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ASSIGNMENT

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

Sperm transport within the female reproductive tract is a cooperative effort between the functional properties of the sperm and seminal fluid on the one hand and cyclic adaptations of the female reproductive tract that facilitate the transport of sperm toward the ovulated egg. Much of the story of sperm transport in the female reproductive system involves the penetration by the sperm of various barriers along their way toward the egg (see Fig. 14.7B).

During coitus in the human, semen is deposited in the upper vagina close to the cervix. The normal environment of the vagina is inhospitable to the survival of sperm, principally because of its low pH (<5.0). The low pH of the vagina is a protective mechanism for the woman against many sexually transmitted pathogens, because no tissue barrier exists between the vagina (outside) and the peritoneal cavity (inside). The acidic pH of the vagina is bacteriocidal and is the reflection of an unusual functional adaptation of the vaginal epithelium. Alone among the stratified squamous epithelia in the body, the cells of the vaginal lining contain large amounts of glycogen. Anaerobic lactobacilli within the vagina break down the glycogen from shed vaginal epithelial cells, with the production of lactic acid as a byproduct. The lactic acid is responsible for the lowered vaginal pH.

Direct measurements have shown that within 8 seconds from the introduction of semen the pH of the upper vagina is raised from 4.3 to 7.2, creating an environment favorable for sperm motility. Another rapid event is the coagulation of human semen through the actions of semogelin by a minute after coitus. The coagulative function is incompletely understood, but it may play a role in keeping sperm near the cervical os. Thirty to 60 minutes after it coagulates, prostate-specific antigen (PSA), a proteolytic enzyme, degrades the coagulated semen. Within the semen and altered vaginal fluids, the sperm have begun to swim actively. A critical element in sperm motility is the availability of fructose, a nutrient provided by the seminal vesicles, within the semen. Because of their paucity of cytoplasm, spermatozoa require an external energy source. Unusually for most cells, spermatozoa have a specific requirement for fructose rather than glucose, the more commonly utilized carbohydrate energy source.

The next barrier facing sperm is the cervix. The cervical entrance (os) is not only very small, but it is blocked by cervical mucus. During most times in the menstrual cycle, cervical mucus is highly sticky (G mucus) and represents an almost impenetrable barrier to sperm penetration. Around the time of ovulation, however, the estrogenic environment of the female reproductive system brings about a change in cervical mucus, rendering it more watery and more amenable to penetration by sperm (E mucus). Considerable uncertainty surrounds the question of passage of sperm through the cervix. The swimming speed of human sperm in fluid is approximately 5 mm/min, so in theory, sperm could swim through the cervical canal in a matter of minutes or hours. In reality, some sperm have been found in the upper reaches of the uterine tubes within minutes of coitus. These pioneers are likely to have been swept up the female reproductive tract during muscular contractions occurring at the time of or shortly after coitus. Research on rabbits has indicated that most of these sperm have been damaged and would not be able to fertilize an egg. The functional status of early-arriving human sperm is not known. On the other end of the spectrum, viable sperm have been taken from the cervix as long as 5 days after coitus. Between these two extremes, over the course of hours or even days, most of the spermatozoa make their way through the cervical mucus and up the cervical canal and into the uterus, where even less is known about the course of sperm transport in the human. Whether or not sperm are stored in the cervix is still not entirely certain. Sperm transport into and through the uterus is assumed to be assisted by contractions of its thick smooth muscle walls. There may or may not be subtle influences that favor the transport of sperm toward the opening of the uterine tube that contains the ovulated egg.

Of the huge numbers of sperm that enter the female reproductive tract, almost all fail to reach the uterine tubes. The unsuccessful sperm are removed by the infiltration of white blood cells into the cavities of the vagina, cervix, and uterus. These cells, along with certain immunoglobulins, inactivate and degrade foreign invaders, in this case, the excess sperm. Fortunately, the uterine tubes are not subject to this sort of cellular infiltration.

The openings of the uterine tubes into the uterus (uterotubal junction) represent another barrier to sperm transport. With two uterine tubes and usually only one ovulated egg, any spermatozoon that enters the empty uterine tube is automatically doomed to reproductive failure. Roughly 10,000 or fewer sperm cells of the millions in the ejaculate enter the correct tube. These sperm cells collect in the lower part of the uterine tube and attach to the epithelium of the tube for about 24 hours.

