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MATRIC NO: 18/MHS02/174

TOPIC: IMPLANTATION

It is well understood that a myriad of complex steps occur between the time of ejaculation and the union of the haploid number of chromosomes from each partner that results in the final process of fertilization. Consequently, it is easy to imagine the numerous potential problems that can occur at each step, and thus may prevent a successful pregnancy. Often overlooked are the complexities of sperm transport and the steps that must occur in the sperm, a process known as capacitation, before fertilization can occur. These processes of sperm delivery and potentiation are addressed in detail in this chapter.

TRANSPORT

Vaginal Insemination

The complex process of sperm transport through the female reproductive tract begins at the time of ejaculation. During coitus, 1.5- to 5.0-ml of semen containing between 200 and 500 million sperm is deposited at the posterior vaginal fornix, leaving the external cervical os partially submerged in this pool of fluid.1 At this time, some sperm may be passively taken up by the cervix in a process described as “rapid transport;” otherwise, sperm undergo “delayed transport.” Both of these are discussed at length in this chapter.

The optimal pH for sperm viability is between 7.0 and 8.5,2,3,4,5 and a reduction in sperm motility is seen at a pH less than 6.0.6,7,8 Normal vaginal pH is only 3.5 to 4.0,9 and the acidic environment of the vagina is thus toxic to sperm. However, both seminal fluid and cervical mucus present within the posterior vagina are alkaline and act as buffers. Fox and coworkers have shown that vaginal pH rises to 7.0 within just seconds after ejaculation,10 and this decrease in acidity can be maintained for up to two hours after ejaculation.

Within about 1 minute after coitus, the ejaculate undergoes coagulation. This coagulum temporarily restricts movement of sperm out of the seminal clot, thus preventing their passage into the cervical mucus and ascension up the female reproductive tract. Over the next 20 to 30 minutes, however, a seminal-fluid proteolytic enzyme produced by the prostate gland gradually liquefies the clot. At this time, motile sperm may then enter the cervical mucus, leaving behind the seminal plasma. Although there are reports of motile sperm persisting within the vagina for up to 12 hours after ejaculation,11 motility of most vaginal sperm is diminished within about 30 minutes, and after 2 hours almost all sperm motility in the vagina has been lost.

Rapid Sperm Transport

Sperm may begin to undergo the process of rapid sperm transport within seconds after ejaculation. This type of sperm movement is thought to be predominantly passive, resulting from coordinated vaginal, cervical, and uterine contractions. Although these contractions are of short duration, they are believed to be the primary force responsible for the rapid progression of sperm to the upper female reproductive tract—the oviduct. Settlage and coworkers in 1973 reported results of a study in which fertile ovulatory females were intravaginally inseminated with donor sperm at the time of bilateral salpingectomy for sterilization. Within 5 minutes after insemination, sperm were present within the Fallopian tubes, and the number of sperm found there was proportional to the number inseminated. Similar results demonstrating this rapid transport process have also been documented in numerous animal studies.

The Cervix

Several important functions have been attributed to the cervix, and these include15

- Providing a receptive environment for sperm entry near the time of ovulation
- Preventing access of sperm, microorganisms, and particulate matter to the upper reproductive tract and thus, the peritoneal cavity
- Filtering spermatozoa and removal of seminal plasma
- Preventing sperm phagocytosis by white blood cells within the female reproductive tract
- Providing a biochemical environment sufficient for sperm storage, capacitation, and migration

The structure of the human cervix facilitates performance of the these stated functions. The endocervical canal has an average length of 3.0 cm, and it is lined by two types of columnar epithelial cells, ciliated and nonciliated. The cervix does not contain true glandular units; rather, the mucosa is arranged with a series of infoldings that form crypts off the central canal. The nonciliated columnar epithelial cells secrete mucin granules, and the ciliated cells propel the cervical mucus from the crypt of origination toward the external cervicalos. Production of mucus is perhaps the most important function of the cervix, and this is discussed at length later in the chapter. Finally, cervical pH is alkaline, with a peak pH during the periovulatory period. This environment is much more hospitable to spermatozoa than the acidic pH of the vagina.

