, possibly mediated through sperm surface carbohydrate-binding proteins, termed lectins).
6. Acquisition of additional maturational changes, collectively termed capacitation, that permit a spermatozoon to fertilize an oocyte (requires an assortment of signal transduction cascades).
7. Release from oviductal epithelial cells and passage to the vicinity of the oocyte at the isthmic-ampullar junction of the oviduct (requires a coordinated sperm-release mechanism, hyperactivated motility, and possibly chemotaxis and thermotaxis).
8. Penetration through the extracellular matrix of the oocyte cumulus (possibly mediated by hyperactivated motility and redistribution or unmasking of surface-associated hyaluronidase, as the cumulus matrix is rich in hyaluronic acid).
9. Binding to the zona pellucida, a highly glycosylated protein matrix surrounding the oocyte (may involve specific affinity between sperm surface molecules and the zona pellucida components).
10. Acquisition of the acrosome reaction, a regulated form of exocytosis (requires reorganization of the outer acrosomal and overlying plasma membranes necessary for fusion and vesiculation).
11. Penetration of the zona pellucida (release of acrosomal contents is required for this event to occur).
12. Binding and fusion with the oolemma (requires specific region-dependent molecular interactions).
13. Dispersion of nuclear contents (requires specific fusogenic alterations of the lipid membranes of the spermatozoon and oocyte).
14. Oocyte activation (spermatozoon-derived factor (s) is (are) required for activation of the oocyte through spermatozoon-mediated intracellular calcium oscillations).
15. Pronucleus formation (requires rapid decondensation of the highly compact sperm chromatin).
16. Organization of the mitotic spindle for restoration of diploidy (through the formation of the sperm aster which originates from the proximal centriole of the penetrating spermatozoon in most mammals).
17. Contribution to postfertilization

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This article was presented at the 12th International Symposium on Spermatology, August 10–14, 2014, Newcastle, Australia.

events (through transfer of mRNA and microRNAs to the oocyte).

After viewing this lengthy list of functions, one becomes quite appreciative of the highly complex and specialized features of a spermatozoon. In fact, the biochemical and biophysical features are so sophisticated that many of the cellular and molecular mechanisms remain unresolved to this day. Furthermore, spermatozoa (and oocytes) represent some of the most highly differentiated cells in the mammalian body; yet, when sperm and oocyte are combined, they retain their potential for totipotency (ability to divide and produce all cell types of the body) through creation of a zygote.

REGULATION OF SPERMATOGENESIS

The life of a spermatozoon begins within the testes unless one wishes to consider the embryonic origin of the primordial germ cells. The testis, an elaborately designed organ, is classically considered to possess two functions: (1) exocrine - spermatogenesis and (2) endocrine - production of hormones important for spermatogenesis, sexual differentiation, development of secondary sex characteristics, and libido. While this simplistic description provides one with the general concept of testicular function, it does not portray the extremely complex interplay of these two processes. Conventional descriptions convey the role of hypothalamic- and pituitary-derived hormones on the regulation of testicular function, as well as feedback mechanisms required for homeostasis. While such pathways are undoubtedly key orchestrators of testicular function, emerging information is revealing a multitude of molecular-mediated events that "cloud" our understanding of the events that coordinate spermatogenesis. As with any area of study, the more learned we become about a topic, the more queries surface that require additional clarification. Without question, a thorough understanding of testicular function will require a keen appreciation of the mechanisms by which genes and gene products are expressed and repressed. As an example of the genetic complexity surrounding control of testicular function, polymorphism of the gene for a methyltransferase, *DNMT1*, may increase the susceptibility of men to oligospermia.[1] A substantial genotyping study revealed that numerous single nucleotide polymorphisms (SNPs) were associated with oligozoospermia or azoospermia in men.[2] The importance of paracrine, juxtacrine, autocrine, cryptocrine, and lumicrine pathways to testicular and epididymal function has also become quite apparent in recent years. For instance, use of mice with a selective androgen receptor knockout in Sertoli cells revealed that the androgen receptor in the Sertoli cell is an absolute requirement for normal spermatogenesis.[3,4] Furthermore, experimentation with germ-cell specific androgen receptor knockout mice revealed normal spermatogenesis, suggesting that androgen receptor signaling for spermatogenesis may occur through autocrine/paracrine pathways.[5] The role of molecular chaperones in identifying aberrant germ cells for degradation is also being studied. In essence, to more fully understand what makes the testes "tick," we must continue to capitalize on the powerful genetic and molecular tools that have been developed in this capacity.