Two critical events occur during this period of attachment. The first is called capacitation, a reaction necessary for a spermatozoon to be able to fertilize an egg. The first phase of the capacitation reaction is the removal of cholesterol from the surface of the sperm. Cholesterol was introduced onto the sperm head to prevent premature capacitation. The next phase of capacitation is the removal of many of the glycoproteins that were deposited on the sperm head within the epididymis. After their removal, the spermatozoon is now capable of fertilizing an egg. It is likely that covering the sperm cells with glycoproteins and then cholesterol is done to prevent the sperm from prematurely attempting to fertilize other somatic cells that they encounter on their way to meeting the egg. Capacitation removes the molecular shield.

A second phenomenon occurring while the sperm are attached to the distal tubal lining is hyperactivation of the sperm. Hyperactivation is manifest by the increased vigor in their swimming movements and allows the sperm to break free from their binding with the tubal epithelial cells. Hyperactivated sperm are more efficient in making their way up the uterine tube and penetrating the coverings of the egg.

Once capacitated sperm break away from the tubal epithelium, they make their way up the uterine tube through a combination of their own swimming movements, peristaltic contractions of the smooth musculature of the tubal wall and the movement of tubal fluids directed by ciliary activity. In the upper third of the uterine tube, a few hundred sperm approach the ovulated egg. Only one of them out of the millions that left the male reproductive tract will attain is ultimate goal of fertilizing that egg.

Sperm Transport

Sperm transport occurs in both the male reproductive tract and the female reproductive tract. In the male reproductive tract, transport of spermatozoa is closely connected with their structural and functional maturation, whereas in the female reproductive tract, it is important for spermatozoa to pass to the upper uterine tube, where they can meet the ovulated egg.

After spermiogenesis in the seminiferous tubules, the spermatozoa are morphologically mature but are nonmotile and incapable of fertilizing an egg (Figure 2). Spermatozoa are passively transported via testicular fluid from the seminiferous tubules to the caput (head) of the epididymis through the rete testis and the efferent ductules. They are propelled by fluid pressure generated in the seminiferous tubules and are assisted by smooth muscle contractions and ciliary currents in the efferent ductules. Spermatozoa spend about 12 days in the highly convoluted duct of the epididymis, which measures 6 m in the human, during which time they undergo biochemical maturation. This period of maturation is associated with changes in the glycoproteins in the plasma membrane of the sperm head. By the time the spermatozoa have reached the cauda (tail) of the epididymis, they are capable of fertilizing an egg.

