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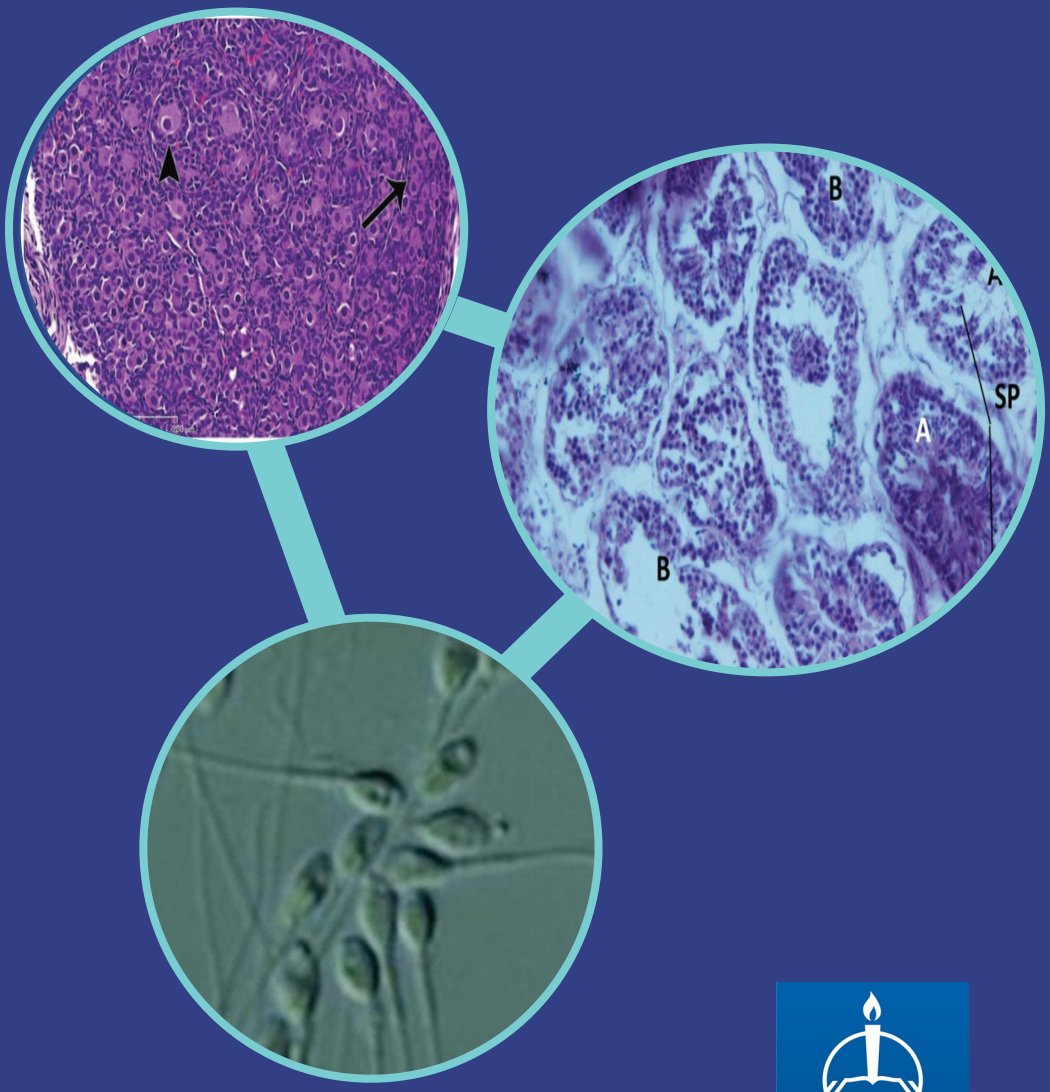


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**Yasemin AFŞİN[1] Umut SARI[2]**

**Editör**

**Aysun EKİNCİ[3]**

IKSAD Publishing House

[1] Private Batman Life Hospital, Department of Obstetrics and Gynecology, Batman, Turkey. Orcid id: 0009-0006-4603-6510 yasminafsin@gmail.com, 05370202708

[2] Gynecology and Obstetrics U Clinic,İstanbul,Turkey. Orcid id: 0000-0002-9593-9904, Umut85143@gmail.com

[3] Dicle University Faculty of Medicine, Department of Medical Biochemistry, Diyarbakır, Turkey. Orcid id: 0000-0002-4139, draysunekinci@gmail.com

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TURKEY TR: +90 342 606 06 75
USA: +1 631 685 0 853
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## PREFACE

Infertility means infertility. Infertility is mentioned in cases where pregnancy does not occur despite unprotected sexual intercourse for 1 year. Not only in Turkey, but all over the world, there is a problem of infertility due to living conditions, unhealthy diet and many other factors. In case of pregnancy, repetition of stillbirth or miscarriage is considered infertility. Along with the changing living conditions, the age of marriage is also increasing day by day. Even early married couples avoid having children before reaching a certain standard of living. For this reason, the number of couples who decide to have children between the ages of 30-40 and even after the age of 40 is increasing day by day. However, women over the age of 35 have a much lower chance of getting pregnant when compared to women who are younger. Studies have shown that 30% of women over the age of 35 have infertility problems. However, infertility problems are also encountered at younger ages. There are other reasons besides age. Therefore, many techniques are applied to achieve a more successful result in patients diagnosed with infertility. These techniques may vary according to the IVF center. We think that this book that we have made will be more widely used in IVF centers and will be effective in the emergence of future methods. I would like to thank my colleagues for their hard work and wish them continued success.

# CONTENTS

## INTRODUCTION

Infertility and childlessness have a very important effect on human life. It is reported that more than 180 million couples worldwide are facing the problem of infertility [1]. Despite one year of unprotected intercourse, approximately 15% of couples cannot conceive. Especially in developing countries, the problem of infertility can be ignored and it does not take an important place in the general health approach of the society. On the other hand, with the developing technologies, a chance for treatment can be provided for many infertile couples. Animal and human studies on in-vitro fertilization (IVF) began in the second half of the 20th century. In 1954, Thibault achieved the first in-vitro fertilization in rabbits, then Chang achieved the first live birth in animals with the development and subsequent transfer of in-vitro fertilized rabbit oocytes into the embryo [2]. A new era in reproductive medicine began with the birth of Louisia Brown on July 25, 1978, as a result of the first IVF method in humans. prof. After these great successes of Edwards and Steptoe, studies on IVF gained momentum in many countries around the world. This first IVF baby was obtained by laparoscopic aspiration of the oocyte in the natural cycle followed by IVF and embryo transfer to the uterus [3]. In 1992, ICSI (Intracytoplasmic sperm injection) method was started to be applied for the first time in the world. With this method, the chance of treatment has begun to be provided for couples with very limited sperm counts. Moreover, pregnancy has started to be achieved after ICSI with spermatozoa obtained from epididymal spermatozoa and testis. Alternative applications have started in cycle controls with medications

such as GnRH antagonists developed for ovulation induction, FSH and LH developed with recombinant technologies. With the developments in laboratory techniques, methods such as in-vitro maturation, pre-implantation genetic diagnosis, freezing of gamete cells and tissues and ovarian tissue transplantation have opened new fields in reproductive medicine. In the developing reproductive medicine, obtaining gamete cells from stem cells is one of the new steps expected to take place in the near future. While the first indication for IVF application was tubal factor, today the indications have diversified considerably and almost all factors that cause infertility have started to constitute a justification for IVF. The incidence of infertility is increasing in the world. Today, it is thought that especially the postponement of child demand to advanced ages and environmental factors lead to an increase in the frequency of infertility. IVF emerges as an effective treatment modality when conventional methods cannot achieve results. Among the female factors, tubal factor, endometriosis, age-related infertility, immunological factors, sperm problems in men and also patients whose cause cannot be determined are candidates for IVF techniques.

infertility; It means that pregnancy does not occur despite unprotected and regular sexual intercourse for at least one year. It is estimated that the infertility rate in the world is 15% [4]. The male factor is responsible for 20-50% in infertile couples, and the cause is idiopathic in 30-40% of infertility due to male factors. Complementary medicine is applications that will provide additional benefit to the current condition of the patient without reducing the effect of modern medicine. NCCAM

(American National Center for Complementary/Alternative Medicine) complementary medicine; defines it as a diverse group of practices, such as medical and health care systems, methods, and products, that are not considered a full part of classical medicine [5]. Today, complementary medicine applications are frequently preferred by patients. Zini et al. In a study they conducted in 2004, it was shown that 31% of 500 male patients who applied to an infertility center in Canada received one or more complementary treatments, and 63% of them took antioxidant vitamin and mineral supplements in complementary treatment areas [6].

Infertility is defined by the World Health Organization (WHO, 2009) as “the inability of a woman to become pregnant despite regular sexual intercourse for at least one year without using any contraception in a couple of reproductive age”. According to WHO, infertility, which has a prevalence of 10-15% in the society, is a life crisis with cultural, religious and class aspects, which brings medical, psychiatric, psychological and social problems, and is a stress factor for family members because it is an individual-specific and uncertain outcome. Guilt, anger, disappointment and hopelessness are the most common emotions experienced during the diagnosis and treatment of infertility and due to the emotional stress associated with not being able to have children, and they affect both couples and cause communication and marital problems between spouses. In studies conducted with infertile women in different countries, it has been reported that women experience depression, fear, loneliness, low self-esteem, guilt, grief,

hopelessness, physical violence and social isolation during the infertility process.

## I-UNFULLY DIFFERENT STAGE

### a) Development of gonads

The gonads (testes and ovaries) originate from three sources. These are the coelomic epithelium lining the posterior abdominal wall, the mesenchyme (embryonic connective tissue) and primordial germ cells under this epithelium. Although the sex is certain at fertilization, both sexes appear similar until the 7th week. The early period is the undifferentiated stage of development. At the beginning of the 4th week, the primordial germ cells of the yolk sac migrate to the developed gonadal ridges at the medial edge of the mesonephron. Coelomic epithelial cords (primary sex cords) enter the mesoderm of the gonadal ridge to form the outer cortex and inner medulla. At 6 weeks, germ cells migrate to the gonads. While the cortex regresses to form the medulla testes in the male, the medulla regresses in the female.

### b) Development of genital organs

Until the 7th week, the external genitalia of both sexes are the same in appearance. First, a genital ridge appears in the midline, extending from the cephalad to the depression of the proctodeum (Week 4). This genital ridge will form the penis or clitoris. On the posterior surface of the genital ridge, there are two urogenital folds reaching the cloacal membrane and two labioscrotal swellings. The genital ridge elongates to form the phallus and is as large in females as in males. As the tubercle

lengthens, it carries with it an extension from the urogenital sinus (urethral groove). In the sixth week, the urorectal septum fuses with the cloacal membrane and divides the cloacal membrane into the ventral urogenital membrane and the dorsal anal membrane. Shortly after the sixth week, the urogenital membrane ruptures and continues with the urethral groove and urogenital hole. The term phallus is used for indistinguishable stages of development.

**c) Development of genital canals**

Two pairs of ducts are formed in both sexes: These are the mesonephric (Wolff's duct) and the paramesonephric (Müller's duct) ducts. Paramesonephric ducts, consisting of coelomic epithelium, run lateral and generally parallel to the mesonephric ducts. The cranial ends of the canals open into the coelomic space, and their caudal ends ventrally cross the mesonephric canals and fuse to form the 'Y' shaped uterovaginal primordium, which in females extends into the urogenital sinus. In the male, the development of the paramesonephric duct is suppressed and the duct forms the appendix of the testis. The Müllerian tubercle is formed where the primordium enters the sinus. The tubercle forms the hymen in the female and the colliculus seminal in the male. The mesonephric ducts enter the sinus from both sides of the tubercle.

**d) Determination of Gender**

Genetic sex is determined by fertilization. If the ovum with the X chromosome is fertilized with the sperm carrying the X chromosome, the female embryo will develop into the male embryo if it is fertilized

with the sperm carrying the Y chromosome. By the seventh week, the appearance of the gonads is similar in both sexes, so they are called 'undifferentiated gonads'. The Y chromosome is required for the development of the male phenotype. The SRY gene required for testicular determining factor (TBF) is located on the short arm of the Y chromosome and is the main determining factor. Two X chromosomes are required for the development of the female phenotype. A series of genes and regions on the X chromosome have special roles in determining sex.

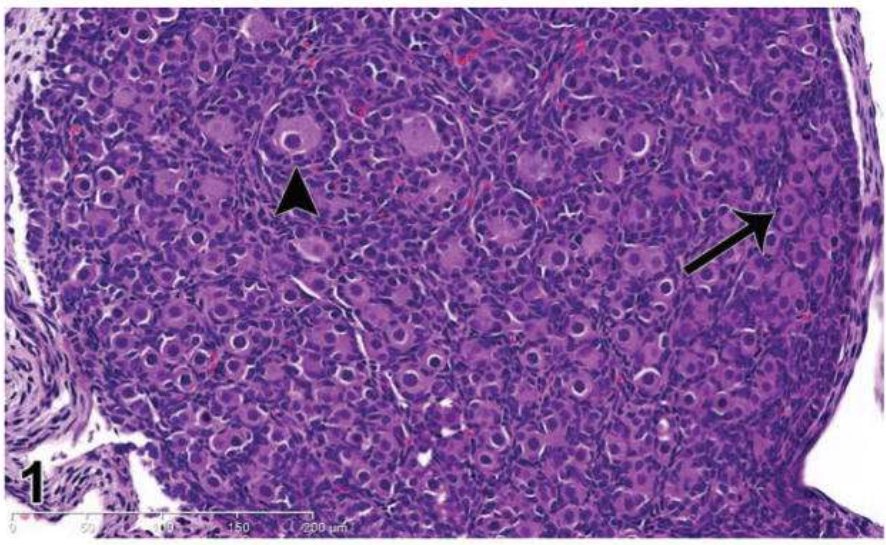


**Figure 1.** PND 6 (neonatal). Note the predominance of primordial follicles near the periphery (arrow) while primary and secondary follicles are formed in the nucleus (arrowhead) [7].

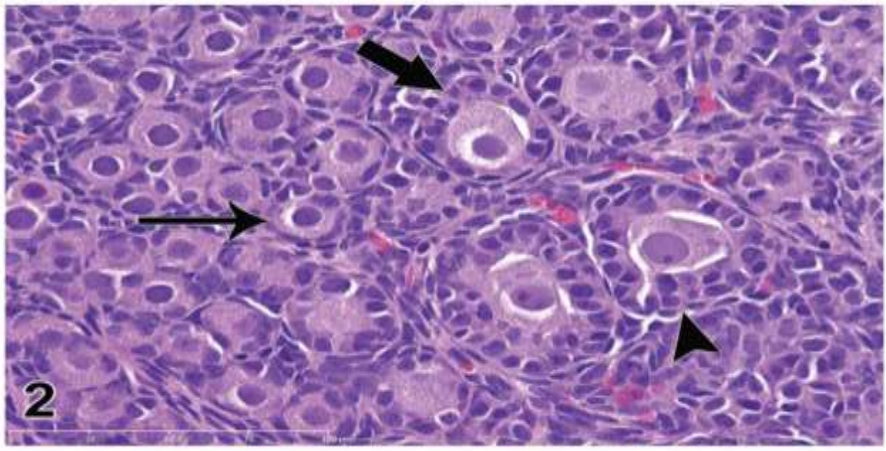


**Figure 2.** PND 6 (neonatal). Note the primitive (thin arrow), primary (thick arrow), and secondary (arrowhead) follicles. Follicle packs surrounded by mesenchymal cells resemble egg nests [7].

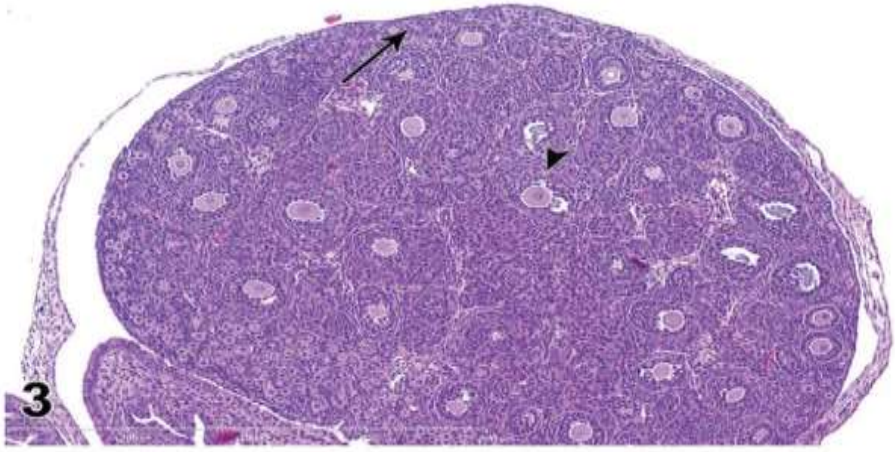


**Figure 3.** PND 10 (early infantile). The nucleus of the ovary is enlarged by immature secondary follicles (arrowhead) with a thin margin of primordial follicle (arrow) [7].

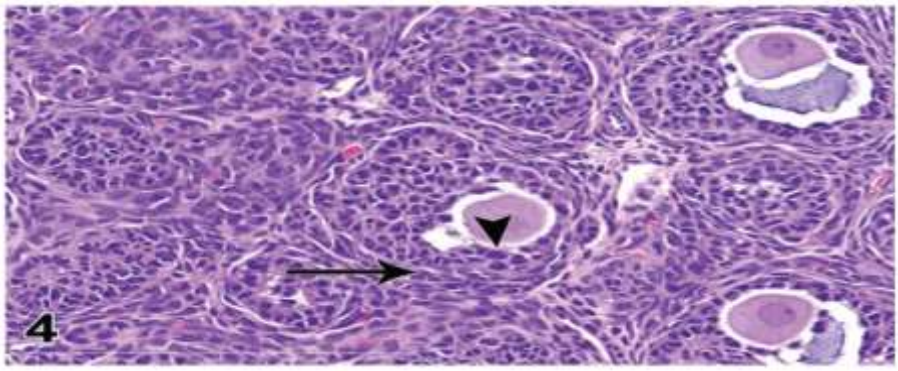


**Figure 4.** PND 10 (early infantile). Note immature secondary follicles with no clear boundary between granulosa cells (arrowhead) and plump theca cells (arrow) and no zona pellucida [7].