SPERMATOGENESIS – THE PROCESS

Testicular parenchyma occupies nearly 90% of the total testicular mass in adult stallions,[6] and over 70% of the testicular parenchyma is occupied by seminiferous tubules.[7] Sperm production, *i.e., spermatogenesis*, occurs within the seminiferous tubules. Both ends of these highly-coiled tubules open directly into the rete testis, such that the products (both cellular and noncellular) of the seminiferous tubules are excreted into the rete testis and delivered to the excurrent duct system (*e.g.,* the epididymis and ductus deferens). The numerous and tortuous seminiferous tubules, with a combined average length of 2419 m per testis in the stallion,[8] are comprised of an epithelial wall, termed the seminiferous or germinal epithelium, and a lumen. The *seminiferous epithelium* consists of germ cells in various stages of development intermingled with Sertoli cells that serve to provide structural support and a nurturing source to the germ cells. The Sertoli cells are anchored to the basement membrane and extend to the lumen, of the seminiferous tubules. The seminiferous tubules are bordered by peritubular myoid cells (myofibroblasts) that through peristaltic contractions, may aid in evacuation of luminal contents into the rete testis. These myoid cells are also considered to be involved in paracrine signaling events.[9–11]

A critical feature of the seminiferous epithelium is the formation of tight junctional complexes that develop between Sertoli cells, thereby physically dividing the seminiferous epithelium into basal and adluminal compartments. This structural complex is termed the *blood-testis barrier* because it offers immune privilege, by restricting direct access of blood-borne substances into the adluminal compartment. The described actions of the blood-testis barrier are: (1) to segregate meiotic (except the earliest preleptotene spermatocytes) and postmeiotic germ cells from immunologic attack, as these germ cells are considered to be in an immunologically privileged site, and (2) to provide a unique microenvironment for the final stages of germ cell development within the adluminal compartment.[12] Many studies involving genetically modified mice support the need for the blood-testis barrier in normal spermatogenesis.[13] Although other blood barriers exist in the general body, there is no apparent counterpart to the blood-testis barrier in the female ovary. In the female, development of primary oocytes occurs prior to the immune system recognizing self, and the female does develop the counterpart to spermatids (*i.e.,* ootids). In the male, germ cell development to spermatocytes and spermatids does not occur until puberty. This is well after the immune system recognizes self and considers these cells as foreign. Especially intriguing is the molecular control over the transient disassembly of the blood-testis barrier to facilitate migration of germ cells from the basal into the adluminal compartment.[12,14]

Spermatogenesis is an extremely complex process that involves germ cell proliferation, germ cell differentiation, and, paradoxically, programmed germ cell death (apoptosis). This lengthy process, 57 days in the stallion,[15] is controlled by a vast array of messengers acting through endocrine, paracrine, and autocrine pathways.[16–20] Considerable species diversity exists in the regulatory mechanisms, and some argue that certain aspects of spermatogenesis are independent of hormonal control.[21]

Spermatogenesis is initiated by the differentiation of spermatogonia from a stem cell pool that is continually replenished for most of a male's adult life. Under normal circumstances, this is a highly productive process, yielding on the order of 5 to 6 billion spermatozoa per day for an adult stallion. This translates into 30 to 40 trillion spermatozoa produced over the course of a stallion's reproductive life. From an equally impressive view, an average healthy stallion produces 60 000–70 000 spermatozoa per s. Newly formed spermatogonia enter a proliferative phase, whereby continuous mitotic amplifications yield a dramatic increase in spermatogonial numbers. Interestingly, the cytoplasmic component of mitotic divisions is often incomplete, resulting in daughter cells that remain connected by intracytoplasmic

