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Sperm transport in the female reproductive tract

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At coitus, human sperm are deposited into the anterior vagina, where, to avoid vaginal acid and immune responses, they quickly contact cervical mucus and enter the cervix. Cervical mucus filters out sperm with poor morphology and motility and as such only a minority of ejaculated sperm actually enter the cervix. In the uterus, muscular contractions may enhance passage of sperm through the uterine cavity. A few thousand sperm swim through the uterotubal junctions to reach the Fallopian tubes (uterine tubes, oviducts) where sperm are stored in a reservoir, or at least maintained in a fertile state, by interacting with endosalpingeal (oviductal) epithelium. As the time of ovulation approaches, sperm become capacitated and hyperactivated, which enables them to proceed towards the tubal ampulla. Sperm may be guided to the oocyte by a combination of thermotaxis and chemotaxis. Motility hyperactivation assists sperm in penetrating mucus in the tubes and the cumulus oophorus and zona pellucida of the oocyte, so that they may finally fuse with the oocyte plasma membrane. Knowledge of the biology of sperm transport can inspire improvements in artificial insemination, IVF, the diagnosis of infertility and the development of contraceptives.

Key words: Fallopian tube/spermatozoa/uterine tube/uterus/vagina

Introduction

Passage of sperm through the female reproductive tract is regulated to maximize the chance of fertilization and ensure that sperm with normal morphology and vigorous motility will be the ones to succeed.

Oocytes are usually fertilized within hours of ovulation (Austin, 1957; Harper, 1994). On the other hand, in some species, sperm may be inseminated days (horses, cattle and pigs) or even months (some bat species) before the arrival of the oocyte. In humans, there is evidence that fertilization occurs when intercourse takes place up to five days before ovulation (Wilcox *et al*., 1995). Because sperm are terminally differentiated cells, deprived of an active transcription and translation apparatus, they must survive in the female without benefit of reparative mechanisms available to many other cells. Sperm are subjected to physical stresses during ejaculation and contractions of the female tract, and they may sustain oxidative damage. Furthermore, because sperm are allogenic to the female, they may encounter the defenses of the female immune system meant for infectious organisms (Menge and Edwards, 1993). Thus, sperm must somehow use their limited resources to maintain their fertility in the face of numerous impediments. As it is, of the millions of sperm inseminated at coitus in humans, only a few thousand reach the Fallopian tubes and, ordinarily, only a single sperm fertilizes an oocyte.

Site of semen deposition

The site of semen deposition is not easy to establish in many species because it must be determined by examining the female immediately after coitus and by considering the anatomy of the penis, vagina and cervix during coitus. However, it has been accomplished for humans, in which semen has been observed pooled in the anterior vagina near the cervical os shortly after coitus. Within minutes of vaginal deposition, human sperm begin to leave the seminal pool and swim into the cervical canal (Sobrero and MacLeod, 1962). In contrast, rodent sperm deposited in the vagina are swept completely through the cervix into the uterus along with seminal plasma within a few minutes (Zamboni, 1972; Bedford and Yanagimachi, 1992; Carballada and Esponda, 1997). Some species, such as pigs, bypass the vagina altogether and deposit semen directly into the uterine cavity, where sperm may quickly gain access to the oviduct (Hunter, 1981; Roberts, 1986).

Whereas most of the semen of murine rodents is rapidly transported into the uterine cavity, some remains in the vagina where it coagulates to form a copulatory plug. The plug forms a cervical cap that promotes sperm transport into the uterus (Blandau, 1969; Matthews and Adler, 1978; Carballada and Esponda, 1992). Ligation of the vesicular and coagulating glands of rats prevented the formation of plugs and the transport of sperm into the uterus (Blandau, 1945). The plugs formed by semen of guinea pigs and

mice extend into the cervical canals and thus could form a seal against retrograde sperm loss (Blandau, 1969).

Male mice deficient for the gene encoding the protease inhibitor known as protease nexin-1 (PN-1) show a marked impairment in fertility (Murer *et al*., 2001). Vaginal plugs formed in females after mating with PN-1 null males were small, soft and fibrous and did not lodge tightly in the dual cervical canals. No sperm could be found in the uterus 15 min after mating with PN-1 null males, demonstrating the importance of the plug for promoting transport of mouse sperm into the uterus (Murer *et al*., 2001).

Human semen coagulates, but it forms a loose gel rather than the compact fibrous plug seen in rodents. The coagulate forms within about a minute of coitus and then is enzymatically degraded in ½ to 1 h (Lilja and Lundwall, 1992). The predominant structural proteins of the gel are the 50 kDa semenogelin I and the 63 kDa semenogelin II, as well as a glycosylated form of semenogelin II, all of which are secreted primarily by the seminal vesicles (Lilja, 1985). The gel is degraded by prostate-specific antigen (PSA), a serine protease secreted by the prostate gland (Watt *et al*., 1986). It has been proposed that this coagulum serves to hold the sperm at the cervical os (Harper, 1994) and that it protects sperm against the harsh environment of the vagina (Lundwall *et al*., 2003).

Seminal gels are not fully successful at holding sperm at the cervical os. In cattle, several studies have demonstrated loss of sperm from the vagina after mating or insemination (reviewed by Hawk, 1987). The fate of spermatozoa that are ejaculated or inseminated into the vagina, but that do not enter the cervix, has not been studied extensively in humans. However, in a 5 year study of 11 female volunteers Baker and Bellis (1993) examined the characteristics of sperm loss from the vagina following coitus (‘flowback’). They found that flowback occurred in 94% of copulations with the median time to the emergence of ‘flowback’ of 30 min (range 5–120 min). Furthermore they estimated that a median of 35% of spermatozoa were lost through flowback but that in 12% of copulations almost 100% of the sperm inseminated were eliminated. This suggests that less than 1% of sperm might be retained in the female reproductive tract and this supports the notion that only a minority of sperm actually enter cervical mucus and ascend higher into the female reproductive tract.

Like humans, some primates produce semen that forms a soft gel. However, in chimpanzees, a species in which females mate with more than one male in a brief time, the semen coagulates into a compact plug resembling that of rodents (Jensen-Seaman and Li, 2003; Kingan *et al*., 2003). The plug may serve to prevent other males from mating with the female. Some carnivores (e.g. domestic dogs, *Canis familiaris*) and some rat and mouse species of the family Cricetidae use the penis as a copulatory plug; i.e. the mating pair remains joined together for a period after coitus (Dewsbury, 1975).

Vaginal defenses against infectious organisms may affect sperm

The vagina is open to the exterior and thus to infection, especially at the time of coitus; therefore, it is well equipped with antimicrobial defenses. These defenses include acidic pH and immunological responses and can damage sperm as well as infectious organisms. To enable fertilization to take place, both the female and the male have adopted mechanisms for protecting sperm. In humans, semen is deposited at the external os of the cervix so that sperm can quickly move out of the vagina (Sobrero and MacLeod, 1962).

