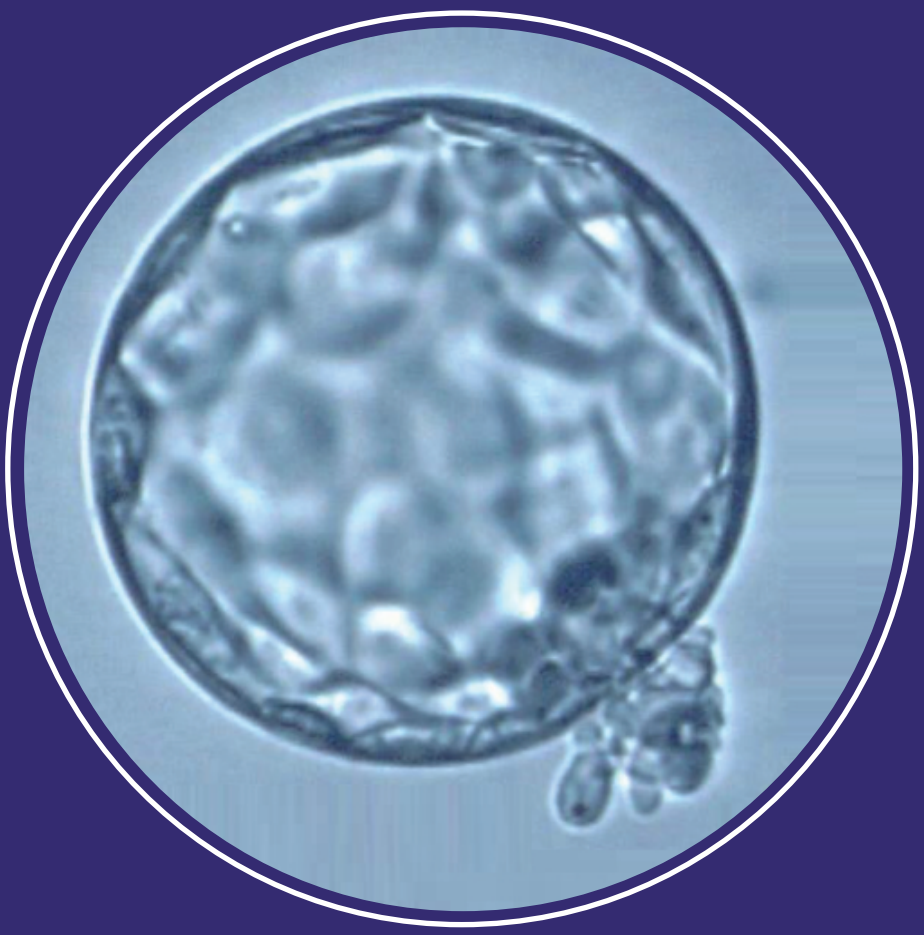
Evaluation of the Effect of Fresh Embryo and Frozen Embryo Transferred on Pregnancy in Infertile Patients

Assoc. Prof. Dr. Cenap EKİNCİ **Lecturer İlhan ÖZDEMİR**



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PREFACE

Infertility is a condition that cannot become pregnant despite regular sexual intercourse for more than 12 months. Infertility is important in all aspects of the world in biological, psychological, economic, ethical, moral, legal and social aspects, as well as for women and men. Assisted reproductive techniques (ART) have made significant technological progress over the last 30 years. One of these steps is the process of freezing the embryos obtained as a result of ovarian stimulation without transfer and transferring it in the following cycles. In the in vitro fertilization process, conventionally ovarian stimulation, egg collection and then purification of the eggs from the cumulus cells after the maturation of the mature eggs are completed and the mature sperm sperm morphology is very good and high quality sperm are selected and processed. At the end of the procedure, fertilization formation, embryo development and quality evaluation, in the last stage, transfer of high quality fresh embryo is achieved and very good pregnancies are obtained. However, in addition to the efficacy and safety of treatment, other approaches have begun to be questioned. One of these new approaches is the process of freezing developing embryos before transferring and resolving and re-transferring them in subsequent cycles. This approach, which has been criticized in terms of pregnancy in the previous periods and which is prejudiced, is now being applied as an alternative treatment option. In this study, we evaluated the effect of embryo transfer on pregnancy rates between fresh and frozen embryos. We urge that the information compiled from current studies will contribute to IVF and the clinic. We would like to thank our esteemed professionals and scientists for their contributions.

Regards.

İÇİNDEKİLER

PREFACE……i

INTRODUCTION……..7

Genital System……7

Female Genital System……7

General Structure of Egg Cell (Ovum)……... 26

Male Genital System……... 30

Infertility……47

Infertility Assessment in Women……52

Evaluation of Ovulation…….. 57

Evaluatıon of Over Reserve…… 61

Special Sperm Analysis Tests……70

Unexplained Infertility……73

Comparison of Obstetric and Neolatal Outcomes of Fresh and Frozen Embryo Transfer Cyclics……77

Comparison of Pregnancy Rates of Fresh Frozen Embryo Transfer……... 79

CONCLUSION…….. 81

REFERENCES……... 83

FIGURE LIST

Figure 1. Female Genital System (https://www.webmd.com/sex-relationships/guide/your-guide-female-reproductive-system#1, Access; 07.11.2020)……...8

Figure 2. Ovarıum (https://tr.pinterest.com/pin/293226625711218879/, Access; 07.11.2020)…… 9

Figure 3. Primary follicle (https://tr.pinterest.com/pin/752241943980929491/, Access; 07.11.2020). 16

Figure 4. Seconder follicle (https://tr.pinterest.com/pin/158329743134385129/, Access; 07.11.2020). 17

Figure 5. Sturcter of Graafian follicle (https://www.toppr.com/ask/question/short-long-answer-question-type-drawa-labelled-diagram-of-a-human-ovum-just-released/ Access; 07.11.2020)……...19

Figure 6. Ovulation chart. Female menstrual cycle (*https://www.news-medical.net/health/What-is-Ovulation.aspx*, Access; 07.11.2020)…….. 21

Figure 7. Oogenesis (https://en.wikipedia.org/wiki/Oogenesis, Access; 07.11.2020)……...25

Figure 8. Histological Structure of Testes (https://teachmeanatomy.info/pelvis/the-male-reproductive-system/testes-epididymis/, Access; 07.11.2020)…… 33

Figure 9. Spermatogenic cells…….. 35

Figure 10. Seminifer tubule……. 35

Figure 11. Schematic diagram shows the arrangement of Sertoli cells (https://www.histology.leeds.ac.uk/male/spermatogenesis.php, Access; 07.11.2020)……...36

Figure 12. Male Reproductive System: *Spermatogenesis*……..... 37

Figure 13. Illusturation of spermatogenesis……. 37

Figure 14. Spermatogenesis (Sharma and Agarwal, 2018)…….. 38

Figure 15. Acrosome Reaction……43

Figure 16. Schematic diagram of the acrosome reaction and fertilization (Price, 2019)…….45

Figure 17. Formation of fertilization……45

Figure 18. Embryo development and fertilization……46

Figure 19. Illustration showing stages in human embryonic development (https://www.123rf.com/photo_16988272_illustration-showing-stages-in-human-embryonic-development.html, Access; 07.11.2020)…….47

INTRODUCTION

Genital System

Female Genital System

The female reproductive system includes a pair of ovaries and tubal uterinas (oviduct or fallopian tubes), which are evaluated as part of the internal genital organs located in the pelvis, and the uterus and vagina. Labia major, labia minor and clitoris are examined within the scope of external genital organs. Although the mammary glands and placenta are not genital organs, they are functionally related to the genital system (Figure 1). Tuba uterinas are the areas where oocyte fertilization takes place, and uterus is the place where fertilized oocytes are kept during pregnancy. The ovaries and uterus undergo recurrent changes at regular intervals, known as menstrual cycles. The vagina is where the internal genital organs open outside the body (1). The development and function of the reproductive system is under the control of gonadotropic hormones. The organs remaining in the resting position until puberty show hormonal, physiological and histological changes in the period of eighteen days with the first menstrual bleeding called menarche. This reproductive cycle, which is entered by menarche between the ages of 9 and 15, ends with the irregularities of menstruation, hormonal and neurological symptoms. After menopause, there is a regression in the reproductive organs. (1).

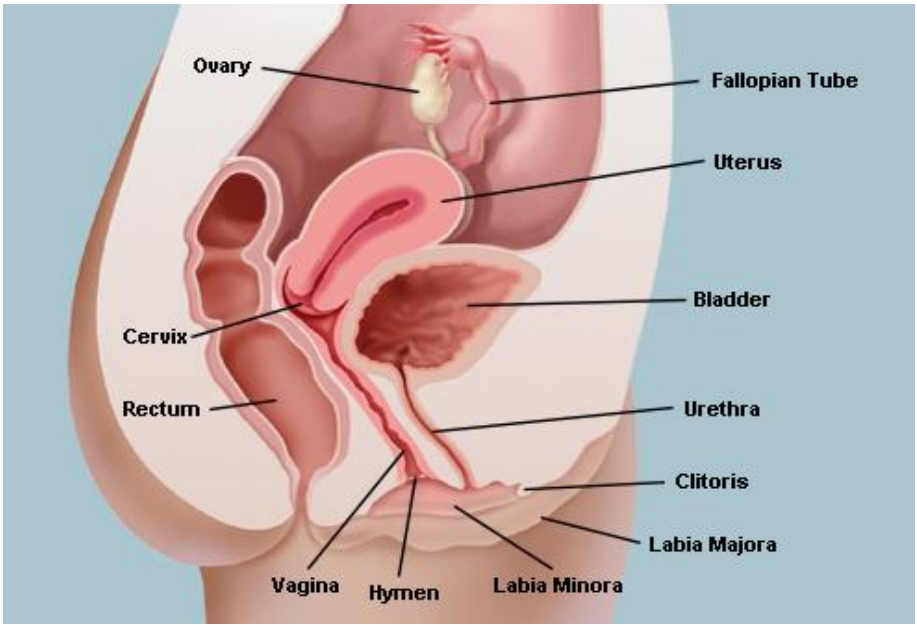


Figure 1. Female Genital System (https://www.webmd.com/sex-relationships/guide/your-guide-female-reproductive-system#1, Access; 07.11.2020).

Ovarium

It is the organs that allow the development of egg cells within specialized structures called follicles. There are two on the right and left of the uterus. A newborn girl has about 300,000-500,000 semi-ripe eggs. From puberty to menopause, 300-500 of these eggs are used. Every month, several oogonium in ovaries produce mitocytes and meiosis and oocytes. One of these cells becomes an egg cell. Others remain around the egg cell as a nutrient. Egg cells were surrounded by follicle cells consisting of epithelial cells. Follicles consisting of only one layer of cells are called primary follicles. Follicle cells both secrete substances taken by the oocyte and help

actively grow the oocyte, as well as synthesize and secrete hormones called estrogens. Estrogen hormones (most importantly estradiol and androstenedione) are secreted by the proliferation of Luteinizing hormone (LH) secreted from the pituitary gland in the blood. Estrogen hormones help the egg ripen. When estrogens reach a certain amount, their synthesis stops and the egg emerges from the follicle and passes into the fallopian tube (Figure 2).

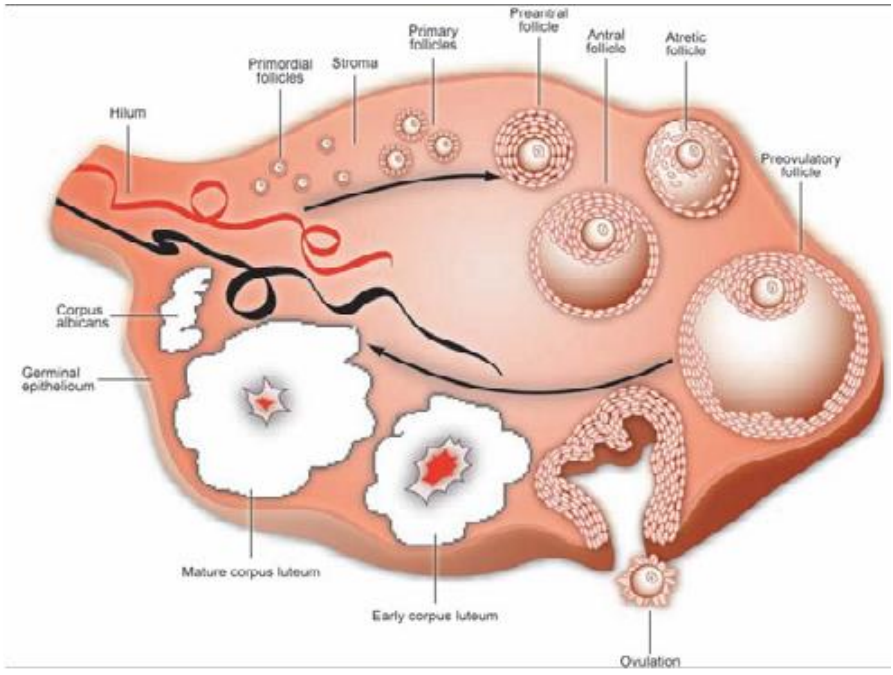


Figure 2. Ovarium (https://tr.pinterest.com/pin/293226625711218879/, Access; 07.11.2020).

The ovary was covered from the outside by a single-layer cubic epithelium layer, with the exception of the hilus region. This epithelium is called germinative 2 epithelium because it develops from the mesodermal coeloma epithelium that makes offspring cords

in the embryo. These epithelials, which are cubic in young and flat in the elderly, sit on a strong basement membrane. Most histological preparations are either partially or not at all, since they are easily spilled during preparation. The tight connective tissue rich in collagen yarns under the germinative epithelium, about 100 microns thick, is called tunica albuginea. The pinkish-gray color of the ovary comes from this layer. In the ovary sections, two areas are divided into the inner medulla (substantia medullaris) and the outer cortex (substantia corticalis). The medulla is surrounded by cortex on all sides except the hilus. Gamet production in women is called oogenesis. Two main hormones, estrogen and progesterone, are secreted from the ovary. (2).

1- Estrogens: It supports the growth and maturation of internal and external sex organs. She is responsible for the female sex characters that develop with puberty. Estrogens also affect the gland 15 and stimulate ductal and stromal growth. They also cause fat accumulation and play an important role in breast development. (3).

2- Progesterones: Prepare the internal sex organs, especially the uterus, for pregnancy. However, they also support lobular proliferation and prepare the mammary gland for breastfeeding. (3). Both hormones play an important role in the menstrual cycle to ensure the implantation of fertilized oocytes into the uterus. If implantation does not occur, the uterine endometrium degenerates and the menstruation cycle continues (4). Neurosecretory cells in the hypothalamus synthesize GnRH (gonadotropin-releasing hormone) and this hormone is delivered to the anterior lobe of the pituitary gland

via the hypophysial portal system. GnRH stimulates the release of two hormones produced in the pituitary and acting on the ovaries(5).

1- **Follicle Stimulating Hormone (FSH):** Stimulates the development of ovarian follicle and estrogen release from follicle cells.

2- **Luteinizing Hormone (LH):** It triggers ovulation and stimulates follicle cells and corpus luteum to produce progesterone (5). In histological sections, the ovary is examined in two

Cortex: The external and functional part of the organ. It includes follicles and corpus luteum structures at different stages of development.

Medulla: Blood and lymph vessels and nerves are found in three parts of the pale. It is not possible to histologically separate the cortex and medulla. The ovarian stroma consists of collagen fibers in the cortex, a network of reticular fibers, elastin lamellae in the vessel walls, and thin-long shuttle-shaped stromal cells. Stroma cells are privileged cells that can be transformed into teka interna cells that secrete hormones unlike fibroblasts. The stroma continues similarly to the medulla. Elastin is composed of fibroelastic loose connective tissue containing smooth muscle cells rich in lamellae. The medulla also contains cells containing oxidation enzymes and other enzymes; their numbers increase with age and are found in 80% of menopause. The interstitial cells are polygonal shaped, epitheloid cells with round

nuclei and prominent nuclei. Cytoplasm contains small fat cells. Since they resemble luteinized cells, it is thought that they are formed from the interna of the follicular goa. They are found individually or in groups in the ovary stroma and secrete estrogenThese cells are abundant in mammals that breed very well, referred to as interstitial glands; It decreases after the first menstruation in human, very little in adult. Hilus cells, another large group of epithelioid cells, is observed in the hilus in the form of small islets. It is similar to Leydig cells of the testis and contains fat droplets, lipofuccin pigment, Reinke crystals in the cytoplasm; secretes androgens. Neuroendocrine (APUD) cells showing argirophilia in the passage of corticomedullary stroma have been shown in 6% of women (6,7, 8).

Ovarian follicles: The ovarian follicles (foliculi ovari) are round or oval structures surrounded by an oocyte in the middle and single or multiple rows of follicle (granulosa) cells around it. Follicles formed during fetal life (primordial follicles, foliculus ovaricus primordialis) are surrounded by single-row flat follicle cells and contain a primary oocyte in the middle. These follicles are located in the uppermost layer of the cortical region. The oocyte in the primordial follicle is approximately 25 μm in diameter and round. It has a large core and a fairly large nucleus. These cells are in the prophase stage of I. meiosis. Chromosomes are usually dissolved and do not stain dark. Organelles in the cytoplasm are located close to the nucleus. The cytoplasm contains numerous mitochondria, several Golgi complexes and endoplasmic reticulum. The basal lamina beneath the follicle cells

forms the border separating the follicles from the surrounding stroma. With puberty, a group of primordial follicles begins a process called follicular growth. This process involves cellular changes in the stroma surrounding oocytes, granulosa cells and follicles. It is not known how the follicles entering the growth process are selected from a large number of primordial follicles (6,9).

Follicle development

Follicular development is continuous from menarche to menopause and continues during pregnancy, ovulation and anovulation. In the embryo, the follicular increases its number in mitoses up to the 5th month and reaches approximately 7 million. This number decreases to 700 thousand in birth and 40-50 thousand in adolescence and 400-500 hundred oocytes thrown by ovulation. There are four types of follicles in different stages of development in the ovary: primordial follicle (primary follicle), single-layer and multi-layer primary follicle (primary follicle), secondary follicle (secondary follicle), Graaf follicle (mature follicle) (7,10,11,12).

Primordial follicle

Differentiation of oogonium from the primordial germ cells in the embryo starts from the 9th week, increasing their numbers with mitosis 12-13. weeks of primary oocytes. Primary oocytes remain and wait at the diplotene stage of the prophase of meiosis. They can wait for many years at this stage, so this period is called the rest (diktiyoten) phase. Primary oocytes waiting in this way under the

tunica albuginea in the ovarium undergo atresia both in the embryo and after birth. Unlike men, oogonium survives only embryonally. Oogonium and primary oocytes are surrounded by single-layer flat stroma cells. This structure, which contains primary oocyte, is surrounded by stroma cells called follicle cells and is called primordial follicle. Follicle cells include desmosomes. The diameter of the oocyte is approximately 18, 25 μm, and the diameter of the follicle is 40-70 μm (12). The core is located close to the edge of the cell and contains one or more nucleoli which are quite prominent. Nuclear plasma is seen as vesicular because of the chromosomes opened. In the oocyte cytoplasm, called Balbiani body; Golgi complex membranes and vesicles, endoplasmic reticulum, many mitochondria and a structure formed by the accumulation of lysosomes (13,14). After puberty, 5-15 of these follicles develop in each menstrual cycle and progress to advanced stages of oocyte meiosis. FSH (Follicle-stimulating hormone) stimulation is required for growth and development to continue, although the stimulus to initiate growth is not known (12).