Cervical Mucus

Cervical mucus is continuously secreted through exocytosis by the nonciliated epithelial cells that line the cervical canal. This biomaterial serves many important functions, including exclusion of seminal plasma, exclusion of morphologically abnormal sperm, and support of viable sperm for subsequent migration to the uterus and oviduct. It is a heterogeneous fluid with both high- and low-viscosity components. The amount of mucus produced and its composition and characteristics fluctuate with circulating progesterone and estrogen levels. As estrogen levels peak at midcycle, cervical mucus is abundant in volume and thin in consistency because of increased water content. Under the influence of progesterone, water content decreases, and the mucus has a much higher viscosity.

Ultrastructurally, cervical mucus can be seen as a complex biphasic fluid with high viscosity and low viscosity components. The high viscosity gel phase is composed of a network of filamentous glycoproteins called mucin. Collectively, mucin macromolecules form a complex of interconnected micelles, which comprise a lattice whose interstices are capable of supporting the low viscosity phase, which is predominantly water. Sperm movement through the cervical mucus is primarily through the interstitial spaces between the mucin micelles, and the sperm's progression depends on the size of these spaces. The size of the interstices is usually smaller than the size of the sperm heads; thus, sperm must push their way through the mucus as they proceed through the lower female genital tract.

Besides hormonal factors, physical processes, such as shearing, stretching, and compression can alter the spaces between molecules and, consequently, orientation of the mucin filaments. These mechanical forces can be imparted by thrusting and pelvic contraction during coitus, and also by cervical contractions in the pericoital period. Additionally, rheologic forces associated with the mucus outflow from the cervical crypts tend to align the mucin filaments in a longitudinal fashion within the cervical canal, thus creating aqueous channels between the filaments. Given this longitudinal orientation, with mucus outflow originating in the crypts of the cervical epithelium, it has been postulated that sperm are constrained to swim in the direction of least resistance, that is, along the tracts of mucus outflow in the direction of the cervical crypts. Using mucus stretched in vitro, several investigators have indeed demonstrated the parallel swimming patterns of sperm.This theory complements the notion that spermatozoa entering the cervix are directed toward the cervical crypts, the site of mucus secretion that serves as a possible storage reservoir. Spermatozoa may retain their fertilizing capacity in human cervical mucus for up to 48 hours and their motility for as long as 120 hours. From their temporary storage location within the cervical crypts, sperm can be released gradually over time, thus enhancing the probability of fertilization.

Another potentially important feature of human cervical mucus is the belief that it is able to restrict migration of human spermatozoa with abnormal morphology. The percentage of

spermatozoa with normal morphology in the cervical mucus and in the uterine fluid is significantly higher than usually seen in semen.Quantitatively, these findings have been demonstrated following artificial insemination in which the percentage of sperm with normal morphology from the inseminated specimen was known ahead of time, thus allowing a more accurate comparison of the postinseminate semen within the cervical mucus.These results suggest that spermatozoa with abnormal morphology may be constrained by a process of restricted entry into cervical mucus. Comparison of morphologically normal versus abnormal human sperm in semen has shown that abnormal sperm are less likely to be motile, and those that are motile tend to swim with a lower velocity than normal cells.Katz and colleagues studied human sperm motility and morphology in vitro and they found that sperm with normal morphology swim faster than sperm with abnormal morphology, despite similar flagellar frequencies and amplitudes.These results suggest that morphologically abnormal spermatozoa may experience decreased movement resulting from increased resistance of mucus.