bridges. Such an arrangement permits direct communication within this syncytium of developing germ cells, thereby assisting in their synchronous development. Following completion of spermatocytogenesis, the spermatocytes enter a meiotic phase, characterized by duplication and exchange of genetic information (*i.e.,* genetic recombination) and two meiotic divisions which reduce the chromosome complement to form haploid round spermatids. It is during the meiotic stage that germ cells pass through the blood-testis barrier to enter the adluminal compartment. During the final phase of development, spermiogenesis, round spermatids undergo a dramatic transformation that includes nuclear reshaping through chromatin compaction, creation of a flagellum, development of the acrosome, and considerable loss of cytoplasm. The fully developed spermatids are released as spermatozoa into the lumen of the seminiferous tubules by a process termed spermiation. It is during spermiogenesis that the germ cells may be most vulnerable to both structural and genetic defects.[22]

GERM-CELL DEGENERATION AND SPERMATOGENESIS

Germ-cell degeneration can be amplified with abnormal testicular function,[23] but it is also a normal phenomenon in spermatogenesis.[24] In fact, "normal" spermatogenesis is a relatively inefficient process, and has been reported to result in an estimated loss of 25% to 75% of the potential number of spermatozoa produced by spermiation in the rat.[25,26] In the stallion, germ-cell degeneration is more profound during the physiologic breeding season than during the nonbreeding season.[27] Developing spermatogonia appear to be the most vulnerable to apoptotic degeneration.[28] It is possible that the "physiological" form of germ-cell degeneration is a homeostatic mechanism that prevents overloading the Sertoli cells, *i.e.,* to maintain a fine balance in the germ cell: sertoli cell ratio. Apoptosis is a well-defined physiological process of cell elimination, and the apoptotic process is required for normal spermatogenesis in mammals.[28,29] Of interest, formation of the developing seminiferous tubules and the Sertoli cell (*i.e.,* blood-testis) barrier coincides with increased germ cell apoptotic rates in stallions, providing evidence that apoptosis may play an intricate role in initiation of spermatgenesis.[30] As expected, these changes are coincident with gene expression patterns.[31]

EPIDIDYMAL TRANSIT AND MATURATION

Mammalian spermatozoa are incapable of the *in-vivo* fertilization upon exiting the testis. The cells must undergo considerable posttesticular remodeling within the epididymis (and the female reproductive tract) to acquire this ability. Each epididymis is an unbranched tortuous duct that spans 70–80 m in the stallion, which is considerably longer than that of man and most other domestic animal species.[32] The epididymis is connected to the rete testis by several efferent ducts. The anatomical features of the rete testis and efferent ductules have been well-characterized in the stallion.[33]

On average, 90 billion spermatozoa reside in the excurrent ducts of reproductively mature stallions when sexually rested, with a lower number (60 billion) reported for 2- to 4-year-old stallions.[34] On average, 62% of the spermatozoa within the excurrent duct system of the stallion are in the caudae epididymides, and 7% are in the ductus deferens. Based on radiolabeled studies, the average time required for sperm transit through the epididymis of the stallion is reported to be 8 to 11 days.[35] Sperm transit time through the caput and corpus segments is constant at 4 days and is unaffected by frequency of ejaculation, age, or season,[35–37] whereas transit time through the cauda epididymis can be accelerated somewhat by increased ejaculation frequency.[38]

Spermatozoa are immotile upon entering the epididymis and do not gain the motility until reaching the distal corpus. A motility pattern consistent with ejaculated spermatozoa is not acquired until spermatozoa reach the cauda epididymis.[39] As such, movement of spermatozoa through the epididymis is primarily attributed to rhythmic contractions of the smooth musculature that surrounds the epididymal duct. In men, both the thickness of the smooth musculature, and its adrenergic innervation, increase from the proximal to the distal end of the epididymis, and into the ductus deferens.[40] Autonomic drugs, both cholinergic and adrenergic) increase contractility of all segments of the epididymis.[41] Prostaglandins (PGF2α and PGE) have been isolated from the testes and excurrent ducts of male rats, with a concentration noted to be lowest in the testes, intermediate in the epididymides, and highest in the ductus deferens.[42] These same workers demonstrated that aspirin, which suppresses the production of PGF2α, strongly inhibited contractility of the caput epididymis *in vitro*. Oxytocin receptors are also present in the epididymal epithelium, and the frequency of caput epididymal contractions increases, and tubule diameter decreases, in a dose-responsive manner to oxytocin exposure.[43] While the epididymis in considered to be an androgen-dependent tissue, estrogens appear to play a more important role than androgens in promotion of epididymal motility. This is achieved through estrogen-mediated up-regulation of both the oxytocin receptor gene and the receptor protein.[44]