Primary follicle

As the oocyte continues to grow, the surrounding squamous epithelial cells become cubic, then prismatic, and are referred to as single-layered primary follicles. When the follicle cells continue to multiply and become multilayered, the follicle is called the preantral follicle because it is the previous period before the formation of the multilayer primary or antrum. The proliferating follicle cells are called granulosa cells. With the innermost cell layer surrounding the oocyte, the row of

cells resting on the outermost basal lamina retains its prismatic shape, the others being polygonal. It provides follicle nutrition by diffusion and lacks vascularity. Granulosa cells are pale cytoplasm, polygonal and hyperchromatic nuclei; The effect of FSH creates corrugated connections between each other and oocytes. Activin secreted from oocytes stimulates proliferation in follicle cells. The primary oocyte in the primary follicle grows and has a large, large core, called the germinal vesicle. When the oocyte reaches a diameter of 60-80 μm, an eosinophilic, homogeneous, PAS (+) layer is selected between the oocyte and the surrounding granulosa cells. This is called shingles pellucida. It is synthesized by oocyte and granulosa cells. The first row of granulosa cells surrounding the zona pellucida is called a solid corona radiati. Zona pellucida is rich in glycoprotein and glycosaminoglycan. Its thickness reaches 10-15 μm upon completion. Shingles pellisuda was shown to contain three glycoproteins. These are ZP-1, ZP-2, ZP-3. The disaccharide sequence in ZP-3 constitutes a receptor for specific membrane 19 proteins on the spermatozoon head. Thus, the entry of more than one spermatozoon into the oocyte cytoplasm is prevented (7,14). The monolithic follicle is formed around the connective tissue sheath, which differs from the stromal cells around the primary follicle with 3-5 rows of granulosa cells. It develops as the follicle develops. It is divided into two parts: teka interna and teka eksterna. There is no strict boundary between the two. Teka interna is separated from the granulosa cells by basal lamina and is made of fibroblast-like spindle-shaped cells, containing abundant capillaries. Teka interna cells that contain LH receptors in their

plasma make androstenodion hormone, which diffuses into granulosa cells and is converted to estrodiol by the aromatase enzyme. Teka externa is mostly fibrous connective tissue; smooth muscle cells, collagen bundles and larger blood vessels 8Figure 3) (6,12).

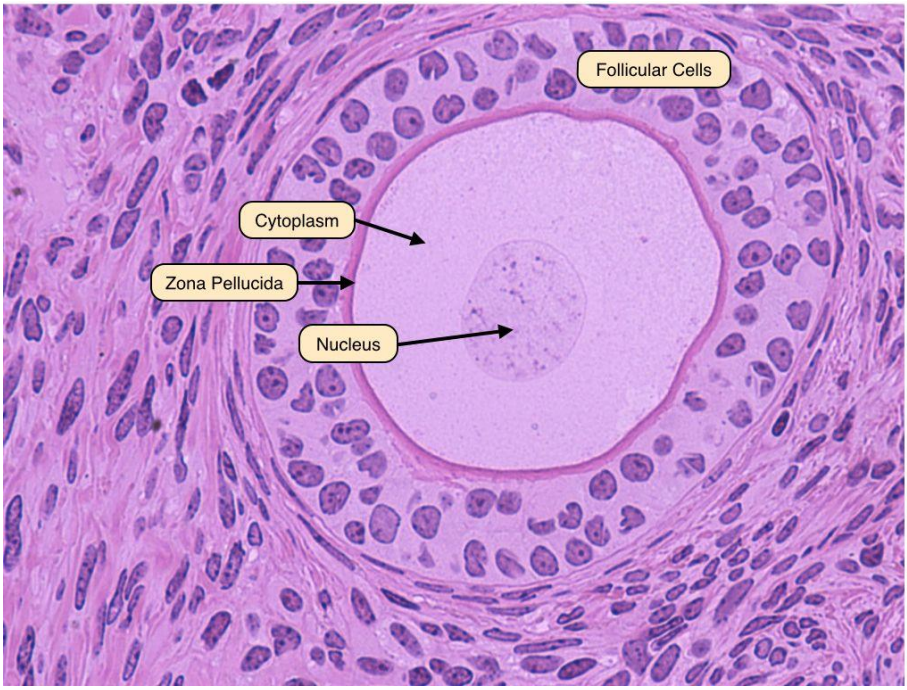


Figure 3. Primary follicle (https://tr.pinterest.com/pin/752241943980929491/, Access; 07.11.2020).

Secondary follicle

When the follicle reaches 200 µm and the granulosa cells reach 6-12 times, the gaps begin to form in the follicle called the antrum. In the cavities, there is the follicle fluid which is secreted from the granulosa cells. The gaps merge to form a single large antrum. The secondary follicle is also called an antral follicle. When the oocyte in the

secondary follicle reaches a diameter of 120-130 μm, its growth stops. However, granulosa cells continue to increase in number with the effect of FSH and follicle diameter increases. Follicle fluid is formed by filtration of blood from the monster at different amino acid concentrations with low lipid and glucose density. Steroid binding proteins include proteoglycans and glycosaminoglycans. The pH of the follicle fluid is 7.3. Mostly estrogens, steroid hormones such as progesterone and androgens, and non-steroid hormones such as FSH, LH, inhibin and activin are found in the follicle fluid. The oocyte controls the formation of follicle fluid (6). Many follicles go through atresia at this stage, but the granulosa cells of some do not degenerate and remain in small groups that secrete androgens. A few secondary follicles develop into mature follicles (Figure 4) (12).

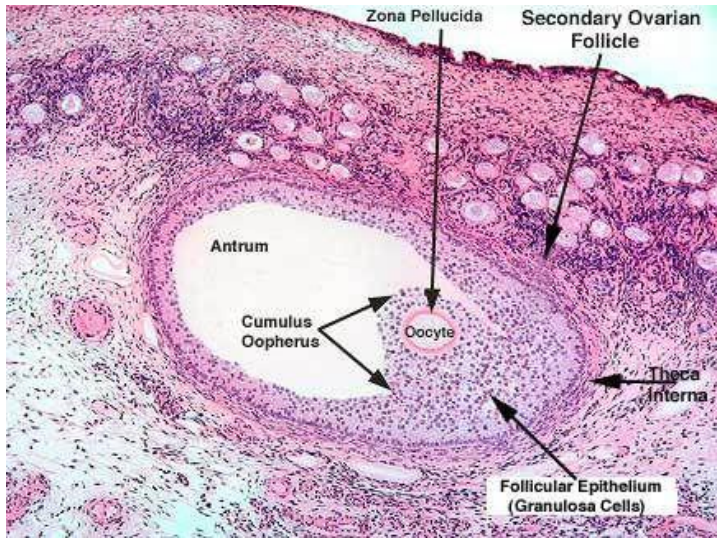


Figure 4. Seconder follicle (https://tr.pinterest.com/pin/158329743134385129/, Access; 07.11.2020).

Mature (Graaf) follicles

As the antrum grows further, the oocyte is pushed into a pole with the surrounding granulosa cells. As the granulosa cells proliferate, they become distant from each other, they are connected to each other by short cytoplasmic extensions and appear as angular or star shaped. The connection of the oocyte to the granulosa cell layers loosens. The protrusion of the oocyte into the follicle cavity, which is wrapped around the corona radiata, is called the egg stigma (cumulus ooforus). In this period, granulosa cells are called cumulus cells adjacent to oocytes and non-adjacent mural granulosa cells. The oocyte controls its microenvironment by suppressing the differentiation of mural cells and promoting the differentiation of cumulus cells. This effect is achieved by secreting paracrine signaling factors. Most mural granulosa cells are associated with the basal lamina. Cells of granulosa and cola follicle are highly differentiated. Teka is the main source of androgens, but also secretes renin, prostaglandin and angiogenic factors. This follicle, which is observed just before ovulation, is also called preovulatory follicle (Figure 5) (6,12).

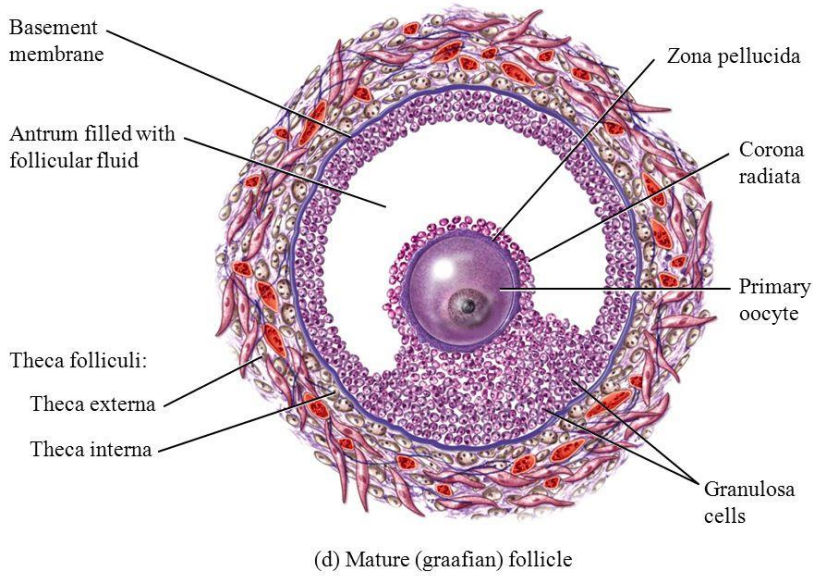


Figure 5. Sturcter of Graafian follicle (https://www.toppr.com/ask/question/short-long-answer-question-type-drawa-labelled-diagram-of-a-human-ovum-just-released/ Access; 07.11.2020).

Ovulation

Response to gonadotropins develops after LH receptors appear in granulosa cells. 24-36 hours after LH release, follicle fluid rapidly increases. Before ovulation, the diameter of the oocyte reaches 120 µm and the diameter of the follicle reaches 15-25 mm. With the release of meiotic stimulant, a local factor under the influence of LH, the oocyte prophase up to the period of the Graaf follicle reaches the prometaphase and metaphase stages 24-36 hours before ovulation. At the time of ovulation, the oocyte rapidly passes through the telophase and anaphase stages, completing the first meiotic division and monitoring the first polar body in the previtellin range. Unlike men,

the cytoplasm distribution in this division is unstable. Polar body 21 is a small cell with a narrow cytoplasm relative to the oocyte. The cell tossed into the tuba uterine by ovulation has secondary follicles; second meiosis expects fertilization at the metaphase of division. Proteoglycans and hyaluronic acid, which are continuously produced by granulosa cells in the graft follicle, retain water, which causes both the follicle to grow and the mural granulosa cells lose contact with each other. Immediately before ovulation, a pale, bloodless, thin-walled swelling is observed in the area of the Graaf follicle on the ovarian surface. It's called a stigma. The thinning of the wall involves cellular displacement and collapse of the collagen bundles in the tunica albugine with the goat. Collagenase was found high in follicles just before ovulation. Follicle fluid contains plasminogen, granulosa cells synthesize plasminogen activator under the effect of LH before ovulation. This helps to convert plasminogen to active plasmid and to break down collagen in the basal membrane, cola and tunica albugine by activating procollagenase. Mature oocytes and some granulosa cells around the perforation of the peritoneal cavity are excreted through the hole drilled in the stigma region by degeneration. This event is called ovulation. Complies with the 14th day of the 28-day menstrual cycle. The discarded oocyte catches the fimbria of the tuba uterina. The journey of the oocyte in the tuba uterina lasts for approximately four days, and if it is fertilized, the second maturity division of the meiosis is completed, the secondary oocyte and the second polar body form. If fertilization does not occur, the secondary follicle degenerates within 24 hours (Figure 6) (6,12).

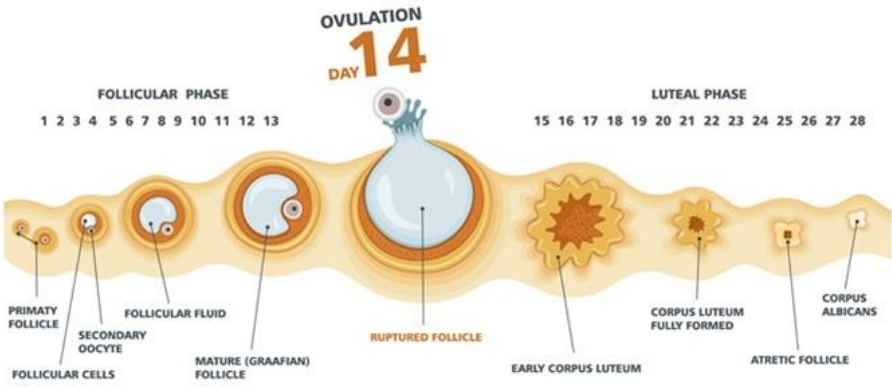


Figure 6. Ovulation chart. Female menstrual cycle (*https://www.news-medical.net/health/What-is-Ovulation.aspx*, Access; 07.11.2020).

Corpus luteum (yellow body)

After ovulation, the follicle wall becomes constricted and curled by the effect of smooth muscles on the externa. The basal lamina between granulosa and teka interna is depolymerized and a clot forms in the middle space with blood leaking from the capillaries of the teka interna. This structure is called the corpus luteun hemorrhagicum. Teka interna cells around; The blood and lymph vessels, fibroblasts and accompanying connective tissue enter the granulosa cells and fill the middle space cleared by macrophages to form a corpus luteum that functions as an endocrine gland. In the corpus 22 luteum, granulosa cells grow, pale stain and make up 80% of the corpus luteum. Polygonal shaped cells containing microvilli. Well-developed granulate-free endoplasm reticulum, numerous small golgi complex, abundant tubular crystal mitochondria, abundant oil droplets observed; light heterochromatin nuclei, granularosa containing prominent nucleoli become lutein cells. Some of the dense granules in them turn

into androgens from the lysosome structure of relaxin and teka lutein cells into estrogens. Progesterone provides the secretion period in the endometrium. On the other hand, changes in the interna cells are observed. These are smaller, dark-stained cells and make up 20% of the corpus luteum. There is no microvillus around it. Endoplasmic reticulum without granules is abundant, fat droplets accumulate in the cytoplasm, underdeveloped golgi complex, heterochromatin core is observed. These are called teka lutein cells. They secrete small amounts of estrogens, progesterone and androgens. Estrogen and progesterone secreted from the corpus luteum inhibit the secretion of LH and FSH secreted from the anterior pituitary. The absence of FSH prevents the development of new follicles and a second ovulation. The condition of the corpus luteum varies depending on whether or not fertilization occurs. Without fertilization, the absence of LH causes the formation of the menstruation corpus luteum. After 10-12 days, granulosa and cola cells shrink and regress, vacuoles form in their cytoplasm, estrogen and progesterone secretions decrease. Fibroblasts in Teka externa synthesize collagen and replace the regressing cells. The cell-free, whitish, small structure consisting of collagen and hyaline remaining from the corpus luteum is called corpus albicans (white body). This gradually shrinks and remains a small scar on the ovary face. If pregnancy occurs, it is called corpus luteum (copus luteum gravidarum) due to hCG (human chorionic gonadotropin) secreted from trophoblasts (12).

Uterine histology

The uterine wall of the tuba consists of 3 layers. These are tunica mucosa, tunica muscularis and tunica serosa layers respectively from inside to outside.

Tunica mucosa

Tunica is composed of mucosa, epithelium and lamina propria. The tunica mucosa is thick in the ampulla region. It contains many longitudinal plicas that make secondary and tertiary branches. Therefore, the transverse (transverse) sections, the lumen is seen as irregular star. The longitudinal mucosa plicas of the ampule extend from the infundibulum to the surface of the ovarium. These are called fimbria (fimbriae ovaricae). Fimbria plays a role in the removal of the oocyte thrown from the ovarium by ovulation into the tuba uterina. From the ampulla to the uterus, longitudinal mucosal folds are lowered and branching is reduced. Mucosal folds in the intramural segment are very low and few, they do not show branching (15,16,17).

Oogenesis

It is called oogenesis that mature egg cell (ovum) occurs in the ovarian follicles first by mitosis and then by meiosis. In the third month of the embryonal phase, the oogonium is divided by mitosis to increase their number. Some of these are surrounded by flat follicle cells in a row from the fetus. These structures are called primordial follicles. The flat shape of the primordial follicle cells is transformed into cubic cell shape, which is called the primary follicle. The human has a total

of 300,000-500,000 primary follicles for both ovaries, and no new ones are added. Primary follicle cells increase their number by increasing the number of oogonium in the middle while increasing the number of mitotic cells. These large and diploid cells are called primary oocytes. In females reaching sexual maturity, one of the primary follicles develops every 28 to 30 days (menstural cycle). Primary follicle cells surround the primary oocyte in two rows, this structure is called the secondary follicle. The secondary follicle continues to develop and becomes graff follicle (mature follicle). The primary oocyte in the mature follicle is surrounded by follicle cells and connected to the surrounding follicle cells by a stem formed from follicle cells (cumulus ooforus). Between the inner follicle cells and the surrounding follicle cells is the antrum fluid made by the follicle cells. In the antrum fluid;

- glycosaminoglycans
- steroid binding proteins
- progesterone
- androgens
- Estrogens are found.

Around the primary oocyte is a layer of zona pellucida consisting of glycoprotein. Surrounding of the surrounding follicles, the teka interna developed from them, and the surrounding teka externa consisting of fibroblasts. When the mature follicle comes into contact with the

germinal epithelium, the germinal epithelium is forced due to the pressure of the antrum fluid in it. At this time, the primary oocyte enters the first meiosis and divides into two cells of different size carrying a haploid number of chromosomes. The larger one is called secondary oocyte and the smaller one is called I. pole cell. Due to the pressure of the antrum fluid, the germinal epithelium is ruptured and some follicle cells and secondary oocytes are thrown into the oviduct (egg channel). This event is called ovulation. If the secondary oocyte encounters and fertilizes with sperm, the maturation continues to divide and undergo the second meiosis. II. At the end of meiosis, two cells of different sizes are formed, again with a haploid number of chromosomes. The larger one is the ootide and the smaller one is II. It's called a polar cell. The mature egg cell develops into the ovum and the pole cells degenerate (Figure 7).