Sperm Transport Through the Uterus

Little is known about sperm transport within the endometrial cavity. Sperm motility does not appear to be the only force directing the sperm toward the oviducts, because inert particles deposited within the uterus are transported to the Fallopian tubes. Uterine muscular contractions likely play a role in this process. Unfortunately, much difficulty has been met in attempts to recover and quantify uterine sperm.45 Moyer and colleagues examined sperm recovered at the time of ovulation from the uterus of women undergoing hysterectomies 25 to 41 hours after intercourse.Sperm was recovered in only 6 of 26 women, and for these women the total number of sperm ranged from 1 to 4. None of the sperm were motile.

A study by Kunz and coworkers used vaginal sonography to demonstrate that uterine peristalsis during the follicular phase of the menstrual cycle exhibits an increasing frequency and intensity of subendometrial and myometrial peristaltic waves as the follicular phase progresses. During this portion of the cycle, the number of contractions propagating in the fundocervical direction decreased, and number of contractions progressing in the cervicofundal direction increased. In another part of this same study, the investigators placed technetium-labeled albumin macrospheres, about the size of spermatozoa, into the posterior vaginal fornix. The ascension of these particles was monitored by serial scintigrams. As soon as 1 minute after placement, the macrospheres reached the intramural and isthmic portion of the oviduct. Quantitatively, the number of macrospheres progressed dramatically as the follicular phase progressed, with only a few particles entering the uterine cavity during the early follicular phase of the menstrual cycle. By the midfollicular phase, the proportion of macrospheres entering the uterine cavity increased dramatically, and by the late follicular phase, the highest level of macrosphere transported to the oviducts was noted. Perhaps the most striking finding of this particular study was the preferential transport of these inert particles to the oviduct ipsilateral to the side of the dominant follicle. Other investigators have shown that near the time of ovulation, the number of spermatozoa is higher in the oviduct ipsilateral to the dominant follicle than in the contralateral

oviduct on the side of the nondominant follicle. Several responsible forces have been proposed, including chemotaxis of the sperm toward the dominant follicle. The results of the above study, however, seem to suggest that lateralizing muscular contractile forces may play a significant role in this preferential movement, in that inert particles are obviously unable to engage in chemotactic migration.

Fallopian Tube

The adult human Fallopian tube, about 9 to 11 cm long, consists of five distinct segments: the fimbria, infundibulum, ampulla, isthmus, and intramural segment. The epithelial lining of the tube is composed of four cell types: ciliated, secretory, intercalary (peg), and undifferentiated cells. Epithelial cells undergo histologic changes in response to cyclic estrogen and progesterone variations, with the height of the epithelial cells being greatest at the time of the estrogen peak near midcycle. Tubal musculature is organized in a spiral fashion, and at the tubouterine junction these muscles become continuous with the myometrium.

Sperm movement through the Fallopian tube relies on a combination of forces: intrinsic sperm motility, tubular muscular contraction, and fluid flow. Tubal fluid production is maximal at the time of ovulation, and this fluid sustains the sperm before fertilization. Tubal fluid may also facilitate both sperm capacitation and acrosomal reaction.

Although the uterotubal junction does not act as a barrier to inert particles, it may serve as an additional functional barrier to sperm with abnormal morphology or motility. The number of sperm that reach the oviduct is many orders of magnitude lower than the total number of sperm in the ejaculate. Although tens of millions to hundreds of millions of sperm are deposited in the vagina at the time of ejaculation, anatomic studies have shown that typically only hundreds of sperm are present in the oviduct at various postcoital timepoints. Williams and colleagues studied the number and distribution of spermatozoa within the human oviduct near the time of ovulation. Parous women undergoing total abdominal hysterectomies for menorrhagia were inseminated with partner or donor semen, and 18 hours later, during surgery, both oviducts were ligated into ampullary, isthmic, and intramural regions. Using flushing techniques, scanning electron microscopy, and homogenization procedures, patients' oviducts were carefully evaluated for the presence of sperm. A median of only 251 total sperm was recovered from the oviducts of these women, and the ampulla near the ovulating ovary contained a significantly higher percentage of spermatozoa than did the nonovulatory side.