In addition to serving as a conduit, the epididymis bestows upon spermatozoa specific structural and physiologic alterations, the result of which yields acquisition of fertilizing potential. Maturational changes in spermatozoa occur predominantly within the caput and corpus, as spermatozoa recovered from the cauda have attained a fertilizing capacity similar to that of ejaculated spermatozoa for most species studied.

A paucity of information exists with regard to the specific mechanisms that control sperm maturation in the epididymis. Studies to date indicate that a salient feature of the epididymis is a continual change in the microenvironment to which spermatozoa are exposed during epididymal transit. This is portrayed by the variation in histomorphologic characteristics of the numerous epididymal cell types that line the various regions of the duct, and the distinct regional and cell-specific patterns of gene expression and product secretion. Although a variety of such localization studies have been conducted, these must be followed by mechanistic and functional approaches before we can fully appreciate the molecular interactions that impart fertilizing power to a spermatozoon.

The unique composition of the epididymal plasma can be attributed, in large part, to the formation of a blood-epididymis barrier.[45,46] This barrier is formed by tight junctional complexes near the luminal border between adjacent principal cells of the epididymal epithelium.[47] This mechanism restricts entry of various blood-borne molecules into the epididymal lumen, thereby allowing spermatozoa to be bathed in a milieu that is controlled locally; and affording sperm protection against immunologic attack. Disruption of the blood-testis barrier[48] and the blood-epididymis barrier[49] may be a contributor to reduced semen quality in older males. The corpus epididymis may be the most vulnerable segment of the epididymis to age-related barrier dysfunction.[49]

PHYSIOLOGICAL CONSIDERATIONS REGARDING EJACULATED SPERMATOZOA

Spermatozoa have only completed a short leg of their journey upon ejaculation. At this juncture, the spermatozoa remain incapable of fertilizing an oocyte under *in-vivo* conditions. Following natural mating, spermatozoa enter harsh conditions within the uterine lumen, and only a small portion of this sperm population gains access into the protective confines of the oviduct where fertilization occurs. Residence time in the reproductive tract imparts a series of maturational changes in the sperm, such that they are finally capable of fertilization. Many attributes are required of spermatozoa to fulfill the mission of migration to the oviduct and fertilization of an ovulated oocyte.

SPERM MOTILITY

Under natural conditions, regulation of sperm motility occurs at three critical points: epididymal reservoir (cauda epididymis and ductus deferens) – suppression of motility; ejaculation – activation of motility; and oviductal reservoir – hyperactivation of motility. Spermatozoa in the cauda epididymis are intrinsically capable of motility but do not exhibit motility until released from the epididymis. Specific motility-inhibiting proteins have been identified in rat cauda epididymal fluid that, when removed, allow initiation of motility.[50,51] A pH-dependent inhibitory factor has been reported in bulls.[52,53] Although such inhibitory factors may exist, it is possible that sperm motility may simply be suppressed by the acidic pH of the epididymal environment. The pH of bull cauda epididymal fluid is reported to be 5.5, and the cytosolic pH of bull epididymal spermatozoa is reported to be 6.5 to 6.6.[52,54] Caudal epididymal fluid of bulls, rams, boars, and stallions does not contain measurable quantities of bicarbonate (HCO_3^-),[55] and HCO_3^- is known to be a key effector of sperm motility. Bicarbonate is present at fairly high concentrations in seminal plasma, and may be higher in seminal plasma of stallions than some other mammals studied.[56] Simple exposure of normal spermatozoa to seminal plasma or physiologic fluids will activate sperm motility that is characterized by a moderate amplitude and symmetrical flagellar beat leading to a forward propulsive trajectory.[56,57]