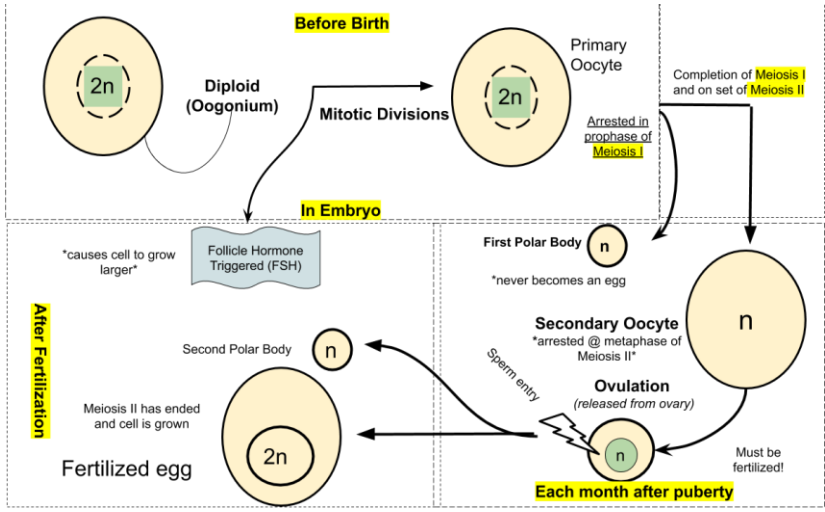


Figure 7. Oogenesis (https://en.wikipedia.org/wiki/Oogenesis, Access; 07.11.2020).

General Structure of Egg Cell (Ovum)

A mature egg cell has a large nucleus and abundant cytoplasm. Centriole disappears. Sometimes the Golgi Complex is not seen, but other organelles, such as granular endoplasmic reticulum, mitochondria, lysosomes, centrioles, are found in the cell. Pigments in the cytoplasm give color to the egg. The egg is surrounded by protective layers. There are different amounts of vitellus in the egg according to their types and they are immobilized. Eggs are generally oval shaped and have two poles due to polarity. The location of the centrosome in each cell relative to the nucleus determines the general shape of the polarity, ie the line joining the centrosome and the nucleus forms the principal axis of the cell. The part of the egg where the polar cells are thrown is called the animal pole and the part that is opposite it is called the vegetative pole. Generally, the activity is greater because the cytoplasm and nucleus are located around the animal pole. The central region of the egg is called the equatorial region. The region between the equatorial region and the animal pole is called the animal region, and the region between the vegetative pole is called the vegetative region.

Vitellus

The nutrient that accumulates in the cytoplasm during the growth of oocytes is called vitellus. Vitellus provides the necessary nutrients in the development of the embryo. Vitellus is found in different amounts and different places in egg types. Vitellus content; A small amount of

protein, phospholipids are contained in neutral oils glycogen, mineral salts, vitamins, enzymes. The amount of the molecule in the content of vitellus is called the name of the molecule. For example, if the protein is high, it is called protein vitellus. In the oocyte of many animals, the mass structure adjacent to the nucleus is called the vitellus nucleus or the Balbiani body. There are two types of eggs depending on the presence of vitellus in the egg:

1- Alesital Eggs: Eggs without vitellus are called. Examples are human eggs.

2- Endolesital Eggs: Eggs with vitellus in the cytoplasm are called. Endolesital eggs are divided into three types according to the placement of the vitellus:

1.Oligolesital (Isolesital, homolesital) Eggs: Vitellus is homogeneously distributed in the egg, but the amount is small. The core is located in the middle of the egg. It is the type of egg seen in many invertebrates such as sea urchin, amphoxus and human.

2. Telolesital (Anisolesital) Eggs: Vitellus is not evenly distributed in the egg. The abundant vitellus is located in the vegetative hemisphere of the egg. The nucleus and cytoplasm are found in the animal hemisphere or animal pole.

3. Sentrolesital Eggs: Vitellus is located in the middle of the egg and is abundant. Vitellus is located between the inner cytoplasm around the nucleus and the peripheral cytoplasm beneath the cell membrane. These two cytoplasms are connected to each other by cytoplasmic

bridges that cross the egg radially. An example of this type of egg is arthropods insect eggs.

Egg Covers

All eggs; like other cells, they are covered by the cell membrane. The eggs of all animals except the sponges and some solenters are surrounded by special egg covers in addition to the cell membrane. These drapes are divided into 3 depending on their origin:

1- **Primary Egg Covers:** It is the covering of the egg cell in the area where microvilli is located between follicles and oocytes during vitellus formation. Previously, they have the property of mucopolysaccharide, and later they become fibrillated protein with the participation of some substances.

Primary Egg Covers are of two types:

a) **Vitellus Membranes:** They are membranes that are condensed outward and gain special structure and are firmly adhered to the oocyte surface. Insects, mollusks, amphibians and birds are seen in the eggs.

b) **Fertilization Membrane:** Thickening of the plasma membrane after fertilization and the outer face is separated from the inner face and is a membrane formed by emptying the cortical granules between these two membranes. The part between the fertilization membrane and the cell is called the perivitellin area.

The gelatinous cover that covers the sea urchin eggs is an example of a primary cover.

2- Secondary Egg Covers: When the egg is in the ovary, they are either formed solely by the follicle cell or jointly by both egg cells and follicle cells. Chorion in insects, some fish and tunicates is the secondary cover of zona radiyata in fish eggs and zona pellucida in vertebrate eggs. The chorion is very hard because it contains chitin and mineral substances in its structure and has various patterns on it. For this reason, sperm enters the egg from the so-called micropylar. In the vertebrates, the cell-free transparent cover on the egg that is detached from the ovary is called zona pellucida. In some animals, this covering has a radial appearance, so it is called shingles. The cell layer consisting of follicle cells on the shingles pellucid is called corona radiate. This layer is temporary, the egg is discarded as it passes through the fallopian tube, and only the zona pellucida remains.

3- Tertiary Egg Covers: After the egg is thrown out of the ovary, they are added to the egg by the fallopian tube (oviduct) or by the helper glands of the genital organs opened to the tubes. They may be in one or more layers. Albuminous part and $CaCO_3$ shell in birds, and gelatin covers in frogs are examples of tertiary egg membranes. Some animals lay their eggs in a spongy substance or a filamentous capsule called cocon. This structure is a tertiary egg cover.

Male Genital System

Histology of Testes

The testes are a pair of endocrine and exocrine glands. It produces testosterone as an endocrine hormone. As an exocrine, it produces a male mature gamet cell, spermatozoon.(18,19). The testes are located in the scrotum outside the abdominal cavity at a temperature of 2-3 ° C lower than the body temperature to provide the required 34-35 ° C for normal spermatogenesis. The testes that are suspended in the scrotum are externally wrapped with testicular capsule. The testicular capsule consists of 3 layers.

a) **Tunika Vaginalis:** A single layer of flattened mesothelial cells on the outermost. The visceral layer of the closed serous sac originates from the peritoneum. It surrounds the front and side surfaces of the testis. It also lies on the surface of the scrotum and forms the parietal layer of the tunica vaginalis. The visceral layer of the tunica 10 vaginalis rests on a basal lamina. The serous space between the visceral and parietal layers allows the testis to move freely.
b) **Tunica Albuginea:** It is the most prominent layer and is separated from the basal lamina and the tunica vaginalis. Tunica albuginea is a dense fibroelastic connective tissue containing smooth muscle cells. These smooth muscle cells are more dense on the posterior surface of the testis adjacent to the epididymis.

c) **Tunica Vasculosa:** The innermost layer of the testicular capsule, the tunica vasculosa, consists of a network of blood vessels embedded in a thin areolar connective tissue.

The tunica albuginea thickens on the posterior surface of the testis and the mediastinum is inserted into the organ as the testis. The mediastinum divides the inner part of the testicle into up to 250 pyramidal compartments in thin, fibrous extensions from the testis to the capsule. These chambers are called lobuli testis (testicular lobules). The apexes of the testicular lobules are directed towards the mediastinum. Each lobule contains highly curved seminiferous tubules ranging from 1 to 4. The flat parts of the seminiferous tubules in the mediastinum testis are called tubulus recti (flat tubules). Tubulus recti mediastinum testis continues with the system of channels showing anastomosis called rete testis. There are many types of cells in the stroma, including vessels, nerves and mainly interstitial cells (Leydig cells). Leydig cells are large and often arranged in groups. They are very important cells due to their endocrine functions (20).

There are two types of cells sitting on the basement membrane on the wall of the seminiferous tubes. These;

a) Sertoli cells (feeder and supporting cells)

Sertoli cells are small in number and have settled at regular intervals between spermatogenic cells. They have a long pyramid shape, their base rests on the basement membrane and their apex extends to the tube lumen. Cell boundaries are ambiguous because their lateral

extension surrounds spermatogenic series cells. Some of the triangular nuclei are in the basal and parallel to the long axis of the cell, are echromatic, so 2-3 nucleoli are easily selected. The apical cytoplasm has recesses and protrusions suitable for burying spermatids. Cytoplasms contain abundant mitochondria, a small number of granular ERs, well-developed golgi apparatus, abundant lysosomes and secretory granules. Only the Sertoli cells and spermatogonium, the first individual of the spermatogenic series, sit on the basement membrane in the tube wall. Adjacent sertoli cells are connected to each other by zonula occludens at the level of spermatogonialar. During the spermatogenesis, the cells of the spermatogenic series consisting of spermatogoniums move along the lateral walls of sertoli cells and move towards the lumen. The presence of zonula occludens between Sertoli cells prevents the passage of macromolecules between intratubular and extratubular spaces. Thus, the proteins of the germ cells in the intratubular space do not reach the blood and no antibodies are made against them. The zonula occludens around the peritubular tissue and sertoli cells together form the blood-testicular barrier. There are also gap junctions between Sertoli cells. In this way, ionic and chemical exchange of cells is provided (Figure 11).

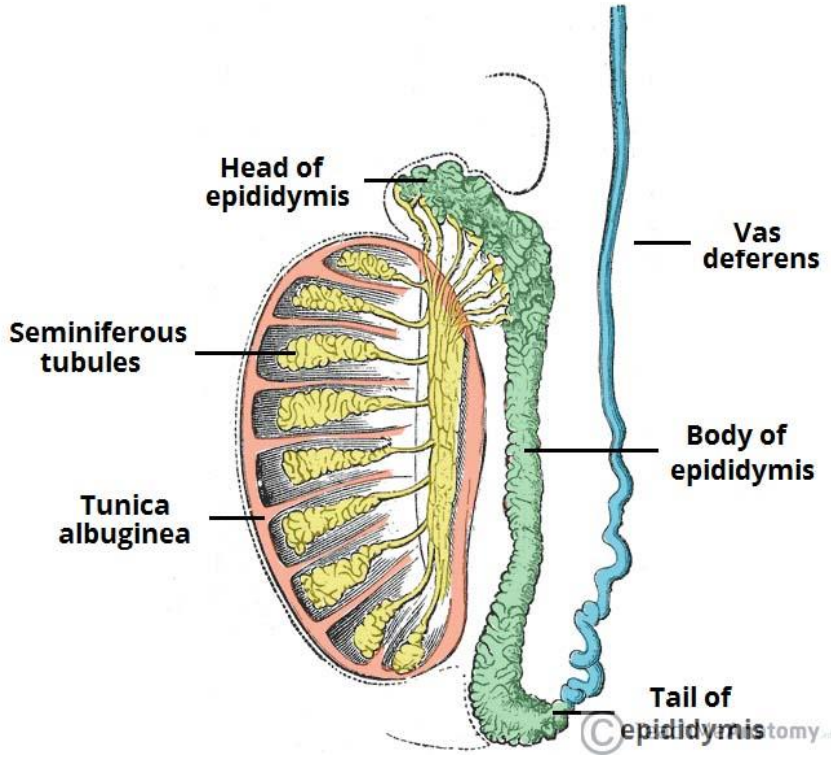


Figure 8. Histological Structure of Testes (https://teachmeanatomy.info/pelvis/the-male-reproductive-system/testes-epididymis/, Access; 07.11.2020).

Spermatogenic cells (Germ cells) are series of 4-8-fold cells located between the basement membrane and lumen of the seminiferous tubule. These cells multiply and differentiate (spermatogenesis event) to form spermatozoa. The spermatogenic series contains the following cells:

a) **Spermatogonium:** They sit on the basement membrane. There are only these cells in the wall of the tube until puberty begins. From puberty, they multiply by hormonal effect and turn into other types of cells. There are two types of spermatogonium that

are smaller than the cells that develop from it: 1. Type A (stem cell); core is oval. When proliferated by mitosis, half remains type A, while the other half converts to Type 2 B. It is larger than Type A, the nuclei are very round, darker. They multiply by mitosis and grow into spermatocytes (Figure 9).

b) Spermatocyte I (primary spermatocyte): Spherical or oval shaped. It's the largest cell on the wall. They enter the prophase phase of the first meiotic division and maintain these conditions for a long time, so they are frequently seen in sections. 46 chromosomes (diploid) carry (Figure 10).

c) Spermatocyte II (Secondary Spermatocyte): These are the cells with 23 chromosomes (haploids), half the size of which are formed by meiotic division of the primary spermatocyte. Since they immediately undergo a second meiotic division, they appear infrequently on the sections. Spermatids are formed by dividing them.

d) Spermatid: They form a cytoplasmic cell cluster that is half the size of Spermatocyte II, has haploid chromosomes and makes cytoplasmic connections with each other (21).

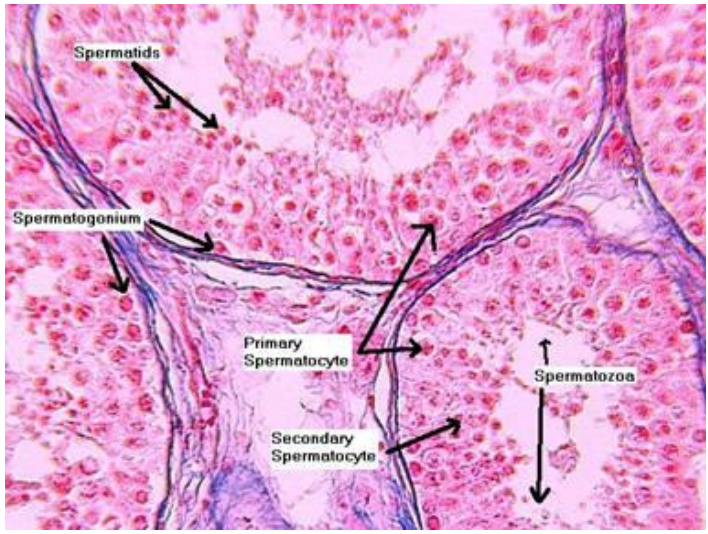


Figure 9. Spermatogenic cells

(https://www.apsubiology.org/anatomy/2020/2020_Exam_Reviews/Exam_5/CH27_Seminiferous_Tubules.htm, Access; 07.11.2020).

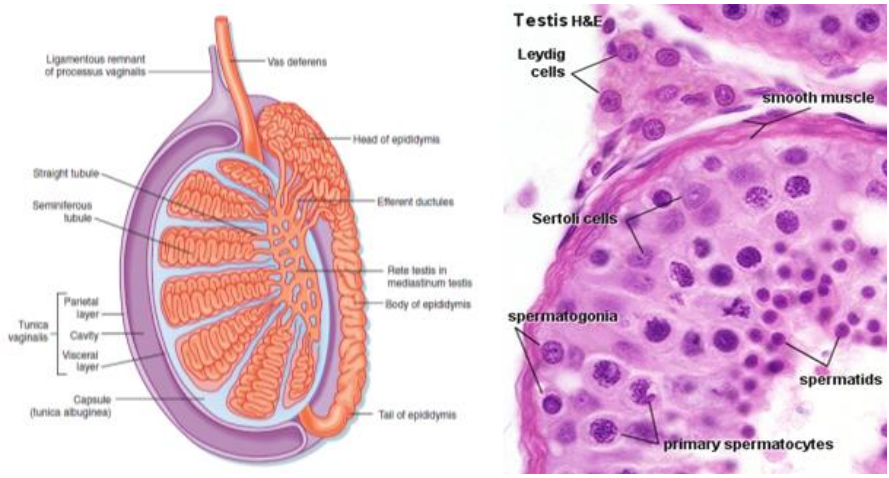


Figure 10. Seminifer tubule

(https://www.memorangapp.com/flashcards/253203/C4T2L27+Male+Reproductive+Physiology/ , Access; 07.11.2020).

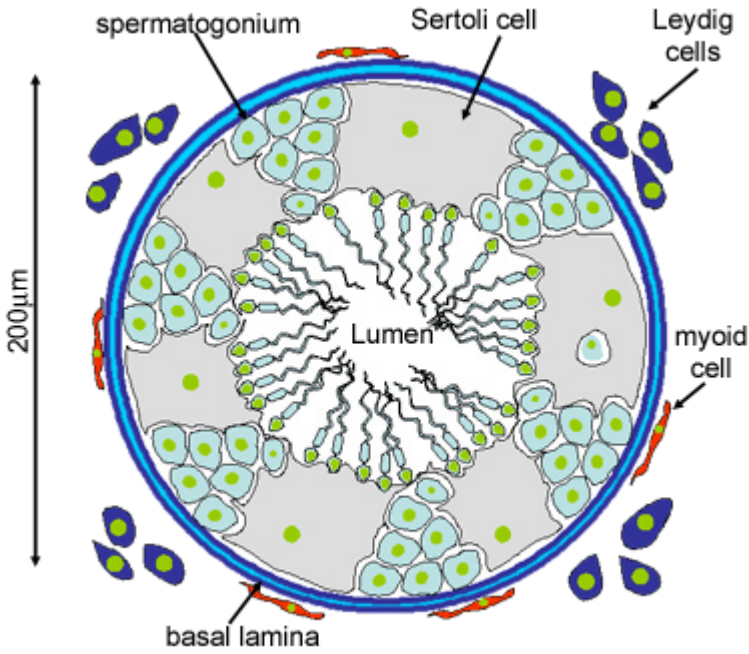


Figure 11. Schematic diagram shows the arrangement of Sertoli cells (https://www.histology.leeds.ac.uk/male/spermatogenesis.php**,** Access; 07.11.2020).