The precise role played by tubal fluid in gamete transport and sperm activation is still not entirely understood. Zhu and colleagues used an in vitro technique to demonstrate that human oviductal fluid maintains sperm motility induced by exposure to follicular fluid longer than does

exposure to a simple salt solution. Furthermore, these investigators reported that the sperm acrosome reaction, which is induced by follicular fluid, is modulated by exposure of spermatozoa to tubal fluid. These findings may suggest that tubal fluid potentiates the motility and viability of spermatozoa, thus enhancing the chances of fertilization. Yao and colleagues used in vitro oviductal cell cultures incubated with spermatozoa to determine that oviductal cells promote capacitation and stabilize the acrosome. There is still much to learn about the dynamics of spermatozoa and the tubal environment. Although done in an in vitro setting, new studies such as the ones already discussed will likely provide clarity to the complex interplay between male gametes and the female reproductive tract.

SPERM CAPACITATION AND THE ACROSOME REACTION

Sperm Capacitation

In 1951 Chang, while studying rabbits, and Austin, while working on rats, each independently reported that mammalian sperm must reside in the female reproductive tract for a finite period of time before they gain the ability to fertilize ova. One year later, Austin introduced the term “capacitation” when he stated that “the sperm must undergo some form of physiologic change or capacitation before it is capable of penetrating the egg”. Capacitation is now commonly regarded as the reversible, prefertilization activation process of sperm which results in the spermatozoa gaining the ability to:

Develop hyperactivated motility, with vigorous nonlinear flagellar motion

Bind to the zona pellucida

Undergo the acrosome reaction

Proceed eventually to fusion with the oolemma and egg fertilization

Initial investigative work in the area of sperm capacitation was performed using animal models such as rabbits, rats, and hamsters. In fact, in 1963, Yanagimachi and Chang broke major scientific ground with their finding that hamster epididymal spermatozoa could be capacitated in vitro. Work soon followed with the demonstration of in vitro sperm capacitation in a large number of other animal species. A significant finding from these collective studies is that capacitation-related changes at the molecular level in the spermatozoa seem to vary from species to species. Temporally as well, there are also differences in capacitation between species with some species capable of much more rapid capacitation in vitro than others.

Studies of capacitation have sometimes met with controversy, largely because of lack of morphologic criteria by which to assess its occurrence. Although sperm capacitation has been induced in vitro, it is not clear whether changes caused by in vitro manipulation are the same as

those that occur in vivo. Despite this, both in vivo and in vitro capacitation enable the spermatozoa to undergo fusion of the plasma and outer acrosomal membrane during the acrosome reaction and thus proceed to subsequent fertilization. These two steps, sperm capacitation and the acrosome reaction, are both essential precursors of normal fertilization. Evidence of this is seen in sperm that have not been incubated in the female reproductive tract or otherwise capacitated cannot effectively fertilize an egg.

Many substances within the female reproductive tract have been examined as potential capacitating factors, but at this time none has been uniquely identified. Nonetheless, we do know that at the molecular level, several key changes are noted to occur in the spermatozoa as a result of capacitation. These changes include:

Alteration or removal of sperm coating materials. These coating materials become adsorbed to or integrated within the sperm plasma membrane during epididymal transport and also during exposure to seminal plasma59,60

A decrease in the net negative surface charge61

Changes in the content and location of surface antigens62

Conformational changes to intrinsic membrane proteins63

Changes in the permeability of the membrane to various ions, especially calcium64

Capacitation in Human Spermatozoa

Very little is known about human sperm capacitation in the female reproductive tract. We do know that human sperm that are recovered from the cervical mucus and placed into a noncapacitating medium are able to penetrate the zona pellucida of the human oocyte and also fuse with zona-free hamster oocytes.65 Thus, it appears that human sperm capacitation can occur in the cervical mucus. Because of the inherent difficulty in manipulating and subsequently evaluating the in vivo environment of the female reproductive tract, much of what we now know about human sperm capacitation is the result of in vitro studies.

