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Micro-architecture of the Female Reproductive System

Arbab Sikandar and Muhammad Ali

Abstract

The female reproductive system consists of the ovary, oviduct, uterus, and vagina. This chapter will discuss how these organs look like under the microscope and what types of ultrastructural tissues are present in it, how the shape and physiology of the tissues/cells change with the physiological activities including reproductive cycles, what type of alterations occurs in the ovary during ovulation and how its follicle and epithelium differ, and how the ovulation takes place. The chapter will also elaborate how the lining epithelium and the tract mucosa facilitate the fertilized ovum and conceptus. Also, the chapter is highlighting the architectural changes within the mucosa of the uterus during and after pregnancy and type of ovary and spermatozoa that is most suitable for fertilization.

Keywords: ovary, reproduction, microstructures, cortex, mucosa, zygote, sperm

1. Introduction

It is important to know the microstructure of the female reproductive system. In this chapter those microarchitectures are highlighted which played an important role in theriogenology right from release to fertilization and care of embryo and infants. The micro-architecture of the ovarian cortex, the microscopic structures of the follicles especially the graafian follicle including mature ova, and the histomorphology of the fimbriae and oviduct and its function in ova transportation, zygote transformation, and embryo implantation were highlighted. The microstructures of various microscopic layers of the uterus and its role in reproduction were addressed. The structure and function of the cervix and vulva and its role in reproduction are mentioned. Also, the most suitable type of ova and sperm for fertilization was also mentioned. The whole reproductive system is concerned with the production and transport of the ovum, facilitation in the transport of spermatozoa, the fusion of both gametes, and the accommodation of the embryo and fetus until birth [1].

2. Microstructure of cortex of the ovary

The cortex is a wide peripheral part of the ovary that contains follicles in various stages [2]. The surface of the ovary is covered by a thin layer of cuboidal cells, also called as germinal epithelium. Next to the germinal epithelium in the inferior side is a thick layer of connective tissue (CT) called the tunica albuginea. And the

remaining portion of the ovarian cortex is covered by the CT which contains the primordial follicles, surrounded by a flat follicular cell (squamous shaped). Another follicle a bit larger is called a primary follicle, internally lined by the simple and stratified cuboidal epithelium termed as granulosa cells. In the connective tissue of the cortex of the ovary, many blood vessels surround the developing follicles. The granulosa cells are more prominent in the larger follicle called as secondary follicle or also called as antral follicle [3]. The antrum is the cavity formation within the follicle, which splits the granulosa cells into two layers. The fluid within the antrum is called as liquor folliculi. A single large ovum is present in the follicle, and a single layer of granulosa cells which surrounds the oocyte is known as corona radiata [4]. There are acellular glycoprotein layers known as zona pellucida between the corona radiata and oocyte. The zona pellucida which is 3–5 μm in thickness is produced by the ova and the granulosa cells. Within the antrum the ova are anchored by a compact mass of cells called as cumulus oophorus. After releasing the ova, the granulosa cells are disorganized within the antrum with pyknotic nuclei (fragmentation of nuclei) in the remaining follicles. This condition of the inactive follicles is called as atretic follicle [5].

3. Microstructure modulation in graafian follicle

A graafian follicle consists of a large cavity called antrum having fluid termed as follicular fluid. Graafian follicle is also known as antral follicle [3]. Before ovulation, secondary follicle undergoes first mitotic division, and as a result the graafian follicle is formed which has two N haploid chromosomes. The characteristic structural feature of graafian follicle is follicular antrum in which granulosa cell and oocyte are present. Antral follicle is a three-dimensional structure having a cavity (antrum) which is surrounded by different types of cells [6]. The antral follicle has six different histologic components which are as follows:

- The theca externa
- The theca interna
- The basal lamina
- The granulosa cell
- The oocyte
- The follicular fluid

3.1 Theca externa

Theca externa consists of smooth muscle cells that are innervated by the autonomic nerve. The functional importance of theca externa is still unclear, but there is evidence that changes in the contractile activity of theca externa are involved in atresia and ovulation [5].

3.2 Theca interna

It is consisting of different cells which are present in the matrix of loose connective tissues and blood vessels. The function of theca interna cells is regulated by

luteinizing hormone (LH). Theca interna is highly vascularized, and its function is to deliver hormone, nutrients, vitamins, and cofactors, which are necessary for growth of the oocyte and granulosa cells [4].