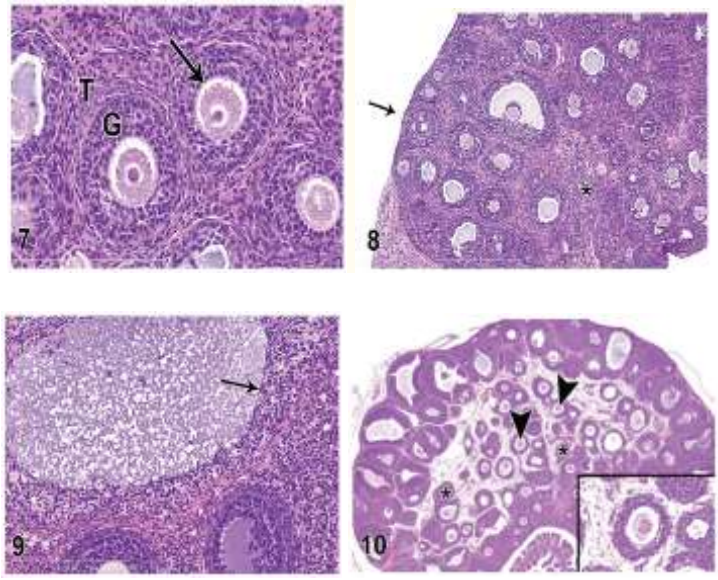


**Figure 5.** Follicle atresia is seen with apoptosis of granulosa cells, interstitial glands (asterisks) and necrotic oocytes (arrowheads) are present. Addendum: Note the atretic follicle with a necrotic oocyte in the middle [7].

The structures that make up the genital system are originally divided into gonads, genital excretory ducts and external genital organs, and follow an interactive development process with the structures around them, depending on genetic sex. While the sex of the individual is determined by the addition of a Y or a new X chromosome to the X chromosome in the egg during fertilization, the phenotypic features begin to separate from each other only at the end of the 6th week. The development of the genital system in men is observed as a process that shares common with the urinary system structures at some points. The process in question shows a closer relationship with the primitive urinary system structures and shows differentiation as structures that gradually separate from each other until the 3rd month. While examining this process, it may be useful to recall basic embryological structures.

In the third week of embryonic life, the mesoderm tissue, which forms the middle one of the germ leaves that develop in three layers, is divided into three sub-sections as paraxial mesoderm, intermediate mesoderm and lateral plate mesoderm on both sides of the midline and specializes to form different tissues and systems [8]. While the paraxial and lateral plate mesoderm forms other structures, the structures of the urinary system, some parts of the gonads and the excretory ducts of the male genital system develop from the intermediate mesoderm. The intermediate mesoderm extends downward as a pair of columns on either side of the dorsal body wall, forming three distinct nephric structures: cervical nephrotomes, mesonephros, and metanephros, each

successively emerging more developed than the previous one. Of these structures, the mesonephros plays an important guiding function in the development of both the urinary and genital systems. The mesonephric ducts (Wolff's ducts), which are located lateral to the mesonephros and also formed from the intermediate mesoderm, are observed as separate structures extending caudally.

Paramesonephric ducts, which are a pair of new channels in the dorsal body wall as a result of the inward folding of the coelomic epithelium lining the inner surface of the abdominal cavity next to the mesonephric ducts, in the form of a column with inward folding of the mesonephric ducts on the outside of the mesonephric ducts on the 44th and 48th days of gestation. ) occurs [9]. The folds formed by the mesothelium, which thickens in the form of plaque, approach towards the mesonephric duct in the form of epithelial cords with the effect of Wnt-4, which is produced by the mesonephros and contributes to the development in the female direction [10]. At the ends of the paramesonephric ducts interacting with the mesonephric ducts, a proliferation center dependent on the Wnt-9b signal sent from the mesonephric ducts is formed, and thus the ducts extend caudally up to the primitive urogenital sinus. If the mesonephric ducts are cut, the caudal extension of the paramesonephric ducts also stops. The lumen does not form inside the paramesonephric ducts until it comes into contact with the urogenital sinus. Its cranial end opens into a funnel-shaped coeloma. Although the shapes of both ducts in the development of male and female genital

systems change, paramesonephric ducts (Müller ducts) do not participate in an important structure in the male genital system.

## Primordial Germ Cells

Primordial germ cells, which begin to migrate from the posterior wall of the vitreous sac in the sixth week, settle in the genital folds that begin to form in the mesenchymal tissue of the posterior body wall via the dorsal mesentery by circulating over the allantois and the intestine from the back with ameboid movements [11]. Experimental animal studies have shown that these cells originate from the epiblast and are dependent on the expression of the BMP-4 (Bone Morphogenic Protein-4) gene in the extra-embryonic ectoderm. Primordial germ cells are attached to each other by cytoplasmic extensions during migration. Approximately 1000-2000 primordial germ cells located in the gonad drafts are totipotent and express the stem cell-specific Oct-4 gene [12] and proliferate in response to mitogen stimuli such as LIF (Leukemia Inhibitory Factor) and Steel Factor [13]. Gonads differentiate by expression of WT-1 from the steroidogenic mesoderm (Mesonephros) along the ventromedial edge of the mesonephros, Steroidogenic Factor-1 (SF-1), which is expressed in cells in the hypothalamo-pituitary system as well as in somatic cells in the gonad, and Lim-1 genes, which are effective in head region development [11]. As the germ cells reach the level of the 10th thoracic segment just medial to the mesonephros, cells of the mesonephros and adjacent coelomic epithelium are induced, and these cells come together to form somatic sex cords that surround the germ cells. Cells in the sex cords differentiate into Sertoli cells in

the male and follicle cells in the female. Cells that will differentiate into Sertoli cells among the cells that settle in the gonad outline from the coeloma epithelium produce Fgf9 in the first stage and increase their number.

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retinoic acid secreted by the mesonephros, thanks to the retinoic acid metabolizing enzyme initially synthesized by Sertoli cells originating from the coelomic epithelium [16]. Although the exact mechanism is not known, the intercellular junctions that provide direct contact between Sertoli cells and primordial germ cells play an important role in the development of male gametes. This interaction takes place shortly after primordial germ cells reach the gonad outline. As a result of this interaction, while the mitosis process in germ cells is suppressed, their entry into meiosis is also prevented. At this stage, no further changes are seen in the remaining cells until the 3rd month after birth. From the 3rd month after birth, differentiation into type A spermatogonia begins.

Differentiation into B type spermatogonia, which are the next stages of male gametogenesis, meiosis and spermatogenesis are delayed until puberty. The phenotypic differentiation of the sex begins with the gonads and proceeds by the action of the gonads on the sex-differentiated ducts. While the natural course of the phenotypic sex is in the female direction, the interactions that will bring the male characteristics originating from the testis into action, reveal the behavioral differentiation by affecting the brain development as well as the external genital and secondary sex characters. Although genetically, the sex differentiation in the embryo is determined by the fusion of the paternal X or Y chromosome with the maternal X chromosome at fertilization, there is no difference between the genital structures of male and female embryos until the end of the 6th week (Ambisexual stage). It has been demonstrated that genes such as Wt1, Steroidogenic

factor-1 (Sf1), Emx2, Lim homeobox protein-9 (Lhx9) and Gata-binding protein-4 (Gata4) are effective in the genetic control of male gonad development [17]. Some of these genes are effective not only in the formation of undifferentiated gonads in the early period, but also in the expression of Sry and genes that are the target of Sry [18]. Although it is debated when the sex difference between male and female embryos first emerged during development, there are studies showing that Sry gene transcription occurs before implantation [19]. In genetically male embryos, the SRY gene, a single copy gene located on the Y chromosome, is briefly activated in somatic cells in the gonad (detected on days 41-44 after ovulation and can be observed until day 52).

On the other hand, findings have been published showing that there is a difference in the rate of development between male and female embryos and that XY embryos develop faster before implantation. The fact that male and female embryos can be differentiated antigenically from each other suggests that the difference in gene expression emerges from the pre-implantation period. The first step in the development of the male genital system is the expression of the SRY protein. At the end of the sixth week, when the SRY (Sex Determining Region of Y) gene, which is a specific gene belonging to the SoxB gene family located on the Y chromosome in the somatic support cells of the sex cords of the XY gonad and encodes a non-histone protein with 223 amino acids, begins to be expressed, the course of sex differentiation changes [20]. Embryos without SRY gene expression develop in the female direction even if the Y chromosome is present. That is, the expression of the SRY

gene is the turning point in the differentiation of the gonads to the male direction. It is currently unknown which genes are the target of SRY. SRY binds to minor regions in DNA, causing DNA to fold in specific ways and perform positional changes suitable for the binding of different transcription regulators. Sox9 (Sry-associated HMG box-9) gene has been identified as the main SRY target known today [21]. The product of this gene comes from the coelomic epithelium and enables cells that settle in the gonad outline to develop into Sertoli cells. Sertoli cells are the main regulators of testicular development. They allow other cells in the gonad to directly differentiate and differentiate into other cell lines. If Sertoli cells remain below a certain number, testicular development is disrupted. On the other hand, it is also important to detect the incoming Sox-9 warning in a timely manner. Primordial germ cells tend to undergo meiosis and differentiate into oogonia, as the differentiation of presertoli cells into Sertoli cells will be disrupted if the stimulus is premature. At the end of the sixth week, the cells that begin to differentiate into Sertoli cells begin to surround the germ cells in the form of testicular cords together with the interstitial cells. The testis begins to round, restricting its contact surface with the mesonephros. As the testis continues to develop, the coelomic epithelium is separated from the testicular cords by a layer of connective tissue called the tunica albuginea. The parts of the sex cords in the testis located in the inner region 5-12. It comes into contact with the mesonephric tubule segments. While the rete testis and efferent ducts develop in this contact area, seminiferous tubules are formed in the near-surface part of the cords. At puberty, the testicular cords take

the form of canals and the seminiferous tubules take their final form. In the germ cell-free region adjacent to the mesonephros, Sertoli cells form a series of thin-walled canals called the rete testis. The rete testis, which connects the seminiferous tubules to the lesser number of mesonephric tubules, takes the form of a canal at puberty. The caudal part of the mesonephric ducts develops as epididymis, sperm ducts or vas deferens and seminal vesicles. The functions of Sertoli cells can be listed as stimulating the migration of mesenchyme cells (Future Leydig cells) in the mesonephros to the testis and enabling them to transform into Leydig cells, preventing male germ cells from entering the meiosis cycle, secreting Müllerian Inhibitory Substance and secreting androgen binding factor. The source of the cells accompanying the germ cells in the gonads is not only the coelomic epithelium. Cells from the mesonephros to the gonad fold in response to signals emanating from Sf1-positive Sertoli precursor cells form precursors of peritubular myoepithelial cells, Leydig cells, endothelial cells, and support cells in the stroma. Such chemical attraction does not occur during ovarian development. Myoepithelial cells together with Sertoli cells and germ cells form epithelium-shaped testicular cords. It has been shown that the vascular network coming to the gonad outline plays an important role in the formation of testicular cords [22].

At the 9th to 10th weeks, the mesenchyme cells collected in the region with the stimulus of Sertoli precursor cells differentiate into Leydig cells. These cells begin to secrete testosterone as an endocrine secretion. Testosterone ensures that the mesonephric duct becomes permanent

and, in later stages, secondary sex characteristics are formed. In the first 12 weeks of development, testosterone secretion is regulated by chorionic gonadotropin, a peptide hormone secreted by the placenta. In the later stages of development, the pituitary gonadotropins take over the control of this secretion. The number of Leydig cells and the amount of testosterone secreted are 14-18. It reaches its peak in weeks under the control of chorionic gonadotropin. Luteinizing hormone (LH) receptors on Leydig cells begin to appear in the 12th week and the amount of steroid enzymes secreted from these cells increases at about the same time. However, from the 16th week, when gonadotropin control passes to the pituitary, the number of Leydig cells and the amount of steroid enzymes begin to decrease. Pituitary gonadotropin secretion begins in the 2nd and 3rd trimesters. Mutations that negatively affect the differentiation and function of Leydig cells lead to pseudohermaphrodism. Two types of Leydig cells carry out androgen biosynthesis during fetal and postnatal periods. Two factors formed by Sertoli cells, Desert Hedgehog and Platelet-Derived Growth Factor (PDGF), and basic regulators such as hepatocyte growth factor trigger the differentiation of fetal Leydig cells (20,21). Fetal Leydig cells produce the testosterone required for the development of male organs (epididymis, seminal vesicle and ductus deferens developing from the mesonephric duct). The 5α-reductase enzyme in Leydig cells leads to the formation of dihydrotestosterone from testosterone, which enables the development of the male urethra, prostate, penis and scrotum and the descent of the testicles into the scrotum. However, fetal Leydig cells regress and degrade towards the end of fetal life. At puberty, Leydig

precursor cells in the peritubular interstitium begin to differentiate to form a new Leydig cell group. Androgens secreted from this cell group are effective in initiating differentiation and spermatogenesis, which will regulate male behavior in the brain. The development of male genital tracts and ducts takes place under the control of hormones secreted from the testicles. The SRY protein, the product of the SRY gene, secretes antiMüller hormone (also called AMH- or Müller inhibitory substance [Mis]), a glycoprotein belonging to the Tgfβ (Transformative Growth Factor-β) family, from cells that will differentiate into Sertoli cells at the 8th week of gestation . This glycoprotein causes degradation of the paramesonephric ducts, leaving residual structures only at the cranial and caudal ends. The Müller inhibitory substance acts not directly on the epithelium, but on the AMH receptor-type II (AMHRII) receptor (also called Misr-II) on the surrounding mesenchymal cells, stimulating the mesenchymal cells to direct the paramesonephric duct epithelial cells to apoptosis or transform into mesenchymal cells. On the other hand, stimulation of the mesenchyme through Wnt-7 expression in the epithelium also plays a role in the continuation of the AMHR-II interaction. When this interaction is interrupted, Amhr-II expression is abolished and Müller duct derivatives remain in the male. It has been determined that AMH and AMHR-II gene mutations are present in cases of cryptoorchitis (undescended testis) or ectopic testis accompanying inguinal hernia within the scope of Persistent Müller Canal Syndrome [23]. Testosterone synthesized by Leydig cells enables the emergence of secondary characteristics of sex. Although the mesonephric kidneys

deteriorate, the mesonephric ducts continue to develop from the level of the gonad under the influence of testosterone and continue to exist as ductus deferens. The mesonephric tubules near the testis form the paradidymis. Hox genes play an active role in this process. The deepest parts of the sex cords that do not contain germ cells differentiate into the rete testis. The rete testis attaches to a limited number of mesonephric tubules and forms the connection of the seminiferous tubules with the mesonephric duct, which will open at puberty. These nephric tubules transform into the efferent ducts of the testis, and the mesonephric ducts form the vas deferens, while the paramesonephric ducts degenerate. In the third month, the seminal vesicle buds from the distal part of the vas deferens, which is the continuation of the mesonephric duct, and the prostate and bulbourethral glands from the adjacent endoderm-derived pelvic urethra. These glands appear as epithelial budding due to the interaction between epithelium and mesenchyme in the duct system where they are located. Androgenic receptors developing in mesenchymal cells enable epithelial cells to acquire glandular epithelial characteristics in a paracrine way in response to androgens in the environment. During prostate development, dihydrotestosterone, acting on receptors on mesenchymal cells, stimulates the expression of FGF-10 and TGF-β1 in the mesenchyme, and this situation leads to the production of sonic hedgehog in the genital sinus epithelium. In response to Shh signals, the urogenital sinus endoderm forms epithelial budding just below the bladder that will develop into prostate gland ducts. BMP-4, which has an inhibitory effect on budding, regulates the level of proliferation.

These molecular interactions have been shown to be regulated by the Hoxa-13 and Hoxd-13 genes. The number of prostate ducts was found to be low in mutants lacking the Hox gene [23]. The fibromuscular tissue surrounding the prostate glands originates from the mesenchyme. The 5α-reductase enzyme synthesized in the tissues around the urogenital sinus in the embryo converts testosterone to dihydrotestosterone, and depending on these two hormones interacting with the appropriate receptors, undifferentiated external genital organs (a pair of urogenital and labioscrotal folds on each side of the urogenital membrane and a single genital tubercle in front) and differentiate into the scrotum. Testes, like kidneys, are retroperitoneal organs and descend into the scrotum behind the peritoneal epithelium. At the beginning of the tenth week, the cranial suspensory ligament of the mesonephros derives from the diaphragmatic ligament of the mesonephros cranially, and the inguinal (caudal) ligament of the mesonephros, which will later be called the gubernaculum, is located caudal to the testicles. The descent of the testicles takes place in three stages between the 10th and 14th weeks. In the first stage, the testicles enlarge while the mesonephric kidneys regress. As a result of the interaction of androgens with their receptors in the cells in the cranial suspensory ligament, the connection between the testis and the diaphragm is destroyed, and the testicles slide caudally. In the second stage, which is defined as transandominal descent, the testicles descend to the level of the inguinal canal, but do not enter the canal with the effect of Insl-3 produced by Leydig cells. In the third stage, called the transinguinal descent, between the 7th and 9th months, the testicles

settle into the scrotum as a result of the shortening of the inguinal mesonephros ligament, namely the gubernaculum, by losing water. On the other hand, it is thought that the testicles are placed in the scrotum as a result of the elongation of the other structures of the body, not shortening the gubernaculum. Hermaphroditism as a result of sex-determining chromosomal anomalies, pseudohermaphroditism due to anomalies in sex hormones or receptors, and pubertal disorders due to hypogonadism are the main causes of sex anomalies.

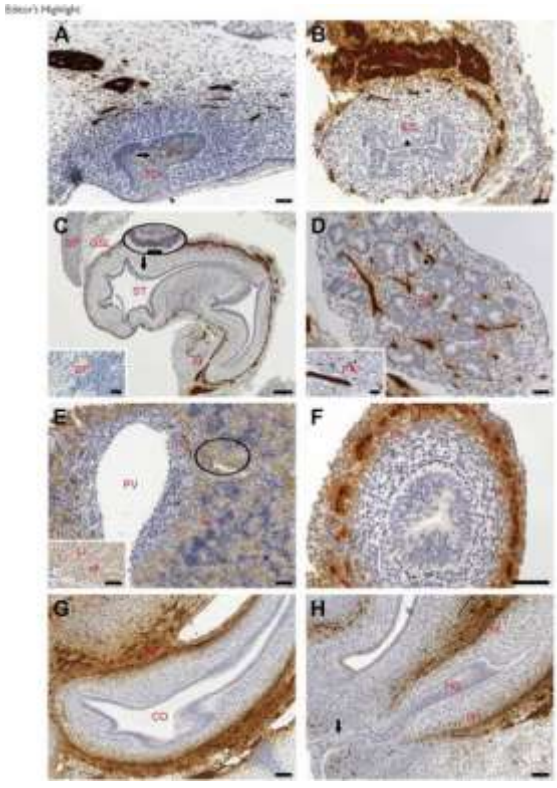


**Figure 6.** L1 cell adhesion molecule (L1CAM) protein localization in digestive organs and adults starting from w8-12 gestational period; sagittal sections (A, CE, GH) and transverse sections [24].