Capacitation in Vitro

Capacitation is associated with significant alteration of the surface of the sperm, with various molecules being removed or rearranged. Substances in the first group are called “decapacitation factors,” because when added to suspensions that have been previously capacitated, they quickly inhibit fertilizing ability. This inhibition, like capacitation, is reversible. Rosselli and coworkers investigated human spermatozoa using transmission electron microscopy and found

that aliquots of spermatozoa incubated with either cervical mucus or a capacitating medium enriched with 3% bovine serum albumin each showed ultrastructural “stripping” of the sperm coat. Yudin and colleagues proposed in 1989 that human sperm may experience physical stresses while moving through cervical mucus which result in a removal of sperm coat molecules from the gamete's surface. Balerna and associates postulated that these sperm coat alterations may result from hydrogen bonding and electrostatic forces by the glycan moiety of the mucin molecules, thus causing the removal of certain sperm surface molecules.66,70

Capacitation is also characterized by a loss or reduction of cholesterol from the plasma membrane of spermatozoa. Benoff and colleagues have shown that a loss of membrane cholesterol is a necessary feature of capacitation in human spermatozoa. Electron microscopy studies have shown a reduction in cholesterol concentration overlying the acrosome cap during in vitro capacitation. Further studies have shown that human spermatozoa can be kept in a noncapacitated state if placed in a suspension saturated with cholesterol. Capacitation in this setting will only occur after the sperm are transferred to an environment containing albumin or a similar molecule that can act as a cholesterol acceptor.71,73

Membranes are a very dynamic collection of proteins and lipids that are capable of responding to various environmental signals that modify cellular activities. Part of this ongoing dynamic process involves alterations of membrane topography, with certain cell surface molecules moving to various locations or domains in response to environmental conditions. Cholesterol has been shown to limit the insertion of proteins into lipid bilayers, to prohibit the movement of receptors in cell membranes and to change membrane protein conformation and thus alter their activity.74,75 The ratio of cholesterol to phospholipid, so important in the sperm membrane, controls fluidity and ion permeability in most biologic membranes, and the proportion of these two components change during capacitation.58,59,76,77 Various studies have shown in vitro that plasma membrane cholesterol content is reduced by 20% to 50%, depending on the makeup of the capacitating medium.58,78 Collectively, these changes in sperm membrane composition are believed to be interrelated to subsequent changes in membrane ion transport and possibly membrane fusion.

Hyperactivation of Motility

This is described as one of the hallmark characteristic changes seen as a result of capacitation. Sperm motility becomes more vigorous with a decreased rate of forward progression. Specifically, the sperm develops:

Wider amplitude of lateral head displacement

Marked increase in flagellar beating

A curved and tortuous trajectory79

Although the functional significance of these changes remains unclear, they may facilitate sperm transit through the oviduct and provide the necessary force needed to penetrate the granulosa cell layer and zona pellucida surrounding the ovum.69A,70A Some studies have shown hyperactivation in about 20% of spermatozoa after a sufficient incubation period with in vitro media, and sperm that display hyperactivated patterns tended to be those with normal morphology.80 Factors determining which sperm incubated in capacitating solutions will ultimately demonstrate hyperactive motility are not well understood.

Sperm Membrane Changes

The sperm plasma membrane is composed of a lipid bilayer interspersed with a number of proteins. Lipid types present include cholesterol, glycolipids, and phospholipids. The proteins found here can traverse the entire membrane from cytosolic compartment to extracellular space. These proteins have important functions, including activation of receptors and transport of ions.

The Acrosome Reaction

The mature human ovum possesses a number of surrounding layers that must be penetrated by the spermatozoa for normal fertilization to occur. To assist with this task, the spermatozoa has a caplike region called the acrosome covering the anterior 80% of its head. This structure contains a number of digestive enzymes, such as hyaluronidase, corona-penetrating enzyme, and acrosin to facilitate membrane fusion and sperm entry into the ovum.

