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Special Issue on Infertility

"Infertility: Current Concepts"

The aim of this special issue is to compile the qualified reviews on the infertility and related issues, treatment options in male and female patients with the interdisciplinary perspective of both Urology and Gynecology.

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From the Editors

Dear Colleagues,

Infertility is a global health issue affecting 15% of reproductive-aged couples worldwide. According to the World Health Organization, available data suggests that between 48 million couples and 186 million individuals have infertility globally. Infertility is not only a reproductive system disease but also it is one of the primary reasons for divorce among couples, cause of psychiatric symptoms such as sexual dysfunction, anxiety, and depression. Also, it is a social problem and an economic burden for societies.

In the last fifty years, there have been dizzying developments in the field of infertility. In this special issue in 2022, we have invited Turkish Andrology leaders to share their experience and discuss the contemporary literature on some of the most topical issues in male infertility. The editorial team is very grateful to all the authors and reviewers who have contributed to this high-quality special issue summarizing the most up-to-date literature. In addition, I would like to send my special thanks to the Editor-in-Chief of Duzce Medical Journal, Assoc. Prof. Mehmet Ali SUNGUR, for his all great effort.

To our readers, we hope you enjoy this issue and the included articles help hone your clinical practice.

Respectfully yours,

Ozan EFESOY, MD, FEBU
Associated Professor of Urology

Dear Colleagues,

Nowadays, infertility is encountered more frequently due to the increase in the desire to have children at an advanced age and the ease of access to the health center. Despite technological advances, a certain percentage of patients still remain under the scope of unexplained infertility. Although Reproductive Endocrinology and Infertility are not recognized as a sub-branch in our country, special issues of meetings and journals are published by many associations and publications to inform physicians of new developments.

There is no doubt that infertility evaluation is a process that requires simultaneous evaluation of men and women. However, current data on infertility in many sources have been evaluated as either male or female. Feedback on this topic encouraged us to make this special issue. We would like to thank all our authors who accepted our invitation and supported our special issue without any expectations at a time when medical practices are getting more and more difficult. Also, we would like to thank Assoc. Prof. Mehmet Ali SUNGUR, the editor-in-chief, for extraordinary work in the preparation of this special issue.

We hope this special issue will be useful for our readers and help to improve their clinical practice.

Sincerely yours,

Mustafa KAPLANOĞLU, MD
Associated Professor of Gynecology

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Evaluation of Male Infertility

Erkek İnfertilitesinin Değerlendirilmesi

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ABSTRACT

Infertility is a condition that has psychological and social effects on couples. Around 50% of fertility problems originate in males. The causes of male infertility are highly variable, and many health conditions, congenital or acquired diseases can affect male fertility. While some causes are reversible, some of the causes are curable but not reversible. The main purpose of a male assessment is to identify correctable causes that may affect the fertility or health status of the patient. Correction of curable or reversible factors can improve both couples to have children and improve the general health of the man. Despite several research in this field, in many cases, the underlying causes are unknown. A proper infertility evaluation is essential to prevent complications associated with assisted reproductive techniques and testicular sperm extraction (TESE), and psychological problems that may result from failure, and to provide accurate preoperative information to patients. Initial evaluation for fertility should include a detailed physical examination along with his medical and reproductive history. Although sperm analysis is still a critical assessment in evaluating male infertility, the introduction of advanced diagnostic tests facilitates the determination of the etiology. This paper discusses the evaluation of male infertility in light of current guidelines.
Keywords: Genetic evaluation; hormonal evaluation; male infertility; semen analysis.