## Male Reproductive System Anatomy

Male reproductive system organs are divided into external and internal genital organs.

external genitalia

Penis

Scrotum

internal genitalia

Testis Epididymis Funiculus spermaticus

Ductus deferens (Vas deferens)

Glandula vesiculosa (Glandula seminalis)

Glandula prostatica (Prostate)

Glandula bulbourethalis (Cowper's glands)

Ejaculate (Semen)

Ductus Ejaculatorius

## Penis

The penis, which is the male mating organ, consists of two parts, the radix penis and the corpus penis. The radix penis is in the superficial perineal space. It consists of two parts called bulbus penis and crus penis [25]. The bulbus penis is the large posterior part of the erectile structure called the corpus spongiosum penis and is covered by M. bulbospongiosus. It is pierced by the urethra. The crus penis consists of

two erectile structures located on the back of the corpus cavernosum penis. It is covered by M. ischiokavernosus. The corpus penis consists of one corpus spongiosum penis and two corpus cavernosum penises. These erectile structures are surrounded by the tunica albuginea on the inside and the fascia penis (Buck's fascia) on the outside. The corpus spongiosum penis enlarges and forms the glans penis. The base of the glans penis is called the corona glandis. The corpus spongiosum passes through the penis into the urethra. The urethra enlarges in the glans penis and forms the fossa navicularis urethra. The skin covering the glans penis is called the preputium penis. Penis, league. fundiforme penis and lig. The suspensorium hangs on the anterior abdominal wall with the penis. As for the vessels, nerves and lymphatic structures of the penis, the penis is a. It is fed by three pairs of arteries, which are branches of the pudenda interna, and these arteries are a. bulbi penis, a. profunda penis and a. dorsalis penis appears to be present. The glans penis stimulated by the N. dorsalis penis is also n. It is also stimulated by a branch of the perinealis. The N. dorsalis penis extends along the ventrolateral of the glans penis [26]. Most of the blood from the erectile structures v. The dorsalis profunda drains the penis. Parasympathetics of the penis come from S2-4 and sympathetics from L1-2. The lymph vessels draining the skin of the penis go to the superficial inguinal lymph nodes, the lymph vessels draining the glans penis go to the deep inguinal and external iliac lymph nodes, and the lymphatic drainage of the erectile structures goes to the internal iliac lymph nodes [27].

## Scrotum

The scrotum, which is a bag-shaped structure containing the testis, epididymis and part of the funiculus spermaticus, is divided into two compartments by the septum scroti from the inside and the raphe scroti from the outside. The septum scroti includes all layers of the scrotum wall except the skin (1). Layers of the scrotum (from outside to inside);

1. Leather

2. Tunica dartos (Musculus dartos); It originates from the camperfascia. The wrinkled appearance of the scrotum skin is due to the contraction of this muscle. M. dartos, n. Sympathetic nerves in the genital branch of the genitofemoralis stimulate.

3. Fascia spermatica externa; M. obliquus derives from the fascia of the externus abdominis.

4. Fascia cremasterica and M. cremaster; It derives from the fascia and fibers of the M. obliquus internus abdominis. Innervation of this layer n. genitofemoralis r. provides the femoralis. Therefore, it constitutes a clinically important reflex pathway. As a result of stimulation of the inner thigh, m. Depending on the contraction of the cremaster, the testicles are pulled upwards towards the lower part of the abdomen (Kremaster reflex). The afferent pathway of this reflex is n. genitofemoralis r. femoralis; efferent pathway of the same nerve r. provides genitalis.

5. Fascia spermatika interna; It derives from the fascia transversalis. Fascia spermatika interna is loosely attached to the parietal leaf of the

tunica vaginalis testis, one of the layers of peritoneal origin that surrounds the testicles. The interna layer of the fascia spermatica also surrounds the funiculus spermaticus, testis, and epididymis.

6. The tunica vaginalis is the parietal leaf of the testis (periorchium); it is the parietal leaf of the peritoneum.

Vessels and nerves of the scrotum

• rr. scrotales anteriores; a. branches of pudenda externa profunda (branch of a. femoralis)

• rr. scrotales posteriores; AA. branches of perineales (branches of a. pudenda interna)

• a.kremasterica; a branch of a.epigastricainferior (branch of a. iliaca externa)

• a. branches from testicularis

Veins are the same name as arteries. Its lymph goes to the superficial inguinal lymph nodes. nerves

• n. genital branch of genitofemoralis; of the anterolateral face,

• nn. scrotales anteriores (branches of n. ilioinguinalis); front and top,

• nn. scrotales posteriores (branches of the perineal branches of n. pudendus); your back,

• nn. perineales (branches of nn. clunium inferiores of n. cutaneus femoris posterior); carries the sense of the lower face.

The nerves from the L1 segment of the spinal cord carry the sensation of the anterior 1/3 of the scrotum, and the nerves from the S3 segment of the posterior 2/3. Therefore, for the anesthesia of the anterior surface of the scrotum, the anesthetic must be administered from above. In the anesthesia of the posterior side, it should be given from below [28].

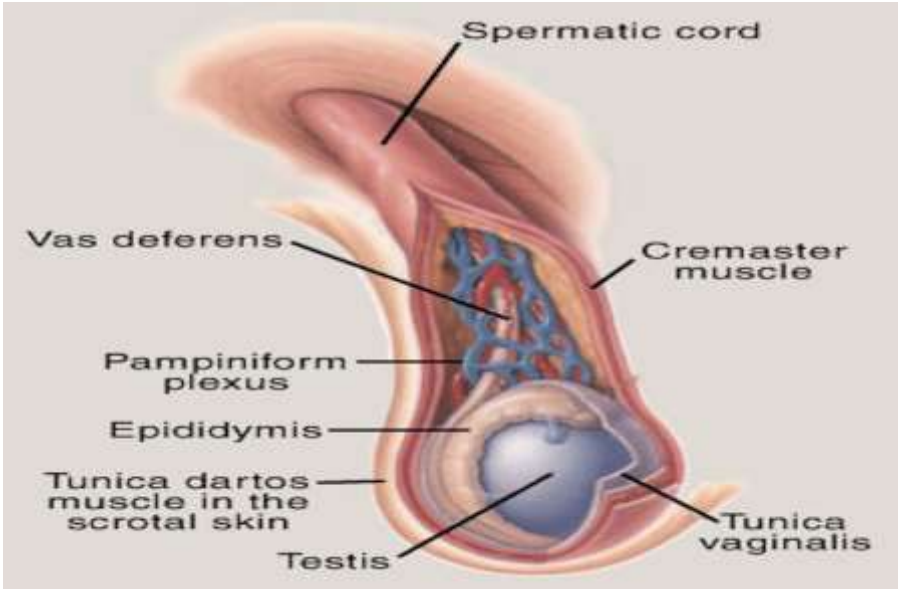


**Figure 7.** Scrotum

**Testis**

The testicles are in the scrotum and suspended from the funiculus spermaticus. Testes, the primary reproductive organ, weigh 10.5-14 g. The testis is 4 cm long, 2.5 cm wide and 3 cm thick. The left testis is lower than the right. It carries out the production of the male sex cells called sperm and the testosterone hormone that provides the male character. Since the tunica albuginea layer of the testis is not elastic and

does not expand, they are very sensitive to pressure and lose their function with pressure. They are also affected by extreme temperature differences. They do not function at normal intra-abdominal temperature and produce sperm at a temperature 2-3 $^{0}$C lower than body temperature [29]. Testis has three layers (Outside to inside);

Visceral leaf of tunica vaginalis testis (epiorchium),

Tunica albuginea

Tunica vasculosa

Tunica albuginea testis; The mediastinum sends septum called septula testis to the testis and divides the testis into lobules. The tunica vaginalis is the remnant of the lower end of the processus vaginalis. Tunica vaginalis testis consists of two layers, lamina visceralis (epiorchium) and lamina pariealis (periorchium). Fluid accumulation in the space between the two layers causes a pathology called hydrocele.

Sertoli cells in the testicles secrete a hormone called inhibin, which provides nutrition to the sperm, performs phagocytosis. In addition, the cells called Leydig cells synthesize the male hormone testosterone, as well as the estradiol hormone, which acts like estrogen. The functioning of these cells is controlled by the pituitary gland [27]. The vessel that feeds the testicles is the testicular artery, which branches off from the aorta abdominalis in the abdomen. Starting from the lower side of the kidneys m. It runs down the anterior surface of the psoas major, passes through the anulus inguinalis profundus, takes its place in the funiculus spermaticus, and extends into the bag and enters the testis from the

mesorchium. Apart from A. testicularis, there are also the following vessels that provide collateral blood supply.

1. A. ductus deferentis, a. iliaca interna a. It is the same branch of the uterina.

2. A. cremasterica, a. It comes from the epigastrica inferior.

3. Aa. pudendae externae, a. They are small branches coming from the femoralis.

Veins providing venous return v. testicularis (v. spermatica interna). On the right v. to the cava inferior; on the left (at a right angle) v. They are poured into the renalis. The beginning of the vein is the venous network (plexus pampiniformis) within the funiculus spermaticus. This venous network is a thermoregulatory system that works in the opposite direction and regulates the testicular temperature to be lower than body temperature. Varicocele is the varicose enlargement of the plexus pampiniformis, which is considered to be one of the important causes of male infertility. Varicocele right testicular vein v. It is stated that there is an angle of opening to the renalis. In addition, it is stated that regional venous hypertension, which occurs as a result of the sigmoid colon being filled with feces and compressing the vein, may also cause varicocele. Varicocele leads to a decrease in the number of sperm due to deterioration in temperature regulation. The lymphatic flow of the testis follows the testicular vein and directly reaches the paraaortic lymphatic vessels. For this reason, retroperitoneal metastases in the abdomen of testicular cancer cases (seminoma) develop rapidly.

Innervation of the testicles: The nerves of the testis originate from the T 10-12 spinal cord segments. The effects of motor autonomic fibers in the testis are not fully known. Afferent fibers follow the sympathetic nerve fibers running parallel to the vessels of the testis, first plexus aorticorenalis; then n. splanchnicus minor et n. They reach the spinal cord via the splanchnicus imus. Testicular pain is felt in the middle and lower parts of the anterior abdominal wall in accordance with the dermatome areas of the nerves coming from the same segments.

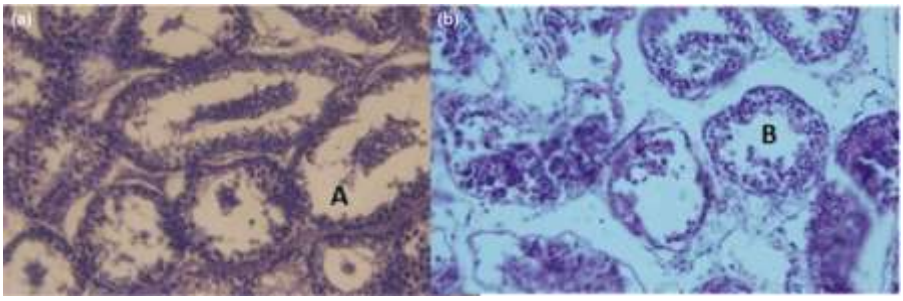


**Figure 8.** Histological section of the seminiferous tubule. 1, seminiferous tubule (A) containing less than three layers of differentiated germinal cells; 2, Seminiferous tubule containing three and/or more than four layers of differentiated germinal cells. (B) Seminiferous tubule containing less than three and/or four times the differentiated germinal cells [30].

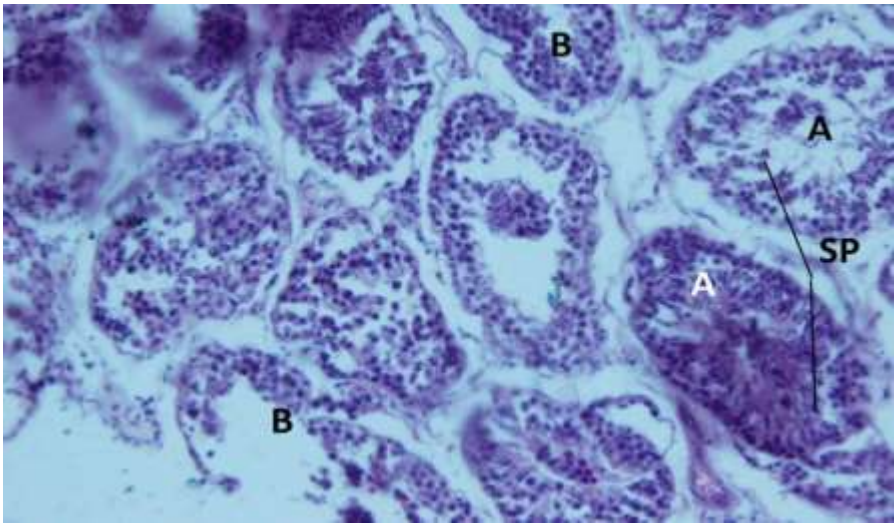


**Figure 9.** SP , spermatozoids. (A) Seminiferous tubules containing spermatozoa. B, Seminiferous tubules without spermatozoa (C), (H&E Staining × 400) [30].

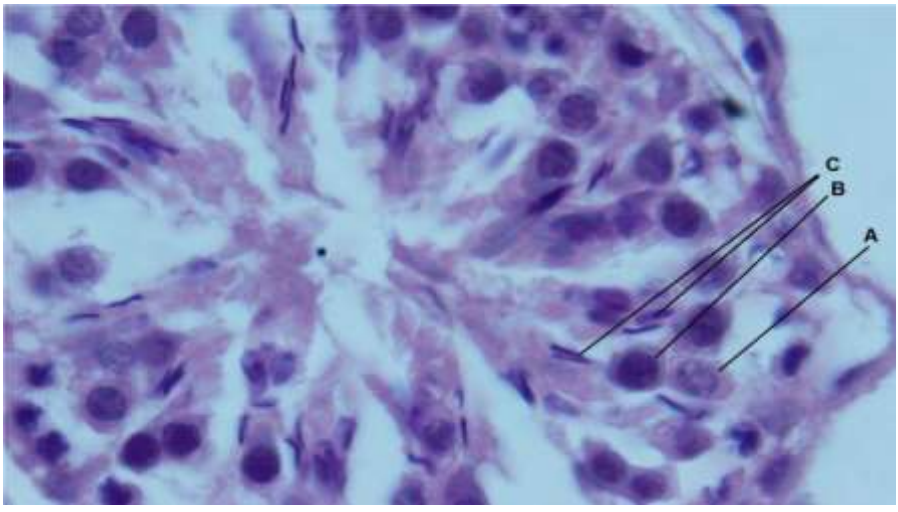


**Figure 10.** Histological section of seminiferous tubule showing positive SPI and positive TDI . spermatocyte (A); Sertoli cell (B); Spermatozoids (C), (H&E staining ×1000) [30].

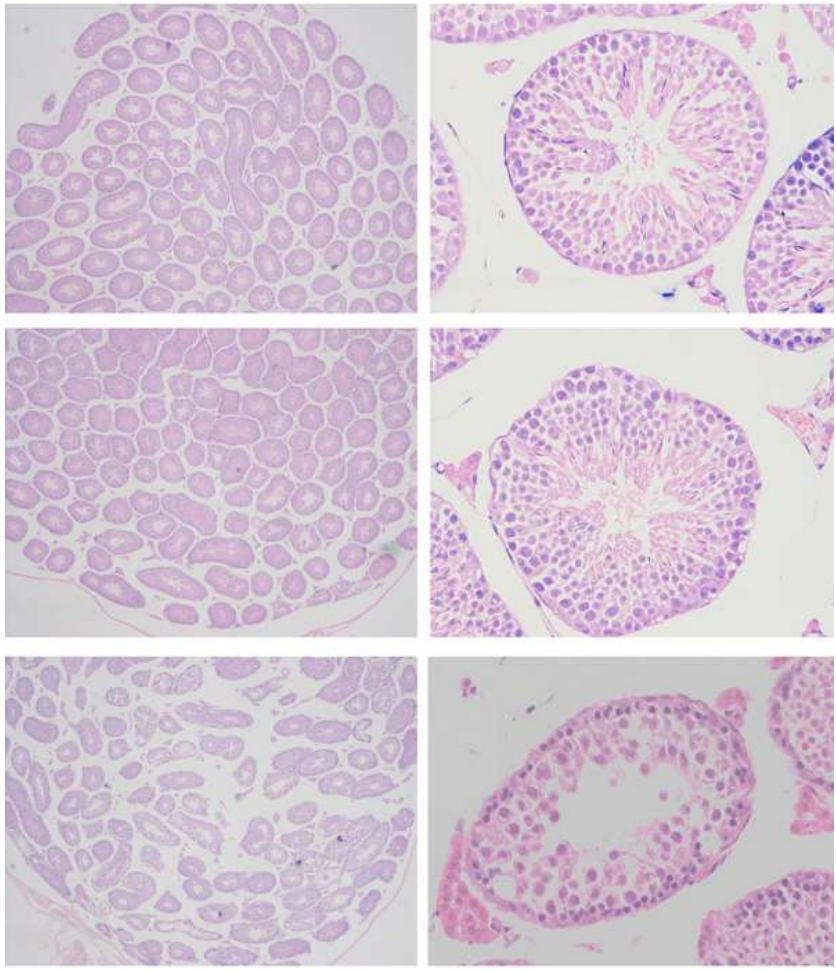

**Figure 11.** Absence of GGNBP2 results in abnormal testicular morphology. (A) Histology of three rodent genotypes of HE testis. HE sections were generated using the standard method. From top to bottom are testicles of wild-type, heterozygous and GGNBP2 KO mice. The left row is 10×10 under the LM and the right row is 10×40 under the LM. (B) Quantification of sperm count per area on the intersecting surface of the testicles (field of view at 400x magnification) [30].

## Epididymis

In the epididymis, located at the upper-posterior edge of the testis, the sperm reach functional maturity and some of them are stored here. The epididymis is a single ball of ducts about 4 cm long. Later, the ducts merge to form a single duct (ductus epididymis). When the ball is opened, its length exceeds 6 meters [27]. The epididymis has three parts: the caput epididymis, the corpus epididymis, and the cauda epididymis. The cauda epididymis is at the lower end of the testis and continues as the ductus deferens. Sperm that have completed their development in the testicles are stored in the epididymis and complete the final stage of their maturation here. When sperm are produced in the testis, they do not have the ability to move, that is, they cannot fertilize the ovum. They acquire the ability to move in the epididymis. With the addition of prostate secretion, mobility takes its final form. The ability of the sperm to move in such a way that they can fertilize the ovum is called capacitation. While some sources accept the feeding of the epididymis by blood vessels and nerves as the same as the testis, other sources state that there is a difference. For example, the main feeder vein a. ductus deferentis [27]. Parasympathetic and visceral innervation of the epididymis nn. erigentes provides. Accordingly, the origin of the nerves coming here is the S2-4 spinal cord segments. Parasympathetic stimuli initiate peristaltic fluctuations that move semen into the ductus deferens. Afferent fibers from the epididymis follow the same path to the plexus prostaticus and from there to nn. They reach the S2-4 spinal cord segments via the erigentes route. The sensation of pain originating from the epididymis (for example, due to a disease such as

epididymitis) is felt in the perineum and posterior regions of the thigh. sympathetic; It comes to the epididymis from T11-12 segments [29].