Spermatogenesis: The stage from spermatogonium to spermatid formation is called spermatogenesis. Spermatids are differentiated by burying in sertoli cell tassels and called spermiogenesis. Newly formed spermatids; a centrally located round nucleus has a well-developed golgi apparatus, a large number of mitochondria and a pair of centrioles. Changes in all these structures occur with spermiogenesis. Spermatids are fed into the cytoplasm recesses (tassels) of the Sertoli cells on the luminal faces, and both feed on it and complete their differentiation. Differences in spermiogenesis are as follows (Figure 12, Figure 13, Figure 14):

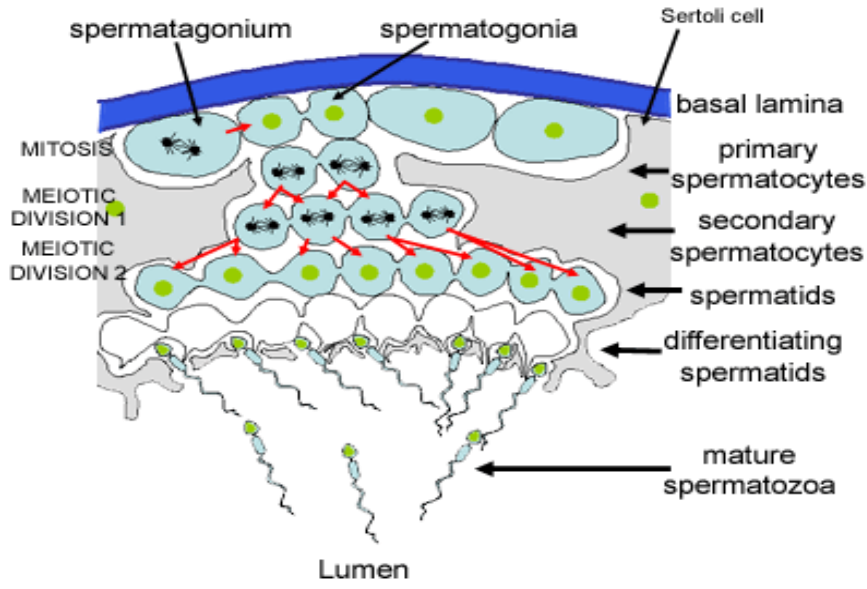


Figure 12. Male Reproductive System: *Spermatogenesis*

*(*https://www.histology.leeds.ac.uk/male/spermatogenesis.php, *Access; 07.11.2020).*

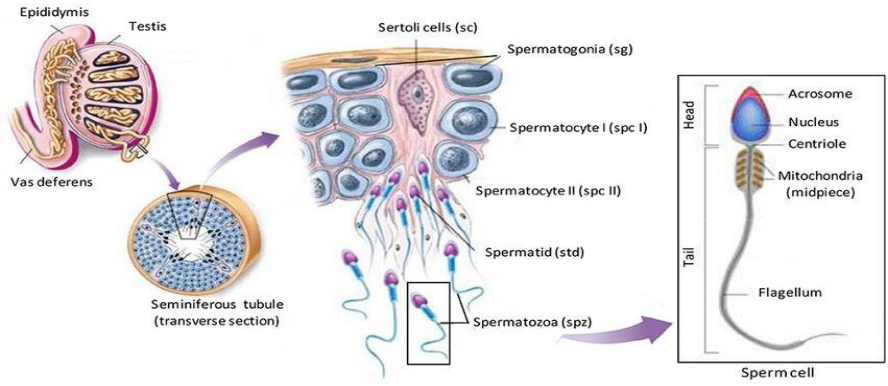


Figure 13. Illusturation of spermatogenesis

(https://www.researchgate.net/publication/267753964_Role_of_the_prion_protein_family_in_the_gonads, Access; 07.11.2020).

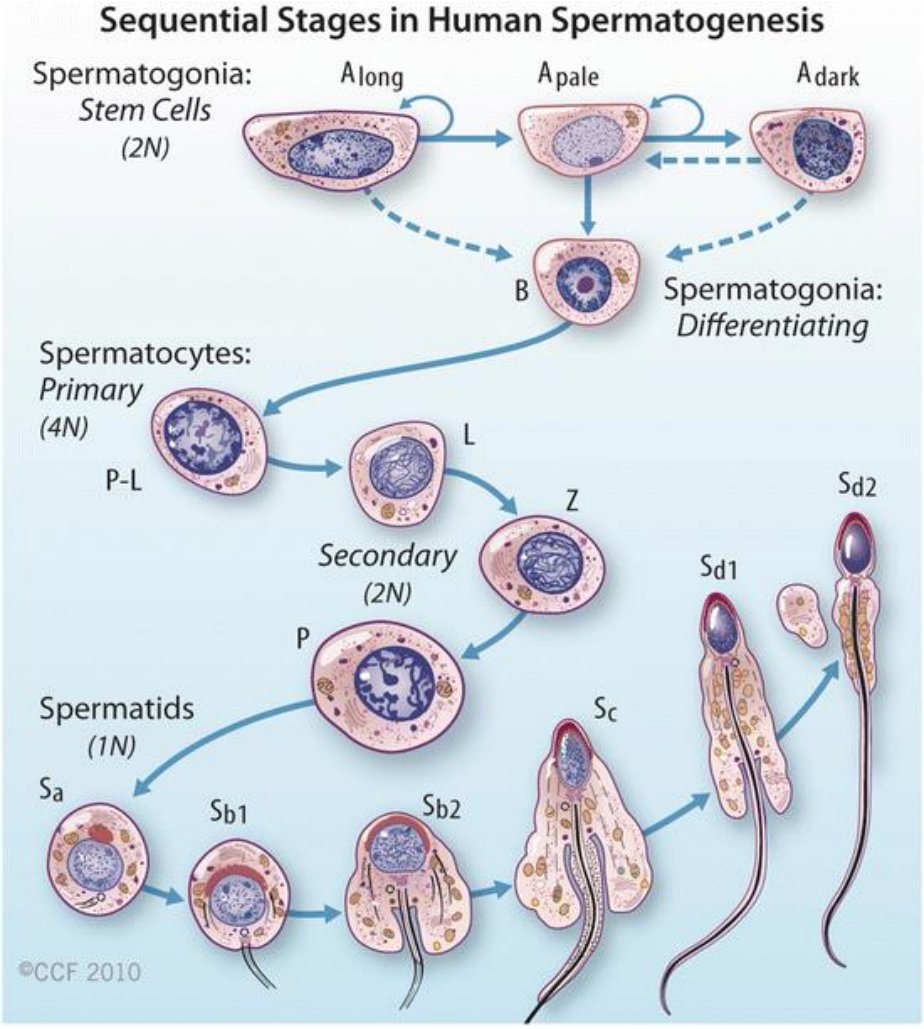


Figure 14. Spermatogenesis (Sharma and Agarwal, 2018).

Sharma R., Agarwal A. (2018) Defective Spermatogenesis and Sperm DNA Damage. In: Zini A., Agarwal A. (eds) A Clinician's Guide to Sperm DNA and Chromatin Damage. Springer, Cham. https://doi.org/10.1007/978-3-319-71815-6_14

1. The carbohydrates-rich granules (preakrosomal granules) synthesized from the Golgi apparatus combine with each other to form a single, coarse granule surrounded by membranes. This is called an acrosomal granule (acrosomal vesicle). The acrosomal vesicle adheres to the outer membrane of the nucleus, wraps it like a cap and is called the acrosome. Acrosome contains many hydrolytic enzymes. These enzymes work during the separation of corona radiata cells and softening of the zona pellucida during fertilization.

2. Acrosome is formed in one pole of the nucleus, while the other pole is divided into two pairs of centrioles (proximal and distal pairs of centrioles). Proximal centriol develops and extends a flagellum. As it extends, a fibrillary sheath forms around it and forms the tail of the spermatozoon. Around the proximal part of it, the mitochondria are arranged in regular order to form the middle part. The distal centriol migrates towards the end of the middle part and surrounds the flagellum as an annulus at the beginning of the tail.

3. A part of the cytoplasm slides in the tail direction and surrounds the middle part. The rest is thrown out. Sertoli cells are phagocytosed and eliminated. As the differentiation progresses, the excess cytoplasm continues to be discarded. Cytoplasm remains only to cover the core, middle part and tail principals of the tail.

4. The core chromatin condenses and the core takes a flat oval shape and forms the head of the spermatozoon. Spermatids are called spermatozoon (spermium) when they mature and are released into the lumen of the tubule. They are morphologically mature but functionally

immature, immobilized, no fertilization ability. They complete their maturation in the epididymis. After ejaculation, the final step of maturation, the enzymatic changes are completed at the female body just before fertilization. Capacitation takes about 7 hours, there is no morphological change. The secreted spermatozoa thus produced in seminiferous tubes are transported outward in the testicular fluid made by sertoli cells and rete testicular cells.

The smooth muscle structure on the wall of myoid cells and other channels outside the seminiferous tubules contributes to this transport. Mature spermatozoa have head-neck, middle parts and tail parts (22). The interstitium between the testicular seminiferous tubes is composed of loose connective tissue. There are abundant blood and lymph capillaries, fibroblasts, macrophages, mast cells, reticular strands and undifferentiated mesenchymal cells. The capillaries are of the window type. After puberty, interstitial cells turn into Leydig cells. Leydig cells begin to secrete testosterone in early fetal life. Testosterone is essential for the development of gonad in the male fetus during the embryonic period. In puberty, it is responsible for the initiation of sperm production, secretion of accessory glands and the development of secondary sex characters. It is necessary for the continuation of spermatogenesis, accessory glands, genital canals and secondary sex characteristics in adults (23,24).

Fertilization

Male and female sexual intercourse is called coitus. Fertilization (fecondation, fertilization); The combination of male and female mature sex cells (spermatozoon and ovum) is the meeting of chromosomes within the same cell. After ovulation, the oocyte II, which is wrapped with corona radiata, is thrown into the egg pathway. The egg pathway (fallopian tube, tuba uterina) is proximal to distal; infundibulum, ampulla, isthmus and interstitial part. For an ideal pregnancy, fertilization should occur in the ampulla (ampulla tuba uterina) (24). Spermatozoa, which are discharged to the vagina during coitus, pass to the cervical uteri within a short period of time because the phina of the vagina is acid. From there, they continue their way to the tuba uterina with the ampulla containing oocyte II. Spermatozoa can take this route (vagina-ampulla anterior approximately 18 cm) at a rate of 3-3.6 mm per minute at an average hour, but morphologically and physiologically active spermatozoa have not yet been able to fertilize (25). This ability is given to spermatozons during their travels in the uterus and oviduct mucosa, a process that lasts approximately 2-6 hours, and is called capacitation. Spermatozoa utilize (+) rheotaxis and (+) chemotaxis abilities in their progression. The role of oxytocin hormone secreted from the posterior lobe of the pituitary during mating (contracting the smooth muscles of the uterus and oviduct wall) is important for this progression of spermatozoa (26,27). At the same time, the striking direction of the cilia of the kinosylated epithelium in the uterus and tuba uterina and the ampulla direction in the stage also play a role in the progression of spermatozoa. In

addition, the spermatozoon-attracting substance called fertilizin secreted by oocyte II also plays a role in this journey (28). It is also known that gymnogamon I, II secreted by oocyte II, and androgamon I, II secreted by spermatozoon 9 have a role in this combination. It is stated that these gamons constitute attractiveness between both genera of cells and some infertilities with unknown reasons and some sexual incompatibilities are related to the insufficiency of these substances. Capacitation and acrosome reaction events are required for spermatozoon to fertilize oocyte II. After these, shingles reaction takes place (Figure 17, Figure 18) (29).

The capacitation; It is the process of removing seminal plasma proteins on the cell membrane of the spermatozoon and various glycoproteins in the cell membrane covering the acrosome region due to secretions produced by the uterus and oviduct mucosa. Spermatozoa reach the location of the oocyte II and begin to accumulate around it. In order for the female cell to be fertilized, the corona radiata cells surrounding it must be removed and the permeability of the zona pellucida increased. This is accomplished by acrosome reaction (30).

Acrosome reaction; spermatozones, which have completed their capacitation and have reached the ampulla, attach to the oocyte II by surface receptors in the cell membranes that have been released during capacitation. In the zona pellucida of oocyte II, there are species-specific binding sites of different types of glycoproteins that are suitable for surface receptors in the spermatozoon membrane. By

providing the linkage, the Ca ion input into the spermatozoon is accelerated and thus the acrosomal reaction begins. The spermatozoon cell membrane merges and fuses with the outer membrane of the acrosome. The cell membrane and the acrosome outer membrane in this region dissolve. This phenomenon is called acrosome reaction. The surrounding corona radiata epithelial dissolves, the zona pellucida is softened, the cell membrane in the postacrosomal region of the first spermatozoon (probably the strongest) passing through this barrier adheres to the cell membrane (oolemma) of the oocyte II, where both cell membranes dissolve, from which point the spermatozoon is exposed. with the tail into the cytoplasm of oocyte II. As soon as the spermatozoon enters, a so-called shingles reaction occurs that prevents the entry of a second spermatozoon (Figure 15) (31).

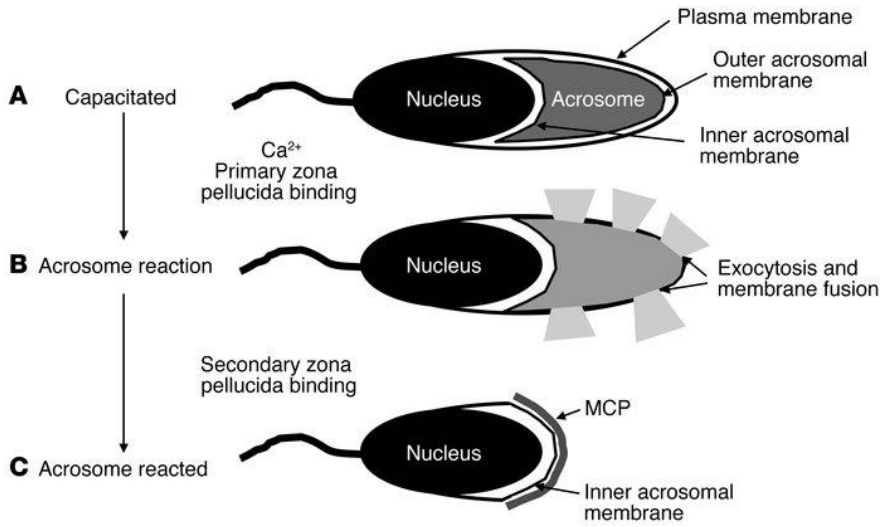


Figure 15. Acrosome Reaction.

Zona reaction; This includes changes in oolemma and zona pellucida to prevent a second spermatozoon from entering the spermatozoon. This event is regulated by chemicals synthesized during the development of oocyte II and stored in cortex granules outside the cytoplasm. These secretions of cortical granules; oolemma's molecular structure, as well as loss of spermatozoon binding sites in the zona pellucida prevents the entry of a second sppermmatozoon. The female cell is still in the oocyte II stage. As soon as the spermatozoon enters, the second maturity completes its division, discards the second pole cell and becomes the mature germ cell, the ovum. With the introduction of the spermatozoon, there is a very severe shrinkage of the ovum, a part of the cytoplasm is thrown out, the zona pellucida regains its original stiffness (shingles reaction), the other spermatozoon cannot enter (monospermia). The nucleus of the ovum is called the female pronucleus. The tail of the spermatozoon breaks, the head swells by absorbing water from the cytoplasm of the ovary, which is called the male pronucleus. As both pronucleus are approaching each other, shuttle threads appear between the irradiations that appear around the male centrosome. The membranes of both nuclei melt, chromosomes appear and meet in the middle. Thus fertilized egg cell, zygote occurs (Figure 16) (32).

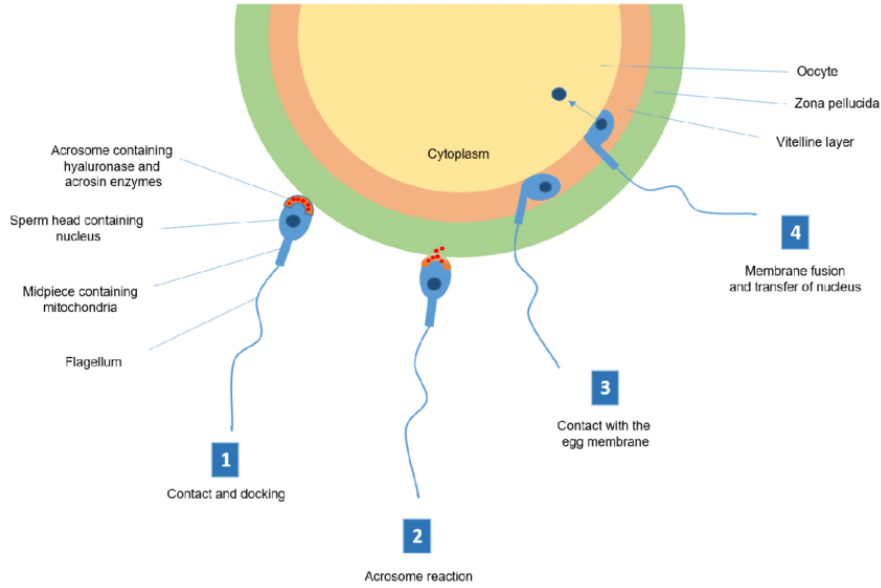


Figure 16. Schematic diagram of the acrosome reaction and fertilization (Price, 2019).

Price L. 2019. Biological effects of nicotine exposure: A narrative review of the scientific literature. F1000 Research 8:1586

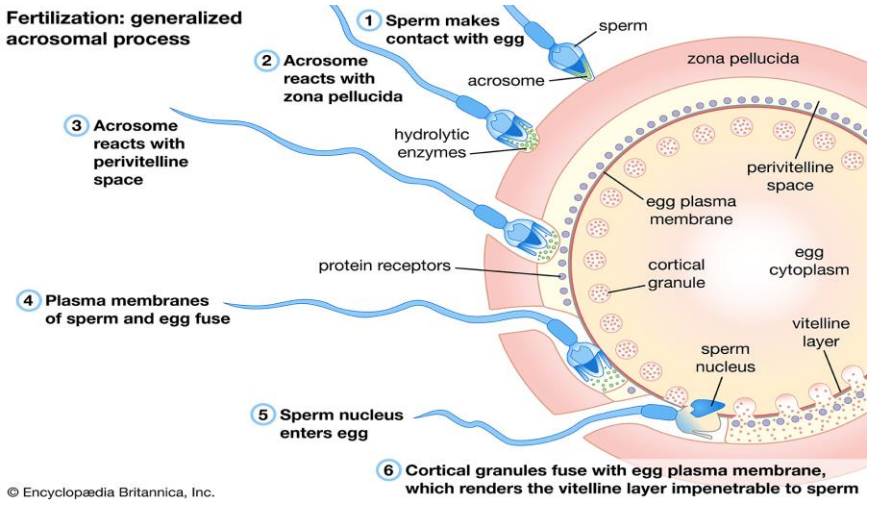


Figure 17. Formation of fertilization.