Human sperm must contend, however briefly, with the acidic pH of vaginal fluid. The vaginal pH of women is normally five or lower, which is microbicidal for many sexually transmitted disease pathogens. Evidence indicates that the acidity is maintained through lactic acid production by anaerobic lactobacilli that feed on glycogen present in shed vaginal epithelial cells (Boskey *et al*., 2001). Lowering pH with lactic acid has been demonstrated to immobilize bull sperm (Acott and Carr, 1984; Carr *et al*., 1985). The pH of seminal plasma ranges from 6.7 to 7.4 in common domestic species (Roberts, 1986) and has the potential to neutralize vaginal acid. Vaginal pH was measured by radio-telemetry in a fertile human couple during coitus. The pH rose from 4.3 to 7.2 within 8 s of the arrival of semen; whereas, no change was detected when the partner used a condom (Fox *et al*., 1973). Vaginal washings of women with high levels of detectable seminal antigens had a median pH of 6.1, whereas the median pH of washings lacking detectable antigens was 3.7 (Bouvet *et al*., 1997). Contraceptive gel designed to maintain a low vaginal pH after coitus has been shown to immobilize human sperm *in vitro* and *in vivo* (Amaral *et al*., 2004).

In additions to pH buffers, seminal plasma contains inhibitors of immune responses, including protective components that coat sperm (Suarez and Oliphant, 1982; Dostal *et al*., 1997). These are most effective when sperm are bathing in seminal plasma and may be gradually shed when sperm leave the seminal plasma behind.

Males may also overcome female defenses by inseminating many sperm. This strategy is particularly effective for overcoming cellular immune responses. In the rabbit, deposition of semen results in an invasion of neutrophils into the vagina. This invasion takes time, however, to build to an effective level. Numerous leukocytes, many containing ingested sperm, were recovered from vaginas of rabbits 3–24 h post coitus (Phillips and Mahler, 1977a,b). By that time, however, thousands of sperm had already reached the Fallopian tubes (Overstreet *et al*., 1978).

Sperm transport through the cervix

In some species, the cervical canal widens under the influence of estrogen. Fluoroscopy and scintigraphy have been used in domestic dogs and cats to examine cervical patency. Opening of the cervix in these species has been correlated with estrus (Silva *et al*., 1995; Verstegen *et al*., 2001; Chatdarong *et al*., 2002). Radioopaque fluid and also human serum albumin radiolabelled with technetium 99 could be seen rapidly passing through the cervix and filling the uterine lumen after deposition in the cranial vagina at estrus.

Sperm of humans and cattle enter the cervical canal rapidly where they encounter cervical mucus (Figure 1A). Under the influence of estrogen the cervix secretes highly hydrated mucus, often exceeding 96% water in women (Katz *et al*., 1997). The extent of hydration is correlated with penetrability to sperm (Morales *et al*., 1993). Coitus on the day of maximal mucus hydration in women is more closely correlated with incidence of pregnancy than coitus timed with respect to ovulation detected using basal body temperature (Bigelow *et al*., 2004).

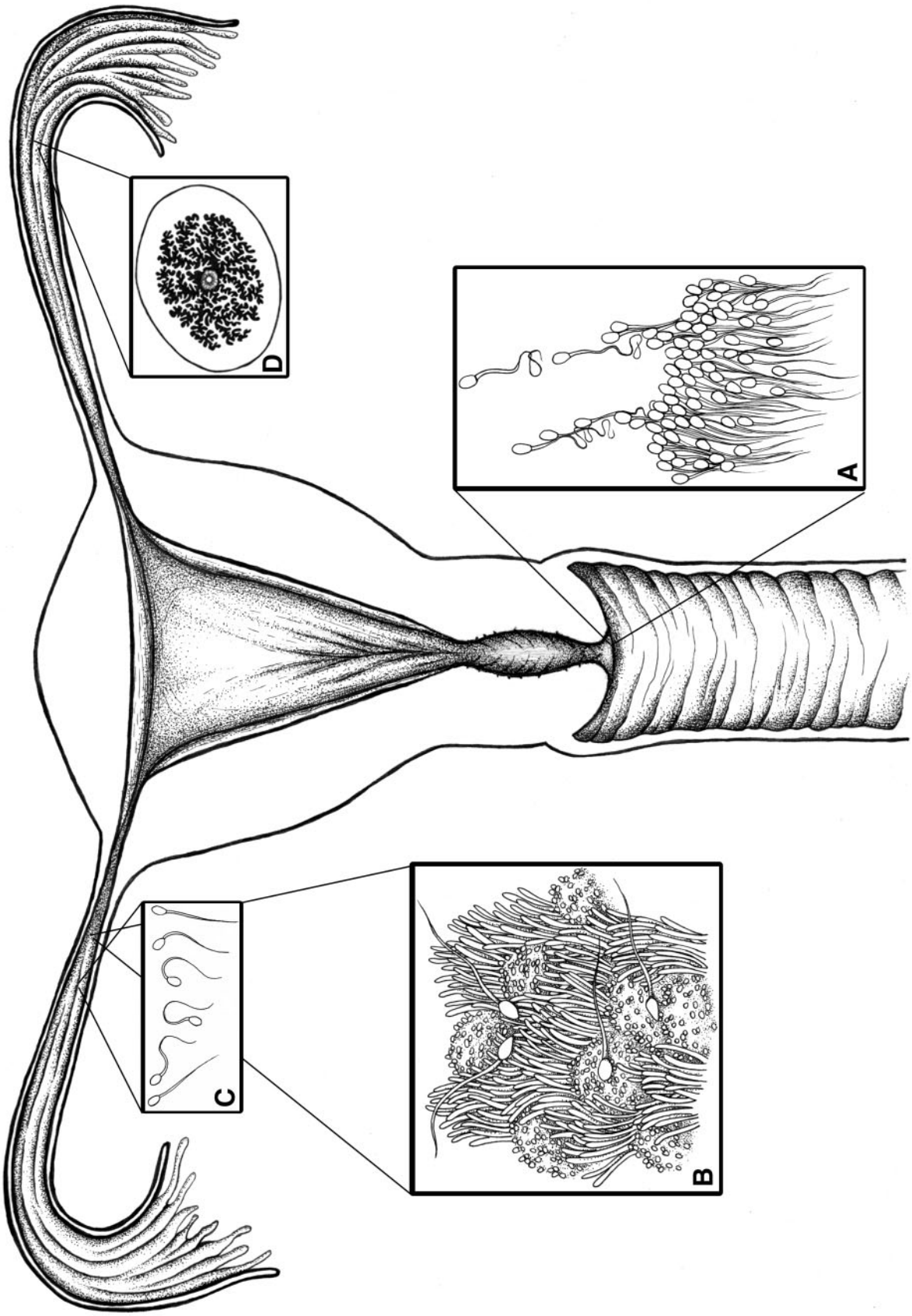

Figure 1. Human female reproductive tract illustrating stages of gamete transport. (**A**) Sperm entering cervical mucus at external os of cervix. The mucus fills the upper half of the inset. (**B**) Sperm interacting with endosalpingeal epithelium in Fallopian tube. (**C**) Hyperactivated motility of sperm in Fallopian tube. (**D**) Oocyte in cumulus within a transverse section of the tubal ampulla. Artwork by C. Rose Gottlieb.

Cervical mucus presents a greater barrier to abnormal sperm that cannot swim properly or that present a poor hydrodynamic profile than it does to morphologically normal, vigorously motile sperm and is thus thought as one means of sperm selection (Hanson and Overstreet, 1981; Barros *et al*., 1984; Katz *et al*., 1990, 1997).

