

NAME; YUSUF JEMIMAH SULE

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ASSIGNMENT

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

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. 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. 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. 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 cervix or shortly after coitus. Within minutes of vaginal deposition, human sperm begin to leave the seminal pool and swim into the cervical canal. 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. 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.

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. Ligation of the vesicular and coagulating glands of rats prevented the formation of plugs and the transport of sperm into the uterus. 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.

Male mice deficient for the gene encoding the protease inhibitor known as protease nexin-1 (PN-1) show a marked impairment in fertility. 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.

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 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. 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.

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.

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. By that time, however, thousands of sperm had already reached the Fallopian tubes .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. 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. The extent of hydration is correlated with penetrability to sperm. 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.

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.

The greatest barrier to sperm penetration of cervical mucus is at its border, because here the mucus microarchitecture is more compact. 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.

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. Neutrophils migrate readily through midcycle human cervical mucus. In rabbits, neutrophils were found to heavily infiltrate cervixes within a h of mating or artificial insemination. 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. 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. 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.

Complement proteins are also present in cervical mucus, along with regulators of complement activity. 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.

An elegant three-dimensional reconstruction of serial sections of the bovine cervix produced by Mullins and Saacke 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 found that when he flushed the cervixes 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. 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. 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, Sheehan and Carlstedt, Sheehan. It is thought that these long molecules become aligned by the secretory flow in mucosal grooves and thus serve to guide sperm. Human Chretien and bull Tampion and Gibons, 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.

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, and the presence of sperm in mid-cycle cervical mucus forms the basis of the 'post coital test' (PCT). Nevertheless, it is not known whether sperm collected from cervixes 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 and those sperm are greatly outnumbered by leukocytes. 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.

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. 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. 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. 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, 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. The uterine contractions occurring in women during the periovulatory period are limited to the layer of myometrium directly beneath the endometrium. 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.

In humans, contractile activity of uterine muscle may draw sperm and watery midcycle mucus from the cervix into the uterus. Fukuda and Fukuda 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 μ l Casslen, and cervical mucus is plentiful enough to fill the lumen.

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. 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. 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. Removal of the seminal vesicles significantly reduced the pregnancy rate in mice. 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-2 α .

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. The leukocytes are primarily neutrophils and have been observed phagocytizing uterine sperm in mice, rats and rabbits. 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.

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. The narrowness of the lumen is especially apparent in living tissue Suarez and in frozen sections, in which tissue does not shrink as it does during standard preparation of paraffin-embedded sections.

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. This arrangement of folds seems designed to entrap sperm and prevent further ascent.

Rapid sperm transport

Sperm have been recovered in the cranial reaches of the tubal ampulla only minutes after mating or insemination in humans and several other species of mammals. 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, 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.

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 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.

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 leukocyte.

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, by resecting the isthmus to bypass the reservoir or by administering progesterone into the muscularis to inhibit smooth muscle constriction of the lumen. 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*. In a variety of non-human species, this phenomenon has been seen *in vitro* or *in vivo*.

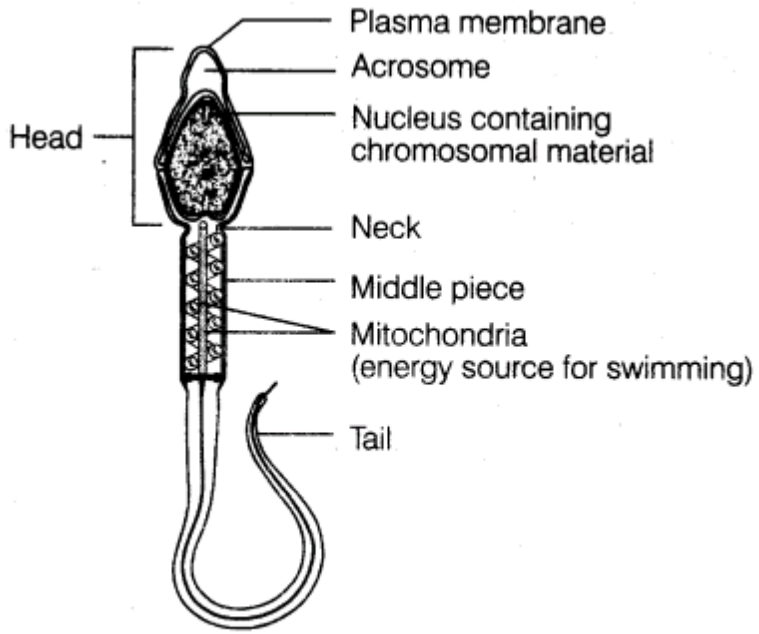
Scanning electron micrograph showing human sperm attached to a ciliated area of Fallopian tube epithelium *in vitro*. Arrows indicate sperm heads associated with cilia. Scale bar, 4 μm . Reproduced from Pacey.

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. Binding of stallion sperm to explants of endosalpinx was inhibited by asialofetuin and its terminal sugar, galactose, while binding of boar sperm was blocked by mannose. Bull sperm binding was blocked by fucoidan and its component fucose and pre-treatment of bovine epithelium with fucosidase reduced binding. 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.

It has not been established whether human sperm attach to the endosalpinx through a carbohydrate, although recent experiments by Reeve *et al.* 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, 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. PDC-109 is an approximately 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 Manjunal. Epididymal bull sperm bind endosalpingeal epithelium in very low numbers, but when they are coated with purified , their binding increases to the level of ejaculated bull sperm.



Structure of a sperm