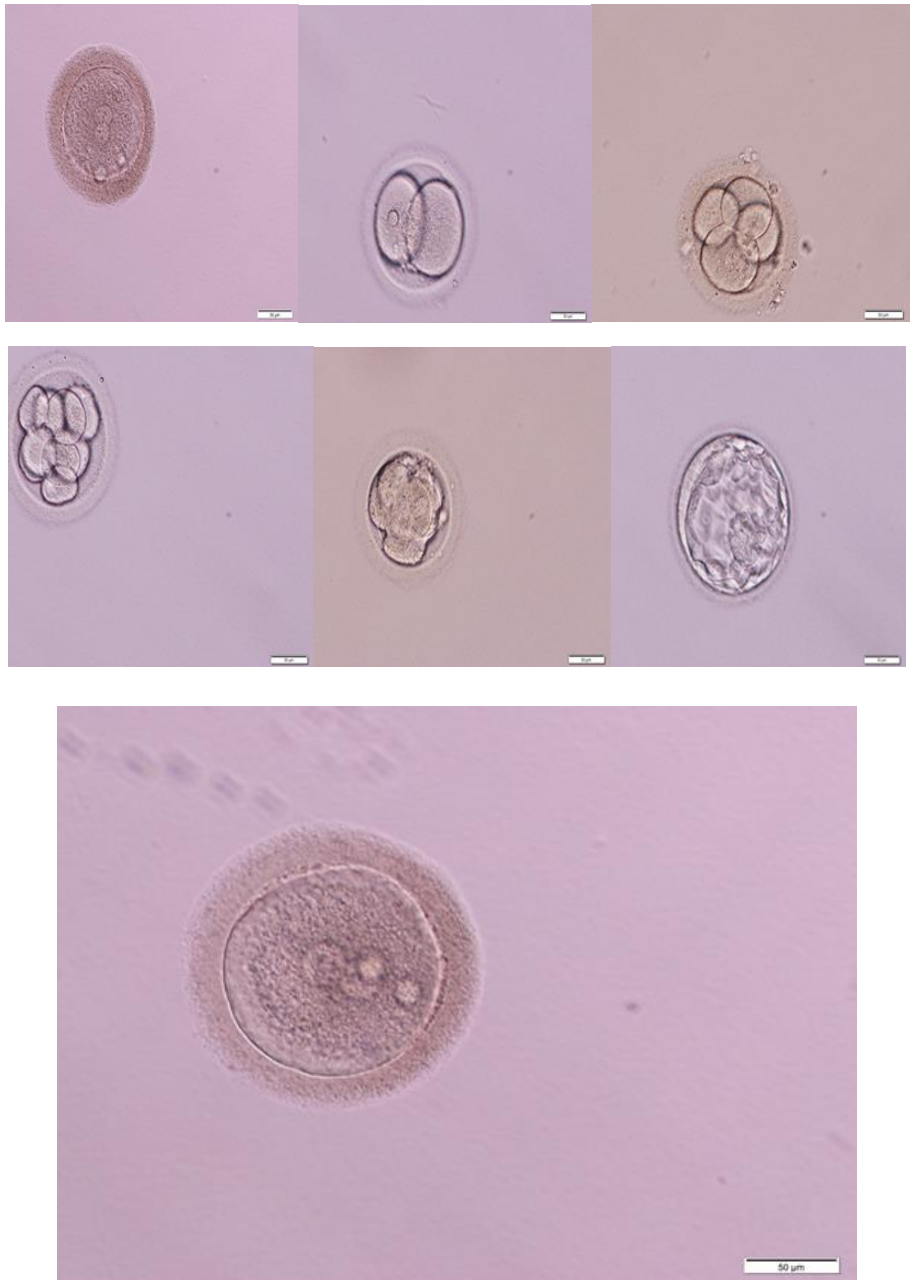


Figure 18. Embryo development and fertilization.

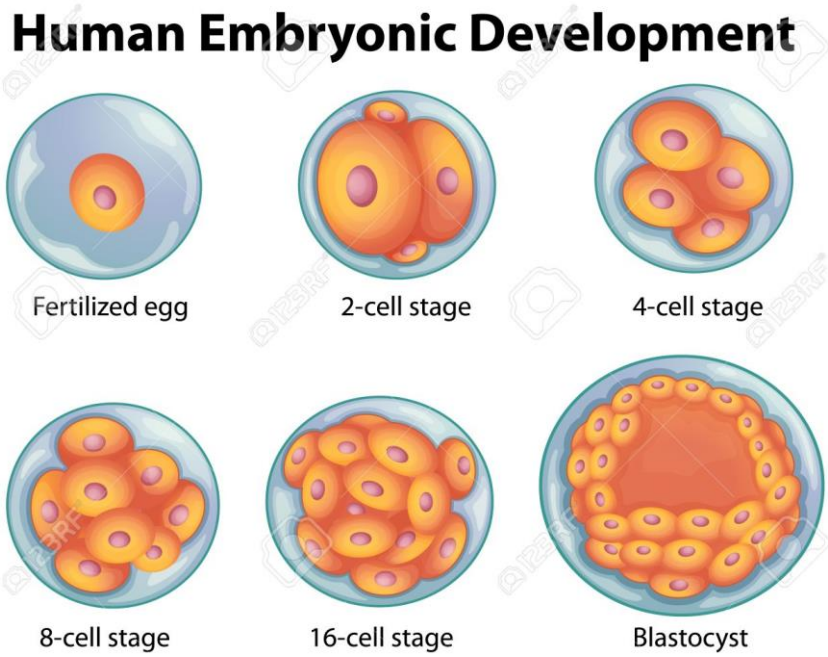


Figure 19. Illustration showing stages in human embryonic development (https://www.123rf.com/photo_16988272_illustration-showing-stages-in-human-embryonic-development.html, Access; 07.11.2020).

Infertility

Infertility was common in developing countries around the world, but did not affect 30% of couples. (33). nfertility causes depression, anxiety, social isolation, sexual dysfunction and decreased quality of life. When infertility is compared with a somatic disease such as cancer, it is stated that it affects the individual's life at a similar level (34). Infertility is defined as a woman's inability to conceive despite regular sexual intercourse for at least one year without any protection.

Infertility is an important problem that brings together medical, psychiatric, psychological and social problems (35). In women, there is a decrease in fertility depending on age, so if patients over 35 years of age do not develop pregnancy within 6 months, infertility is diagnosed and the treatment process begins. If the patient is over 40 years of age, the patient had a history of previous pelvic surgery, history of oligoamenorrhea, chemo-radiotherapy, history of male subfertility, history of suspected utero-tubo-peritoneal disease, and so on. should be started immediately without waiting for 6 months (36). The prevalence of unexplained infertility varies between 22-28%. In a study performed in infertile couples; 21% under 35 years and 26% over 35 years of age (37).

The causes of infertility are caused by 40% of women, 40% of men, 20% of both. Approximately 10-20% of infertile couples have unexplained infertility. Couples of unknown etiology have a 56% chance of fertility within 3-5 years. After 5 years, this rate drops to 30%. Among the causes of infertility, 55-75% of primary infertility and 25-40% of secondary infertility have been reported (38). Invitro fertilization is a technique of ovarian ovulation, laboratory fertilization, and placement in the uterus of the patient. The chance of implantation in assisted reproductive techniques is generally around 30% if 3 or more zygotes are transferred (39). Unexplained causes of infertility include cervical pathologies, transport-related problems, mild ovulatory dysfunction, embryo implantation failure, endometriosis, follicle non-rupture (LUF), antioxidant imbalance,

increased peritoneal macrophage activity, sperm-oocyte or shingles. more prospective studies should be performed on this subject (40). The formation of highly reactive but unstable free radicals that are formed physiologically in the organism and their removal by antioxidant mechanisms are always in balance. Oxidative stress is the disruption of this balance in favor of free radicals (41). Free radicals in physiological doses have important effects on ovulation, folliculogenesis, oocyte maturation, implantation and embryo development. If we look at female infertility; endometriosis, polycystic ovary syndrome and unexplained infertility pathogenesis (42). Therefore, there are studies proving that the addition of antioxidants to culture media in patients who are planned for in vitro fertilization by oral or intravenous administration of antioxidant agents to patients in this group may increase fertility success and assisted reproductive techniques in patients (43). Many new techniques have been developed to minimize the damage of oocytes, sperm and embryo to increase pregnancy outcomes. The most commonly used procedures are in vitro fertilization (IVF) and embryo transfer and intrastoplasmic sperm injection (ICSI) procedure. Oxidative stress is an important factor in the success of IVF. Oocyte and granulosa cells work in correlation with each other. Granulosa cells are useful for growth-maturation of oocytes, fertilization; they produce antioxidants to protect the oocyte from apoptosis, which is enhanced by oxidative stress. Oocytes develop around the follicular fluid. Follicular fluid affects oocyte quality, ability to interact with sperm, implantation and embryonic development of the embryo. In the

absence or absence of antioxidants in the follicular fluid; Causes an increase in ROS (Reactive Oxygen Species) levels. In recent studies, follicular fluid and granulosa cells significantly affect the quality and activity of the oocyte, so the results obtained by examining the follicle fluid content can be used to determine the etiology of unexplained infertility, but also to determine the success of IVF / ICSI (44). In a study, it was stated that the decrease in total antioxidant levels leading to growth of follicles was lower in live pregnancies formed with high quality oocytes (44). Mature oocyte formation and embryo survival depends on pre-existing maternal deposits. mRNA, proteins, and other factors, especially mitochondria, accumulate during oocyte growth and maturation and reduce embryo quality if these components are deficient, dysfunctional or abnormally functioning (45). In another study, abnormal oocyte production, impaired mitochondrial activity, and reduced production of ATP in infertile patients over the age of 35 limit normal embryo development. Mitochondrial pool system deterioration with increased oxidative stress and poor quality embryo development can be said.

If the causes of infertility are classified according to population screening studies:

1. Male factor (26%) (hypogonadism, testicular problems, seminiferous tubule dysfunction)
2. Ovulatory dysfunction 21%
3. Tubal problems 14%
4. Endometriosis 6%

5. Coital problems 6%
6. Cervical factor 3%
7. Unexplained infertility can be evaluated as 28% (46).

Although the distribution of causes of infertility varies demographically (center-to-center, population-to-population), causes of infertility have not changed significantly over the past 25-30 years (47).

The reproductive process in humans is complex. Causes of infertility can be a problem at every stage of the reproductive process. For healthy reproduction:

- The sperm must be present in the cervix or vagina close to the cervix at a time when the oocyte is either ovulated or / or will be ovulated recently, be able to reach and fertilize the oocyte through the cervix, endometrium and tuba (male factor).• Düzenli ve önceden tahmin edilebilir- takip edilebilir şekilde matür bir oosit ovüle edilmelidir (overyan faktör).
- The cervix should filter the incoming sperm and the sperm from the cervical canal must reach the endometrium (cervical factor).
- The uterus should be receptive to the embryo reaching its cavity and be able to provide growth and development for the embryo it will develop (uterine factor).
- The fallopian tubes should be able to capture the ovocyte oocyte, allow fertilization of the oocyte with sperm, and effectively carry the embryo formed by ciliary movements into the cavity

(tubal factor). Each infertility assessment should begin with a careful history and physical examination. A good history and physical examination will help to focus on researching possible factors among the causes of infertility.

Infertility Assessment in Women

Medical history and physical examination in women should include (48).

Story

- Gravida, parity, pregnancy outcomes and associated complications
- Mentral history (menarche age, cycle time, characteristics, dysmenorrhea, amount of bleeding)
- Medical, surgical and gynecological history (Sexually transmitted diseases, pelvic inflammatory diseases, Pap smear results and treatments, symptoms of thyroid diseases, galactorrhea, signs of hirsutism, pelvic or abdominal pain, questioning of past surgeries)
- Sexual history (questioning sexual dysfunction, frequency of sexual intercourse, lubricant use) • Family history (questioning hereditary diseases)
- Personal and lifestyle history (age, occupation, frequency of exercise, stress, diet, smoking, alcohol and substance use, investigation of environmental exposures)
- Medications and allergies used

• Duration of infertility and previous treatments

Physical Examination

• Patient's weight and body mass index
• Thyroid gland enlargement, nodularity or tension
• Breast examination, secretions and characteristics
• Findings of androgenism

Pelvic or abdominal tension, tenderness, organomegaly or mass

• Vaginal or cervical abnormalities, secretions and discharge
• Uterus size, shape, position and mobility
• Adnexal mass or tension
• Mass, tension or nodularity in the sac in the ear

Screening Tests

Pap smear should be recommended to all sexually active individuals with cervix over 21 years of age. Blood group and Rh antibody determination, hepatitis markers should be studied. Other additional screening tests should be performed if necessary in the light of medical history and physical examination.

Evaluatıon of Uterıne Cavıty and Tubal Patency

Tubal pathologies account for about 14% of all causes of infertility (46). Pelvic inflammatory disease (PIH), septic abortion, ruptured appendix, endometriosis, previous tubal surgery and history of ectopic pregnancy are the most common causes. PIH by laparoscopy has been

shown to increase the number and severity of pelvic infection. In general, the rate of infertility increases to 10-12% after one attack, 23-35% after two attacks, and 54-75% after three attacks (49). The mechanism of tubal factor infertility is the anatomical abnormalities that prevent the encounter of sperm and ovum. Tubal blockages may be in the proximal, middle or distal part of the fallopian tubes. Proximal tubal obstructions prevent sperm from reaching the ampulla part of the fallopian tubes where fertilization occurs. Distal tubal obstructions prevent the ovum from being overexpressed.

Transvaginal Ultrasonography and Saline Sonohisterography
Transvaginal Ultrasonography

(Tv-USG) is a frequently used method for the evaluation of uterine differentiators and tubas in infertile women. Saline sonohysterography requires simultaneous Tv-USG delivery of saline into the cavity with a catheter. It is very sensitive and can even show small pathologies (50) however, it is not sufficient to provide useful information about endometrial function and sensitivity.

Hysterosalpingography

It is the most widely used, valid and effective initial test for the evaluation of tubal patency. The advantages of the test are that it is less invasive than laparoscopy, does not require general anesthesia and is cheaper than laparoscopy. Hysterosalpingography (HSG) shows the size and shape of the uterine cavity, most of the uterine developmental abnormalities (unicorn, bikorn, septate uterus, dysmorphic uterine

subtypes, infantile type uterus, septate uterus, bicorporeal uterus, didelfis), submucous shows the internal structure of the lumen. These evaluations cannot be performed by laparoscopy.

Disadvantages of the test are radiation exposure and rare complications related to infection (51). The best time for HSG extraction is to minimize infection and intrauterine hemorrhage or clots to affect the quality of the images, and to prevent shooting during a possible pregnancy. Day. It does not require any preliminary preparation. However, a nonsteroidal anti-inflammatory drug (NSAID) to be taken 30-60 minutes before the procedure can help to reduce the feeling of discomfort associated with the procedure. Infection-related complications are very rare (1-3%), even in high-risk populations (51-52). Postponing HSG exposure for several weeks after an acute PIH attack may minimize the risk of a possible infection. It is controversial whether the contrast agent used in HSG extraction is soluble in water or oil. It is thought by some researchers that oil-soluble substances are insufficient to show the internal structure of the tubes due to excessive thick consistency, their distribution in the pelvis is insufficient, there are risks of granulomatous reaction, intravenous passage and embolism, and therefore, water-soluble substances should be used (53-54). Contrary-looking researchers argue that granulomatous reactions, intravenous passage and embolism are very rare, and refer to studies that show increased fertility in the months following HSG with fat-soluble substances (55, 56). Uterine anomalies were revised and classified in

ESHRE / ESGE (European Society for Human Reproduction and Embryology) in 2013 (57).

The normal uterine cavity is smooth, symmetrical and inverted triangular. It is the largest in the fundus. Dysmorphic uterus is T-shaped and characterized by a narrow cavity from the isthmus to the fundus. Septate 7 uterus can also be partially seen from the fundus to the cavity and may form complete septum extending from the cervix to the vagina. Y-shaped. The diagnosis of bikornu uterus alone cannot be decisive even if there is a high degree of suspicion with HSG. 2D-3D ultrasonography, MRI or laparoscopy are required. Fibers and polyps produce curved and generally smooth surface filling defects according to their size. Adhesions usually produce irregular filling defects. HSG sensitivity in the evaluation of tubal patency in the infertile population is moderate compared to laparoscopy. In 84% of cases, HSG has been shown to miss at least one pathology (58). In addition, abnormal findings were detected by laparoscopy in 21-68% of patients in whom HSG detected normal findings (59-60). HSG has 65% ability to detect when tubes are open and 83% when tubes are closed. In other words, even if bilateral tubes are found to be obstructed in HSG, they are likely to be open. However, if tubes are detected open in HSG, it is unlikely that these tubes will actually become clogged (61,62).

Hysteroscopy

Hysteroscopy is the gold standard method for the diagnosis and treatment of intrauterine pathologies that may lead to infertility. Hysteroscopes with a diameter of 3-4 mm can be used even in polyclinic conditions. For larger pathologies, operative hysteroscopes are used which require more functional features.

Laparoscopy

Laparoscopy is the most reliable diagnostic test available for the evaluation of tubal patency. Negative sides are general anesthesia, expensive and invasive procedure. During laparoscopy, uterus, anterior posterior cul de sac cavities, bilateral tubas, ovaries and ova fossa should be examined systematically. Tubal patens are evaluated by gently injecting methylene blue or indigo carmine with an appropriate cannula or intrauterine manipulator placed in the cervix. Indigo carmine dye should be preferred to avoid acute methemoglobinemia caused by methylene blue in patients with glucose 6 phosphate dehydrogenase deficiency (63).
It has mastery of pelvic anatomy, ability to detect endometriosis, and can be used as a diagnostic and therapeutic tool.

Evaluation of Ovulation

Ovulation normally occurs with the release of a mature oocyte from the ovary every month. Ovulation disorders affect 21-25% of infertile couples (46).

The World Health Organization examines ovulation disorders in three groups:

Group 1: Hypothalamo-pituitary insufficiency (10%). It is associated with low gonadotropin levels. Endogenous estrogen is low. Endometrial proliferation cannot occur. Therefore, it cannot produce a bleeding response to the progesterone withdrawal test. It can be seen in cachectic women with a body mass index below 20 and women who exercise intensively.

Group 2: Hypothalamic-pituitary-ovarian axis dysfunction: Polycystic ovary syndrome and hyperprolactinemic amenorrhea are in this group. It constitutes 85% of ovulation disorders. Gonadotropin levels are normal. Endogenous estrogen can be produced and may respond to the progesterone withdrawal test bleeding.

Group 3: Ovarian failure (5%). It is associated with high gonadotropin levels. In the reproductive age, it usually occurs due to chemotherapy, radiation or surgery that will cause ovarian injury. It may also be idiopathic. These women with low follicle reserve cannot respond to follicle stimulating hormone stimulation. There is no definitive diagnostic criterion in the evaluation of ovulation. No test is the best test either. The only definite criterion of ovulation is pregnancy.

Menstrual history

Menstrual history alone is sufficient to diagnose ovulatory dysfunction. Menstruation of a woman with normal ovulation is regular and consistent. The volume and durations are similar. Most of

the cycles of a woman with regular menstrual cycles are ovulatory. Women who have irregular and sparse menstruation are not regular at ovulation.

Basal body temperature

Basal body temperature (BVS) is the body's resting temperature. It should be measured without waking up after waking up in the morning. It is used with the thermogenic effect of progesterone to detect ovulation. As progesterone increases, basal body temperature also increases. Daily measurements can be easily detected by keeping records. During the luteal phase, the basal body temperature increases by 0.4-0.8 ° C. Menstrual falls again with the onset. Menstruation begins approximately 12 days after the increase in temperature (64). The highest fertility interval in cycles followed by BVS is 9 days just before the mid-cycle increase of BVS, and sexual intercourse is recommended every other day.