ÖZ

İnfertilite çiftler üzerinde psikolojik ve sosyal olarak etkileri olan bir durumdur. Fertilite problemlerinin yaklaşık %50'si erkeklerden kaynaklanmaktadır. Erkek infertilitesinin nedenleri oldukça değişkendir ve birçok genel sağlık durumu, doğuştan gelen veya sonradan kazanılan hastalıklar erkek fertilitesini etkileyebilir. Bazı nedenler geri döndürülebilir iken, bazı nedenler tedavi edilebilirdir ancak geri döndürülebilir değildir. Erkek değerlendirmesinin temel amacı, hastanın fertilitesini veya sağlık durumunu etkileyebilecek düzeltilebilir nedenleri belirlemektir. Tedavi edilebilir veya geri döndürülebilir faktörlerin düzeltilmesi, hem çiftin çocuk sahibi olmasını sağlayabilir hem de erkeğin genel sağlık durumunu iyileştirebilir. Bu alandaki birçok araştırmaya rağmen, çoğu durumda altta yatan etiyoloji bilinmemektedir. Doğru bir infertilite değerlendirmesi, yardımcı üreme tekniklerine ve testiküler sperm ekstraksiyonuna (TESE) bağlı komplikasyonları ve başarısızlıktan kaynaklanabilecek psikolojik sorunları önlemek ve hastalara işlem öncesi doğru bilgi vermek için gereklidir. Fertilite için ilk değerlendirme, tıbbi ve reprodüktif öykü ile ayrıntılı bir fizik muayeneyi içermelidir. Sperm analizi erkek infertilitesinin değerlendirilmesinde halen önemli bir araç olmakla birlikte, ileri tanısal testlerin kullanılmaya başlanması etiyolojinin belirlenmesini kolaylaştırmaktadır. Bu makale, mevcut kılavuzlar ışığında erkek infertilitesinin değerlendirilmesini tartışmaktadır.
Anahtar kelimeler: Genetik değerlendirme; hormonal değerlendirme; erkek infertilitesi; semen analizi.

INTRODUCTION

Infertility is defined as the inability of a couple to conceive despite having frequent, unprotected sex for at least a year for couples (1). About 85% of couples achieve pregnancy within one year of trying. At the end of one year, 15% of couples trying to conceive are diagnosed with infertility. Around 50% of fertility problems originate in males.

Male infertility can occur due to congenital or acquired diseases, as well as in completely healthy men. Routine physical examination findings and normal hormone parameters are found in healthy men, but decreased spermatozoa count (oligospermia), decreased motility (asthenospermia), and multiple abnormal forms (teratozoospermia) in the morphological inspection can be detected. There are many causes of male infertility. Some causes, such as hypogonadotropic hypogonadism and ejaculatory duct obstruction are reversible. Other causes, such as testicular torsion, trauma, or infection-related testicular atrophy are curable but not reversible. Although there is an unexplained decrease in semen quality in 25-30% of men, the etiology of male infertility cannot be determined, and it is called idiopathic male infertility. Unexplained infertility, found in approximately 25% of couples, is a condition in which semen analyses are within normal values and endocrine abnormalities are excluded (2). The etiology of male infertility is summarized in Table 1 in the European Association of Urology (EAU) guidelines (3).

The main purpose of a male assessment is to identify correctable causes that may affect the fertility or health status of the patient. Diagnosing and treating curable conditions can improve a man's fertility and allow the partner to have children through intercourse or assisted reproductive techniques. The testis is a heterogeneous organ. Spermatogenesis can occur in any part of the testis. Therefore, in azoospermic patients, there may be sperm production in the testicles, even in small quantities. Identifying incurable conditions frees couples from unnecessary interventions and allows them to consider options such as adoption or donor insemination. Male infertility may be related to other systemic comorbidities and may sometimes be an emerging symptom of an underlying severe condition (4). Failure to identify disorders such as pituitary tumors or testicular cancer can lead to severe consequences. Identifying genetic etiologies of male infertility permits couples to receive genetic counseling about genetic abnormalities that may affect their children's health. An appropriate male assessment is necessary for the couple to better understand the main cause and consequences of the problem.

EVALUATION

Couples who do not conceive after at least one year of regular, unprotected sexual intercourse should be evaluated for male infertility. Since pregnancy rates can drop severely in women older than 35, male infertility evaluation is recommended six months later for couples with a female partner older than 35. However, for men with concerns about their future fertility, evaluation before one year may be considered (2). Screening initiates with a medical and reproductive history, physical examination, and two semen samples at least one month apart. Depending on the history, physical examination, and semen analysis results, further andrological assessments such as hormonal and genetic evaluation may be recommended.

Medical and Sexual History

A history covering all etiologies affecting male fertility should be taken. The following areas should be considered in order.