Environmental cues activate sperm motility through a signal transduction mechanism. Although the signaling pathways of activated sperm motility are not resolved completely, an ever-increasing body of information indicates that HCO_3^-, calcium ions (Ca^{2+}) and cAMP are key signaling components. Although increased alkalinization of the sperm cytosol is known to activate membrane Ca^{2+} channels, this may be of primary importance in hyperactivation of sperm motility where increased cytosolic Ca^{2+} is required. Sperm motility can be activated and maintained for a short time in Ca^{2+}-free media for many species, but the presence of extracellular Ca^{2+} maximizes sperm motility. The flagellum is known to contain a variety of Ca^{2+} membrane transport channels, including voltage-gated, cyclic nucleotide-gated, transient receptor potential, Ca^{2+}-release, and CatSper channels. While the full roles of some of these channels remain unknown, their mere presence suggests that they probably contribute in some manner. Sperm $[Ca^{2+}]_i$ are also known be regulated by Ca^{2+}-ATPases, Na^+/H^+ exchangers, and Ca^{2+}/H^+ exchangers.

A substantial and continuous supply of energy, in the form of ATP, is required for activated sperm motility, and this requirement is heightened for hyperactivated motility. The mechanisms by which ATP is generated and transferred in the flagellum remain unsolved. Certainly, oxidative respiration within the mitochondria yields a plentiful supply of ATP. Some investigators propose that the ATP produced in this manner is capable of diffusing along the entire length of the flagellum in a manner suitable for initiation and maintenance of sperm motility. Others contend that local glycolysis within the principal piece is critical to the generation and distribution of ATP required for sperm motility. Certainly, a full complement of glycolytic enzymes has been identified in association with the fibrous sheath and/or outer dense fibers. At present, it appears that either oxidative respiration or glycolysis can support the ATP generation and availability needed to drive sperm motility, and the mechanism is species-dependent.[58]

SPERM TRANSPORT IN THE FEMALE REPRODUCTIVE TRACT

Deposition of spermatozoa is typically intrauterine when artificial insemination is used, and a large portion of an ejaculate is deposited directly into the uterine body at the time of the natural coitus for some species, such as equids. Following intrauterine deposition of semen, the spermatozoa are rapidly transported to the oviduct where a sperm reservoir forms and the spermatozoa gain fertilizing potential prior to interaction with the vestments of the oocyte near the ampullar-isthmic junction.

Sperm migration to the oviducts is dependent in large part on uterine contractions. The effect of insemination volume on the frequency of these uterine contractions appears variable, but, within a range tested (5 to 50 × 10^6 spermatozoa per ml), sperm concentration had no apparent effect on sperm number recovered from mare oviducts at 4 h following insemination.[59]

The time required for spermatozoa to gain access into the oviduct has not been studied extensively. Spermatozoa have been detected in mare oviducts as early as 2 h postinsemination.[60] Sufficient equine spermatozoa to establish pregnancy may be transported into the oviduct as early as 30 min following insemination based on extensive lavage of the uterus postinsemination with an iodine-based solution to immobilize intrauterine spermatozoa. Pregnancy rates are maximized by delaying this type of uterine lavage until 4 h postinsemination.[61] The site of intrauterine insemination appears to have a significant impact on the sperm number that gains access into the oviduct. In one study, a higher number of spermatozoa were recovered from the ipsilateral oviduct following deep uterine-horn insemination than resulted from uterine body insemination.[62] Mann *et al.*[62] demonstrated that seminal plasma can also gain access into the oviducts.[63] Only a very small fraction of inseminated spermatozoa gains access into the oviducts. In one report, only 0.0006% to 0.0007% of stallion spermatozoa were recovered from oviductal flushings of mares at 18 h following intrauterine insemination.[62]

The process of sperm passage into the oviduct does not appear to be a passive one, *i.e.*, controlled only through the reproductive tract of the female. Studies to date suggest that a dynamic interaction occurs among spermatozoa, reproductive fluids, and the epithelial surface of the uterotubal junction (UTJ) and caudal isthmus of the oviduct.[64] Spermatozoa tend to associate closely with the epithelium while traversing through the oviductal papilla of the mare, a phenomenon also observed with other species. Cross-talk between spermatozoa and the epithelium is further manifested by studies with transgenic mice lacking a gene for two different sperm surface proteins. In these males, all functional characteristics of the spermatozoa appear normal, except that sperm migration into the oviducts is hampered.[65,66]