The greatest barrier to sperm penetration of cervical mucus is at its border, because here the mucus microarchitecture is more compact (Yudin *et al*., 1989). Components of seminal plasma may assist sperm in penetrating the mucus border. More human sperm were found to enter cervical mucus *in vitro* when an inseminate was diluted 1:1 with whole seminal plasma than when it was diluted with Tyrode's medium, even though the sperm swam faster in the medium (Overstreet *et al*., 1980).

Like the vagina, the cervix can mount immune responses. In rabbits and humans, vaginal insemination stimulates the migration of leukocytes, particularly neutrophils and macrophages, into the cervix as well as into the vagina (Tyler, 1977; Pandya and Cohen, 1985). Neutrophils migrate readily through midcycle human cervical mucus (Parkhurst and Saltzman, 1994). In rabbits, neutrophils were found to heavily infiltrate cervices within a ½ h of mating or artificial insemination (Tyler, 1977). Interestingly, it was discovered that if female rabbits were mated to a second male during the neutrophilic infiltration induced by an earlier mating, sperm from the second male were still able to fertilize (Taylor, 1982). Thus, although the cervix is capable of mounting a leukocytic response, and neutrophils may migrate into cervical mucus, the leukocytes may not present a significant barrier to sperm. It has been demonstrated that neutrophils will bind to human sperm and ingest them only if serum that contains both serological complement and complement-fixing anti-sperm antibodies is present (D'Cruz *et al*., 1992). This can happen *in vivo* if the female somehow becomes immunized against sperm antigens. Altogether, the evidence indicates that leukocytic invasion serves to protect against microbes that accompany sperm and does not normally present a barrier to normal motile sperm, at least not shortly after coitus.

Immunoglobulins, IgG and IgA, have been detected in human cervical mucus. Secretory IgA is produced locally by plasma cells in subepithelial connective tissue. The amount secreted increases in the follicular phase but then decreases at about the time of ovulation (Kutteh *et al*., 1996). The immunoglobulins provide greater protection from microbes at the time when the cervical mucus is highly hydrated and offers the least resistance to penetration. However, when there are antibodies present that recognize antigens on the surface of ejaculated sperm, infertility can result (Menge and Edwards, 1993).

Complement proteins are also present in cervical mucus (Matthur *et al*., 1988), along with regulators of complement activity (Jensen *et al*., 1995). Thus, there is a potential for antibody-mediated destruction of sperm in the cervical mucus as well as leukocytic capture of sperm. Some anti-sperm antibodies are not complement-activating; however, they can still interfere with movement of sperm through cervical mucus by physical obstruction (Menge and Edwards, 1993; Ulcova-Gallova, 1997).

An elegant three-dimensional reconstruction of serial sections of the bovine cervix produced by Mullins and Saacke (1989) led them to conclude that mucosal folds in the cervical canal form channels leading to the uterine cavity. Furthermore, based on histochemical staining characteristics of the mucus, they concluded that, during the follicular phase, mucus deep in the channels is different in composition and less dense than that in the central portion of the cervical canal. They proposed that bull sperm enter deep channels at the external os and travel in them all the way to the uterine cavity, thereby avoiding the more viscous mucus in the centre of the cervical canal that serves to discharge uterine contents. This model is supported by results of earlier studies on farm animals. Mattner (1968) found that when he flushed the cervices of goats and cows 19–24 h after mating he recovered approximately 90% of the mucus and more than 90% of the luminal leukocytes, but only about half of the sperm. The remaining half of the sperm were found deep in the mucosal grooves. These observations also indicate that the cervix supports the passage of normal motile sperm while discouraging passage of microbes and sperm with abnormal form or motility. Normal, fresh, motile sperm can avoid the area most populated by neutrophils and they appear to be resistant to leukocytic phagocytosis anyway, as discussed above. In descriptions of human cervical anatomy, mention is made of cervical crypts that are thought to entrap and store sperm (Fawcett and Raviola, 1994; Harper, 1994). On the other hand, scanning electron microscopy of the human cervix indicates that mucosal grooves forming a preferential pathway for sperm could be present as in the bovine (Figure 2). A comprehensive study of the human cervix is needed to determine whether sperm follow mucosal grooves to traverse the cervical canal.

Sperm may also be guided through the cervix by the microarchitecture of the cervical mucus. Mucins, the chief glycoproteins comprising cervical mucus, are long, flexible linear molecules (molecular weight of human mucins is approximately 10^7 Daltons). The viscosity of mucus is due to the large size of mucins, while elasticity results from the entanglement of the molecules (Carlstedt and Sheehan, 1984, 1989; Sheehan and Carlstedt, 1984; Sheehan *et al*., 1986). It is thought that these long molecules become aligned by the secretory flow in mucosal grooves and thus serve to guide sperm. Human (Chretien, 2003) and bull (Tampion and Gibons, 1962) sperm have been demonstrated to orient themselves along the long axis of threads of bovine cervical mucus. Human sperm swimming through cervical mucus swim in a straighter path than they do in seminal plasma or medium (Katz *et al*., 1978).

Are sperm stored in the cervix?

Little is known about how long sperm spend traversing the cervix or whether sperm are stored there. Vigorously motile sperm have been recovered from the human cervix up to 5 days after insemination (Gould *et al*., 1984), and the presence of sperm in midcycle cervical mucus forms the basis of the 'post coital test' (PCT) (Mortimer, 1994). Nevertheless, it is not known whether sperm collected from cervices this long after coitus would reach the Fallopian tube and succeed in fertilizing, nor could it be known whether these sperm had re-entered the cervix from the uterus. Very few sperm have been recovered from human uteri 24 h after coitus (Rubenstein *et al*., 1951; Moyer *et al*., 1970) and those sperm are greatly outnumbered by leukocytes (Thompson *et al*., 1992). Unless sperm are protected from phagocytosis (and they appear to be), it is unlikely that they could travel from a cervical reservoir to the oviduct 24 h post coitus.