Reproductive and Sexual History

The couple's sexual practices, including frequency of unprotected intercourse and duration, the timing of coitus, miscarriages, fertility treatments, prior conception and use of contraception, and ejaculatory and erectile function, should be assessed. For women with a regular cycle, ovulation typically will occur 14 days before the start of their next period. It is recommended to have intercourse every 48 hours leading up to ovulation to optimize the chances of pregnancy (5). Sexual dysfunctions can be a cause or result of infertility. The absence of sexual satisfaction, hypoactive sexual desire, erectile dysfunction, and premature ejaculation are common sexual disorders in infertile men (6). Therefore, the patient should be questioned about intercourse frequency, libido, quality of erection, possible sexual distress, and ejaculation. A solution should be offered to couples undergoing infertility treatment as soon as a diagnosis of sexual dysfunction is made. In addition, treating male infertility might reverse infertility-related sexual dysfunction (6).

Table 1. Male infertility causes and associated factors and percentage of distribution (EAU, 2021)

Diagnosis	Patients (%)
All	100
Infertility of known (possible) cause	42.6
Maldescended testes	8.4
Varicocele	14.8
Sperm auto-antibodies	3.9
Testicular tumor	1.2
Others	5.0
Idiopathic infertility	30.0
Hypogonadism	10.1
Klinefelter syndrome (47,XXY)	2.6
XX male	0.1
Primary hypogonadism of unknown cause	2.3
Secondary (hypogonadotropic) hypogonadism	1.6
Kallmann syndrome	0.3
Idiopathic hypogonadotropic hypogonadism	0.4
Residual after pituitary surgery	<0.1
Late-onset hypogonadism	2.2
Constitutional delay of puberty	1.4
Others	0.8
General/systemic disease	2.2
Cryopreservation due to malignant disease	7.8
Testicular tumor	5
Lymphoma	1.5
Leukemia	0.7
Sarcoma	0.6
Disturbance of erection/ejaculation	2.4
Obstruction	2.2
Vasectomy	0.9
Cystic fibrosis (CBAVD)	0.5
Others	0.8

EAU: European Association of Urology, CBAVD: congenital bilateral absence of the vas deferens

Medical and Surgical History

It is necessary to question the medical drugs used by the patients and their previous surgeries. Medications may disrupt male fertility by adversely affecting libido, impaired ejaculation and erectile function, damage to the hypothalamic-pituitary-gonadal axis, and direct gonadotoxic effects. The patient's medications that affect testicular function, such as alpha-blockers, immune modulators, chemotherapeutic agents, antiandrogens, testosterone preparations, and antipsychotics, should be questioned. Because of their treatments, testicular germ cell tumors, Hodgkin lymphoma, and leukemia are cancers linked with male infertility during the reproductive period. It is suggested that cancer patients should be informed about the harmful effects of chemotherapy agents on spermatogenesis (2). Scrotal surgeries such as orchiopexy, trauma or torsion, hernia repair, vasectomy, and pelvic surgeries that will affect the male's fertility should be questioned. Sperm cryopreservation should be offered to essential patients before procedures that may affect fertility.

Undescended Testis and Varicocele

Cryptorchidism can be a potential cause of subfertility by impairing spermatogenesis. In addition, it is known that the incidence of testicular germ cell tumors is increased in men with cryptorchidism. Performing undescended testis surgery between 6 and 18 months may preserve spermatogenesis and decrease the risk of testicular tumors (7). Clinic varicoceles may cause secondary infertility by mechanisms including increased intratesticular reactive oxygen species (ROS), hypoxia, sperm DNA damage, and compromised testicular cooling. A recent meta-analysis has recommended surgical varicocelectomy in men with clinically significant varicoceles with affected semen analysis (8).

Childhood

The patient’s degree of virilization should be evaluated by inquiring about the age at puberty and sexual development.

Lifestyle Factors

Male infertility is closely related to lifestyle factors. The most important of these are smoking, excessive alcohol consumption, recreational drug use, and obesity (9). These habits are associated with increased sperm morphological defects, lower sperm motility, and lower sperm concentration. Obesity-induced endocrine changes resulting in the peripheral conversion of testosterone to estrogen are associated with impaired spermatogenesis (10). Obesity has been linked with low total testosterone and serum sex hormone binding globulin (SHBG), with normal serum follicle stimulating hormone (FSH) and luteinizing hormone (LH).