A preferential selection process occurs for the few spermatozoa that successfully pass through the UTJ into the caudal isthmus. Scott *et al.* reported that over 90% of equine

spermatozoa at the UTJ were morphologically normal, even when inseminates contained a high percentage of spermatozoa with morphologic abnormalities.[67] The most common morphologic abnormality noted in the bound spermatozoa was a proximal cytoplasmic droplet. Thomas *et al.* demonstrated that co-culture of equine spermatozoa with oviductal epithelial cell monolayers yielded higher percentages of bound morphologically normal and motile spermatozoa than were present in the neat semen.[68] In addition, more equine spermatozoa were bound to isthmic explants than to ampullar implants, and more spermatozoa were bound to explants procured during the periovulatory period, as compared to luteal phase of the estrous cycle.[69]

The caudal isthmus is generally considered to be the reservoir site for oviductal spermatozoa,[60] although the UTJ also appears to harbor a considerable number of these cells.[70] The existence of a sperm reservoir in the oviduct is further supported by the documentation that spermatozoa of some fertile stallions can persist in the mare for up to 6 days prior to ovulation, yet result in establishment of pregnancy.[71,72] The mechanism of sperm attachment to the oviductal epithelial cells has received considerable study and appears to consist of a specific sperm-ligand interaction involving lectin-like molecules on the surface of spermatozoa and carbohydrate-containing moieties on the surface of oviductal epithelial cells.

This intimate oviductal cell contact with spermatozoa in the oviductal reservoir seems to play two divergent roles: assisting with the final maturational events of spermatozoa that must occur prior to fertilization of an oocyte, while also maintaining spermatozoa in a viable quiescent state to allow for an extended storage period. Binding of equine spermatozoa to oviductal epithelial cells under *in-vitro* culture conditions results in both a quantitative and a qualitative change in protein synthesis and secretion by the epithelial cells.[73] Purified oviductal glycoprotein and polypeptides secreted by oviductal epithelial cells have been shown to have a positive effect on sperm capacitation and sperm-oocyte interactions. Stallion spermatozoa that are bound to oviductal epithelial cells in culture exhibit flagellar motion for up to 4 days, with gradual release of spermatozoa during that time frame.[74]

The mechanism for sperm detachment is speculative, but likely involves acquisition of hyperactivated sperm motility to break the connection with the oviductal epithelial cells.[75] The lectin-like molecules on the sperm surface responsible for specific binding with the oviductal cells may also be released during the capacitation process, thereby assisting with sperm detachment.[76] Detachment of spermatozoa probably yields cells that are both hypermotile and primed for spermatozoon-oocyte interaction. It is of interest that hyperactivated spermatozoa tend to have normal morphologic characteristics.[77] Studies have revealed that the flagellar waveform of hyperactivity actually improves the progressivity of spermatozoa over that of activated motility when spermatozoa are exposed to viscoelastic conditions such as those that exist in the oviduct.[78]

Activated motility and hyperactivated motility likely require different environmental signals. Presumably, a signal for hyperactivated motility is elicited within the oviduct under natural conditions in order to initiate the event at a time that is conducive to fertilitilization. Although the precise signals for initiation of hyperactivation remain unsolved, it is possible that chemotactic and/or thermotactic factors serve in this capacity.[79] It is also possible that sperm exposure to alkalinizing conditions, as exists in the oviduct and above that which initiates activated motility, is the primary initiator of hyperactivated motility. The precise mechanisms controlling hyperactivated motility are subject to continued investigation, but an increasing body of literature suggests that an increase in extracellular pH leads to an increase in $[pH]_i$, thereby potentiating the action of Ca^2 + channels.[80–82] Equine spermatozoa may be unique, however, as *in-vitro* studies have revealed that hyperactivation is associated with an increase in intracellular pH but is inversely related to intracellular Ca^{2+}.[83]