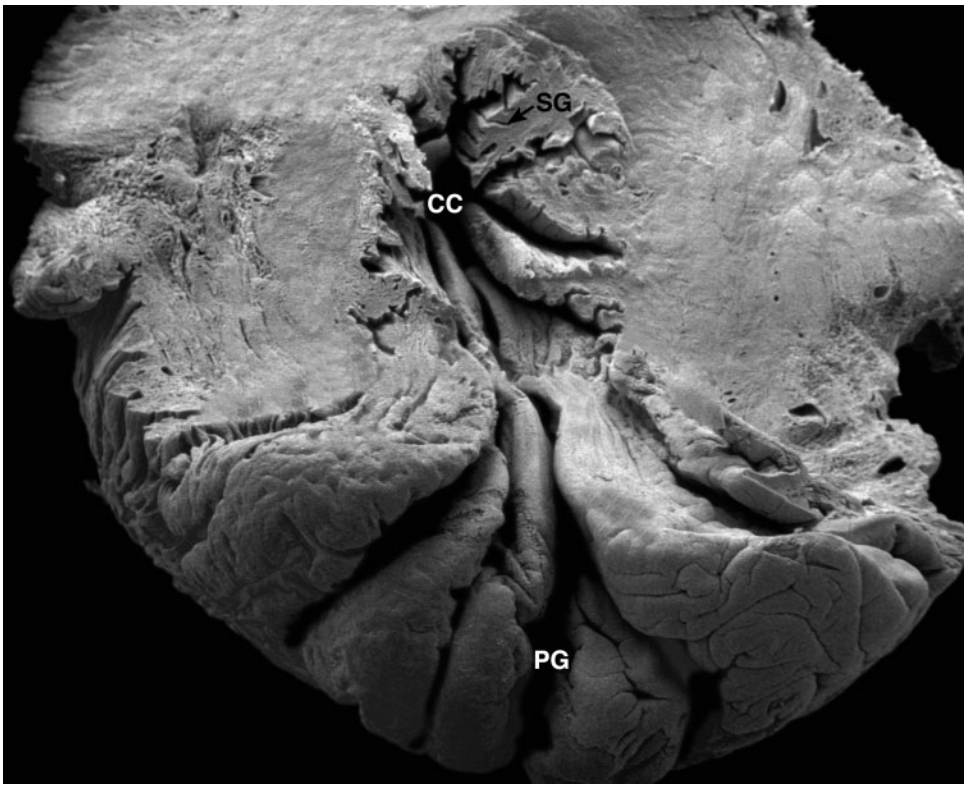

Figure 2. A scanning electron micrograph of the human cervix, illustrating potential passageways for sperm (×67). A portion of the wall has been removed to reveal the architecture of the cervical canal (CC). Large, primary mucosal grooves (PG) can be seen at the external os that extend deep into the cervical canal. Smaller secondary mucosal grooves (SG) branch from the primary grooves. Although the primary grooves appear to form a preferential path for sperm, it is not known whether secondary or even tertiary grooves could end blindly and entrap sperm. Cervical crypts could also trap sperm. Reproduced, with permission, from Kessel and Kardon (1979).

Sperm transport through the uterus

At only a few centimetres in length, the human uterine cavity is relatively small and could be traversed in less than 10 min by sperm swimming at about 5 mm/min, which is the swimming speed of sperm in aqueous medium (Mortimer and Swan, 1995). The actual rate of passage of human sperm through the uterus is difficult to determine due to experimental limitations. Variation is high among women within a study and between studies (Croxatto, 1996). In one set of experiments, fertile women were inseminated into the cranial vagina shortly before surgical excision of both Fallopian tubes. Sperm were recovered from the fimbrial segment of the ampulla in two women whose tubes were removed 5 min after insemination, even though they had been abstinent for at least 16 days. Sperm were recovered all along the tubes of two more women merely 10 min after insemination (Settlage *et al*., 1973). Unfortunately, the motility of these sperm was not assessed; therefore, it could not be determined whether the sperm were capable of fertilizing. In another study (Rubenstein *et al*., 1951), several motile sperm were recovered from Fallopian tubes following hysterectomy 30 min after insemination in one patient and 1 h after insemination in three out of seven patients; however, these women underwent surgery for treatment of fibroids, polyps or endometriosis and therefore sperm transport may have been abnormal.

Transport of sperm through the uterus is likely aided by proovarian contractions of the myometrium. Ultrasonography of the human uterus has revealed cranially directed waves of uterine smooth muscle contractions that increase in intensity during the late follicular phase (Lyons *et al*., 1991; Kunz *et al*., 1996). The uterine contractions occurring in women during the periovulatory period are limited to the layer of myometrium directly beneath the endometrium (Lyons *et al*., 1991; de Ziegler *et al*., 2001). This is in contrast to contractions occurring during menses, which involve all layers of the myometrium. In cows and ewes, electromyography has indicated that strong contractile activity occurs during estrus, while contractions are weak and localized during the luteal phase (Hawk, 1983).

In humans, contractile activity of uterine muscle may draw sperm and watery midcycle mucus from the cervix into the uterus. Fukuda and Fukuda (1994) interpreted ultrasound images of the uteri of women in the late follicular phase to indicate that the uterine cavity is filled with mucus. They proposed that the cervical mucus assists sperm movement through the human uterine cavity. This is possible because the volume of uterine fluid in midcycle women is only about 100 µl (Casslen, 1986) and cervical mucus is plentiful enough to fill the lumen.

Kunz and collaborators (1996) deposited 5–40 µm albumen microspheres radioactively tagged with technetium into the cranial vaginae of women to determine how such contractions might transport sperm. They found that spheres were rapidly and maximally transported into the uterine cavity and even into the tubal isthmus during the late follicular phase. Interestingly, transport of the spheres was greater to the isthmus ipsilateral to the dominant

follicle than to the contralateral isthmus. This preferential transport may result from signals passed through a vascular communication from the pre-ovulatory follicle to the uterus and Fallopian tube. An arterial anastomosis lies between the ovarian and uterine arteries (which also supply the Fallopian tube) in the corneal region of the human uterus. Doppler flow sonography revealed increased perfusion of these anastomosing vessels on the side of the pre-ovulatory follicle (Kunz, 1998). It is thought that these vessels carry hormones from the dominant follicle directly to the uterus and oviduct without first passing through the systemic circulation, because the ovarian artery associates closely with the ovarian vein. This could enable a countercurrent transfer of ovarian hormones from the venous drainage of the ovary to the ovarian artery and then to the arterial supply of the uterus and oviduct (Hunter *et al*., 1983; Stefanczyk-Krzymowska *et al*., 1998). In addition, lymphatic drainage of the ovary might transfer hormones to the ovarian artery and then the oviductal vessels (Stefanczyl-Krzymowska *et al*., 1998).

Studies of uterine contractions during estrus should be interpreted with caution if coitus did not occur. Video-laparoscopic examination of mated and unmated rats revealed significant changes in contractile patterns of the uterine horns after mating. Unexpectedly, the change consisted of several-fold increases in both cranially and caudally propagating circular contractions (Crane and Martin, 1991). Caudally directed peristalsis would be expected to carry sperm away from the uterotubal junction. In estrous domestic cats, both ascending and descending contractions were observed by fluoroscopy (Chatdarong *et al*., 2002). Perhaps the ebb and flow of contractions direct fresh waves of sperm to the uterotubal junction.

Myometrial contractions may be stimulated by seminal components. When vasectomized male rats were mated with females, the incidence of strong uterine contractions declined, indicating that sperm or testicular or epididymal secretions have stimulatory activity (Crane and Martin, 1991). Removal of the seminal vesicles significantly reduced the pregnancy rate in mice (Peitz and Olds-Clarke, 1986). In boars, there is evidence that estrogens, which may reach 11.5 μg in an ejaculate, increase myometrial contraction frequency. Since boar semen is deposited directly into the uterine cavity, the uterus is exposed to the full amount of estrogens in the semen. There is evidence that the estrogens enhance contraction by stimulating secretion of PGF-2α (Claus, 1990).

Rapid transport of sperm through the uterus by myometrial contractions can enhance sperm survival by propelling them past the immunological defenses of the female. As is the case in the vagina and cervix, coitus induces a leukocytic infiltration of the uterine cavity, which reaches a peak several hours after mating in mice (Austin, 1957). The leukocytes are primarily neutrophils and have been observed phagocytizing uterine sperm in mice, rats and rabbits (Austin, 1957; Bedford, 1965). This phagocytosis was observed several hours after insemination and therefore might be directed primarily against damaged sperm. However, normal sperm may also be attacked, particularly in vaginal inseminators like humans, because their sperm have lost much of the immune protection afforded by seminal plasma constituents (Suarez and Oliphant, 1982; Dostal *et al*., 1997).