Serum Progesterone Concentration

Measurement of serum progesterone at appropriate time is a simple and objective test of ovulatory function assessment. Mid-luteal progesterone measurement is the most cost-effective test, although weekly measurements are required in non-regular cycles. Normally, progesterone levels are below 1 ng / mL in follicular phase, gradually increase with LH peak and 7-8 after ovulation. reaches the highest value per day. Progesterone levels below 3 ng / mL in the sample taken at the appropriate time indicate anovulation (65). High levels of

progesterone release indicate sufficient progesterone release from the corpus luteum and retroactive ovulation. The ideal day for serum progesterone level measurement is 7 days before the expected menstrual date. In other words, the ideal test day for a woman with a cycle of 28 days is the 21st day, while the ideal test day for a woman with a cycle of 35 days is the 28th day of the cycle. Progesterone levels should be repeated weekly in women whose cycle day is not known (66). There was no consensus on the lowest progesterone value indicating ovulation. In several studies, progesterone levels predicting ovulation range from 5 ng / mL to 10 ng / mL (67, 68). According to WHO, 5.6 ng / mL is an indicator of ovulation. The commonly used value in daily practice is 10 ng / mL (69). Progesterone levels above these reference values are indicative of luteinization, and without ovulation, their elevation may indicate non-ruptured follicular syndrome.

Urinary Luteinized Hormone excretion

Also known as ovulation prediction kits or luteinized hormone (LH) kits. The LH peak lasts approximately 48 hours and is then rapidly excreted in the urine. With these kits, the LH level exceeding the threshold value detected in the urine after LH peak positivizes the test. Taking into account the total length of the cycle, the test is performed daily starting 2-3 days before the estimated LH peak. The most suitable time for the test is usually between 16.00 and 22.00, since the LH peak is against the morning and the excretion of urine in the first few hours is not sufficient. It is not necessary to repeat the test after

the first positive test (66). 10 Ovulation usually occurs within 48 hours after the LH peak (70). Therefore, the highest fertility time is 2 days after LH peak. The day after the first positive test is the best day for timed intercourse or intra uterine insemination.

Evaluatıon of Over Reserve

The ovarian reserve defines the remaining oocyte pool in the ovaries, predicting the possibility of conception. As all screening tests have a protective disease, ovarian reserve tests consist of tests to detect reduced ovarian reserve (AOR). The ideal ovarian reserve test should predict the primordial follicle pool that constitutes the actual ovarian reserve. However, it is difficult to determine the number and quality of primordial follicles. Follicular development is a long process, which takes 6-8 months from primordial follicle to antral follicle (71). First step screening tests are follicle stimulating hormone (FSH), luteinizing hormone (LH) and estrogen values which provide information about hypothalamo-pituitary axis functions but have historical value in terms of ovarian reserve. Age is the most important indicator of ovarian reserve which decreases rapidly from birth to menopause. Transvaginal ultrasonography-guided antral follicle count (AFS) and anti-mullerian hormone measurement are among the newest and most reliable screening tests that predict the ovarian reserve used recently. In 2011, ESHRE (European Society of Human Reproduction and Embryology) established a consensus for the definition of low ovarian reserve and presented the Bologna criteria. According to Bologna criteria, 2 of the following 3 definitions are

defined as low ovarian reserve and poor response in IVF treatment (72).

1. Having a risk factor for advanced age (<40) or decreased ovarian reserve
2. Previous history of low ovarian reserve (obtaining 3 or fewer oocytes with a conventional stimulation program)
3. Abnormal ovarian reserve test (AFS <5-7 follicle or anti-mullerian hormone
4. (AMH) <0.5-1.1 ng / ml)

Basal Follicle Stimulating Hormone and Estradiol Levels

High FSH is one of the earliest indicators of ovarian aging in women. Since the levels vary throughout the cycle, the most appropriate time for level follow-up is 2-4. the days. As the FSH level increases on the 3rd day, maximum estradiol level, the number of oocytes obtainable by induction, pregnancy or live birth rates decrease (73-74). In current tests, values higher than 10 IU / L have a high prediction of 80-100% in poor response to ovarian stimulation (75). Basal serum estradiol level alone has a low value in predicting ovarian reserve. But basal FSH contributes to the interpretation.

Inhibin B

Inhibin B is released from the granulosa cells of small antral follicles in the follicular phase. It varies greatly in each cycle and in each cycle itself. Therefore, it is not accepted as a reliable marker for predicting ovarian reserve (76, 77).

Ovary Volume

By generalizing, the ovarian volume decreases as the follicle pool in the ovary decreases (78). However, the measurability of this parameter limits its generalizability due to its high variability due to ovarian pathologies such as cycle, endometrioma and polycystic ovary. Ovarian volume is generally correlated with the number of oocytes collected, but is not correlated with pregnancy rates (79-80).

Anti Müllerian Hormone

Anti-mullerian hormone (AMH) is released from granulosa cells of preantral and small antral follicles (81). The number of small antral follicles is associated with ovarian reserve and becomes undetectable until menopause (82). Since AMH originates from preantral and antral follicles, its level is independent of FSH and LH levels. It is unaffected by the cycle phase, with little variation between cycles (83). Low levels were associated with poor response to ovarian stimulation, small numbers of oocytes, and low pregnancy rates (78). In 2011, the limit of AMH was accepted as 0.5-1.1 ng / ml in the Bologna criteria, which defines the reduced ovarian reserve prepared by ESHRE (72).

Number of Antral Follicles

A woman of reproductive age has an average of 20-150 follicles that continue to grow and mature at any given time. Tv-USG can only be seen in patients over 2 mm. Follicles over 2 mm have reached a FSH susceptible stage (84). Histological studies have shown a relationship

between ovarian antral follicles and the remaining primordial follicles (85). Therefore, the number of antral follicles provides an indirect assessment of ovarian reserve. It is determined by calculating the total number of antral follicles measured between 2-10 mm in Tv-USG. Most of the antral follicles with Tv-USG will not go to atresia because they cannot receive FSH stimulation.

The number of anthral follicles is also correlated with the number of oocytes that will respond to ovarian stimulation. This suggests that follicles that will go to atresia with exogenous gonadotropins can be saved in IVF treatment (86).

Several studies have been conducted to evaluate the threshold value of antral follicle counts before IVF treatment in high-risk groups for decreased ovarian reserve. Threshold value When 3-4 follicles were taken, the number of oocytes to be obtained and it was found to be critical in obtaining pregnancy (79). In 2011, the limit criteria of antral follicles were defined as 5-7 follicles according to the Bologna criteria which define the reduced ovarian reserve prepared by ESHRE (72).

Infertility Assessment in Male

Male infertility can be divided into four main groups:

1. Hypothalamic-pituitary disorders (secondary hypogonadism) 1-2%
2. Primary hypogonadism 30-40%
3. Sperm transport disorders 10-20%
4. Idiopathic 40-50% Male medical history and physical examination should include:

STORY

- Frequency and timing of sexual intercourse
- Previous history of fertility, duration of infertility
- Childhood diseases and developmental history
- Previous surgeries and indications
- Questioning of systemic diseases
- Drug use and allergies
- Sexual history and questioning of sexually transmitted diseases
- Questioning exposure to gonodal toxins
- Occupation, smoking, alcohol and substance use

Physical Examination

- Penis examination and urethral opening
- Testicular palpation and testicular volume
- Presence and hardness of vasa and epididymis
- Varicocele presence
- Determination of secondary sex characters such as body posture, hair distribution, breast development
- Rectal examination

If the infertility assessment is performed by the gynecologist, the male physical examination may be postponed unless there is an abnormal history in the infertile couple assessment or a problem is detected in the semen analysis. However, abnormal semen analysis or history

abnormalities are indicative for physical examination and should be examined by the urologist.

Semen Analysis

Semen analysis is the most important and most valuable laboratory test in the evaluation of male infertility. It shows the seriousness of male factor in infertility. Semen collection instructions should be explained to patients in detail. Sexual abstinence period should be 2-5 days. Shorter abstinence causes a decrease in semen volume and density, but sperm motility and morphology do not change (87). For longer abstinence periods, there is an increase in the dead, immobilized and morphologically impaired sperm with an increase in semen volume and density (88). However, in shorter sexual abstinence in severe oligospermia 14 better sperm concentrations can be achieved. The semen sample can be taken into a clean box by masturbation or special semen collection condoms that do not contain toxic substances to sperms can be used during coitus. Semen collection should ideally be in the laboratory, but if it is to be collected at home, it should be brought to the laboratory within 1 hour and stored at body temperature. Regardless of the sample collection method, semen should be analyzed within 1 hour of obtaining.

Normal Reference Values

Normal reference values do not indicate the minimum values required for fertilization. While there are many fertile men whose semen analysis is not normal, there are many male infertile patients with

normal semen analysis (89-90). The World Health Organization (WHO-WHO) defined sperm analysis of 1900 fertile men from 8 countries, who had pregnancy within 12 months, as the sub-reference values in the analysis of 5% (91).

These minimal criteria:

- Volume 1.5 mL
- Sperm concentration 15 million spermatozoa / mL
- Total sperm count 39 million spermatozoa / ejaculate
- Morphology in 4% normal form according to strict criteria
- Vitality 58%

  Progressive motility 32%
- Total (progressive + nonprogressive) motility is 40%.

Semen volume: According to the WHO study, the mean semen volume was 3.7 mL. Obstruction of the genital tract should be considered in low semen volumes associated with azoospermia or severe oligozoospermia. These obstructions may be the congenital absence of vas deferens or seminal vesicles or obstruction of the seminal vesicle or ejaculatory duct. Most of the semen volume is composed of seminal vesicles. Seminal vesicles and vas deferens develop from the same embryogenic origin. Seminal vesicles contain fructose and are alkaline. In obstruction, pH value of semen decreases and does not contain fructose and sperm. Ejaculatory duct obstructions can be diagnosed by showing dilated seminal vesicles on transrectal ultrasonography. 15 Low semen volume with normal sperm concentration is probably caused by sperm collection errors or as a

result of partial retrograde ejection. The diagnosis of retrograde ejaculation is made with the presence of sperm in urine analysis after ejaculation.

Sperm concentration and total sperm count: The lower limit reference value of sperm concentration is 15 million / mL. While men with lower values can be fertile, 10 million spermatozoa / mL is considered sufficient for in vitro fertilization (92). Azospermia is the absence of any sperm on microscopic examination. It is found in 1% of all men (93It is found in 10-15% of infertile men (94). For diagnosis, the semen is highly centrifuged and the pellet is examined at high magnification. The absence of sperm should be demonstrated in at least two examinations. Oligospermia is a sperm density of less than 15 million / mL. Less than 5 million is called severe oligospermia. Endocrine and genetic evaluation should be performed in patients with severe oligosperme. Total sperm count is obtained by multiplying sperm volume and sperm concentration.

Sperm motility, forward motion, total motile sperm count and vitality: Sperm motility is the percentage of motile sperm in the total sperm population. Sperm movements are evaluated with a rating of 0-4. Fast forward movement takes degrees 3-4, slow movement takes degree 2, non-progressive movement takes 0-1 degrees. Total motile sperm count includes advanced motile spermatozoa with grade 2- 3-4. The WHO lower limit of progressive motility is 32%. Poor sperm motility is called asthenospermia and is associated with testicular dysfunction. Antisperm antibodies, genital organ infections, partial

obstruction of ejaculatory ducts, varicocele or prolonged sexual abstinence should be questioned and examined. Immotile cilia syndrome should be considered if excessive viable but non-motile sperm is detected. It is characterized by abnormality of sperm tails and absence of tail movements. The definitive diagnosis is made by examining the sperm under electron microscopy. 16 If no motile sperm can be observed, live sperm and dead sperm can be separated by sperm viability tests. Hypoosmotic swelling and fresh sperm staining tests are among the most commonly used viability tests.

Sperm morphology: Abnormality in sperm morphology is called teratospermia. Varicocele may be associated with primary and secondary testicular insufficiencies. Sperm morphology criteria were previously based on the shape of the sperm, but now the shape, length, width, width ratio, neck and tail defects are also examined. Sperm morphology is examined by the WHO-defined Kruger (Tygerberg) or "strict kriter criteria in 1999 (94, 95). These criteria have a predictive value that is highly correlated with fertilization rate and pregnancy rates after IVF (94, 96). If normal sperm rates are above 14%, fertilization rates are high in in vitro fertilization treatment. If the normal sperm morphology rate is 4% or less, success is greatly reduced. Subsequent studies have confirmed the power of strict criteria to demonstrate the success of IVF, and have emerged as an indication for intrastoplasmic sperm injection (ICSI) in the treatment of IVF in severe teratozoospermia with a morphology rate of 0-4% (97, 98).

Round cells and leukospermia: Leukocytes, as well as immature sperm (spermatogonia, round spermatids, spermatocytes), prostate cells and epithelial cells appear as round cells in routine semen analysis and cannot be separated from each other. When the number of round cells exceeds 5 million / mL, additional tests, such as special stains and immunohistochemical tests, are required to separate the leukocytes for the diagnosis of leukospermia. Although not evidence-based, the recommended limit for leukospermia is 1 million leukocytes / mL (99). It is an indication for semen culture. Although leukospermia may adversely affect sperm morphology and function, new studies have not shown a relationship between leukospermia and abnormal sperm parameters (100).

Semen viscosity: It has little clinical significance. A rating of 0-4 is made depending on the individual. The causes of hyperviscus semen have not been reported. It may be associated with genital infections and abnormalities of the prostate and seminal vesicle ducts.

Special Sperm Analysis Tests

Sperm Autoantibodies: The blood testicular barrier protects the sperm from the immune system. However, as a result of ductus obstruction, genital infections, testicular 17 torsion, trauma or surgical intervention in which this barrier may be impaired, sperm may encounter blood and antibodies to sperm may occur. Antibodies formed against sperm affect sperm motility and impair fertilization. The antisperm antibody test is considered to be significant if more than 50% of spermatozoa are encapsulated by antibodies. In a study

conducted in 2016, when sperm kinematics were compared, no difference was found between positive and negative sperm antibodies (101). The WHO guidelines for human semen examination and evaluation published in 2010 showed that anti-sperm autoantibodies can be seen in normal sperm concentration, motility and morphology, and are clinically insignificant in these situations (102).

Biochemistry of Semen: Clinical use is rare. Sperm creatinine phosphokinase is an important enzyme in sperm production and transport. Fructose level can be used for seminal vesicle functions. The presence or absence of abortion may indicate congenital absence or obstruction of vas deferens and seminal vesicles. Diagnosis is confirmed by the presence of dilated seminal vesicles on transrectal ultrasound.

Semen Culture: It is often applied when leukospermia is detected in the semen sample. It does not carry diagnostic values to a large extent. When sampling, care should be taken to avoid skin contamination.

Sperm Oocyte Penetration Test: In this test, which was defined in the 1970s, human sperm were incubated with oocytes from guinea pigs and penetrating oocytes and penetrating sperm rates were examined. Standardization of the test is difficult. False positive and false negative rates are high. It is useless, expensive, time consuming.

Binding to Human Zone Test: Tests the ability of sperm to penetrate oocytes without shingles. It cannot show penetration against shingles. It is used to predict IVF results, but the difficulty in finding human

shingles and oocytes and the technical difficulties of testing have greatly reduced their clinical use.

Computer Assisted Sperm Analysis: It provides precise, automated and objective evaluations. However, the test results depend on the operator's experience, technical conditions and sperm concentration. Although the results of some studies have been shown to have an effect on the foresight of in vivo and in vitro fertilization, it has not been shown in some studies (103-104).

Acrosome Reaction: Acrosome is a membrane-bound structure that contains proteolytic enzymes in the head region of the sperm. The reaction of the sperm to the shingles pellucida begins. Disorders in acrosome reactions prevent sperm from binding to shingles. The reaction is measured with immune fluorescent and specific monoclonal antibodies.

Sperm Chromatin Structure and DNA: DNA integrity and robustness play an important role in normal embryo development. Sperm DNA integrity is ensured by cross-disulfide bonds between protamines and this structure forms the nucleus chromatin structure. Sperm DNA damage can be caused by intrinsic factors such as protamine deficiency or DNA mutations. Extrinsic factors such as heat, radiation and gonadotoxins can also cause DNA damage. DNA fragmentation is called denatured or damaged sperm DNA that becomes irreparable.

Several tests have been developed to measure sperm DNA fragmentation rates. Direct methods such as single-cell gel electrophoresis assay (Comet) and terminal deoxynucleotidyl transferase dUTP nick and labeling (TUNEL) assay can analyze the number of fractures in DNA. Indirect tests such as Sperm Chromatin Structure Assay (SCSA) detect abnormal chromatin structure (105). The DNA fragmentation index breakpoint was proposed to be ≥ 17.5% for the TUNEL test in a 2013 study (106).

Sperm DNA damage was found in the majority of infertile men. Studies have shown that spermatozoa with a high DNA fragmentation index are associated with decreased oocyte fertilization, poor embryo quality, decreased pregnancy rates, and recurrent pregnancy losses (107, 108). However, despite DNA damage, it has been shown that sperm can be viable and have a normal morphology and may even fertilize the oocyte. Because of this limited relationship between abnormal DNA integrity and reproductive outcomes, sperm DNA fragmentation index could not be a routine test for infertile couples.

Unexplained Infertility

Unexplained infertility is a diagnosis that can be made after a systematic assessment fails to determine the cause. It constitutes 40% of female infertility and 10-30% of infertile couples. The variation in incidence in the studies may be that the selection criteria and ages of the study group differ. In the diagnosis of unexplained infertility, ovulation, tubal patency, normal uterine cavity, normal semen analysis

and adequate oocyte reserve should be documented (109). In addition to these tests, post-coital test for cervical pathologies, tests for dating endometrium 19 and routine laparoscopy have been abandoned due to lack of validity or little efficacy that will not alter the evaluation. There are two possible explanations for unexplained infertility.

Either there is actually no abnormality. However, the fecundity of the couple is at the lowest level of the normal range depending on the age of the possible couples or the age of reproduction. Infertility cannot be diagnosed with the available diagnostic tests. The natural reduction in fertility with increasing age is probably the majority of those diagnosed with unexplained infertility, and is therefore up to 2 times higher in women over 35 years of age (110). Possible causes may be cervical mucus, capacitation, acrosome reactions, problems of penetration to zona pelludis, defective oocytes and / or embryos, abnormalities in tubal motility and implantations. For infertile couples, the unexplained infertility diagnostic process is troublesome, and the lack of a cause for them may also mean that there is no cure.

In fact, the prognosis is worse in couples with infertility duration of more than 3 years or women over 35 years of age (111). If the infertility period is less than 2 years and the female age is under 35 years, the prognosis may be better without any treatment. It is recommended that infertility treatment be started if there is an infertility period of 2 years or more or if the female age is above 35 years of age (112). Every 1-month increase in infertility leads to a 2% monthly decrease in pregnancy probability and a 25% annual

decrease. Every 1-year increase in female age above 30 years of age causes a 9% decrease in pregnancy rate (112). It is important to understand the effect of the duration of infertility. It should be noted that the ratio of spontaneous pregnancies with wait-and-see approach is higher in couples with relatively shorter infertility period, and the probability of pregnancy without treatment decreases rapidly as the infertility period increases and the couple's age progresses.