3.3 Granulosa cells and oocyte

In the graafian follicle, the granulosa cells and oocyte have a specific shape and position [6]. There are four different types of granulosa cell layers:

- The outermost is membrana granulosa.
- The inner most is periantral.
- The intermediate is cumulus oophorus.
- Just opposed to oocyte is corona radiata.

3.4 Follicular fluid

It is rich in hyaluronan and proteoglycans. It helps in follicle ruptures. Ovulation occurs under the influence of follicle-stimulating hormone (FSH) and LH [7]. During ovulation the ova are in the metaphase stage of the second meiotic division.

4. Microstructure and physiology of the oviduct and how it facilitates the sperm, ovum, and early-stage embryo

4.1 Microstructure of the oviduct

The oviduct has several layers including the mucosa, muscular layer, and a connective serosa. The size of these layers depends on different regions of the oviduct. The myosalpinx is comprised of smooth muscle; it is thin in the region of the ampulla and multilayered thick in the region of the isthmus. The endosalpinx is a term used for a mucosa, which is lined with simple columnar ciliated epithelium [8]. In the ampulla, the lining ciliated cells are numerous compared with that of secretory cells. The lining mucosa forms folds upward that fill the tubular lumen [9]. Due to the abundance of upward-directed folds, the lining mucosa of the ampulla increased the surface area, assuming excessive ability for the metabolic conversation between the epithelial, luminal, and the underlining vascular compartments. The mucosa of the isthmus has a smaller number of folds, while the abundant number of secretory cells is present there.

4.2 Function of the oviduct

Sperm and oocyte will not reach the oviduct at the same time. In some animals the oocyte reaches first, and, in some, sperm approaches initially to the site of fertilization. The movement of sperm toward the isthmus is promoted by the ciliary movement of the lining epithelium. The spermatozoa are stored and remain active in the oviduct for up to 72 hours, but in some species the time is extended for a month like in bats. The fallopian tube contains fluid secreted by the glandular epithelium, which has a positive impact on the viability of sperm and provides nutrition to the oocyte [8]. This fluid helps in the maturation of the oocyte in the oviduct. Sperm adhere to the epithelial wall of the fallopian tube which preserves

its viability and stops the premature capacitation. This enhances the chances of fertilization [1].

Fertilization of the ovum results in the formation of zygotes. After all the embryonic changes in the zygote, the later embryo is transferred along the fallopian tube. Some hormone also helps in the transportation of the embryo. The production of the estradiol (E2) by the embryo also helps in its transportation. In the mare, the secretion of prostaglandin (PGE2) during the first week acts on the oviduct locally and causes the transfer of the embryo to the posterior region of the uterus. The 4- to 8-cell stage embryos in hamster secrete platelet-activating factor (PAF). The later factor also acts on the oviduct locally, resulting in the transfer of the embryo toward the uterine horns. It is important to note that in the hamster and the mare, the main factor, decisive whether and when the fertilized ovum will drive toward the uterine horns or not, is based on the condition of the fertilized eggs.

4.3 Transportation of oocyte

During ovulation the fimbriae attached to the surface of graafian follicle and sweep the ova further anterior toward the ampulla [8]. The coordinated contraction of the myosalpinx along the length of the fallopian tube and the ciliary beats of the epithelial cells results in the transportation of the oocyte to the site of fertilization [9].

5. Microstructures and physiology of fimbriae

Fimbriae are finger-like growth processes that are present at the terminal of each fallopian tube. They increase the surface area of the infundibulum and cause it to slip over the surface of the ovary when ovulation occurs. This activity of the fimbriae increases the possibility that the oozed-out oocyte will be captured after ovulation from the graafian follicle and transported through a wider opening called ostium into the ampulla of the oviduct [9]. Above the mucus membrane of the fallopian tube, there are three layers of tissues, viz., the innermost layer consists of spirally arranged fibers, the middle layer consists of fibers with circular arrangement, and the outermost sheath has longitudinally arranged fibers that end in fimbriae near ovaries, forming a funnel-shaped depository structure called the infundibulum. One fimbria out of all fimbriae is long enough to reach the external surface of the ovary, which is called as fimbria ovarica [10]. The fallopian tube at this point is lined by small epithelial cells with small, slender hair-like cilia pulsating unidirectionally inside the fallopian tube to guide and direct the ovum from the ovary to the uterus [9].