When sperm first enter the uterus, they outnumber the leukocytes. As time passes, the leukocytes begin to outnumber the sperm. Also, as sperm lose protective seminal plasma coating, they may become more susceptible to leukocytic attack. At some point, even undamaged sperm may fall victim to the leukocytes. Probably, to ensure fertilization, sperm should pass through the uterine cavity before significant numbers of leukocytes arrive.

Transport through the uterotubal junction

The uterotubal junction presents anatomical, physiological and/or mucous barriers to sperm passage in most mammals. Anatomically, the lumen in species as distantly related as dairy cattle and mice is particularly tortuous and narrow (Hook and Hafez, 1968; Hafez and Black, 1969; Beck and Boots, 1974; Wrobel *et al*., 1993). The narrowness of the lumen is especially apparent in living tissue (Suarez, 1987) and in frozen sections, in which tissue does not shrink as it does during standard preparation of paraffin-embedded sections (Suarez *et al*., 1997).

The entrance to the junction is fairly simple in humans; whereas, it is complicated by mucosal folds in cows, pigs, rabbits and many other species (Hook and Hafez, 1968; Hafez and Black, 1969; Beck and Boots, 1974; Wrobel *et al*., 1993). In mice and rats, the entrance forms a conical projection into the uterus called a colliculus tubarius (Zamboni, 1972; Gaddum-Rosse, 1981; Suarez, 1987).

Within the lumen of the junction, there are large and small folds in the mucosa. In the cow, mucosal folds form cul-de-sacs with openings that face back towards the uterus (Yániz *et al*., 2000). This arrangement of folds seems designed to entrap sperm and prevent further ascent.

A physiological valve may be created by a vascular plexus in the lamina propria/submucosal layer of the wall. When engorged, the plexus can compress the lumen. This plexus has been well described in cattle (Wrobel *et al*., 1993). The walls of the bovine junction and adjacent tubal isthmus also contain a thick muscular layer that could further constrict the lumen. The bovine uterotubal junction is sigmoidal in shape and supported by muscular ligaments that appear capable of increasing the flexure of the curve and thus compressing the lumen (Hook and Hafez, 1968; Hafez and Black, 1969). In the mouse, the junction is reported to be patent shortly after coitus, but to be tightly closed about an hour later (Zamboni, 1972; Suarez, 1987). The human junction traverses a thick muscular layer of uterine wall (Hafez and Black, 1969); however, it is unknown whether the muscle regulates the patency of the junction.

The narrow lumen of the uterotubal junction may be filled with viscous mucus that can impede the progress of sperm. Mucus has been found in the uterotubal junction in humans (Jansen, 1980), as well as in rabbits (Jansen, 1978; Jansen and Bajpai, 1982), pigs (Suarez *et al*., 1990) and dairy cattle (Suarez *et al*., 1997, 1990).

In rodents, it has been demonstrated that sperm with linear, progressive motility are more successful at passing through the uterotubal junction (Gaddum-Rosse, 1981; Shalgi *et al*., 1992).

Male mice that are null mutants for the genes encoding fertilin β (Cho *et al*., 1998), calmegin (Ikawa *et al*., 1997; Yamagata *et al*., 2002) or testis-specific angiotensin converting enzyme (ACE) (Krege *et al*., 1995; Hagaman *et al*., 1998) are infertile because their sperm cannot pass through the uterotubal junction nor bind to the zona pellucida. In these null mutants, both the motility and morphology of the sperm are normal. Fertilin β is localized on the plasma membrane overlying the acrosome on mature sperm from wild-type males, while it is lacking in the null mutants (Cho *et al*.,

1998). As for calmegin, sequence homology indicates that it is a chaperone protein, which would place it in the endoplasmic reticulum of spermatids, assisting in the proper folding of proteins destined for membranes. Both wild-type and null mutants lack calmegin in mature sperm; therefore, its affect on fertility is presumed to be due to the lack of proteins that rely on calmegin for proper placement in the sperm plasma membrane. In the case of ACE null mutants, there is strong evidence that the missing ACE normally acts to release GPI-anchored proteins from the sperm plasma membrane (Metayer *et al*., 2002; Kondoh *et al*., 2005). Thus, the lack of ACE means that some proteins that would normally be shed from sperm are retained. These various strains of null mutant mice indicate that certain epitopes must be available and exposed on the surface of sperm to interact with the uterotubal junction and somehow promote sperm passage.

The role of calmegin in enabling sperm to pass through the uterotubal junction was examined more closely using chimeric males that produced a mixture of germ cells with wild-type and disrupted calmegin genes. The question addressed was whether calmegin-chaperoned proteins are required by individual sperm to pass through the junction, or would the presence of wild-type sperm enable them to do so. Such would be the case, for example, if the proteins on the sperm surface assist passage by signalling the junction to open. Chimeric males were created by fusing embryos from 'wild-type' mice that had normal calmegin genes with those from a double transgenic line of mice that were homozygous null for calmegin and expressed enhanced green fluorescent protein (GFP) in their acrosomes. The resulting chimeric XY/XY males produced a mixture of sperm, about half of which were mutant, as identified by the presence of the fluorescent acrosomes. When these males were mated with wild-type females, only wild-type sperm could be found above the junction (Nakanishi *et al*., 2004). This indicates that normal morphology and motility are not sufficient for enabling sperm to pass through the junction. An additional factor, likely a sperm surface protein or proteins, is required by each sperm for it to pass through the junction.

Rapid sperm transport

Sperm have been recovered in the cranial reaches of the tubal ampulla only minutes after mating or insemination in humans (Settlage *et al*., 1973) and several other species of mammals (Overstreet and Cooper, 1978; Hawk, 1983, 1987). Rapid transport of sperm into the Fallopian tube would seem to counter the proposed model of sperm swimming one-by-one through the uterotubal junction. However, when rabbit sperm recovered from the cranial ampulla shortly after mating were evaluated by Overstreet and Cooper (1978), they found that most were immotile and damaged. They proposed that waves of contractions stimulated by insemination transport some sperm rapidly to the site of fertilization, but these sperm are mortally damaged by the associated sheer stress and do not fertilize. Later, motile sperm gradually pass through the uterotubal junction to establish a tubal population capable of fertilizing. The contractions may serve primarily to draw sperm into the cervix but result in overshooting of some sperm. As described above, motile human sperm have been recovered from Fallopian tubes within an hour of insemination; however, it is not known whether function was normal in these women (Rubenstein *et al*., 1951).

A sperm reservoir in the Fallopian tube

As sperm pass through the uterotubal junction and enter the tubal isthmus, they may be trapped and held in a reservoir. Yanagimachi and Chang (1963) were first to describe a reservoir of sperm in the hamster tubal isthmus. Since then, evidence has been found for the formation of sperm storage reservoirs in a variety of species [mice (Suarez, 1987), rabbits (Harper, 1973; Overstreet *et al*., 1978), cows (Hunter and Wilmut, 1984), pigs (Hunter, 1981) and sheep (Hunter and Nichol, 1983)].

The Fallopian tube provides a haven for sperm. Unlike the vagina, cervix and uterus, the tube does not respond to insemination with an influx of leukocytes (Rodriguez-Martinez *et al*., 1990).

In addition to providing a haven, the storage reservoir maintains the fertility of sperm until ovulation. *In vitro*, sperm fertility and motility are maintained longer when sperm are incubated with endosalpingeal epithelium [human (Kervancioglu *et al*., 1994), bovine (Pollard *et al*., 1991), porcine (Suarez *et al*., 1991a), equine (Ellington *et al*., 1993) and canine (Kawakami *et al*., 2001)].