Toxic Testicular Exposure

Conditions such as sexually transmitted diseases, epididymo-orchitis, and mumps orchitis can significantly impair testicular function. Chlamydia trachomatis and Neisseria gonorrhea may cause urethritis, prostatitis, and epididymo-orchitis. Epididymis inflammation can provoke male infertility through sperm tract obstruction (11). Tuberculosis, Mycoplasma genitalium, and Ureaplasma urealitycum may cause male subfertility. Epidemiological studies have reported that exposure to endocrine-disrupting chemicals such as pesticides, phthalates, and bisphenol A may impair testicular development during the intrauterine period and lead to testicular dysgenesis syndrome (12).

Family History

Genetic diseases such as immotile cilia syndrome, cystic fibrosis, deletions in the Y chromosome, and chromosomal abnormalities are hereditary causes of male infertility. Obtaining information about the reproductive history of the biological parents may help determine the etiology. Pre-pregnancy genetic counseling and preimplantation genetic diagnosis should be offered to these patients.

Physical Examination

One of the indispensable steps of male infertility evaluation is physical examination. Genitalia, body habitus, and secondary sexual characteristics such as pelvic build, hair distribution, facial and chest hair, and upper body muscular build should be evaluated. A eunuchoid body habitus, tall stature, gynecomastia, decreased body hair, or obesity might be seen in patients with Klinefelter syndrome. Because it can reflect prenatal androgen insufficiency, the location of the urethral meatus should be evaluated. Testes should be examined for bilateral presence, size, consistency, and presence of mass. Detailed evaluation should be made for plaques, penile curvature, hypospadias, or epispadias that may impair the semen deposition in the vaginal vault. Epididymides examination can reveal induration or fullness indicative of infections or obstruction. The standard diagnostic method for varicocele is still physical examination. The diagnosis is made by palpating the scrotum during a thorough physical examination. The patient is examined in the standing and supine position, and the scrotum is inspected for distended veins. Varicocele grade 1 is palpable only by the Valsalva maneuver, grade 2 is not visible but palpable without the Valsalva maneuver, and grade 3 is visible through scrotal skin. The vas deferens are palpated during the examination of the scrotum. The absence of the vasa deferentia may indicate the congenital bilateral absence of the vas deferens (CBAVD). Because the rectal examination is essential in identifying large midline cysts or dilated seminal vesicles, men with a low ejaculate volume should have a digital rectal examination. It might indicate ejaculatory duct obstruction.

Semen Analysis

World Health Organization (WHO) and recent guidelines recommend semen analysis as the first laboratory test for evaluating male fertility. The WHO Laboratory Manual for the Examination and Processing of Human Semen and Sperm-Cervical Mucus Interaction was first published in 1980. The most recent manual was published as the sixth edition in 2021. The threshold reference values for semen characteristics as published in WHO guidelines are summarized in Table 2.

Conventional semen analysis provides information about the patency of the duct system, the production of spermatozoa in the testes, and glandular secretory activity with a typical abstinence period of 2-7 days, two semen samples should be requested from all patients in general, 2-3 weeks apart.

The seminal vesicles provide 70% of the ejaculate volume. The etiology of the absence of seminal fluid after orgasm (aspermia) is variable and includes pelvic trauma, surgery or radiation, spinal cord injury, ejaculatory duct obstruction, and neurologic causes. Especially after pelvic surgery, there may be retrograde ejaculation into the bladder. In this case, a post-ejaculatory urine examination

Table 2. Cut-off reference values for semen characteristics as published in World Health Organization manuals

	1st ed. (1980)	2nd ed. (1987)	3rd ed. (1992)	4th ed. (1999)	5th ed. (2010)	6th ed. (2021)
Volume	—	≥2.0 mL	≥2.0 mL	≥2.0 mL	≥1.5 mL	≥1.4 mL
Sperm concentration	20-200x10^6 / mL	≥20x10^6 / mL	≥20x10^6 / mL	≥20x10^6 / mL	≥15x10^6 / mL	≥16x10^6 / mL
Total sperm count	≥40x10^6 / mL	≥40x10^6 / mL	≥40x10^6 / mL	≥40x10^6 / mL	≥39x10^6 / mL	≥39x10^6 / mL
Sperm motility (% progressive)	≥60%	≥50%	≥50%	≥50%	≥32%	≥30%
Sperm vitality (%)	—	≥50%	≥75%	≥75%	≥58%	≥54%
Sperm morphology (% normal)	≥80.5%	≥50%	≥30%	≥15%	≥4%	≥4%

should be performed after ejaculation. Ejaculate volume <1.4 mL is most likely due to incomplete orgasm, incomplete collection, or incorrect abstinence. However, it may also be observed in males with the absence of vas deferens, sympathetic dysfunction, retrograde ejaculation, ejaculatory duct obstruction, and hypogonadism.

