NAME: OKOLO KOSISOCHUKWU ANN

COLLEGE: MEDICINE AND HEALTH SCIENCES

DEPARTMENT: NURSING SCIENCES

MATRIC NO: 18/MHS02/134

COURSE: PHYSIOLOGY (PHS212)

**ASSINGNMENT**

Discuss the factors facilitating the movement of sperm in the female reproductive tract.

**ANSWER**

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 allogeneic 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 so 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 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 (‘flow back’). They found that flow back occurred in 94% of copulations with the median time to the emergence of ‘flow back’ of 30 min (range 5–120 min). Furthermore they estimated that a median of 35% of spermatozoa were lost through flow back 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 enters cervical mucus and ascends 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).

## 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).

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 an 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.

Immunoglobulin’s IgG and IgA have been detected in human cervical mucus. Secretory IgA is produced locally by plasma cells in sub epithelial connective tissue. The amount secreted increases in the follicular phase but then decreases at about the time of ovulation (Kutteh et al., 1996). The immunoglobulin provides 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 center 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 was 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 107 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).

## Sperm transport through the uterus

At only a few centimeters 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 fimbria 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 pro-ovarian 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 per ovulatory 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.

## 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/sub mucosal 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 effect 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 signaling 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.