Entrapment and storage of sperm in the initial segment of the tube may serve to prevent polyspermic fertilization by allowing only a few sperm at a time to reach the oocyte in the ampulla. Sperm numbers have been artificially increased at the site of fertilization in the pig by surgical insemination directly into the ampullar lumen (Polge *et al*., 1970; Hunter, 1973), by resecting the isthmus to bypass the reservoir (Hunter and Leglise, 1971) or by administering progesterone into the muscularis to inhibit smooth muscle constriction of the lumen (Day and Polge, 1968; Hunter, 1972). In each of these cases, the incidence of polyspermy increased.

There is strong evidence from multiple species of eutherian mammals that the tubal reservoir is created when sperm bind to the epithelium lining the tube. In humans, motile sperm have been observed to bind their heads to the apical surface of endosalpingeal epithelium *in vitro* (Figures 1B and 3; Pacey *et al*., 1995a;

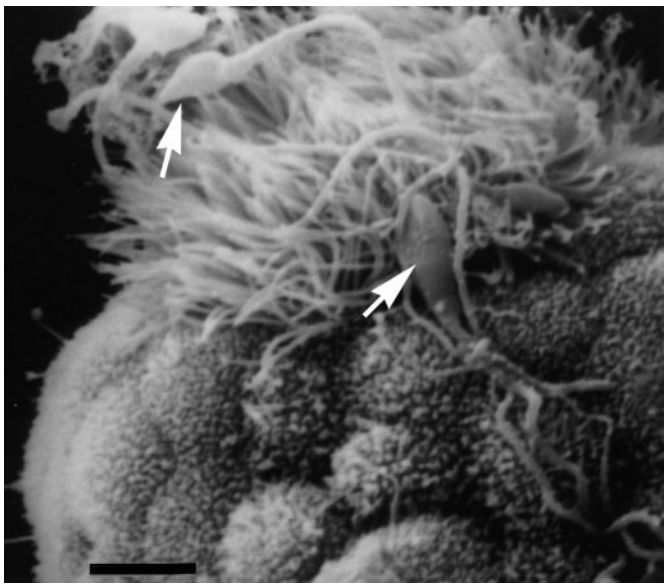

Figure 3. Scanning electron micrograph showing human sperm attached to a ciliated area of Fallopian tube epithelium *in vitro*. Arrows indicate sperm heads associated with cilia. Scale bar, 4 μm. Reproduced from Pacey *et al*. (1995b).

Baillie *et al*., 1997; Reeve *et al*., 2003). In a variety of non-human species, this phenomenon has been seen *in vitro* or *in vivo* (Suarez, 1987; Suarez *et al*., 1990, 1991a; Smith and Yanagimachi, 1991; Thomas *et al*., 1994; Petrunkina *et al*., 2004).

Sperm binding to endosalpingeal (oviductal) epithelium of non-human eutherian mammals studied to date involves the binding of sperm to carbohydrate moieties on the epithelium. Fetuin and its terminal sugar, sialic acid, were found to competitively inhibit binding of hamster sperm inseminated into oviducts, whereas desialylated fetuin did not. Fetuin binding sites were localized over the acrosomal region of the sperm head, which is the region by which sperm bind to epithelium (DeMott *et al*., 1995). Binding of stallion sperm to explants of endosalpinx was inhibited by asialofetuin and its terminal sugar, galactose (Lefebvre *et al*., 1995b; Dobrinski *et al*., 1996a), while binding of boar sperm was blocked by mannose (Green *et al*., 2001; Wagner *et al*., 2002). Bull sperm binding was blocked by fucoidan and its component fucose and pre-treatment of bovine epithelium with fucosidase reduced binding (Lefebvre *et al*., 1997). In each of the species studied so far, a different carbohydrate inhibited binding *in vitro*. These species differences may not seem so unusual when one considers that a single amino acid residue can determine the carbohydrate binding specificity of a lectin and that closely related animal lectins have different carbohydrate specificities (Kogan *et al*., 1995; Revelle *et al*., 1996).

It has not been established whether human sperm attach to the endosalpinx through a carbohydrate, although recent experiments by Reeve *et al*. (2003) have implicated the amino acid sequence Arg-Gly-Asp (RGD) in sperm binding to isthmic but not ampullary endosalpingeal epithelium. Interestingly, human sperm–endosalpingeal interaction *in vitro* appears to be disrupted in tissue donated from women who have had a previous diagnosis of endometriosis (Reeve *et al*., 2005), suggesting for the first time that poor sperm interaction with the endosalpingeal epithelium might be associated with reduced fertility, such as that often observed in women with endometriosis.

A protein responsible for binding bull sperm to endosalpingeal epithelium has been identified as PDC-109, also called BSP-A1/A2 (Ignotz *et al*., 2001; Gwathmey *et al*., 2003). PDC-109 is an approximately 16 kDa protein consisting predominantly of two fibronectin type II domains. It is produced by the seminal vesicles and coats epididymal sperm when they come into contact with seminal secretions (Desnoyers and Manjunath, 1992; Müller *et al*., 1998; Ramakrishnan *et al*., 2001). Epididymal bull sperm bind endosalpingeal epithelium in very low numbers, but when they are coated with purified PDC-109, their binding increases to the level of ejaculated bull sperm (Gwathmey *et al*., 2003).

Preserving sperm fertility during storage

Sperm–endosalpingeal contact somehow preserves sperm during storage. Human sperm incubated with epithelium *in vitro* remain viable longer than when they are incubated in medium alone (Kervancioglu *et al*., 1994), as do sperm from other mammals (Suarez *et al*., 1990; Pollard *et al*., 1991; Ellington *et al*., 1993; Kawakami *et al*., 2001). Viability of human sperm (Murray and Smith, 1997) and other species (Dobrinski *et al*., 1996b; Smith and Nothnick, 1997) can be extended by incubating them with vesicles prepared from the apical membranes of the endosalpinx, indicating that the epithelium can produce the effect by direct contact rather than by secretions. It was reported that equine sperm binding to epithelium or membrane vesicles maintain low levels of cytoplasmic Ca^{2+}, compared to free-swimming sperm or sperm incubated with vesicles made from kidney membranes (Dobrinski *et al*., 1996b, 1997). Human and equine sperm incubated with endosalpingeal membrane vesicles capacitate more slowly than sperm incubated in capacitating medium alone (Dobrinski *et al*., 1997; Murray and Smith, 1997). Possibly, viability is maintained by preventing capacitation and its concomitant rise in cytoplasmic Ca^{2+}. The mechanism for preventing rises of cytoplasmic Ca^{2+} in sperm are not known, but one suggestion is that catalase, which has been detected in the bovine tube, serves to protect against peroxidative damage to the sperm membranes, perhaps preventing inward leakage of Ca^{2+} (Lapointe *et al*., 1998).

The endosalpingeal binding protein on bull sperm, PDC-109, probably acts to stabilize sperm membranes. PDC-109 reduces membrane fluidity and immobilizes cholesterol in phospholipid membranes, including those of epididymal sperm (Greube *et al*., 2001; Müller *et al*., 2002). PDC-109 can also contribute to membrane stability by inhibiting the activity of phospholipase A_2 (Manjunath *et al*., 1994b; Soubeyrand and Manjunath, 1997). Thus, PDC-109 may play a role in preserving bull sperm fertility while they are stored in the reservoir.