All of the treatments given for unexplained infertility, except IVF, are similar in success, and therefore the option of IVF should be prioritized for couples with prolonged infertility and less likely to become pregnant without treatment. Since the cause of unexplained infertility is unknown, the treatments given are also empirical. Although methods change, the aim is to bring more sperm and oocytes together at the right place and at the right time.

Cryopreservation Techniques

Especially in the mid-1980s with the use of cryotechnology, the concept of freezing embryos without transfer came to the agenda. Initially, the slow freezing technique used successfully cleaved embryos or blastocysts, and pregnancy was achieved in the following period. However, low fertilization (approximately 50%) and pregnancy rates in oocyte freeze thaw cycles have led to new technological searches (113).

Rapid crystal freezing using high concentrations of cryoprotectant, called vitrification, resulted in less crystal formation in the cell, resulting in less cell damage and better results. According to the results of a population-based cohort study of approximately 30,000 cases, more live birth rates were reported with vitrified blastocyst transfer than slow freezing (114).

As a result, in today's practice, vitrification has become almost standard practice for both embryo and oocyte freezing.

When to Transfer Frozen Embryo?

In the presence of acceptable success rates of fresh cycles for more than 30 years, it took time for the DET cycles to find a place. With the introduction of recombinant technologies and GnRH antagonists since 1990s, ART has gained a new dimension in terms of safe and patient-friendly approaches. Specifically, the concept of DET has been questioned again, as the concept of preimplantation genetic testing has been brought up and frequently implemented. More patient-friendly approaches have been brought up with antagonists used to prevent premature luteinization, and iatrogenic complication rates called ovarian hypersituration syndrome (OHSS) have been reduced by almost half (50%) compared to agonist cycles (115). In the presence of this condition, a decrease in implantation and pregnancy rates has been proposed due to a shift in the implantation window (116). In cases with premature P elevation, freezing embryos without fresh transfer and postponing transfer to a later date increases pregnancy and live birth rates (117). The use of pre-implantation genetic testing,

especially screening (PGD), has become a popular topic in recent years. PGD, which is widely used for advanced female age, recurrent implantation failure, recurrent pregnancy losses and severe male infertility, has undergone various modifications with the development of technology. Because of the possible embryo damage and mosaicism, which is revealed by blastomer biopsy + FISH technology, called version 1, 23 chromosome analysis has been brought up by biopsy from trophectoderm cells. Especially with the introduction of technologies such as CGH, NGS, which allow 23 chromosome analysis, the concept of DET has become even more important. Since most of these technologies rarely yield results within 24 hours, the process of freezing the biopsied embryo is frequently used. Biopsy of the obtained embryos, followed by freezing, dissolving and transferring the euploid embryos after the result has become almost routine practice today. Another use of DET cycles is to pool the embryos obtained by stimulating weak ovarian reserve cases in successive cycles. It is known that the embryos obtained by this method are then thawed and transfer rates are almost as normal as the cases with normal response (118).

Comparison of Obstetric and Neolatal Outcomes of Fresh and Frozen Embryo Transfer Cyclics

One of the topics discussed today is obstetric and neonatal outcomes of ART pregnancies. Large observational studies have shown that ART pregnancies are more risky in terms of low birth weight (LBW), SGA infant, preterm birth (PTD) and perinatal mortality compared to

natural pregnancies (119). tetric and neonatal outcomes have also been discussed in fresh and DET cycles. In a retrospective large Scandinavian cohort study, a total of 6647 single singleton live births were compared with 42.242 fresh cycles and 288.542 natural conception babies, resulting in fewer LBW (OR: 0.8), PTD (OR: 0.8) and SGA (OR: 0.7) (120). However, postterm pregnancy (OR: 1.49), LGA (OR: 1.4) and macrosomia (OR: 1.5) were more common. The risk for LBW, SGA and LGA was found to be higher with DET than natural conception babies. In another large cohort study, a total of 277,042 singleton live births were evaluated; Less PTD, LBW and SGA were detected by DET (121). However, the prevalence of LGA was found to be high in babies born with DET, and placenta accreta (OR: 3.1) and gestational hypertension (OR: 1.5) were significantly higher. According to the results of another observational study, both gestational age and birth weight were found to be higher with tekDET than fresh cycles. 19 However, hypertensive complications were observed more frequently in DET results and there was no significant difference in terms of major congenital malformations with fresh-cycle infants (2.6% vs 2.8% -OR: 0.9). According to the results of meta-analysis in which single embryos obtained from fresh embryo transfer and DET cycles were reviewed and a total of 11 observational studies were compiled, PTD, SGA, LBW parameters were found to be less in DET babies (122). Table 1 summarizes the clinical outcomes of DET cycles.

TABLE 1: Clinical results of DET cycles.	
Decreasing frequency with DET	Increased frequency with DET
Antepartum hemorrhage (RR: 0.6)	Post-term pregnancy (OR: 1,4)
Preterm labor (RR: 0.8)	LGA (OR: 1.4)
SGA (RR: 0.4)	Macrosomy (OR: 1.5)
Low birth weight (OR: 0.69)	Placenta location anomalies

Comparison of Pregnancy Rates of Fresh Frozen Embryo Transfer

It has been suggested that frozen embryo transfer cycles offer higher pregnancy rates compared to fresh cycles due to the fact that they provide more optimal uterine microenvironment and receptivity for various reasons. According to the results of a recent meta-analysis, DET cycles and clinical and ongoing pregnancies were reported to be higher than fresh cycles (RR: 1.3 95% CI: 1.1-1.5) (123This meta-analysis is based on the results of 3 randomized controlled trials of a total of 633 subjects. When the analysis is examined more closely, it is observed that high response cases were handled in 2 studies, slow freezing in 2PN stage and vitrification in 2PN stage in 1 study. In summary, based on the results of quite heterogeneous and inadequate power studies, the assumption that better pregnancies can be achieved with DET remains in the air. In high-response cases, freezing of all obtained embryos without transfer, ie segmentation, is almost the only alternative approach in terms of patient safety. However, more

randomized controlled trials are needed to adopt the DET policy instead of the fresh cycle in cases with normal response, taking into account live birth rates and cost. In the literature, there are several recent studies in which normal-response subjects are dealt with in terms of DET vs. fresh cycles. Table 2 summarizes these studies. In summary, there is insufficient evidence to adopt DET policy as a first-line approach in patients with normal ovarian reserve. However, in high-response cases, DET policy may be preferred to prevent more optimal pregnancies and OHSS.

TABLE 2: Results of current studies in the literature.[124-125]

	Celada 2015[13]	Roque 2015[14]	Roque 2016[15]	
Number of cases	882 (364 fresh-518 DET)	530 (351 fresh-179 DET)	938 (523 fresh-415 DET)	
Method	-	Cleavage embryo vitrification	Cleavage embryo vitrification	
Age	20-44	35	33-36	
Number of oocytes	4-20	7-8	Group I: 4-9 Group II: 10-15 (12)	
Ongoing pregnancy%	%33,2 (DET) %32,9 (fresh) p>0,05	%39,7 (DET) %31,1 (fresh) p=0,04	Group I: %33 (DET) %31 (fersh) p=0,5	Group II: %47 (DET) %34 (fersh) p=0,02

Table 3: Clinical results of DET cycles.	
Decreasing frequency with DET	Increased frequency with DET
Antepartum hemorrhage (RR: 0.6)	Post-term pregnancy (OR: 1,4)
Preterm labor (RR: 0.8)	LGA (OR: 1.4)
SGA (RR: 0.4)	Macrosomy (OR: 1.5)
Low birth weight (OR: 0.69)	Placenta location anomalies

CONCLUSION

Infertility is defined as being unable to conceive despite regular and unprotected sexual intercourse for 1 year. Pregnancy occurs in 80-85% of couples during this period and pregnancy cannot be achieved in 15%. In women, a decrease in fertility is observed depending on age, so if patients over 35 years of age do not develop pregnancy within 6 months, they are diagnosed with infertility and proceed to treatment. Infertility is increasing day by day and it is developing in the techniques used in embryology laboratory. However, not every innovation is good, and sometimes it can cause harm. Treatment efficacy and safety, especially in ART, should be considered as an integral whole. As a result of our study, frozen embryo transfer is preferred more frequently in some cases due to OHSS and PGT. Today, fresh embryo transfer is routinely performed and frozen embryo transfer is applied as an alternative to treatment. According to

the obtained data, the results of pregnancy of frozen embryo transfer were obtained. Today, however, its routine use is still questioned, except in special cases where it is an alternative to fresh transfer cycles. Although it brings some more optimal obstetric-neonatal outcomes compared to fresh cycle results, there is no consensus that unconditional DET is performed in each case.

REFERENCES

1- William, K. ve Patrick, C., 2009, Netter temel histoloji, (Çev: Müftüoğlu, S., Kaymaz, F., Atilla, P.,), Güneş tıp kitapevleri, Ankara.

2- Topal, F., 2010, Ovaryum dokusunu, vitrifikasyon ve yavaş soğutma teknikleriyle korunurluğunun histopatolojik yönden karşılaştırılması, Tıpta Uzmanlık Tezi, Tıp Fakültesi, Ankara Üniversitesi, Ankara.

3- Başar, M., 2006, Süperovulasyonun ovaryum dokusu yapısı üzerine etkilerinin morfolojik yönden araştırılması, Yüksek Lisans Tezi, İstanbul Üniversitesi, Sağlık Bilimleri Enstitüsü, İstanbul.

4- Ross, M.H. and Pawlina, W., 2006, Histology, a text and atlas with corralated cell and molecular biology, 5th ed. Philadelphia, Lippincott Williams & Wilkins.

5- Ross, M.H., 2003, Histology: A text and atlas, 4th ed, Philadelphia, PA: Lippincott Williams & Wilkins, 726-742p.

6- Abraham, L., 2006, Histoloji ve hücre biyolojisi patolojiye giriş, (Çev: Demir, R.,) Pame yayıncılık.

7- Aksoy, A., 2008, Yaşlanmaya karşın sıçan ovaryumunda follikül ince yapısı ve follikül uyaran hormon reseptörlerinin (FSHR), östrojen reseptörlerinin (ER), progestron reseptörlerinin (PR) immünohistokimtasal olarak belirlenmesi, Yüksek Lisans Tezi, Gazi Üniversitesi, Sağlık Bilimleri Enstitüsü, Ankara.

8- Chang, S.T. and Buswell, J.A., 1996, Mushroom nutriceuticals, World J. Microp, Biotechnol., 12, 473- 476p.

9- Junqueira, C.L. ve Carneiro, J., 2003, Temel histoloji, 10. Baskı, Noel tıp kitapevleri, Brezilya, 449- 459s.

10- Mills, S.E., 2007, Histology for pathologists, 3. Baskı, Lippincott Williams & Wilkins, A.B.D.

11- . Şahinarslan, N., 2009, Nitrik oksitin sıçan ovaryumunda folikül gelişimi üzerine etkilerinin ışık ve elektron mikroskopik deneyde incelenmesi, Yüksek Lisans Tezi, İstanbul Üniversitesi, Sağlık Bilimleri Enstitüsü, İstanbul.

12- Tekelioğlu, M., 2002, Özel histoloji ince yapı ve gelişme, Antıp yayın, Ankara, 215s.

13- Gartner, P.L. and Hiatt, L.J., 2001, Color textbook of histology, 2. Baskı, W.B. saunders company, A.B.D.

14- Uzun, D., 2010, Gebelikte olaylanan deneysel hipokside Ginkgo biloba 'nın ovaryon dokusuna etkilerinin belirlenmesi, Yüsek Lisans Tezi, Gazi Üniversitesi, Sağlık Bilimleri Enstitüsü, Ankara.

15- Erdoğan, D., Hatipoğlu, M.T., Görgün, M., Ilgaz, C., 2007, Özel histoloji. 2. Baskı, Hatipoğlu yayınevi, Ankara.

16- Kierszenbaum, A.L., 2006, Histoloji ve hücre biyolojisi patolojiye giriş, 2. baskı, Palme yayıncılık, Ankara.

17- Şahin, Ş., 2008, Yaşlanmaya koşut sıçan tuba uterinasında cinsiyet hormonlarının immünohistokimyasal olarak ve ultrastrüktürel değişimlerinin incelenmesi, Yüksek Lisans Tezi, Gazi Üniversitesi, Sağlık Bilimleri Enstitüsü, Ankara.

18- Hussein, S. M., El-Fadaly, A. B., Metawea, A. G., & Khaled, B. E. A. (2019). Aging changes of the testis in albino rat: light,

electron microscopic, morphometric, immunohistochemical and biochemical study. Folia Morphol (Warsz). doi:10.5603/FM.a2019.0102

19- Khalil, I., Khan, M. R., Ghani, M., & Akbar, F. (2019). Abutilon pannosum stem bark enhances the aphrodisiac activities and spermatogenesis in rat. Andrologia, e13404. doi:10.1111/and.13404

20- Kauerhof, A. C., Nicolas, N., Bhushan, S., Wahle, E., Loveland, K. A., Fietz, D., . . . Fijak, M. (2019). Investigation of activin A in inflammatory responses of the testis and its role in the development of testicular fibrosis. Hum Reprod. doi:10.1093/humrep/dez109

21- Malolina, E., & Kulibin, A. (2019). The rete testis harbors Sertoli-like cells capable of expressing Dmrt1. Reproduction. doi:10.1530/rep-19-0183

22- Wu, H., Jiang, X., Gao, Y., Liu, W., Wang, F., Gong, M., . . . Han, D. (2019). Mumps virus infection disrupts blood-testis barrier through the induction of TNF-alpha in Sertoli cells. Faseb j, fj201901089R. doi:10.1096/fj.201901089R

23- Aldahhan, R. A., Stanton, P. G., Ludlow, H., de Kretser, D. M., & Hedger, M. P. (2019). Acute heat-treatment disrupts inhibin-related protein production and gene expression in the adult rat testis. Mol Cell Endocrinol, 498, 110546. doi:10.1016/j.mce.2019.110546

24- Kauerhof, A. C., Nicolas, N., Bhushan, S., Wahle, E., Loveland, K. A., Fietz, D., . . . Fijak, M. (2019). Investigation of activin A

in inflammatory responses of the testis and its role in the development of testicular fibrosis. Hum Reprod. doi:10.1093/humrep/dez109

25- Johnson, M. (2019). Human in vitro fertilisation and developmental biology: a mutually influential history. Development, 146(17). doi:10.1242/dev.183145

26- Kalaitzaki, A. E., Mavrogiannaki, S., & Makrigiannakis, A. (2019). A prospective, cross-sectional study of the protective and risk psychological factors of successful in vitro fertilisation outcome: preliminary results in a Greek sample. J Obstet Gynaecol, 1-6. doi:10.1080/01443615.2019.1631766

27- Maheshwari, A., Hamilton, M., & Bhattacharya, S. (2008). Effect of female age on the diagnostic categories of infertility. Hum Reprod, 23(3), 538-542. doi:10.1093/humrep/dem431

28- Latif Khan, H., Bhatti, S., Latif Khan, Y., Abbas, S., Munir, Z., Rahman Khan Sherwani, I. A., . . . Aydin, H. H. (2019). Cell-free nucleic acids and melatonin levels in human follicular fluid predict embryo quality in patients undergoing in-vitro fertilization treatment. J Gynecol Obstet Hum Reprod. doi:10.1016/j.jogoh.2019.08.007

29- Chung, J. Y., Brown, S., Chen, H., Liu, J., Papadopoulos, V., & Zirkin, B. (2019). Effects of Pharmacologically Induced Leydig Cell Testosterone Production on Intratesticular Testosterone and Spermatogenesis. Biol Reprod. doi:10.1093/biolre/ioz174

30- Llamas Luceno, N., de Souza Ramos Angrimani, D., de Cassia Bicudo, L., Szymanska, K. J., Van Poucke, M., Demeyere, K., .

. . Van Soom, A. (2019). Exposing dairy bulls to high temperature-humidity index during spermatogenesis compromises subsequent embryo development in vitro. Theriogenology, 141, 16-25. doi:10.1016/j.theriogenology.2019.08.034

31- Gleicher, N., Kushnir, V. A., & Barad, D. H. (2019). Worldwide decline of IVF birth rates and its probable causes. Hum Reprod Open, 2019(3), hoz017. doi:10.1093/hropen/hoz017

32- Mu, T., Feng, Y., Che, Y., Lv, Q., Hu, J., Yang, Q., & Yang, J. (2019). Taurine Promotes In-vitro Follicle Development, Oocyte Maturation, Fertilization and Cleavage of rats. Adv Exp Med Biol, 1155, 197-203. doi:10.1007/978-981-13-8023-5_18

33- Gleicher, N., Kushnir, V. A., & Barad, D. H. (2019). Worldwide decline of IVF birth rates and its probable causes. Hum Reprod Open, 2019(3), hoz017. doi:10.1093/hropen/hoz017

34- Lau, B. H. P., Yao, S. H., Tam, M. Y. J., Chan, C. L. W., Ng, E. H. Y., & Chan, C. H. Y. (2019). Gratitude in infertility: a cross-sectional examination of dispositional gratitude in coping with infertility-related stress in women undergoing IVF. Hum Reprod Open, 2019(3), hoz012. doi:10.1093/hropen/hoz012

35- Nassan, F. L., Arvizu, M., Minguez-Alarcon, L., Gaskins, A. J., Williams, P. L., Petrozza, J. C., . . . Chavarro, J. E. (2019). Marijuana smoking and outcomes of infertility treatment with assisted

36- Tifrea, D. F., Pal, S., & de la Maza, L. M. (2019). A recombinant Chlamydia trachomatis MOMP vaccine elicits

cross-serogroup protection in mice against vaginal shedding and infertility. J Infect Dis. doi:10.1093/infdis/jiz438

37- Maheshwari, A., Hamilton, M., & Bhattacharya, S. (2008). Effect of female age on the diagnostic categories of infertility. Hum Reprod, 23(3), 538-542. doi:10.1093/humrep/dem431

38- Jin, L., Wang, M., Yue, J., Zhu, G. J., & Zhang, B. (2019). Association between TSH Level and Pregnancy Outcomes in Euthyroid Women Undergoing IVF/ICSI: A Retrospective Study and Meta-analysis. Curr Med Sci, 39(4), 631-637. doi:10.1007/s11596-019-2084-5

39- Gozuyesil, E., Karacay Yikar, S., & Nazik, E. (2019). An analysis of the anxiety and hopelessness levels of women during IVF-ET treatment. Perspect Psychiatr Care. doi:10.1111/ppc.12436

40- Espino, J., Macedo, M., Lozano, G., Ortiz, A., Rodriguez, C., Rodriguez, A. B., & Bejarano, I. (2019). Impact of Melatonin Supplementation in Women with Unexplained Infertility Undergoing Fertility Treatment. Antioxidants (Basel), 8(9). doi:10.3390/antiox8090338

41- Drakopoulos, P., Garcia-Velasco, J., Bosch, E., Blockeel, C., de Vos, M., Santos-Ribeiro, S., . . . Polyzos, N. P. (2019). ICSI does not offer any benefit over conventional IVF across different ovarian response categories in non-male factor infertility: a European multicenter analysis. J Assist Reprod Genet. doi:10.1007/s10815-019-01563-1

42- Buckett, W., & Sierra, S. (2019). The management of unexplained infertility: an evidence-based guideline from the Canadian Fertility and Andrology Society. Reprod Biomed Online. doi:10.1016/j.rbmo.2019.05.023

43- Zimon, A. E., Shepard, D. S., Prottas, J., Rooney, K. L., Ungerleider, J., Halasa-Rappel, Y. A., . . . Oskowitz, S. P. (2019). Embryo donation: Survey of in-vitro fertilization (IVF) patients and randomized trial of complimentary counseling. PLoS One, 14(8), e0221149. doi:10.1371/journal.pone.0221149

44- Oyawoye, O., Abdel Gadir, A., Garner, A., Constantinovici, N., Perrett, C., & Hardiman, P. (2003). Antioxidants and reactive oxygen species in follicular fluid of women undergoing IVF: relationship to outcome. Hum Reprod, 18(11), 2270-2274. doi:10.1093/humrep/deg450

45- Santos, T. A., El Shourbagy, S., & St John, J. C. (2006). Mitochondrial content reflects oocyte variability and fertilization outcome. Fertil Steril, 85(3), 584-591. doi:10.1016/j.fertnstert.2005.09.017

46- Hull M, Glazener C, Kelly N, Conway D, Foster P, Hinton R, Coulson C, Lambert P, Watt E, Desai K, Population study of causes, treatment, and outcome of infertility. Br Med J (Clin Res Ed), 1985. 291(6510): p. 1693-1697.