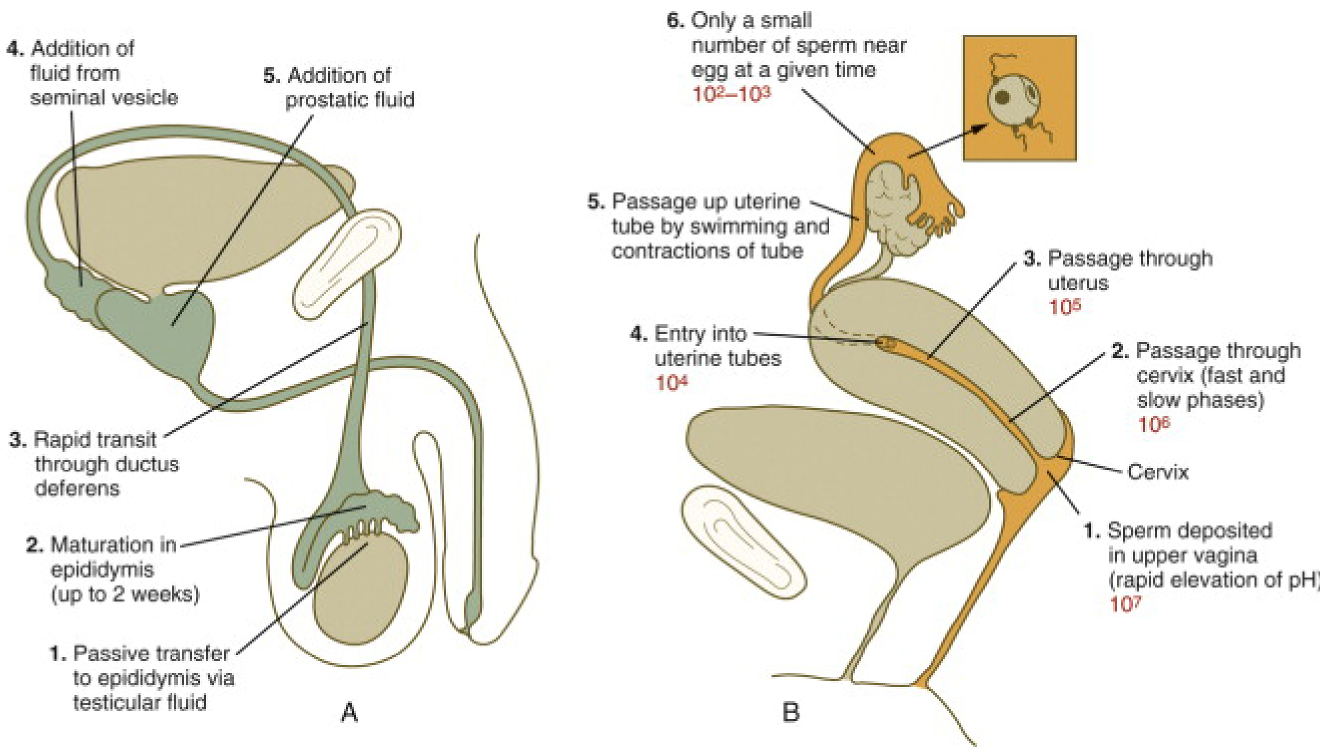

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Figure 2. Sperm transport in (a) the male and (b) the female reproductive tracts. In (b), numbers of spermatozoa typically found in various parts of the female reproductive tract are indicated in red.

On ejaculation, the spermatozoa rapidly pass through the ductus deferens and become mixed with fluid secretions from the seminal vesiclesand prostate gland. Prostatic fluid is rich in citric acid, acid phosphatase, zinc, and magnesium ions, whereas fluid of the seminal vesicle is rich in fructose (the principal energy source of spermatozoa) and prostaglandins. The 2–6 ml of ejaculate (semen, or seminal fluid) typically consists of 40–250 million spermatozoa mixed with alkaline fluid from the seminal vesicles (60% of the total) and acid secretion (pH 6.5) from the prostate (30% of the total). The pH of normal semen ranges from 7.2 to 7.8.

In the female reproductive tract, sperm transport begins in the upper vagina and ends in the ampulla of the uterine tube, where the spermatozoa make contact with the ovulated egg. During copulation, the seminal fluid is normally deposited in the upper vagina, where its composition and buffering capacity immediately protect the spermatozoa from the acid fluid found in the upper vaginal area. The acidic vaginal fluid normally serves a bactericidal function in protecting the cervical canal from pathogenic organisms. Within about 10 seconds, the pH of the upper vagina is increased from 4.3 to as much as 7.2. The buffering effect lasts only a few minutes in humans, but it provides enough time for the spermatozoa to approach the cervix in an environment (pH 6.0–6.5) optimal for sperm motility.

The next barriers that the sperm cells must overcome are the cervical canal and the cervical mucus that blocks it. Changes in intravaginal pressure may suck spermatozoa into the cervical os, but swimming movements also seem to be important for most spermatozoa in penetrating the cervical mucus.

The composition and viscosity of cervical mucus vary considerably throughout the menstrual cycle. Composed of cervical mucin (a glycoprotein with a high carbohydrate composition) and soluble components, cervical mucus is not readily penetrable. Between days 9 and 16 of the cycle, however, its water content increases, and this change facilitates the passage of sperm through the cervix around the time of ovulation; such mucus is sometimes called E mucus. After ovulation, under the influence of progesterone, the production of watery cervical mucus ceases, and a new type of sticky mucus, which has a much decreased water content, is produced. This progestational mucus, sometimes called G mucus, is almost completely resistant to sperm penetration.

There are two main modes of sperm transport through the cervix. One is a phase of initial rapid transport, by which some spermatozoa can reach the uterine tubes within 5–20 min of ejaculation. Such rapid transport relies more on muscular movements of the female reproductive tract than on the motility of the spermatozoa themselves. These early-arriving sperm, however, appear not to be as capable of fertilizing an egg as do those that have spent more time in the female reproductive tract. The second, slow phase of sperm transport involves the swimming of spermatozoa through the cervical mucus (traveling at a rate of 2–3 mm h^{-1}), their storage in cervical crypts, and their final passage through the cervical canal as much as 2–4 days later.