Measuring pH in the ejaculate is a part of the basic semen analysis. Prostate secretion is acidic and seminal vesicle secretion is also alkaline. An acidic seminal pH in patients with azoospermia may specify obstruction. A basic seminal pH (pH is >8.0) may point out inflammation or accessory gland impairment.

The semen liquefies within a certain period with the effect of prostate enzymes. Hyperviscosity is characterized by a thick and coagulated appearance of seminal fluid. It can be caused by dysfunction or infection of the male accessory glands. Insufficient secretion of prostate proteolytic enzymes results in failure of liquefaction.

Accurate measurement of sperm concentration in ejaculates is important diagnostically. Oligospermia, also known as oligozoospermia, is considered cases under 14 million sperm per milliliter of semen. The complete absence of sperm in the ejaculate is called azoospermia. To confirm azoospermia, on two separate occasions, semen should be centrifuged and evaluated for the presence of sperm under a light microscope.

The motility of each spermatozoon is graded as progressive motility, nonprogressive, and immotility. Progressive motility is defined as sperm that move actively, regardless of speed, either linearly or in a large circle. Nonprogressive indicates movement in place or in small circles. Immotility is defined as no movement. It is known that the percentage of progressively motile sperm is associated with pregnancy rates (13). A decrease in total motility or progressive motility is defined as asthenospermia.

Sperm morphology is routinely examined with Papanicolaou, Diff-Quik, or Shorr smears on an air-dried, fixed, and stained semen smear. After examination, smears are scored according to Kruger's strict criteria classification (14). Papanicolaou, Diff-Quik, or Shorr smears can both stain spermatozoa and allow the differentiation of "round" cells. Anormal morphology is decided based on head, midpiece, and tail abnormalities. There are some criteria for the definition of normal spermatozoa: 1) the head should be smooth oval-shaped, 4-5 µm long, and 2.5-3 µm wide. It should also have a visible acrosome covering 40-70% of the sperm head. 2) The midpiece should be approximately one and a half times the length of the head and slender. 3) The tail should be uniform, straight, uncoiled, and not curled. Teratozoospermia is a condition in which less than 4% of sperm have normal morphology.

Head defects: small, large, tapering pyriform, amorphous, and vacuolated heads, double heads, small acrosomal area, or any combination of these.

Mid-piece defects: thin, thick, or irregular mid-piece, a 'bent' neck, asymmetrical insertion between the mid-piece-the head, thin, thick, or irregular mid-piece, or any combination of these.

Tail defects: short, hairpin, multiple, broken or bent, irregular width, or any combination of these.

Hormonal Assessment

The hypothalamic-pituitary-gonadal axis is the key regulator of sex development and reproduction. Gonadotropin-releasing hormone (GnRH) is a very important hormone in the hypothalamic-pituitary-gonadal axis in males and its pulsatile secretion defines the pattern of secretion of the FSH and LH, which then regulate both the spermatogenesis in the testis and the endocrine function.

FSH affects the Sertoli cells, which accelerate spermatogonial maturation. The number of spermatogonia negatively correlates with the levels of FSH (15). In Leydig cells, LH effects cause the synthesis and release of testosterone. Hypergonadotropic hypogonadism, also called primary hypogonadism, is characterized by elevated FSH and LH, as well as low or normal testosterone. A state of reduced testosterone production caused by low levels of FSH and LH is known as hypogonadotropic hypogonadism. In addition, prolonged prolactin excess affects gonadic function, reducing testosterone levels and impairing spermatogenesis.

Endocrine evaluation is not recommended as an initial evaluation in infertility evaluation. It is suitable to perform an endocrine evaluation whenever clinical findings or accompanying sexual dysfunction suggest a defined endocrinopathy and in the presence of oligospermia. In general, endocrine testing is suggested for men with sperm concentrations below 10 million/mL. Hormone analysis is critical, especially in patients with suspected nonobstructive azoospermia. Even though the initial hormonal assessment consists of total serum testosterone and FSH, the accompanying assessment of prolactin, estradiol, and LH lets for a more comprehensive analysis of the patient's endocrine status.