47- Bhattacharya S, Porter M, Amalraj E, Templeton A, Hamilton M, Lee A, Kurinczuk J, The epidemiology of infertility in the North East of Scotland. Human reproduction, 2009. 24(12): p. 3096-3107.

48- Medicine PCotASfR, Optimal evaluation of the infertile female. Fertility and sterility, 2006. 86(5): p. S264-S267.

49- Westrom L, Effect of pelvic inflammatory disease on fertility. Venereology: official publication of the National Venereology Council of Australia, 1995. 8(4): p. 219-222.

50- Breitkopf D, Goldstein S, Seeds J, ACOG technology assessment in obstetrics and gynecology. Number 3, September 2003. Saline infusion sonohysterography. Obstetrics and gynecology, 2003. 102(3): p. 659-662

51- Forseyl J, Caul E, Paul I, Hull M, Chlamydia trachomatis, tubal disease and the incidence of symptomatic and asymptomatic infection following hysterosalpingography. Human reproduction, 1990. 5(4): p. 444-447.53

52- Dabekausen Y, Evers J, Land J, Stals F, Chlamydia trachomatis antibody testing is more accurate than hysterosalpingography in predicting tubal factor infertility. International Journal of Gynecology & Obstetrics, 1994. 47(3): p. 318.

53- Moore D, Segars Jr J, Winfield A, Page D, Eisenberg A, Holburn G, Effects of contrast agents on the fallopian tube in a rabbit model. Radiology, 1990. 176(3): p. 721-724.

54- Grosskinsky CM, Clark RL, Wilson PA Novotny DB, Pelvic granulomata mimicking endometriosis following the administration of oil-based contrast media for hysterosalpingography. Obstetrics & Gynecology, 1994. 83(5): p. 890-892.

55- Nunley Jr WC, Bateman BG, Kıtchın III JD, Pope Jr TL, Intravasation During Hysterosalpingography Using Oil-Base Contrast Medium-a Second Look. Obstetrics & Gynecology, 1987. 70(3): p. 309-312.

56- Johnson N, Vandekerckhove P, Watson A, Lilford R, Harada T, Hughes E, Tubal flushing for subfertility. Cochrane Database Syst Rev, 2007. 11.

57- Grimbizis GF, Gordts S, Sardo ADS, Brucker S, De Angelis C, Gergolet M, Li TC, Tanos V, Brölmann H, Gianaroli L, The ESHRE–ESGE consensus on the classification of female genital tract congenital anomalies. Gynecological surgery, 2013. 10(3): p. 199-212.

58- Karande VC, Pratt DE, Rabin DS, Gleicher N, The limited value of hysterosalpingography in assessing tubal status and fertility potential. Fertility and sterility, 1995. 63(6): p. 1167-1171.

59- Henig I, Prough S, Cheatwood M, DeLong E, Hysterosalpingography, laparoscopy and hysteroscopy in infertility. A comparative study. The Journal of reproductive medicine, 1991. 36(8): p. 573-575.

60- Corson SL, Cheng A, Jacqueline NG, Laparoscopy in the "normal" infertile patient: a question revisited. The Journal of the American Association of Gynecologic Laparoscopists, 2000. 7(3): p. 317-324.

61- Evers J, Land JA, Mol BW. Evidence-based medicine for diagnostic questions. İn Seminars in reproductive medicine. 2003.

62- Swart P, Mol BW, van der Veen F, van Beurden M, Redekop WK, Bossuyt PM, 3. The diagnostic performance of hysterosalpingography in the diagnosis of tubal pathology, a meta-analysis. Fertility and sterility, 1995. 64: p. 486-91.

63- Bilgin H, Özcan B, Bilgin T, Methemoglobinemia induced by methylene blue pertubation during laparoscopy. Acta anaesthesiologica scandinavica, 1998. 42(5): p. 594-595.

64- Bates G, Garza D, Garza M, Clinical manifestations of hormonal changes in the menstrual cycle. Obstetrics and gynecology clinics of North America, 1990. 17(2): p. 299-310.54

65- Wathen N, Perry L, Lilford R, Chard T, Interpretation of single progesterone measurement in diagnosis of anovulation and defective luteal phase: observations on analysis of the normal range. Br Med J (Clin Res Ed), 1984. 288(6410): p. 7-9.

66- Fritz MA, The modern infertility evaluation. Clinical obstetrics and gynecology, 2012. 55(3): p. 692-705.

67- Hull M, McLeod F, Joyce D, Ray B, McDermott A, Human in-vitro fertilisation, in-vivo sperm penetration of cervical mucus, and unexplained infertility. The Lancet, 1984. 324(8397): p. 245-246.

68- Landgren B-M, Undén A-L, Diczfalusy E, Hormonal profile of the cycle in 68 normally menstruating women. Acta Endocrinologica, 1980. 94(1): p. 89-98

69- Jordan J, Craig K, Clifton DK, Soules M, Luteal phase defect: the sensitivity and specificity of diagnostic methods in common clinical use. Fertility and sterility, 1994. 62(1): p. 54-62.

70- Miller PB, Soules MR, The usefulness of a urinary LH kit for ovulation prediction during menstrual cycles of normal women. Obstetrics & Gynecology, 1996. 87(1): p. 13-17.

71- McGee EA, Hsueh AJ, Initial and cyclic recruitment of ovarian follicles 1. Endocrine reviews, 2000. 21(2): p. 200-214.

72- Sallam HN, Ezzeldin F, Agameya A-F, Abdel-Rahman AF, El-Garem Y, The definition of 'poor response': Bologna criteria. Human reproduction, 2011: p. der398.

73- Barroso G, Oehninger S, Monzó A, Kolm P, Gibbons WE, Muasher SJ, Clinical Assisted Reproduction: High FSH: LH Ratio and Low LH Levels in Basal Cycle Day 3: Impact on Follicular Development and IVF Outcome. Journal of assisted reproduction and genetics, 2001. 18(9): p. 499-505.

74- Bukman A, Heineman M, Ovarian reserve testing and the use of prognostic models in patients with subfertility. Human reproduction update, 2001. 7(6): p. 581-590.

75- Scott Jr R, Hofmann G, Prognostic assessment of ovarian reserve. Fertility and sterility, 1995. 63(1): p. 1-11.

76- Broekmans F, Kwee J, Hendriks D, Mol B, Lambalk C, A systematic review of tests predicting ovarian reserve and IVF outcome. Human reproduction update, 2006. 12(6): p. 685-718.

77- McIlveen M, Skull J, Ledger W, Evaluation of the utility of multiple endocrine and ultrasound measures of ovarian reserve

in the prediction of cycle cancellation in a high-risk IVF population. Human reproduction, 2007. 22(3): p. 778-785.

78- Muttukrishna S, McGarrigle H, Wakim R, Khadum I, Ranieri D, Serhal P, Antral follicle count, anti - mullerian hormone and inhibin B: predictors of ovarian response in assisted reproductive technology? BJOG: An International Journal of Obstetrics & Gynaecology, 2005. 112(10): p. 1384-1390.

79- Kupesic S, Kurjak A, Bjelos D, Vujisic S, Three-dimensional ultrasonographic ovarian measurements and in vitro fertilization outcome are related to age. Fertility and sterility, 2003. 79(1): p. 190-197.

80- Mercé LT, Barco MJ, Bau S, Troyano JM, Prediction of ovarian response and IVF/ICSI outcome by three-dimensional ultrasonography and power Doppler angiography. European Journal of Obstetrics & Gynecology and Reproductive Biology, 2007. 132(1): p. 93-100.

81- Weenen C, Laven JS, von Bergh AR, Cranfield M, Groome NP, Visser JA, Kramer P, Fauser BC, Themmen AP, Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Molecular human reproduction, 2004. 10(2): p. 77-83.

82- Sowers MR, Eyvazzadeh AD, McConnell D, Yosef M, Jannausch ML, Zhang D, Harlow S, Randolph Jr JF, Anti-mullerian hormone and inhibin B in the definition of ovarian aging and the menopause transition. The Journal of Clinical Endocrinology & Metabolism, 2008. 93(9): p. 3478-3483.

83- Tsepelidis S, Devreker F, Demeestere I, Flahaut A, Gervy C, Englert Y, Stable serum levels of anti-Müllerian hormone during the menstrual cycle: a prospective study in normo-ovulatory women. Human reproduction, 2007. 22(7): p. 1837- 1840.

84- Reuss ML, Kline J, Santos R, Levin B, Timor-Tritsch I, Age and the ovarian follicle pool assessed with transvaginal ultrasonography. American journal of obstetrics and gynecology, 1996. 174(2): p. 624-627.

85- Scheffer GJ, Broekmans FJ, Dorland M, Habbema JD, Looman CW, te Velde ER, Antral follicle counts by transvaginal ultrasonography are related to age in womenwith proven natural fertility. Fertility and sterility, 1999. 72(5): p. 845-851.

86- Hendriks DJ, Mol B-WJ, Bancsi LF, te Velde ER, Broekmans FJ, Antral follicle count in the prediction of poor ovarian response and pregnancy after in vitro fertilization: a meta-analysis and comparison with basal follicle-stimulating hormone level. Fertility and sterility, 2005. 83(2): p. 291-301.

87- Tyler J, Crockett N, Driscoll G, Studies of human seminal parameters with frequent ejaculation. I. Clinical characteristics. Clinical reproduction and fertility, 1982. 1(4): p. 273-285.

88- Pellestor F, Girardet A, Andreo B, Effect of long abstinence periods on human sperm quality. International journal of fertility and menopausal studies, 1993. 39(5): p. 278-282.

89- Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST, Coutifaris C, Carson SA, Cisneros P, Steinkampf MP, Hill JA, Sperm morphology, motility, 56 and concentration

in fertile and infertile men. New England Journal of Medicine, 2001. 345(19): p. 1388-1393.

90- Swan SH, Brazil C, Drobnis EZ, Liu F, Kruse RL, Hatch M, Redmon JB, Wang C, Overstreet JW, Group SFFFR, Geographic differences in semen quality of fertile US males. Environmental Health Perspectives, 2003. 111(4): p. 414.

91- Cooper TG, Noonan E, Von Eckardstein S, Auger J, Baker HG, Behre HM, Haugen TB, Kruger T, Wang C , Mbizvo MT, World Health Organization reference values for human semen characteristics. Human reproduction update, 2009: p. dmp048.

92- Meeting JFWECoFA, Organization WH, Evaluation of Certain Food Additives: Seventy-first Report of the Joint FAO/WHO Expert Committee on Food Additives. Vol. 71. 2010: World Health Organization.

93- Willott G, Frequency of azoospermia. Forensic science international, 1982. 20(1): p. 9-10.

94- Jarow J, Espeland M, Lipshultz L, Evaluation of the azoospermic patient. The Journal of urology, 1989. 142(1): p. 62-65.

95- Menkveld R, Stander FS, Kruger TF, van Zyl JA, The evaluation of morphological characteristics of human spermatozoa according to stricter criteria. Human Reproduction, 1990. 5(5): p. 586-592.

96- Kruger TF, Acosta AA, Simmons KF, Swanson RJ, Matta JF, Veeck LL, Morshedi M , Brugo S, New method of evaluating

sperm morphology with predictive value for human in vitro fertilization. Urology, 1987. 30(3): p. 248-251.

97- Hernandez M, Molina R, Olmedo J, Olmedo SB, Coetzee K, Estofan D, Prognostic value of the strict criteria: an Argentinian experience. Archives of andrology, 1996. 37(2): p. 87-89.

98- Kihaile PE, Misumi J, Hirotsuru K, Kumasako Y, Kisanga RE, Utsunomiya T, Comparison of sibling oocyte outcomes after intracytoplasmic sperm injection and in vitro fertilization in severe teratozoospermic patients in the first cycle. International journal of andrology, 2003. 26(1): p. 57-62.

99- Wolff H, The biologic significance of white blood cells in semen. Fertility and sterility, 1995. 63(6): p. 1143-1157.

100- Ludwig M, Vidal A, Huwe P, Diemer T, Pabst W , Weidner W, Significance of inflammation on standard semen analysis in chronic prostatitis/chronic pelvic pain syndrome. Andrologia, 2003. 35(3): p. 152-156.

101- Veron GL, Molina RI, Tissera AD, Estofan GM, Marin-Briggiler CI , VazquezLevin MH, Incidence of Sperm Surface Autoantibodies and Relationship with Routine Semen Parameters and Sperm Kinematics. Am J Reprod Immunol, 2016.

102- Organization WH, WHO laboratory manual for the examination and processing of human semen. 2010.

103- Barratt C, Tomlinson M , Cooke I, Prognostic significance of computerized motility analysis for in vivo fertility. Fertility and sterility, 1993. 60(3): p. 520- 525.7

104- Macleod I , Irvine D, Andrology: The predictive value of computer-assisted semen analysis in the context of a donor insemination programme. Human Reproduction, 1995. 10(3): p. 580-586.

105- Evenson D, Jost L, Marshall D, Zinaman M, Clegg E, Purvis K, De Angelis P, Claussen O, Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Human Reproduction, 1999. 14(4): p. 1039-1049.

106- Hosseinifar H, Sabbaghian M, Chehrazi M, Modarresi T, Alipour FJ, Gilani MAS, Assessment of deoxyribonucleic acid fragmentation index, testicular volume, semen parameters, and hormone profile in gonadotropin-treated men with hypogonadotropic hypogonadism. Urology, 2013. 82(6): p. 1291-1295.

107- Aktan G, Doğru-Abbasoğlu S, Küçükgergin C, Kadıoğlu A, Özdemirler-Erata G , Koçak-Toker N, Mystery of idiopathic male infertility: is oxidative stress an actual risk? Fertility and sterility, 2013. 99(5): p. 1211-1215.

108- Wdowiak A, Bakalczuk S , Bakalczuk G, The effect of sperm DNA fragmentation on the dynamics of the embryonic development in intracytoplasmatic sperm injection. Reproductive biology, 2015. 15(2): p. 94-100

109- workshop RotE, on 'Unexplained Infertility'. Anacapri AY, 1993;8:977Y980 HR

110- Maheshwari A, Hamilton M , Bhattacharya S, Effect of female age on the diagnostic categories of infertility. Human reproduction, 2008. 23(3): p. 538-542.

111- Collins JA, Burrows E , Wilan A, The prognosis for live birth among untreated infertile couples. Fertility and sterility, 1995. 64(1): p. 22-28.

112- Ray A, Shah A, Gudi A , Homburg R, Unexplained infertility: an update and review of practice. Reproductive biomedicine online, 2012. 24(6): p. 591-602.

113- Gook DA, Edgar DH. Human oocyte cryopreservation. Hum Reprod Update 2007;13: 591-605

114- Li Z, Wang YA, Ledger W, Edgar DH, Sullivan EA. Clinical outcomes following cryopreservation of blastocysts by vitrification or slow freezing: a population-based cohort study. Hum Reprod 2014;29:2794-801.

115- Al-Inany HG, Youssef MA, Aboulghar M, Broekmans F, Sterrenburg M, Smit J, et al. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. Cochrane Database Syst Rev 2011;(5): CD001750.

116- Al-Azemi M, Kyrou D, Kolibianakis EM, Humaidan P, van Vaerenbergh I, Devroey P, et al. Elevated progesterone during ovarian stimulation for IVF. Reprod Biomed Online 2012; 24:381-8.

117- Healy MW, Patounakis G, Connell MT, Devine K, DeCherney AH, Levy MJ, Hill MJ.Does a frozen embryo transfer

ameliorate the effect of elevated progesterone seen in fresh transfer cycles? Fertil Steril 2016;105(1):93-9.e1.

118- Cobo A, Garrido N, Crespo J, Jose R, Pellicer A. Accumulation of oocytes: a new strategy for managing low-responder patients. Reprod Biomed Online 2012;24:424-32.

119- Henningsen AK, Pinborg A, Lidegaard O, Vestergaard C, Forman JL, AndersenAN. Perinatal outcome of singleton siblings born after assisted reproductive technology and spontaneous conception: Danish national sibling-cohort study. Fertil Steril 2011;95:959-63.

120- Wennerholm UB, Henningsen AK, Romundstad LB, Bergh C, Pinborg A, Skjaerven R, Forman J, Gissler M, Nygren KG, Tiitinen A.Perinatal outcomes of children born after frozen-thawed embryo transfer: a Nordic cohort study from the CoNARTaS group. Hum Reprod 2013;28(9):2545-53

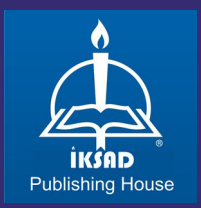
121- Ishihara O, Araki R, Kuwahara A, Itakura A, Saito H, Adamson GD. Impact of frozenthawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. Fertil Steril 2014;101:128-33.

122- Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and metaanalysis. Fertil Steril 2012;98:368,77.e1-9.

123- Roque M, Lattes K, Serra S, Sola I, Geber S, Carreras R, et al. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. Fertil Steril 2013;99:156-62.

124- Celada P, Bosch E, De Los Santos MJ, Giles J, Pellicer A. Elective frozen embryo transfer does not improve reproductive outcome in normo-responder patients. Hum Reprod 2015; 30 Suppl 1:i112.

125- Roque M, Valle M, Guimarães F, Sampaio M, Geber Freeze-all cycle for all normal responders? S. Assist Reprod Genet 2016.



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