Relatively little is known about the passage of spermatozoa through the uterine cavity, but the contraction of uterine smooth muscle, rather than sperm motility, seems to be the main intrauterine transport mechanism. At this point, the spermatozoa enter one of the uterine tubes. According to some more recent estimates, only several hundred spermatozoa enter the uterine tubes, and most enter the tube containing the ovulated egg.

Once inside the uterine tube, the spermatozoa collect in the isthmus and bind to the epithelium for about 24 h. During this time, they are influenced by secretions of the tube to undergo the capacitation reaction. One phase of capacitation is the removal of cholesterol from the surface of the sperm. Cholesterol is a component of semen and acts to inhibit premature capacitation. The next phase of capacitation consists of removal of many of the glycoproteins that were deposited on the surface of the spermatozoa during their tenure in the epididymis. Capacitation is required for spermatozoa to be able to fertilize an egg (specifically, to undergo the acrosome reaction). After the capacitation reaction, the spermatozoa undergo a period of hyperactivity and detach from the tubal epithelium. Hyperactivation helps the spermatozoa to break free of the bonds that held them to the tubal epithelium. It also assists the sperm in penetrating isthmic mucus, as well as the corona radiata and the

zona pellucida, which surround the ovum. Only small numbers of sperm are released at a given time.
On their release from the isthmus, the spermatozoa make their way up the tube through a combination of muscular movements of the tube and some swimming movements. The simultaneous transport of an egg down and spermatozoa up the tube is currently explained on the basis of peristaltic contractions of the uterine tube muscles. These contractions subdivide the tube into compartments. Within a given compartment, the gametes are caught up in churning movements that over 1 or 2 days bring the egg and spermatozoa together. Fertilization of the egg normally occurs in the ampullary portion (upper third) of the uterine tube. Estimates suggest that spermatozoa retain their function in the female reproductive tract for about 80 h.

Preparation of Recipient Mares

During OT, sperm transport, capacitation, fertilization, and embryo development occur within the recipient's reproductive tract; therefore, it is very important that a selection of good quality recipient mares be used in an OT program. Young mares (3 to 10 years) are selected after a complete clinical and reproductive examination. During the reproductive examination, it is important to evaluate the length of the broad ligaments to determine if the ovaries can be easily exposed during OT. Oocyte recipients can be cyclic or non-cyclic mares. Use of cyclic mares as oocyte recipients involves estrous cyclesynchronization of donor and recipient mares and the removal of the recipient's oocytes to be sure that the pregnancy will result from fertilization of the donor oocyte. Recipient mares receive 2000 IU of hCG at the same time as the donors, and the recipient's oocyte is collected approximately 24 hours after hCG administration. Only recipient mares from which an oocyte is collected are used as oocyte recipients. Use of non-cyclic recipients eliminates the need to synchronize donors and recipients and eliminates the need to retrieve the pre-ovulatory oocytes from the recipients before transfers. Non-cyclic recipients receive 3 mg of estradiol benzoate daily for approximately 2 to 5 days before transfer. Following the estradiol treatment 200 mg per day of injectable progesterone in oil or 0.044 mg/kg of oral progestagen(Altrenogest), supplementation is required until OT. Regardless of whether the mares are cycling or not, progesterone supplementation must be continued for pregnancy maintenance until day 110 to 120. Although a corpus luteum forms after aspiration of the preovulatory follicle,[65] progesterone secretion can be delayed or reduced in cyclic mares. In non-cyclic mares, the absence of corpus luteum obviously requires progesterone supplementation.
Oocytes are transferred into the oviduct of the recipient mares preferably by standing flank laparotomy. After sedation and local anesthesia, an incision is made between the last rib and the tuber coxae. Prior to OT the ovary and oviduct are exposed through the incision. The oocyte is loaded into a fire-polished glass pipette with a low volume of medium (<0.1 ml). The pipette is introduced approximately 3 cm into the infundibular

end of the oviduct and the oocyte is gently deposited (Fig. 18-4). The ovary is returned into the abdominal cavity, and the muscle layers and skin are sutured separately. Recipients are routinely treated with parenteral non-steroidal anti-inflammatory drugs and broad spectrum antibiotic for 5 to 7 days after surgery.