5.1 Role of fimbriae in directing the ova to area of fertilization

As there is no direct connection between ovaries and the oviduct, the egg is transported to the uterus in peritoneal fluid produced by fimbriae on the terminal of tube's opening. The specialized granulosa cells of oocyte (cumulus oophorous) at ovulation are sticky and help in adhering to the surface of fimbriae. After this the fimbriae through muscular control create negative pressure that picks up oocyte and moves it to the fallopian tube [9]. There are also some hormonal changes that control the picking up of oocyte [7].

5.2 Process of fertilization within the fallopian tube

At the site of ampulla-isthmus junction, the capacitated and hyperactive spermatozoa crossed the layer of corona radiata and pierced the glycoprotein layer of zona pellucida. Afterward the fusion of the spermatozoa with the ova takes place [11], which results in the formation of zygote.

6. Histological layers, physiology of the uterus, and its role in reproduction

If we see the cross section of the uterus, it consists of four layers including the innermost mucosa, the submucosa, the muscularis, and the outermost serosa [9].

6.1 Mucosa

It is the innermost layer facing the lumen. It is lined with secretory layers of columnar epithelium. The embryo after a series of changes including zygote, two-cell (blastomeres) stage, morula (16-cell stage), blastula (inner cell mass leads to embryo, and the outer cell mass leads to the formation of the placenta, with the formation of fluid filled cavity), and gastrula (where formation of three characteristic embryonic germ layers occurs) is implanted in the endometrial mucosa with a layer of trophoblast [1]. The trophoblastic cell layers are proliferated in the lamina propria (uterine stroma) forming lacunae. The lacunae form an open connection with the maternal sinusoid in the uterine stroma, which results in the establishment of the uteroplacental circulation. The endometrial glands develop from the mucosa and invaginate inward into the submucosa adopting a coiled shape. The endometrial glands secrete PGF2 alpha at a critical time during estrous cycle that causes luteolysis of corpus luteum if the animal is not pregnant [12]. The endometrium of ruminants has caruncles that are highly vascularized and provide a maternal portion of the placenta if attachment of the embryo occurs. While these caruncles are absent in sow and mare, instead endometrial folds are present. These endometrial folds provide uterine surface for the attachment of the placenta. So, the uterus is the place where attachment and nourishment of the fertilized ova take place, and it helps in pushing the baby during parturition [8].

6.2 Submucosa

After the mucosa, a layer of submucosa is present. It houses a rich blood supply, nerve endings, and the lymphatics in addition to the rich supply of mononuclear cells. It has a role in providing nutrition and maintaining coordination and has supporting effect to the mucosa [9]. The muscularis is present beneath the submucosa and consists of double arranged layers of smooth muscles like longitudinal muscles (outer layer) and inner circular muscles (inner layer). A very prominent third layer of smooth muscle in oblique arrangement can also be seen in the gravid uterus. These layers played a very important function by providing the uterus with the ability to contract, and that contraction is very important on the following basis:

- Transportation of the secretory products
- Transportation of the gametes (sperm and ova)

- Movement of early embryo to the appropriate location
- Maintaining and nourishment of the embryo and fetus

It also has a key role in the expulsion of the fetus and fetal membranes at the time of parturition.

6.3 Serosa

It is the outer covering of the uterus that consists of a single layer of squamous cells.

7. Gross and microscopic structures and functions of the cervix

The whole reproductive tract is tubular except the ovary. The cervix is a thick-walled tubular structure where mucosa-submucosa-derived folds or rings are present, but their number varies from species to species. Only onefold is seen in bitch and queen, but multiple folds are seen in cow, ewe, sow, and mare. In cow and ewe, rings/folds are having interlocking finger-like projections. In pigs, rings interdigitate so interdigitation is seen in them. The cervix is soft in mare due to the presence of loose folds of the mucosa. In a bitch the cervix is smooth due to lack of folds. The primary function of the cervix is lubrication of the canal which is required during copulation [8]. Lubrication is by two types of secretions, e.g., the sulfomucin which is viscous (mobility is directed toward exterior (vagina) and inhibits sperm transport in the uterus), and the sialomucins which facilitate the sperm to move into the uterus. The cervix isolates the conceptus inside the uterus from the outer environment by the formation of cervical seal under the action of progesterone which thickens the mucus produced in the cervix and glues the folding together to prevent entry of microbes during pregnancy.