**Funiculus spermaticus**

The cord containing the vessels, nerves and ductus deferens of the testis, extending between the canalis inguinalis and the testis, and the funiculus spermaticus, one each on the right and left, hang the testicles in the scrotum. As the testicles descend into the scrotum in fetal life, they also carry the veins, nerves, and ductus deferens. These structures come together in the anulus inguinalis profundus to form the funiculus spermaticus. The funiculus spermaticus begins at the anulus inguinalis profundus and ends at the posterior margin of the testis. The funiculus spermaticus of the left side is slightly lower than that of the right side. Funiculus spermaticus, after coming out of the anulus inguinalis superfisialis, m. until the scrotum. It courses in front of the adductor longus. During its course, a. pudenda externa superfisialis with a. It passes between the pudenda externa profundus.


**Figure 12.** Funiculus spermaticus

**Ductus Deferens (Vas Deferens)**

The duct thickens in the tail (cauda) part of the epididymis and is called the ductus deferens. It turns upward and enters the abdominal cavity by passing through the inguinal canal in the funiculus spermaticus. The vesika comes to the lower back of the urinara. It joins with the canal of the seminalis vesicle on the lateral side and is called the ductus ejaculatorius and passes through the prostate and opens into the pars prostatic urethra. The length of the ductus deferens is 45 cm and it has four parts called pars scrotalis, pars funicularis, pars inguinalis and pars pelvica. The ductus deferens rises on the posterior surface of the testis and enters the inguinal canal through the anulus inguinalis superficialis. It passes through the anulus inguinalis profundus and enters the abdominal cavity. It passes in front of the external iliac vessels and enters the small pelvis. After crossing the ureter anteriorly, it reaches the fundus part of the bladder. Here, the ampulla shows an enlargement called the ductus deferentis and joins with the ductus excretorius, which is the terminal of the glandula vesiculosa, and opens into the prostatic part of the urethra under the name of the ductus ejaculatorius. Most of the sperm are stored in the ductus deferens. It can remain fertile for about a month in the epididymis and ductus deferens. If they are not excreted by ejaculation within a month, they degenerate and are absorbed by the body. Ductus deferens a. from the umbilicals a. It is fed by the ductus deferentis (corresponding to a. uterina). Venous blood is carried by the vein of the same name. Lymph goes to the external iliac lymph nodes. Its sympathetics come from T11-12.

**Glandula Vesiculosa (Glandula Seminalis)**

It is a pair of tubular glands located in the posterior lower part of the bladder. Since they are curved, their length, which is 5 cm, will be tripled if they are extended. Their ducts (ductus excretorius) combine with the ductus deferens on their side to form the ductus ejaculatorius. The ductus ejaculatorius is about 2.5 cm long. It opens into the colliculus seminalis or utriculus prostaticus in the prostatic urethra (Figure 6). The secretion of this gland constitutes the largest part of the ejaculate (about 60%). Its secretion containing fructose, citric acid, fibrinogen, prostaglandins and specific proteins activates spermatozoa. In addition, it is important in fertilization as it has an immunosuppressive effect on the female genital tract. Fructose is the main nutritional source of sperm. Prostaglandins react with cervical mucus and create a suitable environment for sperm motility. It also causes contractions in the tuba uterina and uterus, allowing the sperm to reach the ovum.

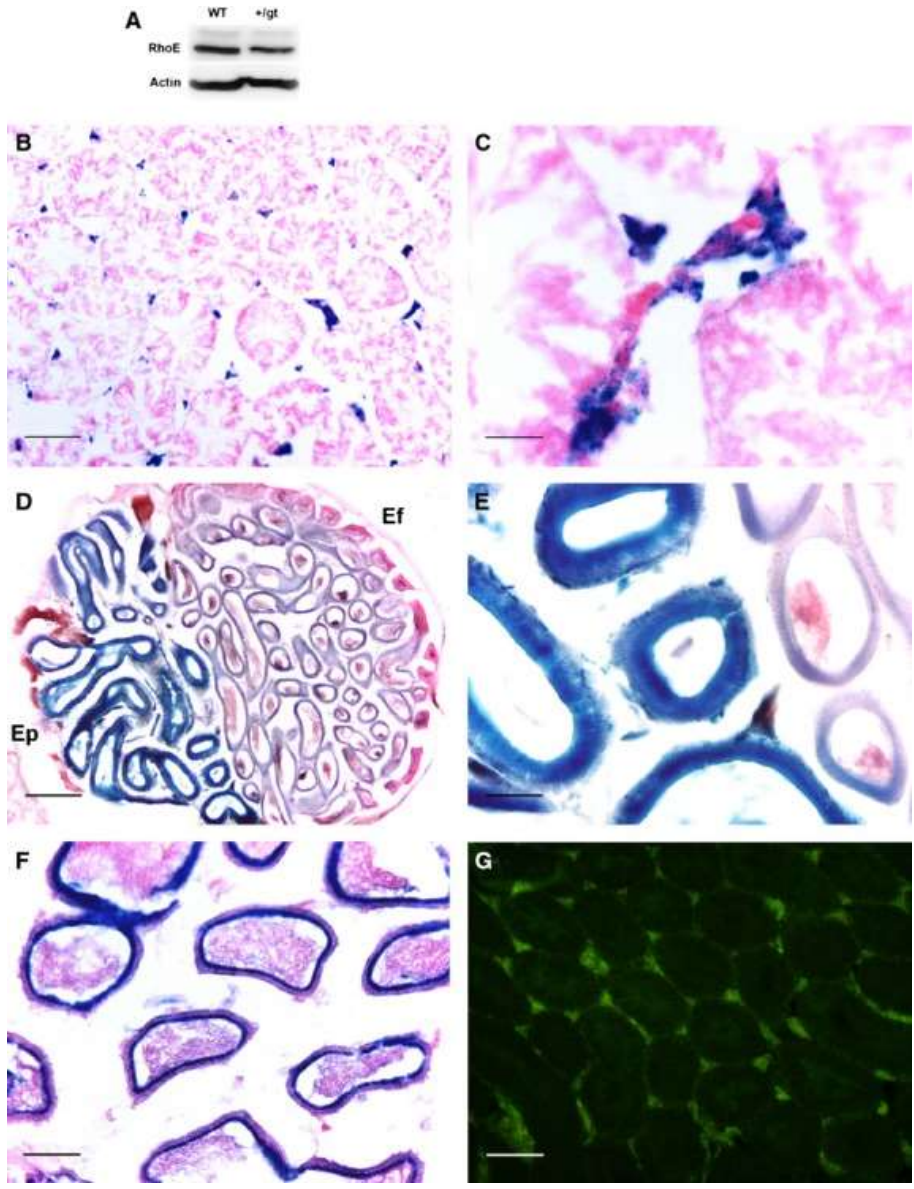


**Figure 13.** X-gal staining of adult testicles of RhoE+/gt mice. (A) Western blot showing RhoE expression in testes of wild type (WT) and RhoE +/gt (+/gt) mice. (B) X-gal staining in adult testicles shows that RhoE expression is localized in Leydig cells as in P15 mice. (C) A

higher magnification showing Leydig cell localization of X-gal staining. (D) Capillary pole section of testis. Ductuli efferentes (Ef) shows a slight X-gal staining (right of section) compared to the high levels observed in the ductus epididymides at the head of the epididymis (Ep; left section of section). (E) A higher magnification showing the different intensity of labeling. (F) Tail of an adult epididymis with high X-gal staining in epithelial cells. Note that the sperm cells in the lumen are not stained. (G) Immunodetection of β-gal in Leydig cells matched to X-gal staining. Scale bars: 100 μm (A, F); 25 micron (B); 200 micron (C); 50 microns (D, E) [31].

**Glandula Prostatica (Prostata)**

It is an organ in the small pelvis, behind the symphysis pubis and arcus pubis, above the urogenital diaphragm, below the bladder and in front of the rectum, in the shape and size of a chestnut, weighing about 20 g, made of smooth muscle fibers, connective tissue and glandular tissue. It surrounds the initial part of the urethra. It can be palpated with an examination from the rectum. The prostate, a roughly chestnut-shaped, purple-colored organ measuring 3x4 cm, is surrounded by a true capsule of solid connective tissue and a false capsule (prostatic fascia) that is an extension of the endopelvic fascia. Due to this capsular structure, it can be easily removed surgically (Figure 7). The parenchymal structure of the prostate is divided into five lobes. The apex (apex prostate) is below; its base (basis prostate) is located above. The excretory ducts of the gland (ductuli prostatici) open into the prostatic urethra. Its secretion is about 0.50 ml per day and constitutes 15-25% of the ejaculate. It has an

alkaline (basic) structure to ensure the fertilization of the ovum. The prostate is supplied mainly by branches from the inferior vesical artery. Its veins form a plexus called the plexus venosus prostaticus. Veins starting from here v. They open into iliaca interna or plexus venosus vertebralis. This last link explains the metastases of prostate cancers to the spinal canal and even to the brain. Lymph vessels go to the internal iliac lymph nodes. The sympathetic nerves of the prostate come from T11-L1. Sympathetic nerves contract the smooth muscle of the prostate, causing the gland to secrete continuously. Afferent pain fibers plexus pelvicus, nn. It reaches S2-4 segments via erigentes (nn. pelvici splanchnici).

**Glandula Bulbourethralis (Cowper's Glands)**

It is a pair of yellow, pea-like glands, one cm in diameter, on both sides of the membranous urethra in the deep perineal space. The compound is tubuloalveolar type. Its ducts open into the proximal part of the cancellous urethra. mucous secretion; It contains substances such as sialic acid, galactose and galactosamine. The secretion enters the urethra during sexual stimulation and neutralizes the remaining urine in the urethra. In addition, it facilitates the passage of ejaculate through the urethra and sexual intercourse. During sexual stimulation, this gland is activated first. The clear secretion at the tip of the penis belongs to this gland. ductus gll. They are called bulbouretrales and are about 2-3 cm long. Sympathetic fibers L1- 2; parasympathetics come from segments S2-4.

**Ejaculate (Semen)**

Epididymis, ductus deferens, vesicle seminalis, prostate and gl. It is the liquid that is formed by the mixing of the secretions of the bulbourethralis and contains the sperms. It is fragrant and sticky. It is approximately 3-4 ml and contains 300-400 million sperm. Sperm make about 10% of the ejaculate. The rest is made up of glandular secretions. The largest part (about 60%) is secreted by glandula vesiculosa. Prostate 30%, ductus deferens 10%, glandula bulbourethralis and secretions from other glands. More than 90% of the ejaculate is water. However, it contains many substances. Fructose is especially abundant. It contains two vitamins, vitamin C and inositol. Elements such as calcium, zinc, magnesium, copper and sulfur are found in the ejaculate. Also, the greatest concentration of prostaglandins in the body is in the ejaculate. The amines produced in the testicles give the ejaculate its smell. Ductus Ejakulatorius is a narrow (0.5 mm) and thin-walled canal, two cm in length, formed by the union of the ductus deferens and the ductus excretorius. It passes through the parenchyma tissue of the prostate gland and opens into the prostatic urethra in the colliculus seminalis. The next part of the uretra is dual-functional to be used for urine and ejaculate transport. Parasympathetic effect causes secretion. Slow peristaltic waves occur along the ductus deferens. Sperm are transported to the ampulla to be stored in the epididymis. The sympathetic effect causes strong contractions in the smooth muscles of the canal, thus causing ejaculation.

## Blood-testicular Barrier

Occlusive junctions (occlusive junctions) between adjacent Sertoli cells form the blood-testicular barrier. The barrier prevents proteins, including antibodies, from reaching the developing spermatogenic cells. Conversely, the barrier prevents protein from leaking from developing spermatogenic cells and triggering an immune response. The blood-testicular barrier is similar to the blood-brain barrier, except that the latter's main element is the occlusive junctions of capillary endothelial cells. Occlusive connections are very important in the blood-testicular barrier; because they divide the seminiferous epithelium into the basal compartment below the junction and the adluminal compartment above the junction. Spermatogonia are located in the basal compartment and spermatocytes and spermatids fill the adluminal position [32]. Delay in postnatal development between the cells forming the blood testicular barrier creates meiotic arrest. In studies conducted in experimental animals, it was determined that disruption of the cellular organization of the blood testicular barrier stops the differentiation stages during spermatogenesis. Filamin A is a molecule in the actin structure and participates in the structure of intercellular cross-links by forming the cytoskeleton. Recent studies have shown that Filamin A in Sertoli cells plays an active role in the organization of the blood testicular barrier and protects the actin filament network in postnatal development [33].

## Spermatogenic Cells

Spermatogonia are diploid spermatogenic cells that are in direct contact with the basal lamina in the basal compartment. They lie beneath the occlusive connections between Sertoli cells. Therefore, they are outside the blood testicular barrier. These cells have stem cell properties. For this reason, it has important effects on male infertility. Spermatogonia resistant to radiotherapy and chemotherapy divide mitotically. Spermatocytes and differentiating spermatids divide meiotically and are sensitive to radiotherapy and chemotherapy. In patients undergoing radiotherapy and chemotherapy, spermatogonial stem cells can regenerate the spermatogenic process after treatment is terminated. Postmitotic Sertoli cells are highly resistant to these treatments [32]. Type B spermatogonia that complete mitosis division enter the prophase of meiosis after completing the S phase (DNA synthesis phase). Thus, they form primary spermatocytes with 46 chromosomes (44+XY) containing twice the DNA (4N) compared to the spermatogonia. These cells remain in prophase for a long time. For this reason, most of the cells seen in histological sections are spermatocytes waiting in prophase. They are the largest cells of the spermatogenic series. The cells formed at the end of the first meiosis are secondary spermatocytes and are smaller cells containing 23 chromosomes (22+X or 22+Y). This decrease in the number of chromosomes (from 46 to 23) is accompanied by a decrease in the amount of DNA in each cell (from 4N to 2N). They are difficult to see in histological sections, they are short-lived cells that remain in the interphase for a short time and quickly enter the second meiotic division. With the division of

secondary spermatocytes, the amount of DNA is halved and haploid (1N) spermatids containing 23 chromosomes are formed. Cells reaching this stage have completed the spermatogenesis stage, which is defined as the spermatozoon production process. After this stage, spermiogenesis begins. Spermiogenesis is the final stage of spermatozoon production and is the process by which spermatids transform into spermatozoon, which are highly specialized cells for transferring male DNA into the ovum. Cell division does not occur in this process. It is a complex process that includes acrosome formation, nuclear condensation and elongation, flagella development, and loss of most of the cytoplasm. Mature spermatozoa are formed, which are deposited into the lumen of the seminiferous tubule.

**Interstitial Tissue**

The space between the seminiferous tubules is filled with connective tissue, nerves, windowed capillaries, and lymph vessels. In this part, besides the cells of the connective tissue, another cell becomes functionally evident after adolescence. This cell is polygonal, round in shape, and has a central nucleus. This cell with small, lipid droplets-rich eosinophilic cytoplasm is known as the Leydig cell. The testosterone hormone they secrete is important in terms of spermatogenesis, sex differentiation during embryonal and fetal life, and control of gonadotropin secretion.

## Sperm Structure

The mature sperm consists of two elements, the head and the tail. The head is connected to the tail with a connecting piece. The tail part is also divided into three parts: the middle part, the main part and the last part. A plasma membrane surrounds the head and tail of the sperm. The head consists of the nucleus surrounded by the acrosome. The nucleus is a flattened dense structure. The anterior half of the nucleus is covered by the acrosome and contains hydrolytic enzymes (Proteases, acid phosphatases, hyaluronidase as well as neuraminidase) commonly found in lysosomes. Often referred to as acrosome, a special type of lysosome acceptable. Acrosomal enzymes are released at the time of fertilization to facilitate sperm entry into the corona radiata and zona pellucida that surround the oocyte. The connecting piece is a narrow piece with a pair of centrioles. The distal centriole originates from the axoneme, the central part of the sperm tail. The middle part of the tail consists of a layer of helically arranged mitochondria, 9+2 microtubular axonemes, and nine columns extending along the tail from the connecting part in the sperm neck called the outer dense fibers. The lower border of the midpiece is marked by the termination of the mitochondrial helix at the annulus. The main piece is the longest part of the tail. It consists of a central axoneme and a fibrous sheath, surrounded by seven outer dense fibers (different from the nine fibers in the middle part). The fibrous sheath is formed by the circular skeleton protruding from equidistant longitudinal columns. Both the outer dense fibers and the fibrous sheath contain keratin, proteins that form a solid skeleton for microtubular gliding and coiling during the forward

movement of the sperm. The last segment is a very short segment of the tail with only the axoneme due to the outer dense fibers and premature termination of the fibrous sheath.

**Molecular Spermatogenesis Regulation and Male Fertility**

Spermatogenesis takes place in the seminiferous tubules in the testis, which contains germ cells and Sertoli cells. Somatic Sertoli cells form the microenvironment necessary for lifelong sperm production. Interstitial tissue between seminiferous tubules; blood vessels, lymph vessels, macrophages and Leydig cells that produce growth factors and testosterone. Peritubular myoid cells surround the seminiferous tubules, provide structural support, are a source of growth factors, and facilitate fluid and sperm movement in the seminiferous tubule lumen [34]. Spermatogenesis is a complex developmental process in which germ cells in the spermatogonial stem cell (SSC) pool enter the differentiation processes and produce sperm. Germ cell development in the testis consists of three important stages, in order: i) primordial germ cells (PGH), which are the first cells of the germ cell line developing in the embryonic period, ii) SSCs and iii) spermatozoa. The first two periods cover fetal and neonatal processes and provide the formation of SCH. Cells that play a leading role in spermatogonial stem cell development are PGH and gonocytes, respectively. PGHs, which migrate from the wall of the vitreous sac wall to the gonads in the fetal period, differentiate into gonocytes, and type A spermatogonia are formed from the gonocytes in the neonatal period. The third period is the beginning of the spermatogenic cycle and encompasses a well-

ordered chain of events. This period; It involves a differentiation process called mitosis and meiosis and spermiogenesis, and spermatozoa are produced as the final product from spermatogonia differentiated from the SSC pool [34]. Studies with mouse models have revealed many genes and molecular mechanisms that are important in elucidating the chain of signals that play a role in the spermatogenesis process [35]. Important information has been obtained during spermatogenesis with many mouse models created with the principle of deletion or overexpression of genes. Failure in spermatogenesis can occur at different levels. These may result from failure of PGHs to migrate to the gonads, loss of SSCs, arrest of spermatogenesis, inadequate spermiogenesis, or an impaired microenvironment. All these disorders can result in azoospermia, severe oligozoospermia, asthenozoospermia, or teratozoospermia. In this section titled Molecular regulation of spermatogenesis and male fertility, we will introduce the reader to the process of entering puberty, which is characterized by the formation of the SSC pool, starting from the germ cell precursors (PGH first and then gonocytes) that emerge during the fetal period, and then the formation of germ cells that are selected from this pool and begin the first meiosis, and play a role in spermiogenesis. Up-to-date information on basic molecular mechanisms will be presented. Finally, by associating this information, mainly obtained from mouse models, with fertility problems in humans, future research in male fertility will be briefly mentioned.