Homologues to PDC-109 have been identified in many species (Villemure *et al*., 2003; Boisvert *et al*., 2004; Greube *et al*., 2004; Barrios *et al*., 2005; Bergeron *et al*., 2005); however, a functional equivalent has yet to be identified in humans.

The storage reservoir in humans

Aggregation of sperm in a distinct reservoir have not been seen in the Fallopian tube of human as in other species (Williams *et al*., 1993). Furthermore, while associations of human sperm with the endosalpinx have been observed *in vitro* (Yeung *et al*., 1994; Pacey *et al*., 1995a,b; Baillie *et al*., 1997; Murray and Smith, 1997), the sperm do not seem to bind as quickly or as tightly to the epithelium as those of some non-primate mammals (Pacey *et al*., 1995b). On the other hand, human sperm viability is maintained by incubation with endosalpingeal epithelium (Murray and Smith, 1997), as it is in species in which there is a distinct reservoir and strong binding of sperm to epithelium. Therefore the protective effects of sperm–epithelial contact in the human does not seem to be as dependent on tight cell–cell interactions as in other species.

Although a visibly distinct sperm reservoir has not been seen in the Fallopian tubes of humans, a functional reservoir may be created by detaining human sperm in the tubal isthmus. Human sperm do stick to endosalpingeal epithelium, although they seem to do so intermittently *in vitro* (Pacey *et al*., 1995b). While intermittent sticking would not hold sperm in a distinct reservoir, it would certainly slow their progress towards the ampulla. Sperm progress would also be slowed by the mucus in the lumen. Finally, the architecture of the mucosal lining of the human Fallopian tube must act to slow sperm progress. The mucosal folds increase in height and complexity towards the ovary, thus offering increasingly greater obstacles to the advancement of sperm into and through the ampulla. Slowing the advancement of human sperm in these various ways could serve the function of a reservoir; that is,

prolonging the availability of sperm in the Fallopian tube and avoiding polyspermic fertilization by ensuring that only a few reach the ampulla at any one time.

Overall, data of human sperm distribution in the Fallopian tubes of women have not provided a clear picture of the events of sperm transport. Sperm recovered at various times in different regions of the Fallopian tube have varied so much in numbers that the data do not permit the construction of a model for the pattern of tubal sperm transport (Williams *et al*., 1993). Nevertheless, since pregnancy has been shown to result from intercourse as long as five days before ovulation (Wilcox *et al*., 1995) human sperm must be stored somewhere in the female tract and the fact that human endosalpingeal epithelium prolongs survival of sperm *in vitro* indicates that the Fallopian tubes are strong candidates for storage sites.

Advancement of sperm beyond the tubal reservoir

Theoretically, sperm could be released from the reservoir either through loss of binding sites on the epithelium or by alterations in the sperm themselves. Changes in the hormonal state of endosalpingeal epithelium related to impending ovulation do not affect the density of binding sites for sperm in many species (Suarez *et al*., 1991a; Thomas *et al*., 1994; Lefebvre *et al*., 1995a), and in women relatively equal numbers of sperm bind to endosalpingeal explants recovered at different times of the ovarian cycle (Baillie *et al*., 1997). Thus, it appears that the epithelium does not release sperm by reducing binding sites. Instead, current evidence indicates that changes in sperm bring about their release.

Sperm undergo two changes in preparation for fertilization: capacitation and hyperactivation. Capacitation involves changes in the plasma membrane, including shedding of proteins and cholesterol, that prepare sperm to undergo the acrosome reaction and fertilize oocytes (reviewed by De Jonge, 2005) and therefore loss or modification of proteins on the surface of the plasma could reduce affinity for the endosalpingeal epithelium. Hyperactivation, on the other hand, is a change in flagellar beating that typically involves an increase in the flagellar bend amplitude (Figure 1C). This can provide the force necessary for overcoming the attraction between sperm and epithelium (Ho and Suarez, 2001; Suarez and Ho, 2003). Hyperactivation has been considered to be part of the capacitation process; however, there is evidence that hyperactivation is regulated by a separate or divergent pathway from that regulating acrosomal responsiveness (Marquez and Suarez, 2004). For the sake of clarity in the following discussion, we will use the more restricted definition of capacitation as the process of gaining acrosomal responsiveness.

Reproductive tracts removed from mated female mice can be transilluminated to examine the behavior of sperm within the reservoir. Under these conditions, it was noted that only hyperactivated sperm detach from endosalpingeal epithelium (DeMott and Suarez, 1992). In other experiments, when hamster sperm were infused into hamster tubes, the sperm bound to the epithelium unless they had been capacitated and hyperactivated *in vitro* before infusion (Smith and Yanagimachi, 1991). When bull sperm were capacitated before being added to explants of oviductal epithelium, subsequent sperm binding was significantly reduced (Lefebvre and Suarez, 1996). In this case, the bull sperm were capacitated but not hyperactivated; therefore, it was concluded that capacitation reduces binding affinity of sperm for epithelium. Taken together, these observations indicate that capacitation-induced changes in the sperm head surface are responsible for reducing binding affinity, although the pull produced by hyperactivation can almost certainly hasten the detachment of bound sperm from the epithelium.

While evidence is lacking for a release mechanism involving reduction in binding sites on the epithelium, the epithelium may still play a role in sperm release by secreting factors that alter sperm. For example, hormonal signals that induce ovulation or signals from the pre-ovulatory follicle could stimulate the epithelium to secrete factors that trigger sperm capacitation and hyperactivation, thereby bringing about sperm release. In support of this concept, tubal fluid and medium conditioned by cultured endosalpingeal cells have been demonstrated to enhance capacitation of bull sperm *in vitro* (Chian *et al*., 1995; Mahmoud and Parrish, 1996).

Capacitated bull sperm show reduced binding to endosalpingeal epithelium as well as to the carbohydrate ligand involved in sperm binding (Revah *et al*., 2000; Ignotz *et al*., 2001). The loss of binding affinity for epithelium can be accounted for by a shedding of the adsorbed seminal plasma protein PDC-109 from the sperm head during heparin-induced capacitation (Gwathmey *et al*., 2003). PDC-109 possesses heparin-binding sites (Calvete *et al*., 1999; Wah *et al*., 2002) and heparin is used to capacitate bull sperm *in vitro* (Parrish *et al*., 1988, 1989; Galantino-Homer *et al*., 1997). Furthermore, addition of heparin to bovine sperm bound to cultured endosalpingeal epithelium enhances the release of sperm from epithelium (Bosch *et al*., 2001). *In vivo*, the increased heparin-like glycosaminoglycans detected in the bovine tube during the periovulatory period (Parrish *et al*., 1989) could serve to release sperm.

Although a distinct tubal sperm reservoir has not been reported for humans, hyperactivation and capacitation could serve to speed sperm movement into the ampulla as the time of ovulation approaches.