Like other tubular organs, it consists of four tunics/laminae, i.e., mucosa, submucosa, muscularis, and serosa/adventitia [9]. The mucosal tunics of the endocervix are lined by simple columnar epithelium along with the presence of mucus-producing cells called goblet cells. In some animal species, a few columnar cells are found ciliated and in simple tubular glands may be seen in the ruminant's mucosa. In the case of pigs, up to 90% of the mucosal lining epithelium is stratified squamous. Most of the ectocervical portion of the cervical mucosa is lined by the stratified squamous epithelium. Mucosa and the submucosa form folds into the cervical lumen. These folds vary in height, width, and thickness from species to species. The lamina propria of the mucosa and submucosa combines to form propria-submucosa. This propria-submucosa is heavily infiltrated by dense irregular connective tissue and supply of rich blood vessels extended deep in the submucosa. The muscularis layer is composed of two smooth muscle layers. The inner layer is circular layer and the outer layer is longitudinal in arrangements. The myenteric plexus is present in the muscular layers. Some elastic fibers are also observed in this layer. Serosa/adventitia is a loose connective tissue layer present in the outer surface. This layer is surrounded by the mesothelium which is a layer of simple squamous epithelium. Gartner's ducts may be seen in serosal layer unilateral or bilateral.

7.1 Functions of the cervix

During estrus stage of estrous cycle, the mucosa is responsible for mucus secretion. Dense irregular connective tissue becomes edematous and forms areolar structure, i.e., loose connective tissue. Circular muscular layer along with elastic fiber is responsible for the involution of the cervix after parturition [8].

8. Gross and microscopic anatomy of the vulva and its role in reproduction

The vulva is the most outer part of the female reproductive track immediately external to the vaginae. It is composed of various structures, including the mons pubis, clitoris, labia minora, labia majora, vulvar vestibule, vestibulovaginal bulbs, urethral meatus, hymen, and Bartholin and Skene glands and ducts. Bartholin glands are like the bulbourethral glands in male. The female clitoris is like the penis of the males [13]. The hymen is the stratified squamous nonkeratinized epithelium. The lateral vulvar surfaces are made up of labia majora and are comprised of fibrous and adipose folds. The labia minora also consists of connective tissue in two folds containing few (if any) adipose tissues. The labia minora is bifurcated anteriorly. The vestibule is the zone between Hart's line and the hymen, which consists of stratified squamous nonkeratinized epithelium [9]. The following are the glands present in the vulvar region of the female reproductive system:

- Apocrine glands (scent glands)
- Skene glands
- Sebaceous glands
- Eccrine glands (sweat glands)

8.1 Microscopic anatomy of vulva

Vaginal lining epithelium is stratified squamous and nonkeratinized, whereas the epithelium of the labia majora is stratified squamous and keratinized. Microscopic layers of the vulva are the mucosa, submucosa, muscularis, and serosa [14, 15]. Glands are present in the submucosal layer and the innermost layer is the mucosa.

8.2 Role of the vulva in reproduction

Being a gateway to the uterus, its primary role is to offer protection by closing the labia [16]. The external uterine orifice is protected by the vulva supported by thick large lips of labia majora along with small lips of labia minora. The urethra also opens in the vulva that is known as the urethral meatus, thus performing a function of urine passage. It also gives the pathway for sperm for entry in the body. Sometimes due to the folds and the moisture, the fungal infection may occur. The area between the opening of the vagina and the anus, below the labia majora, is called the perineum [12]. By observing the vulva, we can detect if the female is in estrous or not. If the vulva is thick and edematous, it is in the heat, and if not the female is not in the heat; this is due to the estrogen level in the female body. The penis like clitoris detects the nerve stimuli and performs the following functions:

- Enabling sperm to enter in the body of female
- Protecting the internal genital organs from infection causing organisms
- Providing the sexual pleasure

