NAME: OKOR PRECIOUS EIKHOMUN

MATRIC NO: 17/MHS01/248

DEPARTMENT: PHARMACOLOGY

COURSE: PHS212

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

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). 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 (‘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.

## 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 onglycogen 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 . 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 a 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).

## 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 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 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.

## 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).

## 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; 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.