**Primordial Germ Cells Migrating to Bipotential Gona**

Although the sex of the embryo is determined genetically at the time of fertilization, the gonads do not have male or female morphological characteristics until the 7th week of development in humans. In mice with a total gestation period of approximately 19-21 days, 10-11 days after fertilization. Gonad morphology has not yet differentiated into male or female direction until days. This is because the germ cells have not yet reached the gonads. Primordial germ cells appear in the extra-embryonic mesoderm in the wall of the yolk sac during early embryonic development (days 7-8 of gestation in the mouse). Differentiation of PGHs from epiblast cells developing from the inner cell mass of the embryo (Embryoblasts) depends on the “Bone Morphogenetic Protein” (BMP) and “Wingless Integration 3” (WNT3) signal molecules expressed from the extra-embryonic ectoderm [36]. "Positive Regulatory Domain Zinc Finger Protein 1" (Prdm1), also known as Blimp transcription regulator, is needed for the formation of PGHs that begin to differentiate from epiblasts on embryonic 6th day in mice [37]. This molecule is expressed from epiblast cells and is required for PGH differentiation. Thus, the expression of both cell extrinsic factors (such as BMP factors) and cell intrinsic factors (such as Blimp1) is important for determining the fate of germ cells. On the one hand, PGHs, which increase their number by repeatedly undergoing mitotic divisions, on the other hand, migrate through the midgut and dorsal mesentery of the embryo and reach the gonad primordium. This process takes about 10-11 in the mouse. occurs in days [38]. At the 4th week of human embryo development, PGHs that appear in the yolk sac proliferate and begin to

migrate to the morphologically undifferentiated gonads and settle in the gonads at the 7th week. PGHs that deviate from the gonad path and migrate to the wrong places or cannot reach the gonad on time are destroyed by apoptosis [39]. Some pr expressed by primordial germ cells Proteins are essential for the migration and survival of these cells. E-cadherin expression is important in cell migration, and if this expression is suppressed, migration of PGHs to the gonad primordium and colonization to the gonads does not occur. In addition, the loss of Oct-4 and Nanos-3 genes, which are germ cell progenitor and pluripotency markers, results in a decrease in the total number of PGHs that migrate and the absence of germ cells in male adults [40]. After the PGHs reach the gonads, the process of gonad differentiation, in other words, determination of testicular morphology and development different from ovarian morphology, begins. The PGHs reaching the male gonad are now called "gonocytes" after this stage.

**Gonocytes**

**Determining Gender**

When primordial germ cells reach the gonad primordium, they are surrounded by somatic Sertoli cells and “seminiferous cords” form. The reason why these formations were named as seminiferous cords in this period is that these structures are in the form of solid masses filled with gonocyte and Sertoli cells, so no lumen formation has yet been observed in these structures. Sertoli cells, which are the somatic cells of the seminiferous cords, are formed by differentiation of coelomic epithelial cells lining the outer surface of the gonad primordium and facing the

abdominal cavity and migrate into the gonad and surround the gonocytes that have reached the gonad. When somatic cell proliferation is inhibited, the formation of testicular cords is impaired [41]. Sertoli cells continue to proliferate within the newly formed seminiferous cords. At this stage, some differences occur depending on whether the gonad is male or female. In the male gonad, 12-14 days of embryonic development. As the gonocytes continue to proliferate, germ cells reaching the female gonae begin meiosis and form oogonia. This process is called the "gonad sex determination" process. Testicular development begins in many mammals when expression of the sex determining region (Sry) gene of the Y chromosome begins in somatic Sertoli cells (Embryonic from day 10.5). The XX gonads in which the Sry gene is not expressed will be differentiated towards the ovary. Sry expression in male gonad Sertoli cells E11.5. reaches its highest level on the day and E12.5. ends before the day. In cases where Sry gene expression is not uniform, somatic support cells differentiate into granulosa cells of the ovary. With the upregulation of the Sry-box 9 (Sox 9) gene, Sry-positive cells differentiate into Sertoli cells [42]. In mice, Sry works with steroidogenic factor 1 (SF1) to activate Sox9 expression and differentiate into the bipotential gonad testis. Approximately E14 in mouse. from day E15-16. In these days, gonocytes exit the cell cycle, terminate their mitotic division, and wait without proliferating until birth. Meanwhile, Sertoli cells continue to proliferate. Signals such as termination of PPA2 expression, expression of activin A subunit and TGF-beta (Transforming Growth Factor) regulating the proliferation of these cells are important when gonocytes

exit the cell cycle and enter the mitotic silencing process [43]. Immediately after this human sex determination process, testicular size is twice the size of the ovary because somatic Sertoli cells continue to proliferate. Proliferation of gonocytes reaching the gonads occurs between the 3rd and 6th weeks of pregnancy in humans. It takes place between the 6th and 6th months of life, and the gonocytes in the male fetus enter into mitotic silence until birth after the 6th month. Germ cells multiplying by mitosis in female or male gonads during embryonic and fetal period; During sex determination, they come to a decision stage to form either the female gonad by starting meiosis, or the male gonad, which will avoid meiosis and enter mitotic silence until birth [44]. It has been revealed that meiosis is initiated by retinoic acid (RA), an active derivative of vitamin A, and is largely inhibited by the P450 enzyme, CYP26B1, which has RA degrading activity. While CYP26B1 is expressed in developing mouse gonads of both sexes, its expression is increased in male gonads and suppressed in female gonads, so it is considered as a male gona-specific meiosis inhibitory factor. In addition to RA degradation in the male gonad, non-retinoid factors secreted by the somatic cells of the testis also prevent the entry of the male gonad into meiosis [45]. Thus, while the female gonad initiates meiosis and forms oogonia during the fetal period, the gonocytes, the germ cells of the male gonad, avoid meiosis and undergo mitotic divisions for a while, then enter mitotic silence until birth. At birth, in both humans and rodents, gonocytes continue to multiply by resuming mitotic divisions, and on the other hand, they migrate towards the base of the

seminiferous cords and differentiate into type A spermatogonia. This migration process requires the c-kit/SCF signal.

**Neonatal Gonocyte Proliferation, Migration and Differentiation into Spermatogonia**

Testicular gonocytes are the precursors of spermatogonial stem cells (SSCs) and are located in the phase between PGHs and SSCs. Postpartum (PP) in the mouse 1-4. Neonatal gonocytes begin to proliferate between days. Among the mechanisms regulating gonocyte proliferation, Platelet-Derived Growth Factor (PDGF), 17β-estradiol (E2), Leukemia Inhibitory Factor (LIF) and RA are known. PDGF and E2 are produced by Sertoli cells and PP3 in the mouse. They are required for the activation of gonocyte proliferation per day [46]. LIF increased the proliferation of gonocytes co-cultured with Sertoli cells for three days from postpartum day 1, while PP3. LIF did not affect gonocyte proliferation in cultures performed after the day. Retinoic acid, PP3. stimulates rat neonatal gonocyte proliferation per day. Retinoic acid induces gonocyte proliferation after 4 days of organ culture in 6-10 week old human fetuses [47]. In a study conducted in the literature in 2012, it was shown that rat gonocytes do not undergo apoptosis in the first week after birth, their distribution changes mainly along the growing seminiferous cords, and their differentiation into spermatogonia by starting their proliferation is concurrent with increasing Oct4 expression. The main difference known between neonatal gonocytes and spermatogonia formed by the differentiation of these cells by migrating to the base of the seminiferous cords is that the

morphological images of these cells and their localization in the seminiferous cords are different from each other. While gonocytes are large round cells located in the middle of the seminiferous cord, spermatogonia are smaller, crescent-shaped cells located close to the basement membrane at the base of the seminiferous cords. In fact, location within the seminiferous cord is not a sufficient criterion because many proteins expressed by gonocytes are also expressed in spermatogonia. Recent findings have identified some gene markers to distinguish between these two cell populations. Stimulated by Retinoic Acid 8 (Stra8), one of these genes, is a marker of gonocyte-spermatogonium transition and is expressed only in spermatogonia and premeiotic cells [48]. In postnatal mouse testis, PP5. Stra8 expression, which starts to be expressed in a small number of spermatogonia from day one, increases in an increasing number of preleptotene and early leptotene spermatocytes. In recent years, RA has been demonstrated to be a key regulator of gonocyte differentiation in both rat and mouse. Retinoic acid has this effect; It does this by decreasing the Growth Factor Receptor alpha 1 (GFRα1) transcript in differentiated gonocytes, decreasing the rate of SSCs and increasing the rate of type A spermatogonia by increasing c-kit mRNA. It can be predicted that the same mechanisms may play a role in the differentiation of gonocytes into spermatogonia in humans. In order for gonocytes to differentiate into spermatogonia, they must migrate from the center of the seminiferous cords to the basement membrane. In some gonocytes that begin to migrate towards the basement membrane by forming pseudopods, c-kit is transiently expressed, so it is thought that c-kit

regulates gonocyte migration [49]. When gonocytes reach the basement membrane of the seminiferous cords and differentiate as SSC cells, c-kit expression is abolished in SSCs. Migrating gonocytes can proliferate and differentiate thus corresponding to the pool of gonocyte that will differentiate into SSCs. Thus, there are at least 2 types of neonatal gonocyte populations in the neonatal testis; The first population of gonocytes form SSCs (Atek spermatogonia) and make the stem cell niche, while the second population of gonocyte differentiates into Aquiferous (Aç) spermatogonia, which have lost their stem cell characteristics and will pass through various stages and form sperm [50]. Testicular cancer is known to be the most common malignant condition in young men. These cancers develop from carcinoma in situ lesions, known as pre-invasion lesions, and there are problems in the successful differentiation of germ cell precursors or gonocytes into spermatogonia in the emergence of their pathology [51]. Therefore, in order to better understand the etiology of testicular germ cell tumors, it is important to elucidate the mechanisms involved in the differentiation of gonocytes into spermatogonia.

**Spermatogoniums: Self-Renewal, Differentiation and Stem Cell Niche Establishment**

With the migration of gonocytes from the middle of the seminiferous cords to the basement membrane, the lumen begins to form in the seminiferous cords, and after this stage, the cords are called seminiferous tubules. Type A spermatogonia, self-renewal and differentiation of spermatogonial stem cells located in the basement

membrane of seminiferous tubules play a key role for normal spermatogenesis. A good understanding of the molecular mechanisms involved here is important in terms of determining the factors that cause male infertility and testicular cancer. There are different subtypes of type A spermatogonia in rodents; While Atek (At ) are stem cells that have the ability to renew themselves, the others formed by differentiation from these are called Asiftli (Aç), Asıral (As), A1-4 spermatogonia. This classification was made on the basis of the morphological and phenotypic differences of type A spermatogonia [52]. Atek spermatogonia are stem cells, whereas spermatogonia that are connected to each other by cytoplasmic bridges (Aç -A1-4) are spermatogonia that have lost their stem cell potential and have begun to differentiate. Intermediate and then type B spermatogonia are formed from A1-4 spermatogonia, and type B spermatogonia give rise to spermatocytes, spermatids and mature sperm. Cytoplasmic bridges between staggered spermatogonia are probably important for these cells to initiate meiosis simultaneously. In humans, Akoyu and Asoluk spermatogonia were described by Clermont years ago [53]. It is thought that Akoyu cells form the stem cell reserve and Asoluk cells are differentiated spermatogonia. These cells are named because the nuclei of these cells are observed as dark or light at the cross-sectional level. However, the functional identities of human SDGs are not yet fully known. In contrast, a study conducted in 2012 revealed that LIN28 molecule is expressed in adult human testis and its expression is limited to a small number of spermatogonial stem cells, in contrast to two studies published in 2011. LIN28 is a highly expressed pluripotency

marker in pluripotent mouse embryonic stem cells, mouse embryonal carcinoma cells and human teratocarcinoma cells (58). LIN28 is a supplement, even a necessary molecule, in induced pluripotent cell production in both humans and non-human primates [54]. Strict regulation of the balance between the ability of spermatogonial stem cells to renew themselves and the differentiation process plays an important role in maintaining spermatogenesis throughout life. If this balance shifts towards differentiation, the stem cell reserve will be depleted. Conversely, an imbalance shifting to cell regeneration will result in stem cell accumulation and reduction of differentiated spermatogenic cells. Studies in mice led to the discovery of some genes that regulate this balance. These; It is known as Inhibitor of DNA Binding 4 (ID4), Promyelocytic leukemia zinc finger (Plzf) (62,63) and Nanos2. ID4 is currently the only marker that plays a role in the balance of self-renewal and differentiation of spermatogonia in mice and is only known to be expressed in Atek spermatogonia. In-vitro suppression of the expression of ID4 inhibits the proliferation of spermatogonial stem cells, and deletion of the ID4 gene causes progressive germ cell loss in the mouse, resulting in male sterility. Plzf, a transcriptional repressor, is expressed in At/Ac/As spermatogonia. In genetic deletion of Plzf, spermatogenesis occurs initially, then gradually ends within months, revealing that Plzf has a role in maintaining the existence of stem cells, not in stem cell formation. Consistent with these, Plzf suppresses the expression of c-kit, a marker of early spermatogonial differentiation, at the transcriptional level [55]. Another newly discovered molecule, Nanos2, is expressed in At and hungry spermatogonia and its deletion

causes loss of undifferentiated spermatogonia, while its overexpression causes accumulation of spermatogonia and inhibition of cell differentiation [56]. While the molecules described above are some of the "intrinsic" factors expressed by spermatogonia that control the balance between self-renewal/differentiation, it is important to mention additionally the "extrinsic" factors that control the persistence and differentiation of the SSC pool. Glial cell line-Derived Neurotrophic Factor (GDNF) is a molecule secreted by Sertoli cells and peritubular myoid cells that regulates the proliferation of SSCs. Overexpression of GDNF causes undifferentiated spermatogonia to accumulate in the seminiferous tubules, while heterozygous deletion of the GDNF gene causes spermatogonia.

**The effect of Endometrial injury In addition to the methods that affect infertility success**

Scientific and technological developments in infertility treatment have led to an increase in success rates. Assisted Reproductive Technology (ART) covers all of the methods that provide embryos by fertilization of human reproductive cells (oocyte and sperm) outside the body. These developing technologies play an important role in realizing the dreams of couples to have children. assisted reproductive techniques; IUI (Intrauterine Insemination), IVF (In Vitro Fertilization), GIFT (Gamete Intrafallopian Transfer), ZIFT (Zygote Intrafallopian Transfer), PZD (Partial Zona Dissection), SUZI (Subzonal Insemination), TET (Tubal Embryo Transfer) ve POST (Peritoneal It includes various developed methods such as Oocyte and Sperm Transfer). The first and still the

most frequently used method is IVF (In Vitro Fertilization). Other methods are more invasive and their usage areas are decreasing nowadays. In addition, sperm retrieval technique and 4 sperm injections are now another component of ART. Injection of a single sperm into the oocyte cytoplasm, ICSI (Intracytoplasmic Sperm Injection); sperm aspiration from testicles, TESA; sperm extraction from testicles, TESE; epididymal sperm aspiration with microsurgery, MESA; Assisted hatching and pre-implantation genetic diagnosis (PGD) are other technologies developed. IVF begins with controlled ovarian hyperstimulation (KOH) using exogenous gonadotropins. Afterwards, the developing follicles are collected under the guidance of transvaginal ultrasonography. Oocytes obtained from follicles are fertilized in laboratory environment with appropriate sperms taken from the partner. Embryos obtained as a result of the procedure are transferred to the uterus of the expectant mother by transcervical route. As of today, around 3 million children around the world have been born using assisted reproductive techniques [57]. If we examine the historical development of assisted reproductive techniques; The first embryo transfer studies started with rabbit experiments in the 1890s. Embryo transfer studies have been carried out in farm animals since 1949, and it has been thought to increase the genetic potential of animals. Today, in vitro fertilization is widely used for this purpose in the world. The first in vitro fertilization was carried out in rabbits after it was understood that sperm must pass the stage of capacitation in the female genital organs for fertilization ability. In late 1969, Dr. Edwards et al. reported that they performed the first successful in vitro fertilization

with human oocytes. The first IVF pregnancy was conceived in 1976 by physiologist Dr. Edwards and gynecologist Dr. Pregnancy performed by Steptoe. However, it is unfortunate that the pregnancy that occurs is an ectopic pregnancy. Finally, nearly 21 years ago, in 1978 at Cambridge, Dr. Edwards and Dr. An intrauterine pregnancy was provided by Steptoe with in vitro fertilization method and a healthy baby named Louise Brown was born. Over time, various modifications of IVF treatment such as GIFT, ZIFT, TET, ICSI have emerged and have been used in appropriate patients. In 1983, Dr. The first GIFT baby (Asch et al.) was born in 1984 and the first ZIFT baby was born in 1986 (Devroey et al.) by Trounson et al. There are many obstacles on the way from a single cell to an organism. Current studies are aimed at increasing the rates of pregnancies and live births created by assisted reproductive techniques. There are cases in which pregnancy could not be achieved and the cause of which could not be explained despite proper procedures at all stages. However, developing technology has opened the door to some chances for us, albeit a little. Each new study sheds light on our understanding of the infertility system, which still has many dark sides. All studies are aimed at revealing and explaining the unknown. Every new study in this field aims to increase implantation and pregnancy rates.

Primordial germ cells; The yolk sac, the allantois, and the posterior part of the intestine originate from the endoderm. At six to eight weeks of gestation, germ cells rapidly proliferate by mitosis and initiate ovarian differentiation. Approximately 6-7 million oogonium is reached in the

sixteenth to twentieth gestational week. After this point, germ cells decrease continuously until menopause. In the eleventh-twelfth week, oogonia transform into oocytes with the first meiotic division and wait in prophase. A single ovum is formed by the second meiotic division. The first meiotic division occurs before full ovulation and the second during sperm penetration. Excess genetic material is excreted by the polar body formed in each meiotic division [57]. After the formation of the oocyte after the first meiotic division, the number of germ cells decreases to 1-2 million at birth and 300-500 thousand at the beginning of puberty. ge of life In the remaining 35-40 years, only 400-500 oocytes will develop, the rest will undergo atresia. The rate of follicular loss will remain the same until the age of 37-38 years, and the rate of loss will increase 10-15 years before menopause [58]. In menopause, less than a thousand follicles will remain. infertility; It was defined as the absence of pregnancy despite the couple's regular and unprotected sexual intercourse for more than one year. It is known that 15% of couples are infertile in developed countries. Changes in population demographics are causing women who are less biologically active and older to try to conceive as well.

Since the fact that fertility decreases with age is now well known, infertile couples try all treatment options.

• The increase in women's desire to work for education and career and the increase in the age of childbearing in relation to this,

• Increasing frequency of late marriages and divorce,

• Development of contraception techniques and family planning services,

• Increase in sexually transmitted diseases that can cause infertility,

• The prevalence of obesity, which causes impaired reproductive functions and abnormal ovulation, has led to a decrease in fertility across populations.

The relationship between female fertility and aging is the best defined among the causes of infertility. Studies conducted in communities that choose natural life where contraception is prohibited are the best evidence to show that fertility decreases with increasing age (4). A normal couple has a 30% chance of conceiving in an ovulatory cycle.