Genetic Evaluation

Genetic abnormalities associated with male infertility are found in approximately 15% of infertile men (16). Patients with genetic abnormalities are frequently related to impaired spermatogenesis and increased aneuploidy. While the most common type of genetic disorder is karyotype anomalies, Klinefelter syndrome is the most common karyotype anomaly. Karyotype anomalies are seen in 12-15% in azoospermia, 5% in severe oligozoospermia,

and less than 1% in normal semen (17). While the American Society for Reproductive Medicine (ASRM) recommends karyotype analysis for patients with sperm count <5 x 10^6/mL, EAU recommends for patients with sperm count <10 x 10^6/mL (2,3). Y chromosome microdeletions identified within the azoospermia factor (*AZF*) gene region are highly associated with severe oligozoospermia or azoospermia and male infertility. These microdeletions are referred to as *AZFa*, *AZFb*, or *AZFc*, based on the genomic region deleted. Both ASRM and EAU point out that Y chromosome microdeletion analysis is indicated in patients with a sperm count less than 5 x 10^6/mL (2,3). In addition, EAU guidelines recommend that Y chromosome microdeletion testing be made mandatory for patients with sperm concentration <1 x 10^6/mL. Although sperm may be found through testicular sperm extraction (TESE) in approximately 50% of men with an *AZFc* deletion, spermatozoa cannot be retrieved by TESE in men with *AZFa* or *AZFb* microdeletions. Because of *AZFc* microdeletions can be transmitted to male offspring, counseling couples is recommended before assisted reproductive techniques.

Cystic fibrosis, manifested by multisystem organ dysfunction, is a rare autosomal-recessive disease. Mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene are a cause of male infertility although spermatogenesis is mostly normal, nearly all men with cystic fibrosis have abnormalities of the mesonephric duct, including atrophic seminal vesicles and vas deferens, and the absence of ejaculatory ducts. Current guidelines recommend CFTR screening in all men with clinical cystic fibrosis or CBAVD. The CFTR test is not recommended as a routine screening for men for whom obstruction is not considered for the etiology of infertility.

Specialized Sperm Evaluations

Since semen analysis does not define defects associated with the functional aspects of spermatozoa, sperm function tests were needed. Although defective sperm-zona interaction is the main cause of fertilization failure, with the widespread use of intracytoplasmic sperm injection, sperm DNA fragmentation tests have now been instead of hemizona or acrosome function assays in clinical practice. Currently, the sperm chromatin structure assay, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling, and sperm chromatin dispersion are commonly used sperm DNA fragmentation tests. Due to the absence of strict standardization and clear cutoff values, ASRM does not recommend routine use of sperm DNA fragmentation testing. Sperm DNA fragmentation testing is recommended by the EAU only in couples with recurrent pregnancy loss or in men with unexplained infertility.

Another way to evaluate sperm function is to measure seminal oxidative stress. Excessive amounts of ROS cause oxidative stress and cause DNA, lipid, and protein damage (18). However, the EAU has recommended that these tests remain experimental until the tests used to measure ROS are validated in randomized controlled trials.

Imaging

Scrotal ultrasound (US) is an available imaging method in evaluating infertile men in some cases due to its safety, noninvasive nature, and low cost. The US provides information about testicular size, volume, and echogenicity, blood flow, epididymal structure, and the presence of varicocele. Current guidelines do not recommend scrotal color Doppler for subclinical varicocele screening. The EAU suggests performing the scrotal US in men with infertility, as they have a higher risk of testicular cancer. The transrectal US is required to evaluate seminal vesicle and ejaculatory duct dilation and midline prostate cysts in patients with suspected genital tract obstruction. If more detailed imaging is needed, a pelvic magnetic resonance imaging (MRI) can be performed. In men with hyperprolactinemia, cranial MRI can diagnose pituitary pathology. Today, vasography is used intraoperatively during reconstructive microsurgery.

CONCLUSION

Initial evaluation of the male for fertility should include a detailed physical examination along with his medical and reproductive history. Both male and female partners should be evaluated concurrent and hormonal or genetic evaluation should be done after detailed history, physical examination, and sperm analysis results. Proper evaluation of male infertility will not only reveal the etiology of infertility but also provide the emergence of pathologies that threaten male general health, such as testicular tumors and endocrinopathies.