SPERM-OOCYTE INTERACTION

Under natural conditions, interactions with an ovulated oocyte require sperm migration to the ampullar region of the oviduct, and only a small percentage of spermatozoa that gain access into the oviduct will eventually arrive at this fertilization site. Such spermatozoa are thought to have achieved full fertilizing potential. The precise mechanisms by which spermatozoa migrate to the ampullar region of the oviduct remain speculative, but contractile movements of the oviduct and hyperactivated sperm motility are thought to play key roles in this migratory phase. Chemotactic, rheotactic, and thermotactic factors may also be important to directional migration of oviductal spermatozoa.[84–87]

The signaling pathways and cellular events leading to hyperactivated motility and to the acrosomal exocytosis are different, and the events of each can occur independently. Certainly, critical sperm priming is required for interaction with the oocyte. The various events of the capacitation process do not appear to be tightly coupled in the laboratory setting, but this may not be the case under *in-vivo* conditions. While an assortment of signaling pathways are reported to be involved in hyperactivated motility and acrosome exocytosis, intracellular pH appears to be a common regulator of these events.[88]

Before a spermatozoon can interact directly with the oocyte, it must first negotiate passage through the cumulus complex and the zona pellucida. Despite decades of study, the mechanism (s) and site (s) of acrosomal exocytosis, and species differences during this entry phase, remain unresolved.[89] Once situated within the perivitelline space, the spermatozoon binds to, and fuses with, the egg plasma membrane (oolemma), leading to internalization of the sperm haploid chromosomal complement by the oocyte. The molecular interactions involved in fusion of spermatozoa with the oolema are also not completely understood; however, recent studies suggest that the proteins Izumo1 (sperm) and Juno (oocyte) are key to this interaction.[90–92]

Entry of the sperm into the ooplasm elicits an initial increase in intracellular Ca^{2+} concentration, followed by repetitive Ca^{2+} oscillations that stimulate activation of the oocyte. The factor responsible for this activation is derived from the sperm cytosol, and a spermatozoon-specific phospholipase C is considered to be the most likely candidate molecule.[93]

TRANSLATION TO THE CLINIC

Despite the wealth of information derived from countless studies relating to sperm function, the direct application of these insights to the clinical arena remains fairly marginal. As an often-confused equine veterinary clinician, some questions that I continually ponder include the following:

1. What is the impact of specific sperm morphologic defects, such as nuclear vacuoles or protoplasmic droplets, on fertility?
2. What improved methods are available for cooled or frozen preservation of sperm such that the procedures can be successfully applied to a larger percentage of males?
3. With our continually expanding knowledge base, why do we continue to

label so many subfertility cases in males as idiopathic?
4. What is the most appropriate battery of tests to assess sperm function with good predictability of case outcome?
5. What are the treatment options for a growing subpopulation of stallions with apparent abnormal sperm acrosomal exocytosis?
6. What is the diagnostic value/reliability of re-sequencing studies for SNP relationship to subfertility in clinical cases?
7. What risk does intracytoplasmic sperm injection (ICSI) impose for propagation of genetic defects associated with subfertility?
8. What are the most appropriate species-specific protocols for in-vitro fertilization (IVF), and why are results involving horses quite poor, in comparison with other agricultural animals or humans?
9. Does the centrifugal fractionation of spermatozoa lead to improvements in some cases of male-directed subfertility?
10. Can we implement treatment strategies to delay age-related testicular dysfunction (degeneration)?

An appreciation of the molecular basis of sperm function, sperm-oviductal interactions, and spermatozoon-oocyte engagement will hopefully lead to many practical applications in the clinical front, such as assemblage of a battery of in-depth laboratory tests to assess sperm function, expanded treatment options for subfertile males, improved methods for preservation of semen, and heightened applications for assisted reproductive technologies such as conventional IVF and ICSI techniques. The bottom line is that the more we learn, the more informed we can be in decision-making regarding diagnostic and therapeutic strategies as they relate to sperm function and reproductive health in males. It becomes incumbent upon us, and clinicians and scientists, to convert these opportunities into practical applications.

COMPETING FINANCIAL INTERESTS

The author declares no competing financial interests in relation to the work described.

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