9. Type of sperm which is most favorable for fertilization

As we know around 250 million sperm cells enter the female external genitalia, but just a few thousands can enter the fallopian tube, and only a single sperm will fertilize the ova [14]. Several problems and barriers came in the pathway of the spermatozoa to touch the final goal. These range from the low pH in and around the vagina, the mucus of the cervix, the narrowness of the uterotubal junction (the entrance of the cervix), the WBCs of the immune system which treat the spermatozoa as a foreign entity to destroy, cell-to-cell interactions, gene expression, phenotypic sperm traits, sperm motility defects, DNA status, lack of capacitation or morphological normality, and failure of abnormal spermatozoa to reach to the site of fertilization. The wall of seminiferous tubules which are the coiled structure is responsible to produce the sperm. For a spermatozoon, around 28–42 days is lapsed to cross the male reproductive system. In the female reproductive tract, sperm undergo changes that help in fertilization called activation and capacitation, but all sperm are not capacitated at the same time; therefore, all sperm are not able to fertilize the cells [16]. However, several mechanisms that aid this process are good motility, adequate morphology, and normal DNA status of the cell. A sperm which has normal head, nucleus, and tail and has a moderate motility is considerable to fertilize the egg [17]. The sperm are guided upon their journey by the chemical and the temperature signals. A sperm reservoir in the fallopian tubes, where sperms bind to the epithelial lining (columnar in shape) of the tubes, reduces the chance of fertilization by multiple sperm. During ovulation, the sperm are hyperactivated to help them to the penetration of the mucus in the fallopian tubes and the outer coating of the egg. Sperm outer membrane fuses with egg outer membrane to facilitate fertilization. Acrosomal reaction occurs in the sperm head once the spermatozoa reached the ova. Acrosin enzyme helps in acrosomal reaction. Hyaluronic acid enzyme has an important role in the permeability and motility of sperms and their interactions with gametes. Formation of a functional reservoir through epithelial binding of sperm to the oviductal isthmus reduces the likelihood of the polyspermic fertilization. Motility or hyperactivation helps sperm in penetrating the mucus in the fallopian tubes and the cumulus oophorus (corona radiata), and the acrosome exocytosis may assist penetration of oocyte zona pellucida that proceeds the fusion within oocyte plasma membrane. Zona pellucida then undergoes biochemical changes so that further sperm cannot penetrate the cell called zona block [17]. In addition to alteration of zona pellucida, the cortical reaction reduces the ability of oocyte plasma membrane to fuse with additional spermatozoa, thus causing vitelline block. Both zona block and vitelline block prevent polyspermy.

10. The type of ova that is most favorable for fertilization

Fertilization is a process in which male and female gametes fuse to form a zygote. Ovum is the female gamete, and its selection is the criteria of fertilization. There are many factors that are responsible for its selection including follicular dominance, cumulus oophorus formation, cumulus oocyte complex formation, fimbrial supportive structure, and zona pellucidal layer that enable sperm to fuse with ovum.

Follicular dominance is based on E2 production. The follicle, which has greater ability of E2 production, has greater capability of its dominance. Dominancy is responsible for onward ovulation. Ovulated egg is surrounded by two protective layers [9]. Corona radiata is the outermost layer containing granulosa cells, and

zona pellucida is the innermost thick transparent glycoprotein membranes surrounding the plasma membranes. With a maturation of oocyte, multiple granulosa cells enlarge called cumulus oophorus [3]. This oocyte and granulosa cells form complex known as cumulus oocyte complex (COC). The cumulus of mature COC adheres to the surface of fimbriae as sticky substances. After the sex hormones signal the fimbriae, it contracts and releases oocyte. After release, oocyte has only 24–28 hours to fuse with sperm; otherwise, it is lost. Oocyte releases some chemicals that attract the sperm, and the sperm tries to penetrate two protective layers of oocyte. First it burrows to the cell of corona radiata and then move toward zona pellucida layer. Zona pellucida is composed of four glycoproteins ZP1, ZP2, ZP3, and ZP4. The primary ligand of sperm oocyte binding is ZP3 and ZP4. Both induce acrosomal reaction of sperm to complete fusion reaction and form a zygote [18]. These factors make the ovum gamete most favorable for fertilization.

11. Conclusion

The microarchitectural examination of the reproductive organs at microscopic level is very much important to recognize its structure and function during reproductive physiology. Furthermore, this chapter highlighted the dealing of the lining mucosa with the fertilized ovum and conceptus.

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