It can be said that aging has no negative effect on the uterus. Although there is an increase in benign uterine pathologies (leiomyoma, endometrial polyps, adenomyosis) with age, there is little evidence to show its negative effect on female fertility. As the duration of infertility gets longer, it is evaluated considering that the couple has organic or functional problems. Unexplained infertility groups often have a long infertility time. The presence of a previous pregnancy is a finding that shows the adequacy of the anatomical and hormonal system in women and men during that period. Regular menstruation indicates that ovulation is normal. Irregular or infrequent menstrual periods are considered ovulatory disorders. Dysmenorrhea, dyspareunia, focal tenderness and ear-de-sac nodularity suggest peritoneal pathologies and endometriosis. History of previous operation and/or pelvic

inflammatory disease, septic abortion, ruptured appendicitis, previous ectopic pregnancy, abdominal myomectomy or adnexal surgery suggest tubal or peritoneal pathologies. Thyroid diseases that may cause ovulatory problems, systemic diseases such as diabetes mellitus and endocrine disorders that may cause symptoms such as galactorrhea and hirsutismus should be questioned. It is evaluated whether pregnancy has occurred before or not. In secondary infertility, treatment is easier and prognosis is better. Previous infertility treatments and results, drugs administered and the ovarian response to these drugs are evaluated. Smoking and alcohol use adversely affect reproductive organ physiology. Questioning them is important in terms of approach to ovulatory problems.

**Physical Examination**

Pathologies to be detected in the thyroid examination (enlargement of the thyroid gland, nodule, tenderness) or detection of secretion (galactorrhea) in the breast are warning signs in terms of endocrine problems. The presence and degree of hirsutismus is important for the follow-up of hyperandrogenism [59].

**Gynecological Examination**

Organic or anatomical disorders to be detected in gynecological examination may play a role in the explanation of infertility. In addition to the routine gynecological examination, the transition from the cervical os to the uterine cavity, determining the distance from the internal os to the fundus provides 10 information about the uterus.

Presence of vaginal infection and cervical erosions are evaluated during gynecological examination. Cervix should be investigated for premalignant lesions with Pap smear test. Vaginal infections detected while planning the infertility treatment of the patient should be treated. Studies have shown that chlamydial infections reduce the chance of success in assisted reproductive techniques and increase the risk of early pregnancy loss [60].

**Ultrasonography**

In the evaluation of infertility, transvaginal ultrasonography is used to view the uterus and ovaries from the closest distance. Uterine dimensions, position, contour arrangement and homogeneity of the myometrium, the relationship of myomatous structures with the endometrial cavity, if any, and their dimensions are examined. Endometrial thickness, structure, consistency with the phase of the cycle, presence of intracavitary pathology (endometrial polyp, submucous myoma) are evaluated [61]. Both adnexal areas are visualized for pathology. The location of the ovaries, the number of antral follicles, cystic or solid formations are evaluated. Observed in the ovaries by transvaginal ultrasonography performed in the early proliferative phase.

The number of antral follicles determines the response of the ovaries when stimulated with gonadotropins. If a total of 15 or more antral follicles are observed in both ovaries, it is predicted that the response of the ovaries will be good. In cases where a total of 5 or less antral follicles are observed in both ovaries, the ovarian response to

gonadotropin stimulation remains very limited [62]. If pathology is detected in the ultrasonographic examination of the pelvis, surgical procedures such as hysteroscopy and laparoscopy may be required. Laboratory investigations. To evaluate the functions of the hypothalamus-pituitary-ovarian axis and to screen for the ovarian reserve, it is used in the 2nd-3rd phase of menstrual bleeding. Follicular Stimulating Hormone (FSH), Luteinizing Hormone (LH), estradiol (E2) and inhibin B levels are determined. Basal serum FSH levels >15-20 IU/l are associated with decreased fertility. Detection of high FSH levels even once reduces success. A serum E2 value of >50 pg/ml on the 2nd day is associated with a poor prognosis in ovulation induction. High E2 levels are the result of increased FSH. It should be kept in mind that increased E2 level may suppress FSH falsely. Therefore, E2 and FSH should be evaluated together. Inhibin B is produced by ovarian granulosa cells during the follicular phase of menstruation and suppresses FSH production. Increased circulating FSH is associated with decreased inhibin B levels. Inhibin B > 45 pg/ml on the 3rd day of the cycle is an indication of good prognosis [63].

**Hysteroscopy**

Intrauterine anomalies such as submucous fibroids, endometrial polyps, adhesions and septum are detected in 10-62% of infertile couples. These pathologies negatively affect endometrial receptivity and implantation. When pathologies in the cavity are suspected, it is the gold standard in the diagnosis and treatment of diagnosed pathologies by clearly evaluating the cavity. Endoscopic surgery directly shows the size, shape

and location of intrauterine pathology. It is a method that provides the evaluation and solution of pathology in cases with recurrent implantation failure or early pregnancy loss. Laparoscopy is a method that enables the diagnosis and treatment of other anomalies associated with the uterus, ovaries and tubes in the diagnosis of pelvic pathologies such as fibroids, adhesions, tubal damage, endometriosis, as well as the evaluation of the passage of methylene blue administered by the cervical route through the tubes. Uterus, anterior and posterior cul-de sac, ovarian surfaces, ovarian fossa and tubes are observed. Laparoscopy is the definitive definitive intervention for the evaluation of tubal factors. With methylene blue administered by the cervical route, it is observed whether the tuba is open or blocked. It also provides diagnosis and treatment opportunities for pathologies that affect infertility and cannot be detected by HSG, such as mild distal tubal diseases (fimbrial obstruction, phimosis), pelvic or adnexal adhesions, and endometriosis. However, since it is an invasive method, it should not be the first-choice diagnostic method in the evaluation of infertile couples [64].

**Epididymal sperm aspiration with microsurgery (MESA)**

It is a method of obtaining sperm by microsurgery in cases with congenital bilateral absence of vas deferens or uncorrected occlusions. In the MESA method; After applying local or general anesthesia, a small incision is made on the scrotum, the tunica vaginalis is opened and the epididymis is exposed. The epididymal tunica is opened with the help of scissors suitable for microsurgery under 8-15 magnification

with the operating microscope, and the dilated tubules that carry the most motile sperm in the proximal part of the occluded epididymis are reached. The tubule contents are aspirated. The sperm obtained are evaluated, and if their immotile or low motility is low, aspiration is performed again from the proximal. When suitable sperm are obtained, hemostasis is ensured and the tissues are closed in accordance with the anatomy [65].

**Testicular sperm extraction (TESE) and aspiration (TESA)**

In non-obstructive azoospermia or in cases where epididymal sperm aspiration has failed, percutaneous biopsy or testicular aspiration for freezing with open microsurgery are the methods in which sperm are obtained. In the TESE method; After local anesthesia is applied, first the scrotum and then the tunica vaginalis are opened. The testicular tissue, which is squeezed out through a 2-3 mm incision made on the tunica albuginea, is cut with fine tissue scissors and a piece of rice grain size is obtained and placed in 1 ml HEPES wash medium for evaluation. If sperm cannot be found, the process is continued by taking parts from other parts of the testis. When the sperm is found, hemostasis is performed and the tissues are closed in accordance with the anatomical structures. In the TESA method; After applying local anesthesia, the testicular tissue is compressed between the two fingers of one hand, and then the testis is entered with a 19-21 G butterfly needle, into which a medium drawn insulin injector with HEPES is inserted. Aspiration is done. The obtained material is examined for the presence of sperm (51.52).

## CAUSES OF INFERTILITY

Fertility is the ability to reproduce. fecundability; probability of getting pregnant in one menstrual cycle (0.22/month) and fecundity; It was defined as the ability to achieve a live birth in one menstrual cycle (0.15-0.18 months). There is an annual pregnancy rate of 85%. Approximately 10-15% of couples are diagnosed with infertility. Pregnancy occurs as a result of the production of healthy oocyte and sperm, the coming together of gametes in the reproductive canals, and the implantation of the formed embryo into the endometrium by reaching the uterine cavity. Couples with long-term infertility usually have multiple and severe pathologies. The problem in one or more of the following steps will cause infertility [66].

• Sperm must be stored in the cervix during ovulation periods, must travel to the fallopian tubes, and have the oocyte fertilization capacity (male factor).

• There should be regular mature oocyte ovulation (ovarian factor).

• The cervix must hold the sperm, mature it and allow it to pass into the fallopian tubes (cervical factor).

• Fallopian tubes must catch the oocyte and provide the transport of sperm and embryo (tubal factor).

• The uterus and endometrium must be ready for implantation for the formed embryo (uterine factor).

**Uterine Causes**

It is congenital or acquired and constitutes 2 - 5% of all infertility causes. Congenital defects; There may be anomalies such as the complete absence of the mullerian ducts that provide the formation of the uterus, fallopian tubes, cervix and upper vagina (Rokitansky - Küstner - Hauser Syndrome), arcuate uterus, uterus septatus, vaginal septum. Partial or total occlusion of the endometrial cavity may occur, especially in secondary infertility with dilatation and curettage, difficult delivery, intrauterine device, endometritis after previous surgery, adhesions or synechiae (Asherman's Syndrome). Intramural and submucous fibroids cause cavity compression, causing implantation failure and obstetric complications.

**cervical causes**

Infertility associated with cervical factor is 5-10%. The cervix plays an important role in sperm transport and capacitation. Cervical mucus changes 24-48 hours before ovulation and becomes thinner, watery, acellular, elastic, alkaline, facilitating sperm transport. Abnormalities in sperm-mucus interaction are considered as cervical factors in infertility [67]. Endometrial causes

The endometrium changes in response to hormonal secretions during the menstrual cycle, preparing for implantation of the embryo. Implantation problems occur as a result of not supporting the luteal phase of the endometrium due to progesterone deficiency. In terms of endometrial receptivity, defect in the secretion of endometrial proteins,

abnormal integrin/adhesion molecules, T cell and natural killer activities, secretion of embryotoxic factors, uterine perfusion abnormalities can be counted among the endometrial causes.

**Tubal Causes**

Tubal or peritoneal factors are detected in 30% of infertile couples. Proximal and distal tubal occlusions, hydrosalpinx, pelvic adhesions, mild il Causes such as advanced endometriosis, previous tubal surgery, pelvic inflammatory disease are among tubal and peritoneal factors. Tubal status is evaluated by HSG or laparoscopy. Surgical success in distal tubal occlusions varies between 10 - 30%. This rate is less than IVF success. Ectopic pregnancy rate is higher. Although tubal re-mouthing with microsurgery is possible in cases with tubal sterilization, IVF is an alternative to surgical treatment in patients who have a poor surgical prognosis and do not want surgery [68]. Treatment options in detected hydrosalpinx are drainage, proximal tubal ligation with or without distal tubal fenestration, and salpingectomy. Randomized controlled studies have shown that salpingectomy before IVF - ET increases pregnancy and delivery rates in patients with hydrosalpinx. Periadnexal adhesions are generally associated with infertility, and it has been reported that pregnancy rates increase after adhesiolysis [69]. Most spontaneous pregnancies after surgery are 6-12 years old. It happens every month, there is no use waiting longer. Especially if the woman is older and has other infertility factors, IVF treatment should not be delayed. Endometriosis is a cause of infertility manifested by impaired adnexal anatomy and decreased endometrial receptivity by

preventing effective oocyte involvement, proper oocyte development or early embryogenesis. IVF is the appropriate treatment following conservative surgery in advanced endometriosis. Treatment is planned by considering age and concomitant infertility causes in patients.

## INFERTILITY DEPENDING ON OVULATUARY DISORDERS

Most women with regular menstruation in 21-35 days ovulate. Anovulation or ovulation dysfunction affects menstrual cycle frequency and duration. The most common cause of oligomenorrhea is polycystic ovary syndrome (PCOS), and it is found in 14% of women of reproductive age and can cause infertility frequently. Anovulation results in amenorrhea. Primary amenorrhea may be associated with gonad development defects, Turner Syndrome (45,XOs). Secondary amenorrhea is the absence of menstruation as a result of anovulation for at least 6 months. Diseases of the thyroid, adrenal, pituitary (hyperprolactinemia) glands that cause severe endocrine dysfunction can be caused. However, premature ovarian failure is encountered most frequently [70].

IVF/ICSI-ET; It is one of the important methods in the treatment of female infertility and male sterility, but the success rate is only around 30%. How to improve clinical pregnancy rates in human reproduction is one of the most important questions. The embryo implantation step in the IVF procedure is a complex and multistage process that can lead to IVF failure [62]. endometrium; It is a complex tissue with different cellular compartments containing epithelial, stromal, endothelial cells and leukocytes. In addition, it is a dynamic tissue with serial changes in

45 morphological, biochemical and molecular areas during the menstrual cycle. In order for pregnancy to occur, the endometrium becomes receptive to embryo apposition, adhesion, and trophoblast invasion between epithelial cells, within an interval defined as the implantation window, by the action of the ovarian steroids estrogen and progesterone. On the other hand, even if embryos of good quality (with good shape and cell count) are used in IVF-ET cycles, implantation may fail. Therefore, for a successful implantation, it is important to develop the receptive endometrium, which is a step limiting success in the IVF procedure. Studies are aimed at identifying biological markers that can predict implantation ability and interfering with the development of the endometrium [71]. However, obtaining the midsecretory endometrium for clinical diagnosis or research is difficult. Implantation failure is the failure of successful implantation and pregnancy despite repeated transfer of embryos of good morphology to a normal uterus. Several approaches have been proposed to increase implantation rates. These approaches are; thinning of the zona pellucida of the embryo by mechanical, chemical or laser method (such as assisted hatching), using various types of media such as fibrin sealate and hyaluronic acid to increase the adhesion of the blastocyst and implantation with the ZIFT technique, in which the zygote is placed laparoscopically in the fallopian tube changing the circumference can be listed as. The true impact of all these procedures in improving implantation rates and pregnancy outcomes has remained controversial [72]. Ultimately, successful implantation is not only dependent on embryo quality, but also on endometrial receptivity. Therefore, the focus was on

endometrial receptivity around the actual timing of embryo transfer. Receptive endometrial formation encompasses morphological and functional changes induced by estrogen and progesterone sex steroids. Estrogen is secreted in the first half of the menstrual cycle, called the proliferative phase. Progesterone is the predominant sex steroid in the secretory phase of the cycle; It is characterized by the formation of large glands that secrete large amounts of cytokines and growth factors. In humans, during the midsecretory phase of the menstrual cycle (days 19-23), the uterus becomes receptive. This interval lasts 7-10 days after the LH peak and is known as the implantation window. Morphological changes during the implantation window include the transformation of fibroblast-like endometrial cells into larger and rounded decidual cells (decidualization) and the appearance of microvilli and large apical protrusions (pinopod) in the luminal epithelium. In parallel, modulation of the expression of different cytokines, growth factors, transcription factors and prostaglandins 46 takes place. Increased expression of IL-11 and LIF was observed in human endometrial cells during the midsecretory phase. According to this line, in vitro studies support that PGE2 and relaxin act via the IL-11 pathway by expanding progesterone-induced decidualization in cultured human stromal cells [73]. Endometrial expression of the gap junction protein Cx43 is a possible parameter for successful implantation . On the other hand, reductions in HOX cluster genes such as hoxc10, hoxc 11, hoxd 10 and hoxd 11 were observed during the implantation window, supporting that transcriptional repressors may interfere with the preparation of the endometrium for implantation.

Since the damage applied to the endometrium was not very deep, it increased the receptivity of the endometrium. Animal studies have shown beneficial effects of trauma-induced decidualization of the endometrium during the luteal phase. However, further studies are needed to determine how many times the endometrial injury should be done optimally in cases with recurrent implantation failure and when the best timing should be. The decidualization and subsequent increase in uterine receptivity shown in animal studies on the development of implantation and scratch damage to the endometrium is based on old evidence. Loeb reported that by drawing the guinea pig uterus, rapid growth of endometrial cells similar to decidual cells in pregnancy was provoked [74]. Damage to the endometrium can also induce decidualization, as has been shown to induce decidualization by injection of fat into the mouse or rat uterus . In experiments in rats, it was stated that histamine secreted by the uterus in response to trauma may possibly be relevant [75]. It has been supported that the decidual response induced by local damage can be prevented in rats by antihistamine treatment. As another mechanism that increases endometrial receptivity, the 'wound healing' effect created by endometrial sampling can be mentioned. Cytokines and growth factors secreted during the wound healing process may additionally exert an appropriate effect on uterine receptivity, thereby enabling blastocyst implantation and pregnancy [75]. Many cytokines and growth factors such as interleukin-6, interleukin-11, leukemia inhibitory factor (LIF), heparin binding epithelial growth factor-like growth factor and amphiregulin have been found to participate in the implantation process

in the paracrine and autocrine system. Local surgery to damage the endometrium may induce expression of these regulatory factors that would be beneficial for embryo implantation [76]. Local injury in the proliferative phase of the endometrium can stimulate decidualization and increase implantation rates.