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Current Approach to Genetic Causes of Male Infertility and Genetic Counseling

Erkek İnfertilitesinin Genetik Nedenlerine Güncel Yaklaşım ve Genetik Danışmanlık

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ABSTRACT

Generally, infertility is defined as the inability of couples who have had unprotected and regular intercourse for at least 12 months or longer to conceive naturally. When all societies in the world are examined, infertility is an important health problem affecting approximately 48 million couples, and it also has socio-cultural, economic, and psychological effects. While 8-12% of reproductive age couples have infertility problems, when gender-related reasons are examined, 20-30% of these reasons are male, 20-30% are female, and 25-40% are together with couples. While the etiology of 60% of male infertility causes has not been clarified yet, congenital urogenital anomalies are the most common causes, and genetic causes are the second most common cause among the known causes. While investigating genetic etiology in patients, chromosomal anomalies and Y microdeletions are at the forefront; however, the importance of monogenic causes has increased as some known genes have been associated with infertility because of familial segregation studies and the whole exome analyses with the development of new generation sequencing technologies. In this review, genetic causes of male infertility, diagnosis, and treatment approaches for genetic causes were examined by the current literature, and the importance of providing the proper genetic counseling to infertility patients was mentioned.
Keywords: Genetics; male; infertility; genetic counseling.

ÖZ

Genel olarak infertilite, en az 12 ay veya daha uzun süre korunmasız ve düzenli cinsel ilişki yaşayan çiftlerin doğal yollarla gebe kalamaması olarak tanımlanmaktadır. Dünyadaki tüm toplumlar incelendiğinde, infertilite yaklaşık olarak 48 milyon çifti etkileyen önemli bir sağlık sorunu olmanın yanı sıra, sosyo-kültürel, ekonomik ve psikolojik etkileri de bulunmaktadır. Üreme çağındaki çiftlerin %8-12'si infertilite problemi yaşarken, cinsiyete bağlı sebepler incelendiğinde, bu nedenlerin %20-30'unu erkek cinsiyet, %20-30'unu kadın cinsiyet ve %25-40'ını çiftler beraber oluşturmaktadır. Erkek cinsiyet kaynaklı infertilite nedenlerinin %60'ının etiyolojisi henüz aydınlatılmamışken, bilinen nedenler arasında doğumsal ürogenital anomaliler en sık nedenler ve genetik nedenler ise ikinci sıklıktaki nedenlerdir. Hastalarda genetik etiyoloji araştırılırken kromozomal anomalileri ve Y mikrodelesyonları ön planda izlenmektedir, ancak, yeni nesil dizileme teknolojilerinin gelişmesiyle birlikte yapılan ailesel segregasyon çalışmaları ve tüm ekzom analizleri sonucunda bilinen bazı genlerin infertilite ile ilişkilendirilmesiyle monogenik nedenlerin önemi artmıştır. Bu derlemede, erkek infertilitesinin genetik nedenleri, genetik nedenlere yönelik tanı ve tedavi yaklaşımları güncel literatürle uyumlu bir şekilde incelenmiş ve infertilite hastalarına doğru genetik danışmanlığın sağlanmasının öneminden bahsedilmiştir.
Anahtar kelimeler: Genetik; erkek; infertilite; genetik danışmanlık.

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INTRODUCTION

Infertility, with its general definition, is the inability to conceive naturally after at least 12 months or more of unprotected and regular sexual intercourse (1). When all societies in the world are examined, infertility is a severe problem which an estimated 48 million couples are affected by (2), which has medical, psychological, economic, and socio-cultural effects and has affected societies in every period of history, the cause of which is still unsolved. Although the prevalence of infertility in reproductive ages varies between 8% and 12%, it is responsible for infertility of 20-30% in men, 20-30% in women, and 25-40% in both sexes (3). Considering the importance of having a child in the majority of cultures, infertility, which is very common, is a significant health problem that affects the society and family socio-cultural structure and causes substantial economic loss for most societies.

When the etiological causes of male infertility are examined, it is 10.7% congenital urogenital anomalies, 5.9% obstruction of the seminal tract, 4.4% severe sexual dysfunction, 3.4% oncological diseases, 6.6% other testicular factors, 7.8%, known genetic factors, 1.3% of them are known as secondary hypogonadism. Today, the cause of 60% has not been revealed (4). Since many factors cause male infertility, patients should be examined in pairs, if possible, and evaluated with a detailed anamnesis in which all systems are questioned.