Hyperactivation of sperm and the final stages of transport

At some point in the female tract, most likely in the Fallopian tubes, sperm become hyperactivated. In aqueous media *in vitro*, hyperactivated sperm swim vigorously but in circular or erratic patterns. *In vivo*, the physical environment encountered by sperm is quite different and evidence indicates that hyperactivation is required by sperm to progress towards the oocyte and penetrate its vestments. As discussed above, hyperactivation may assist sperm in detaching from the endosalpingeal epithelium. In addition, hyperactivation enhances the ability of sperm to swim through viscoelastic substances such as mucus in the tubal lumen and the extracellular matrix of the cumulus oophorus. Mucus fills the uterotubal junction and extends into the isthmus in humans (Jansen, 1980), rabbits (Jansen, 1978; Jansen and Bajpai, 1982), pigs (Suarez *et al*., 1991a) and dairy cattle (Suarez *et al*., 1997). Hyperactivated sperm penetrate artificial mucus, such as viscoelastic solutions of long-chain polyacrylamide or methylcellulose, far more effectively than non-hyperactivated sperm (Suarez *et al*., 1991b; Suarez and Dai, 1992; Quill *et al*., 2003).

Hyperactivation also endows sperm with greater flexibility for turning around in pockets of mucosa (Suarez *et al*., 1983;

Suarez and Osman, 1987). In the human Fallopian tube, as discussed above, mucosal folding increases in height and branching from the isthmus to the ampulla and thus hyperactivation may assist sperm in navigating the increasingly complex maze (Figure 1D).

The most convincing evidence of the importance of hyperactivation in these final stages of a sperm's journey comes from experiments with mice in which the gene for CatSper1 or CatSper2 has been disrupted. Sperm from these animals do not reach the oocytes in the oviductal ampulla (Suarez, personal observations). Although they show normal vigorous progressive motility, they cannot hyperactivate and do not penetrate artificial mucus as well as wild-type sperm (Quill *et al*., 2001, 2003; Ren *et al*., 2001; Carlson *et al*., 2003).

In addition to assisting sperm in reaching the oocyte, hyperactivation also aids sperm in penetrating the zona pellucida. When hyperactivation was blocked in capacitated, acrosome-reacted hamster sperm bound to the zona, they were unable to penetrate it (Stauss *et al*., 1995). Also, sperm from male mice that are null mutants for CatSper1 or CatSper2 genes and cannot hyperactivate also cannot penetrate the zona (Ren *et al*., 2001; Quill *et al*., 2003).

Taxis of sperm towards oocytes

Although the existence of a guidance system to help mammalian sperm reach the unfertilized oocyte has been debated over the years, stronger evidence for such a system has surfaced recently. There is evidence for the existence of two complementary guidance mechanisms operating within the Fallopian tube. The first (long-range) mechanism is where capacitated sperm—released from intimate contact with the endosalpinx (see above)—are guided by thermotaxis towards the site of fertilization. A temperature difference of up to 2°C between the cooler tubal isthmus and the warmer tubal ampulla has been detected in rabbits and there are indications that capacitated rabbit sperm tend to swim towards warmer temperatures (Bahat *et al*., 2003). Once in the tubal ampulla, and at a closer proximity to the oocyte, a second (short-range) chemotactic mechanism may guide sperm closer to the oocyte (Eisenbach, 1999; Babcock, 2003).

Sperm are equipped with a mechanism for turning towards the oocyte in response to chemotactic factors; that is, they can switch back and forth between symmetrical flagellar beating and the asymmetrical flagellar beating of hyperactivation. Hyperactivation is reversible (Suarez *et al*., 1987), so sperm can alternate between turning and swimming straight ahead. Mammalian sperm have been reported to turn towards, or accumulate in, a gradient of follicular fluid (Ralt *et al*., 1991, 1994; Cohen-Dayag *et al*., 1994, 1995; Fabro *et al*., 2002), which could accompany the oocyte into the Fallopian tube. Nevertheless, the chemotactic agent in follicular fluid has not been identified, nor has its presence in the Fallopian tube been detected. Odorant receptors unique to sperm have been localized to a spot on the base of the flagellum of human (Spehr *et al*., 2003), canine (Vanderhaeghen *et al*., 1993) and rat sperm (Walensky *et al*., 1995). Placing human sperm in a gradient of the odorant bourgeonal caused them to orient into the gradient and triggered a calcium and cAMP-mediated signalling cascade (Spehr *et al*., 2004). Nevertheless, a chemotactic odorant has yet to be identified in humans or other mammals. If one were found, it could have vast implications for the development of contraceptives, as well as assessment and treatment of infertility.

The fate of non-fertilizing sperm

After fertilization, any sperm remaining in the female reproductive tract may be phagocytosed by isthmic epithelial cells (Chakraborty and Nelson, 1975; Rasweiler, 1987) or may be eliminated into the peritoneal cavity (Mortimer and Templeton, 1982) where they are phagocytosed. Phagocytosis within the Fallopian tubes may be primarily employed by species, such as mice, which have an extensive ovarian bursa that would limit passage of sperm into the peritoneal cavity. In species where the passage of sperm into the peritoneal cavity is possible, this does not quickly render sperm non-functional as evidenced by the numerous case reports of human tubal pregnancies that arose in spite of lack of access of sperm from the uterus into the oviduct on the side of ovulation (Metz and Mastroianni, 1979; Brown *et al*., 1987; Ansari and Miller, 1994). In these cases, the only route available to the sperm was through the peritoneal cavity.

Lessons for assisted conception

Assisted conception in humans and other mammals relies on techniques to either assist sperm to reach the site of fertilization (by techniques of insemination) or generate embryos in the laboratory (by means of IVF) that can then be returned to the uterine cavity at a later time. Although all of these techniques are well established, they do not guarantee success. Understanding the biology of sperm transport and storage in the female reproductive tract could inspire technological improvements in assisted conception.

Simple insemination techniques, bypassing the vagina and cervix, have proved advantageous for artificial insemination in dairy cattle (*Bos taurus*). Whereas, a bull normally deposits several billion sperm into the vagina, artificial inseminators deposit 5–20 million frozen/thawed sperm directly into the body of the uterus leading to successful conception about 70% of the time (Foote and Kaproth, 1997; López-Gatius, 2000; Vishwanath, 2003). Similar techniques are used in human females where washed preparations of human sperm are inseminated directly into the uterine cavity or the Fallopian tube (Cantineau *et al*., 2004). Often this is in combination with ovarian stimulation of the female partner (Balasch, 2004). Although moderate success rates can be achieved with these techniques, it is unknown whether there are effects of sperm preparation or ovarian stimulation on how sperm behave or respond to the biology of the female reproductive tract before fertilization. Clearly, such information could be useful for those who are interested in the improvement of these techniques.

Similarly, there has been a long held assumption that, because fertilization can now be achieved *in vitro*, there must be little influence of the reproductive epithelium on the physiology of gametes and embryos. However, in IVF many thousand sperm are required to be incubated with oocytes in order to achieve fertilization (Elder and Dale, 2000), but evidence from most mammalian species suggest that only a few sperm are present at the site and time of fertilization in the Fallopian tube (reviewed by Eisenbach, 1999). This staggering difference almost certainly reflects our inability to adequately mimic *in vitro* the environment and sperm

selection mechanisms of the female reproductive tract during natural unassisted conception. Again, clearly if we knew more of the basic biology that underpins these events *in vivo* then there is the potential for improvements to be made to IVF procedures that could impact on the clinical outcome.

Finally, diagnosis of the causes of infertility could be greatly improved if more were known of the means by which sperm travel through the female reproductive tract and the mechanisms that regulate the movement of sperm.

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