The last mechanism is the backward development hypothesis. KOH administered during IVF treatment may negatively affect embryo implantation. Mirkin et al. reported that when compared to natural cycles, KOH cycles result in different structural and functional changes, including histological progression, progression of pinopod maturation, and steroid receptor downregulation [77]. Histological development and pinopod maturation may progress more rapidly in the endometrium of patients treated with high concentrations of sex steroids in KOH cycles. Zhou et al. They argued that the damage applied to the endometrium during the KOH cycle caused the retardation of endometrial development due to the created wound. Thus, they stated that endometrial development may be more equivalent to embryo development and receptivity may increase. Further studies such as immunohistochemistry, histological observation of endometrial biopsy material, scanning with electron microscopy for pinopods, and molecular biological studies are needed to support this hypothesis. Local damage can upregulate the expression of various genes required in preparation of the endometrium for embryo implantation. Zhou et al. They established endometrial damage before embryo transfer in the KOH cycle and analyzed the gene expression profile from the day 10

endometrium. By comparing the endometrial biopsy materials of 10 pregnant and non-pregnant patients, a statistically significant difference was found in the mRNA expression of a total of 218 genes. They showed that 41 genes were up-regulated and 177 genes were down-regulated in pregnant women compared to those who could not conceive. When the regulated genes are divided into 10 groups according to their molecular functions; 14 genes that bind ion, 12 genes that bind protein, 11 genes that bind nucleic acid, 7 genes that bind nucleotides, 5 genes that show transferase activity, 5 genes that show hydrolase activity, 4 genes that show receptor activity, 3 genes that show GTPase regulator activity, from channels or pores They identified 3 genes showing transporter activity, 3 genes showing transcription factor activity, 3 genes showing oxidoreductase activity, and 3 genes showing ion transporter activity. According to the biological functions of the genes; 71.15% in the cellular physiological process, 42.31% in metabolism, 25% in cell exchange, 21.15% in the regulation of the cellular process, 21.15% in localization, 19.23% in the regulation of the physiological process, 9.62% in the downstream of the biological process. In regulation, 9.62% plays a role in the development of the system, 9.62% in the physiological physiological process, 9.62% in the stress response, 5.77% in cell differentiation, 5.77% in sensory perception and 5.77% in the response to abiotic stimuli [78]. Among the genes that are regulated in pregnant women, which are shown to be important in the implantation process at the 10th day endometrium; It is the LNα4 (Laminin alpha 4) gene, which is up-regulated (increased) by 2.61-fold, and the MMP 1 (matrix metalloproteinase 1) gene, which

is up-regulated by 15.95-fold, and the ITGα6 (Integrin alpha 6) gene, which is down-regulated (decreased) by 4.92-fold [78]. 61 Laminins are a major part of the basement membrane in regulating a variety of cellular functions including adhesion, migration, proliferation, differentiation and cell survival [79]. Integrins are heterodimeric glycoproteins that play a role in cell-cell and cell-extracellular matrix adhesion. The LNα4 subunit is a major component of the cell membrane in blood vessels, and recent data indicate that α3β1 and α6β1integrin heterodimers can function as cell surface adhesion receptors for LNα4 [79]. Takeyama et al. They reported that ITGα6 subunit expression is associated with gastrointestinal stromal tumors. Previous studies have shown that integrins interact with laminins and play a key role in the embryo implantation process. However, the exact function of LNα4 is unknown. Zhou et al. They showed different up-regulation and down-regulation of LNα4 and ITGα6 expressions, which supports the important role of both genes in the development of the endometrium through different pathways [78]. The MMP family plays a role in the disruption of the extracellular matrix in normal physiological processes such as embryonic development, reproduction and tissue remodeling. Most MMPs are secreted as inactive proproteins and are activated by cleavage by extracellular proteinases. The MMP 1 gene encodes the secretion of the enzyme that breaks down interstitial collagen. Hurskainen et al. They stated that one of the key enzymes playing a role alone in the trophoblast invasion process or cell surface activator of other proteinases. Kalma et al. compared the endometrium using microarray technology in biopsied and non-biopsied patients to identify

genes in endometrial receptivity induced by local damage. It showed that the expression of 183 genes increased 2-10 times and the expression of 39 genes decreased in patients who underwent endometrial biopsy who will receive IVF treatment in the next cycle. The most conspicuous and up-regulated is the transmembranal protein uroplakin Ib (UPIb), a member of a family of four glycoproteins that strengthen and stabilize the apical surface in the mammalian bladder. UPIa, UPIb, UPII and UPIII are 4 glycoproteins that build the asymmetric membrane in the mammalian bladder [80]. The asymmetric membrane unit plays an important role in the physiology of the normal bladder epithelium. Plaques covering the apical surface of the urinary epithelium form 62. It is the UPIa/UPII and UPIb/UPIII complexes that form plaques in the asymmetric membrane unit. These plaques protect the cells from lysis during bladder distension, stabilize the urinary epithelium and shape the permeability barrier [81]. The UPIb/UPIII complex is also expressed in non-mammals such as Xenopus eggs. In this system, the UPIb/UPIII complex plays a role in sperm-egg membrane interaction and subsequently in egg activation via the tyrosine kinase pathway that activates phospholipase c in fertilization [82]. This was recently supported by egg activation via the ganglioside GM1 pathway by interaction with UPIII in the complex (205). UPIII also has a tyrosine phosphorylation site in its carboxy terminus cytoplasmic sequence, which is important in sperm-induced egg activation. Kalma et al. they did not detect increased expression of UPIII in the human endometrium and thought that it had no role in the pathway to prepare the endometrium for implantation [83]. The

localization of UPIII in the membrane is completely dependent on its dimerization with UPIb. Whereas, UPIb may originate from the endoplasmic reticulum and migrate to the plasma membrane as a monomer. This special feature of UPIb allows its localization at the apical membrane of the glandular epithelium.

UPIb is the only member of the entire uroplakin family that is expressed from the endometrium. The localization of the UPIb protein in the secretory vesicles of the glandular epithelium may support its activity in the endometrial glands. Kalma et al. They showed first evidence of UPIb expression from the human endometrium, and then an increase in UPIb expression after biopsy treatment, and that this increase persisted and remained elevated during the subsequent menstrual cycle. Elevated UPIb mRNA levels are observed during the implantation window period of the spontaneous menstrual cycle (days 21-24), in addition to the 20-21 days of the cycle following biopsy treatment. detected during the day. In immunohistochemical analyzes, UPIb has been shown in the endometrial gland epithelium, which increases from the proliferative phase of the cycle to the secretory phase [83]. Phospholipase A2 (PLA2), adipose differentiation-related protein (ADFP), mucin1 transmembrane (MUC1), lysosomal-associated membrane protein (LAMP2) are other genes that have been shown to increase in endometrial samples of patients receiving biopsy treatment [83]. Changes in these genes from the proliferative to the secretory phase have been reported in previous studies using DNA microarray analysis. Levels of MUC1 in the endometrium increase during the secretory

phase in response to elevated blood progesterone levels [84]. MUC1 plays a central role in the acquisition of endometrial receptivity and the regulation of 63 implantation. MUC1 continues to be secreted during early pregnancy and is taken up by the syncytiotrophoblast via the phagocytotic mechanism [85]. Furthermore, in experiments in mice, the absence of PLA2 resulted in the absence of prostaglandins, indicating a delay in implantation and a reduction in offspring. Zhou et al. and Kalma et al. investigated whether there is a relationship between local damage to the endometrium and gene expression and pregnancy outcomes. This information may lay the groundwork for future evaluation of the gene expression profile in the human endometrium to predict IVFET results. All these results support that local damage increases endometrial receptivity by regulating the expression of various genes required in the preparation of the endometrium for embryo implantation. There may be a problem with increased spontaneous expression of endometrial receptivity-related genes in IVF-failure patients undergoing high-quality embryo transfers. In such cases, local damage to the endometrium may facilitate the endometrial response. The mean age of the patients with endometrial damage in the studies; Barash et al. 33.8±5.8, Li et al. 31.15±4.23 and Raziel et al. It was 33.1±4.9 in Zhou et al., 31.6±3.72 in Zhou et al., and 29.6±3.8 in our study. While the number of failed cycles was 4.0±2.0 (range 1-10) in Barash et al., 7.0±1.9 (range 4-11) in Raziel et al., it was 1.44 in our study. Total number of previously transferred embryos; While Raziel et al. had an average of 22 (range 13-42), Barash et al. did not find any data for this. Both groups were observed to be quite similar. However,

there are still unanswered questions regarding endometrial injury in IVF treatment. Should endometrial biopsy be repeated before subsequent IVF treatment after a successful pregnancy and delivery? Another question is whether there is a time gap between biopsy and ovarian hyperstimulation as we do, or if there will be a time gap, is there any disadvantage or how long should it take until the KOH protocol is started after endometrial damage?

Total fertilization failure can be caused by many factors originating from oocyte or sperm after intracytoplasmic sperm injection (ICSI). The failure of fertilization after microinjection is mostly attributed to the inability of the sperm cells to activate the oocyte [85]. For oocyte activation to occur, the sperm must have completed its capacitation. In order for the sperm hyperactivation and acrosome reaction to occur, which is expected to occur later, Ca+2 ions must be released. It is thought that the realization of oocyte activation is dependent on the release of calcium (Ca+2) [86]. Artificial oocyte activation methods are used to provide oocyte activation in cases where calcium release does not occur. Calcium ionophore, which is used to provide oocyte activation by supplementing with calcium (Ca+2), is one of the artificial oocyte activation (AOA) methods. It is aimed to contribute to the solution of the infertility problem of patients with severe oligoasthenoteratozoospermia (OAT) by using the calcium ionophore in cases with oocyte activation disorder, by ensuring the activation of the oocyte. In this study, based on the knowledge that oocyte activation is provided by calcium, 50 patients who underwent calcium ionophore

procedure after ICSI were identified from the files of 100 patients with severe oligoasthenoteratozoospermia (OAT). As a result of comparing the oocyte fertilization rates, fertilization percentage, embryo quality, percentage of blast progression, clinical pregnancy percentage, live birth rate, biochemical pregnancy and abortion rates of the determined patients with 50 patients who did not undergo calcium ionophore treatment, it was investigated whether the results were better in patients who used calcium ionophore.

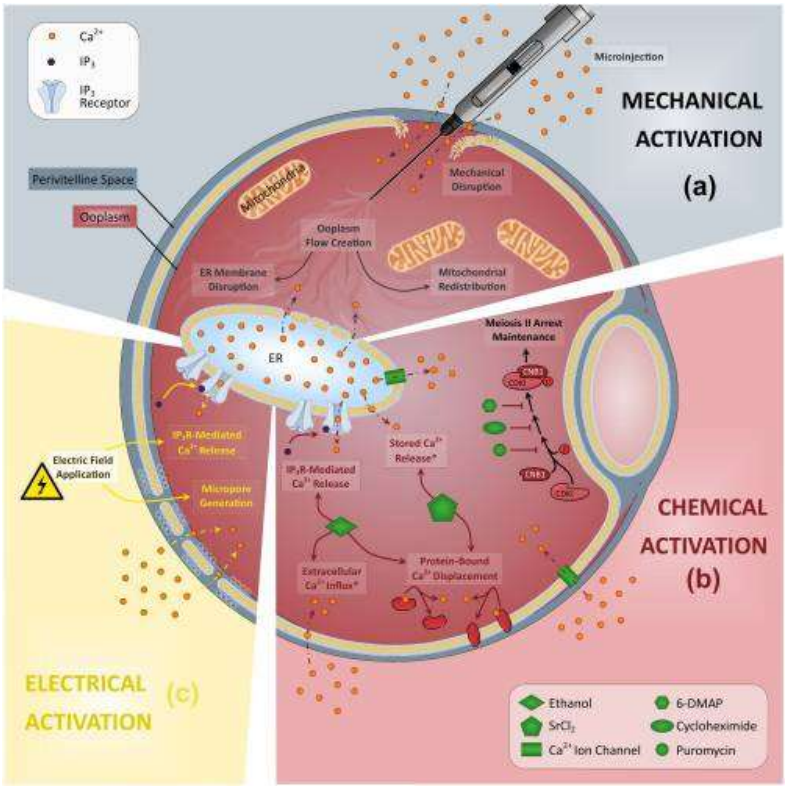


Figure 14. Schematic representation of the pseudo-mechanisms underlying the three most commonly applied methods of assisted oocyte activation.

(a) Mechanical activation usually involves disruption of the plasma membrane and/or components within the oolemma, leading to increased Ca2+ in the oocyte due to Ca2+ influx and/or disruption of Ca2+ storage membranes; as the endoplasmic reticulum (ER). (b) The mechanisms underlying chemical activation vary with the type of agent used, but generally involve facilitated transport of extracellular Ca2+ into the oocyte, either directly or via transport channels. (C) Electrical activation involves the creation of pores within the oocyte membrane through the application of alternating electric fields that allow extracellular Ca2+ to enter the oolemma [87].

Studies show that failure in oocyte activation is the main cause of recurrent fertilization failure after ICSI. It has also shown that calcium activation is an important factor in successfully inducing oocyte activation. When the sperm enters the oocyte, it induces the Ca2+ signal and Ca2+ signals begin to be produced in the oocyte cytoplasm. A rapid depolarization in the oocyte oocyte membrane potential occurs, the release of cortical granules and chromosomal dissociation, the second pole body, decrease in the activity of kinases such as MPF/MAPK, translation of egg mRNA, formation of pronucleus and DNA replication occur. The regular occurrence of these molecular events is also a hallmark of successful oocyte activation. Therefore, induction of high intracellular Ca2+ concentration in oocytes by AOA is an alternative therapy for patients with recurrent fertilization failure in ICSI and may result in normal embryos.

We can say that calcium ionophore (A23187) is widely used in clinical practice for AOA because it provides convenience in the procedures and is easily obtained. When we look at the working system of the calcium ionophore (A23187), it primarily increases the permeability of the cell membrane in order to allow the flow of extracellular Ca2+ into the cell. Thus, it causes an increase in the Ca2+ concentration in the oocyte and thus the oocyte activation. In addition, calcium ionophore (A23187) is known to induce Ca2+ release from the Ca2+ pool in the endoplasmic reticulum present in the oocyte, similar to the IP3-mediated Ca2+ elevation induced by sperm. Many studies show that calcium ionophore (A23187) has good success in terms of good fertilization and implantation in patients with sperm abnormalities and very low numbers. Embryologists focus on the safety of calcium ionophore (A23187) activation, especially since the amplitude and frequency of calcium oscillations caused by the calcium ionophore differ depending on physiological conditions, and calcium release has a significant effect on chromosome segregation and other changes.

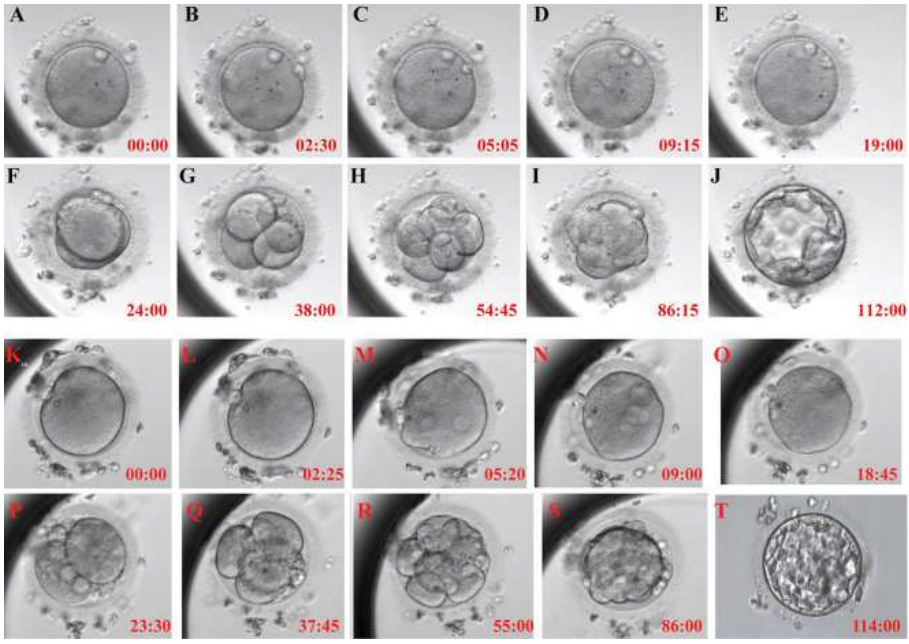


**Figure 15.** Typical embryonic developmental pattern of calcium ionophore (A23187) rescue activation. Each figure shows the activation process (A), second polar body appearance (B), pronucleus appearance (C), pronucleus volume largest and most prominent (D), pronucleus disappearance (E), first division (F) for 0 h. . ), 4-cell stage (G), 8-cell stage (H), morula formation (I) and blastocyst formation (J) in the RA-T group; and 0h (K), second polar body appearance (L), pronucleus appearance (M), pronucleus volume is largest and most prominent (N), pronucleus disappears (O), first division (P), 4-cell stage RA-C group (Q), 8-cell stage (R), morula formation (S) and blastocyst formation (T) [88].

Studies have shown that the use of calcium ionophores to optimize oocyte activation after ICSI with the sperm of men diagnosed with severe OAT contributes positively to the improvement in fertilization rates and the increase in the number of quality embryos. However, these results did not have a positive effect on live birth rates. Fertilization failure is an event that is generally seen in approximately 30% of the oocyte after ICSI. Total fertilization failure after ICSI is seen in the range of 1.29%-3% [89]. The inability of the sperm to activate the oocyte is seen as one of the main causes of fertilization problems. Oocyte activation is a very important event that occurs after sperm-oocyte fusion takes place. In order for oocyte activation to occur in vivo and for embryogenesis to begin, for nuclear and cytological developments in fertilized oocytes to be complete, intracellular calcium release in the oocyte must occur properly [90]. Looking at the literature, calcium ionophore was first used for oocyte activation in the 1990s [91]. Montag et al. found that the application of calcium ionophore for artificial oocyte activation in cases with a fertilization rate of less than 30% after ICSI increased the fertilization rates [92] Similarly, Hoshi et al. performed an oocyte-activating calcium ionophore application and reported the first pregnancy with a successful result [93]. Heindryckx et al. reported that calcium ionophore was effective in the initiation of fertilization in patients with failure after ICSI in their 2005 study. Fertilization rates have improved with the activation process. In fact, one of the goals of the activation process is to improve clinical outcomes in OAT subjects with a previous history of TFF. However, since TFF was not observed in any group in our series, no clear

comments can be made on this issue. It was reported in the cytogenetic analyzes of human and mouse oocytes, which were performed in 70-80% of the oocytes activated with calcium ionophore and had normal morphology in 70-80% of the oocytes. no significant difference was found [94]. This can be interpreted as the use of Ca does not have an obvious toxic effect in early embryogenesis and late perinatal periods. However, toxicity may occur not only in the short-medium term but also in the long term, and it would be beneficial to monitor the babies born with this method in terms of diseases and malignancies for many years. According to a meta-analysis result of the cases in which assisted oocyte activation was applied, it was reported that this procedure did not cause a major defect in children. group, although there is no statistically significant difference. The most likely explanation for this situation is that the overall number of subjects of the study was low and it did not have sufficient statistical power. If sufficient power analysis had been made and the number of subjects had been clarified, this difference might have been significant. Another explanation for this result is that in cases where oocytes are selected according to a certain standard, there may be a problem in the genetic structure of the embryos, although fertilizations are monitored and optimized by Ca administration due to severe OAT. This may adversely affect implantation and live birth rates. In particular, the implantation potential of blastocysts with 23 chromosome analysis and known to be euploid, and blastocysts without genetics are in favor of euploid blastocysts. In OAT cases, it is possible that the embryos are genetically problematic, especially in preimplantation genetic screening studies, an

increase in mosaic embryo rates was found in those with male factor infertility. A morphologically good embryo may also be aneuploid, and this rate was found to be over 40% in one series [95].