Ultrastructurally, the acrosome reaction involves regional fusion of areas of the outer acrosomal membrane and the overlying sperm plasma membrane. These fused areas then lyse, serving as portals through which soluble contents of the acrosome can be dispersed to act on the vestments of the ovum.

The acrosome reaction is initiated as the spermatozoa arrives at the ovum. The outermost covering of the ovum, the cumulus oophorus, is degraded by hyaluronidase located on the plasma membrane of the spermatozoa. Subsequently, corona-penetrating enzyme is released to facilitate spermatozoal transit through the corona radiata. After transit through the corona radiata is completed, the sperm binds to the zona pellucida. Next, proacrosin, a zymogen within

the acrosomal region, is converted to acrosin, and this facilitates breakdown of the zona pellucida glycoproteins. For this biologic process to occur, the spermatozoa plasma membrane and the outer acrosomal membrane must be removed. This, in essence, is the hallmark of the acrosomal reaction. After the spermatozoa has proceeded through the zona pellucida, the sperm head crosses the perivitelline space and attaches to the cell membrane of the ovum. Subsequently, the sperm and ovum plasma membranes fuse, the sperm enters the ovum, and fertilization follows.

The acrosome reaction is a key component of the fertilization process, and its proper timing is essential. Inappropriately early release of the acrosomal enzymes within the female reproductive tract would result in spermatozoa being unable to fertilize. Initiation of the acrosome reaction seems to hinge specifically on spermatozoal binding to the zona pellucida. Although the human model is not entirely understood, the murine model has been extensively studied. With the murine model, spermatozoal exposure and binding to the structural zona glycoproteins, ZP3 (zona pellucida protein #3) have been identified as the molecule that sets the events of the acrosome reaction into motion. After this binding has occurred, several changes follow:

- Influx of calcium into the spermatozoa
- Activation of the adenylate cyclase, adenosine 3',5'cyclic phosphate (cAMP), protein kinase pathway
- Activation of the guanylate cycle, cyclic guanosine monophosphate (cGMP), protein kinase pathway
- Activation of the phospholipase C, diacylglycerate, protein kinase C pathway

Together, these pathways likely share a complex regulation of the events collectively called the acrosomal reaction.

CLINICAL APPLICATION

Extensive clinical application has been made of the large body of information accumulated to date regarding sperm transport and capacitation. The most notable utilization has come with the widespread use of in vitro fertilization techniques since the early 1980s for couples with otherwise untreatable infertility. In particular, spermatozoa capacitation techniques in vitro are now performed readily in the laboratory as a routine part of the in vitro fertilization (IVF) treatment for both male and female infertility.

Because of the large number of sperm required for standard IVF as well as the modest initial fertilization and pregnancy rates associated with IVF, several gamete micromanipulation techniques were developed over the next decade in an attempt to improve successful outcomes. The first advance involved creation of a nick in the zona pellucida, followed by standard IVF. This was called partial zona dissection (PZD). Another advance, called subzonal insertion of sperm (SUZI), involved placing the sperm directly into the perivitelline space, the region between the zonal pellucida and the ovum. Both of these techniques have been used successfully in humans but did not give acceptable success rates.

Since the first report of success with intracytoplasmic sperm injection (ICSI) by Palermo and researchers in 1992, this form of treatment has drastically changed the options available to the infertile couple.84 This approach obviates many processes, such as the acrosomal reaction, that are essential components of sperm-ovum interaction, during normal fertilization and IVF.

Despite these advances in gamete micromanipulation techniques, it is clear that further investigative work regarding sperm transport, capacitation, and sperm-ovum interaction will help to further advance efforts to efficiently treat infertile couples. Although ICSI certainly is a viable and effective treatment option, less invasive approaches for both male and female factor infertility may be developed as a result of further research in techniques for in vivo enhancement of sperm function. Expanded use of cell culture techniques and use of in vivo experimental models will likely be of great benefit in attempts to better understand the processes of sperm transport, capacitation, and ultimately, fertilization.