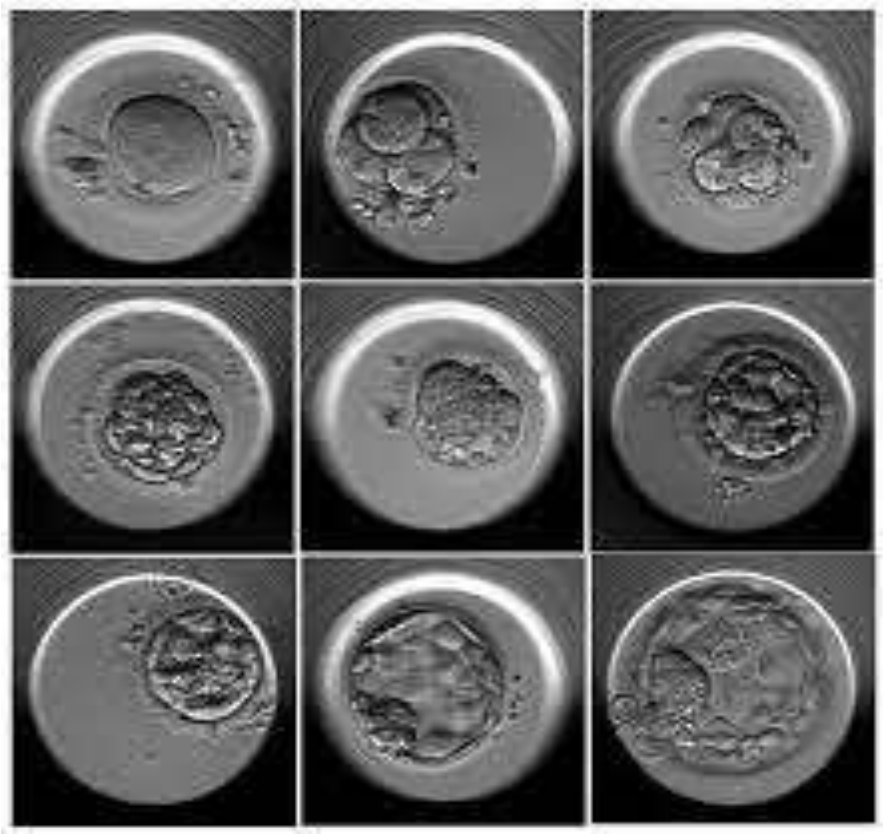

**Figure 16.** Development of an embryo without egg activation

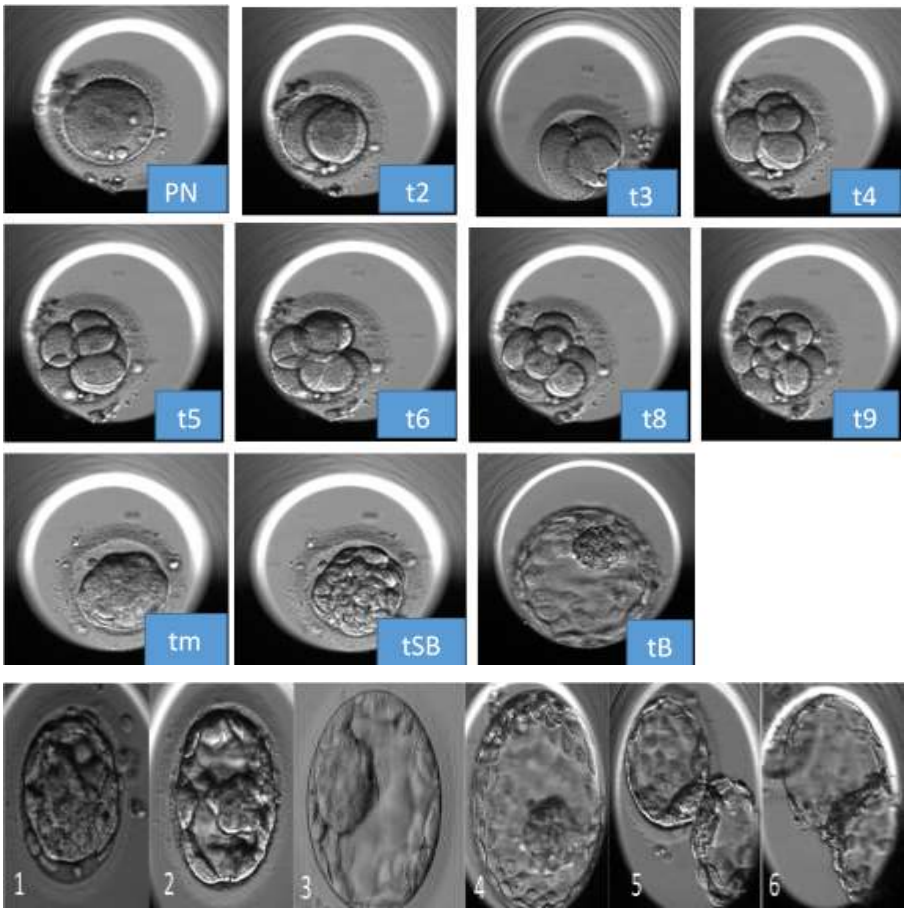


**Figure 17.** Embryo quality developed by fertilization of the egg applied calcium ionophore

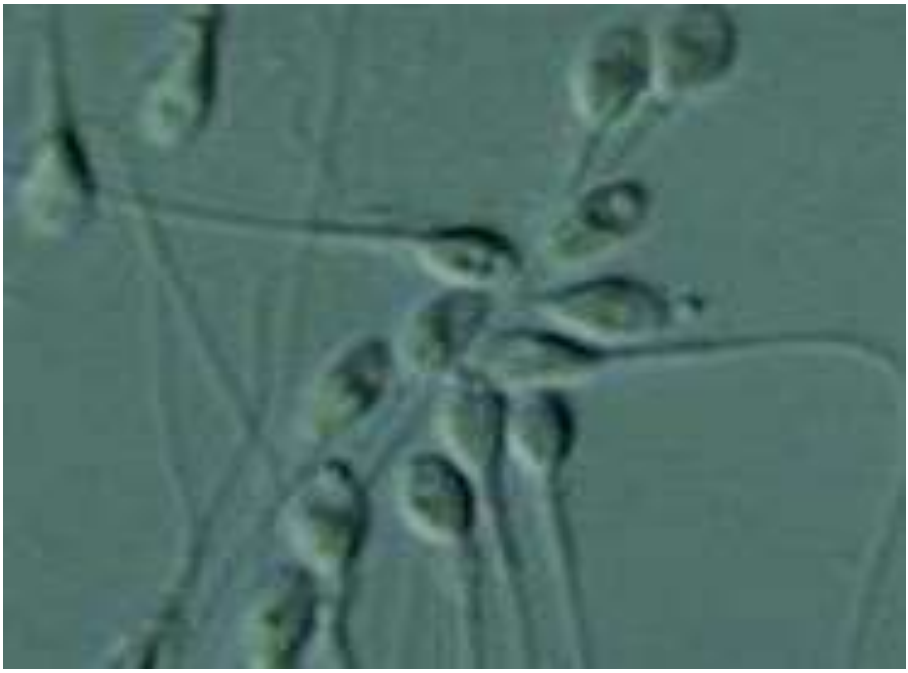

**Figure 18.** Sperm with defective sperm morphology and vacuoles.

In conclusion, genetic screening results of Ca application for severe OAT cases are needed in large series. As a result of the evaluation of the data obtained from the research, the comparison of fertilized oocyte counts, fertilization percentage, 3rd day embryo embryo counts, grade 1 cleavage embryo counts, 5th day embryo counts, good quality blastocyst counts of the experimental group using calcium ionophore and the control group not using calcium ionophore. has been made. In addition, clinical pregnancy rate, live birth rate, biochemical pregnancy rates and abortion rates are also evaluated. It was observed that there was a statistically significant increase in the numbers of good quality blastocysts compared to the numbers of blastocysts. Although there was no statistically significant increase in the experimental group compared

to the control group in clinically compared clinical pregnancy and live birth rates, an increase in percentage was observed. Thus, it is thought that this study can contribute to the literature. It has been understood that there is a need for more detailed studies on embryo development and clinical effects of cases treated with calcium ionophore in severe OAT patients. Therefore, in the light of all these studies, it is recommended that more research should be conducted in the future in order to prove that the calcium ionophore is reliable.

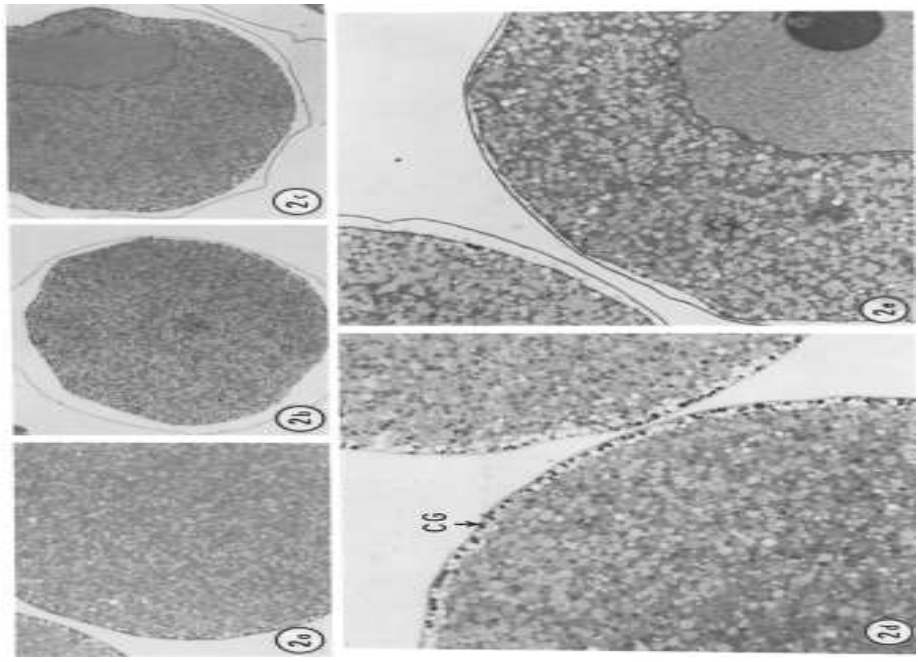


**Figure 19.** The results show that ionophore and sperm-induced vitelline membrane elevations are associated with cortical granule unloading rather than detachment of the vitelline membrane from the oocyte surface. Thus, a true activation response appears to occur in the surface and cortex of immature oocytes.

The first stage of IVF treatment is counseling for prospective mothers and fathers, and the second stage is the process of obtaining suitable eggs for fertilization by stimulating the ovaries of the mother-to-be with hormone drugs. In this way, sufficient number and quality of eggs can be obtained from the expectant mother. Micro-injection, which is the process of injecting the best quality sperm into the egg, is used in cases of infertility caused by the sperm count, maturity and vitality of the man. In this way, fertilization is carried out with sperm that are not sufficient to fertilize the egg spontaneously. However, in cases where fertilization cannot be achieved with micro-injection, fertilization with calcium ionophore is used. Because, for fertilization to occur, the sperm must enter the egg and leave the enzyme in the head part of the egg, thus increasing the amount of calcium inside. If the folfolipase C substance in the sperm is missing or absent, the egg cannot be fertilized. Because without phospholipase, the calcium content in the egg does not increase. Sperm that are strong enough to fertilize the egg may not be obtained from men with low sperm count, quality, maturity, and structural defects in their sperm. In particular, fertilization problems can be experienced in men with consecutive IVF failure, problems in the number or quality of eggs, low vitality and maturity in sperm, and structural defects. In such cases, Mechanical, Electrical and Chemical Activation techniques are used to increase the activation of the egg.

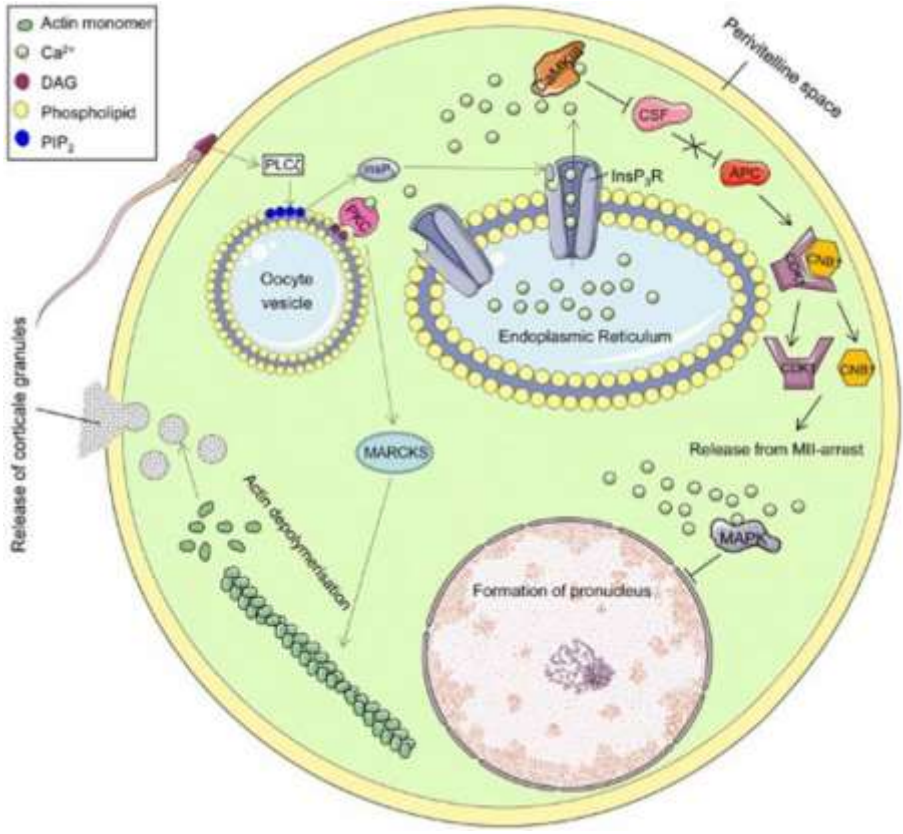


**Figure 20.** Schematic summary of the proposed mechanism underlying Ca2+ release in oocyte activation.

Fertilizing sperm triggers Ca2+ following delivery of sperm-specific phospholipase C zeta (PLCζ) to the oolemma during or after oocyte-sperm membrane fusion. PLCζ interacts with the yet unknown oocyte-derived factor(s), facilitating the hydrolysis of PIP2 to DAG and InsP3, which then triggers Ca2+ released from intracellular stores, attenuating MII arrest. The proposed mechanism mediates cortical granule exocytosis, MAPK deactivation, and subsequent pronuclei formation and CaMKII activation, inhibiting CSF (Emi2) and releasing APC. This

reduces Cyclin B1 levels in the maturation promoting factor (MPF) complex consisting of CDK1 and Cyclin B1; APC, anaphase promoting complex/cyclosome; CaM/CaMKII, calcium/calmodulin dependent protein kinase II; CSF, cytostatic factor; CNB1, cyclin B1; CDK1, cyclin-dependent kinase 1; DAG, diacylglycerol; InsP3, inositol 1,4,5-trisphosphate; InsP3R, InsP3 receptor; MAPK, mitogen-activated protein kinase; PIP 2, phosphatidylinositol 4,5-biphosphate; [96].

**What is mechanical activation?**

Mechanical activation is one of the methods applied in case the fertilization process cannot occur during the fertilization phase of IVF treatment. In mechanical activation, tiny holes are made on the egg membrane and an environment is created for calcium to pass through these holes into the egg. In this way, fertilization can occur.

**What is electrical activation?**

In sperm problems where it is difficult to get pregnant with classical in vitro fertilization treatment, fertilization is tried to be carried out with the micro-injection technique. However, the process of activating the eggs and obtaining fertilization in this way by giving a low voltage electric current to the eggs during the micro-injection process is called electrical activation.

**What is chemical activation?**

Chemical activation, on the other hand, is the process of using some chemical substances that will increase the amount of calcium in the egg after the micro-injection process. Calcium ionophore is the most

commonly used chemical substance for the fertilization process in today's IVF treatment. Calcium ionophore is very suitable for obtaining fertilization in cases where fertilization cannot be achieved with classical IVF treatment or micro-injection treatment alone.

In the fertilization process with a calcium ionophore, micro-injection is first performed and then the eggs are kept in the calcium ionophore for a few minutes. With this process, a healthy fertilization and therefore pregnancy can be achieved.

**Who gets fertilization failure in IVF treatment?**

IVF treatment is applied to couples who cannot conceive naturally. In this context, it can be said that those who apply for IVF treatment already have reproductive problems in varying amounts. As with many infertility problems, pregnancy can be achieved with IVF treatment in case of unsuccessful fertilization due to egg and sperm problems. So much so that in IVF treatment, if the sperm of the man is not sufficient to fertilize the egg, the sperm is injected directly into the egg with the micro-injection process.

In this way, when fertilization does not occur, the egg is kept in a calcium ionophore for a few minutes together with the injected sperm and fertilization is ensured. Since fertilization cannot be performed in approximately 3% of couples who receive IVF treatment with micro-injection, fertilization is performed with calcium ionophore. In some patients, fertilization failures are experienced, although the egg and sperm reserves are at the expected level.

In such cases, the activation of the egg is provided in the laboratory environment and thus fertilization is achieved. Based on all of these; It is possible to say that egg activation with calcium ionophore is suitable for couples who do not have a baby due to sperm and egg related reasons.

Calcium ionophore therapy is a treatment method applied to ensure a healthy fertilization and to obtain a high pregnancy rate. In order to talk about a healthy fertilization, when the sperm enters the egg, the enzyme in the head part of the egg must be released into the egg and the amount of calcium inside must increase.

If the amount of folfolipase C in the sperm is low, the egg cannot be fertilized because without phospholipase, the calcium rate in the egg does not increase.

Calcium ionophore therapy is an application that increases the amount of calcium in the egg. In the calcium ionophore application, the egg and sperm are first fertilized by micro-injection. After this process, the eggs are kept in a calcium ionophore for a few minutes. The main purpose here is to increase the calcium level that needs to be increased in the egg. In other words, calcium ionophore therapy helps the fertilization of the egg by supporting the reactions that should start in the egg.

In order for a healthy fertilization to take place, the sperm must enter the egg and release the enzyme in the head into the egg, thereby increasing the amount of calcium inside. If the folfolipase C substance in the sperm is missing or absent, the egg cannot be fertilized. Because

without phospholipase, the calcium content in the egg does not increase. Sperm that are strong enough to fertilize the egg may not be obtained from men with sperm count, quality and structural defect (morphology). In particular, fertilization problems may be experienced in men with consecutive IVF failure, problems in egg number or quality, sperm motility and morphological development disorders.

Calcium Ionophore is an application that increases the amount of calcium in the egg after the micro-injection process. In the calcium ionophore application, first the egg and sperm are fertilized by micro-injection (ICSI) process, then the eggs are kept in the calcium ionophore for a few minutes. The main purpose here is to artificially increase the calcium level, which should increase in the egg after the sperm enters the egg, if it does not increase. In other words, by supporting the reactions that should start in the egg, the egg is forced to be fertilized. This process provides a healthy fertilization and therefore a high pregnancy rate.

**New Hope in Fertilization Problem Ca-Ionophore**

When the in vitro fertilization applications made using this technique are evaluated, it is seen in the publications that while the fertilization rates were 35% in the previous trial of the same patient, it could be increased to 57% with this application. More importantly, pregnancy rates increase from 7.6% to 46.6%, and live birth rates increase from 1.3% to 34.2%. In addition, there is no increase in the risk of both major and minor anomalies in babies born as a result of IVF performed by applying this technique.

When we make a clinical evaluation in the book we have written, it is seen that it affects the implantation success positively in patients diagnosed with infertile and endometrial injury. In addition, we can say that calcium ionophore applied in patients with severe sperm factor and low ovarian reserve supports a very serious quality embryo development and success of conception. As a result, it will be possible to say that it both increases the implantation rate and increases the live birth rate in certain patient groups such as endometrial injury and calcium ionophore.

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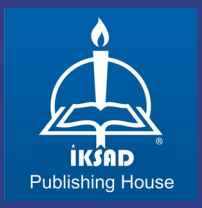


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