In the anamnesis, family history, consanguineous of the couples, history of unsuccessful pregnancy, and known chronic diseases should be carefully examined. If the condition is suspected to be inherited genetically, the inheritance pattern should be revealed, and the cause of the disease should be clarified by performing a complete systemic examination. Before the genetic examination, a spermiogram should be performed; psychological, structural, autoimmune, oncologic, and endocrinologic causes should be excluded, and necessary radiographic imaging, hormonal, and biochemical tests should be evaluated. While examining the patient's spermiogram, sperm count, motility, and morphology must be assessed and classified by WHO 2010 criteria (5, Table 1).

If oligospermia, asthenospermia, and teratozoospermia coexist, it is diagnosed as oligo-astheno-teratozoospermia (OAT). In terms of their etiology, 77.9% of mild oligospermia, 17.1% of azoospermia cases, and strikingly, approximately 0.1% of aspermia are idiopathic. The genetic cause of 25.8% of azoospermia cases can be revealed, while the genetic cause has been shown in only 1% of mild oligospermias (4). These results indicate that as the phenotype of the patients worsens, the causes of infertility are determined more clearly, and the weight of genetic causes increases compared to other reasons. It is essential to determine the cause of male infertility:

- Determining the cause of infertility in couples in the socio-cultural conditions in Turkey, arranging the treatment of patients for them and protecting them from unnecessary interventions, providing the proper treatment of the patient, and preventing unnecessary economic losses.
- A better understanding of the genes and mechanisms that cause infertility can prevent diseases and improve treatment possibilities.
- Evaluation and pre-detection of systemic diseases that may cause infertility and arranging the treatment and follow-up of the patients.
- Providing preimplantation genetic diagnosis (PGD) to infertile patients with monogenic and chromosomal causes and providing genetic counseling to the family regarding genetic diseases transmitted to the offspring.

As the sperm count decreases, the role of genetic etiology increases, and azoospermia is a disorder that causes the most severe infertility genetic causes are better known and are seen together as OAT syndrome in spermiogram. Azoospermia can be divided into obstructive azoospermia (OA) and non-obstructive azoospermia (NOA). OA develops due to obstruction in the ejaculation tract. Congenital bilateral absence of the vas deferens (CBAVD) or inflammation is the cause of other obstructive factors such as fibrosis. NOA is caused by sperm production and development disorders. NOA develops due to primary testicular failure, hypothalamus-pituitary-adrenal axis disorders (secondary testicular failure), and spermatogenesis defects (6). Chromosomal anomalies and causes that make sperm failure monogenic generally cause NOA, and mutations in *CFTR* and *ADGRG2* genes cause OA. If we look at azoospermia from a genetic point of view, the most common cause will be chromosomal anomalies, especially Klinefelter Syndrome. *AZFa*, *AZFb*, *AZFc* microdeletions of the Y chromosome, and *CFTR* mutations are the second and third most frequent reasons (7).

An important monogenetic group, including congenital hypogonadotropic hypogonadism (CHH) and disorders of sexual development (DSD), causes infertility accompanied by other system disorders. However, with the development of genetic techniques such as genome research and next-generation sequencing, the number of genes associated with spermatogenesis errors increases rapidly. Altogether, 104 genes associated with infertility and genitourinary development errors have been identified (8). The inheritance of these genes is quite complex and can be autosomal recessive (AR), autosomal dominant (AD), X-linked, and Y-linked, besides oligogenic and digenic inheritance patterns.

In this review, current developments and approaches in the genetic etiology of infertility will be explained. The causes will be examined under three main headings: quantitative disorders of spermatogenesis, qualitative disorders of spermatogenesis, and other reasons.

QUANTITATIVE DISORDERS OF SPERMATOGENESIS

Quantitative defects of spermatogenesis due to primary testicular disorders are thought to have a range of clinical manifestations, from azoospermia (no spermatozoa in the ejaculate) to oligozoospermia (<39 million spermatozoa per ejaculate), as well as different histological implications. These histologic types of testicular tissue can

Table 1. Assessment of spermiogram analysis

Variables	Normal	Pathologic
Volume (ml)	1,5-6,8	<1,5: Hypospermia 0: Aspermia
Concentration (million/ml)	15-213	<15: Oligospermia 0: Azoospermia
Vitality (%)	58-91	<58: Necrospermia
Total motility (%)	40-78	<40: Asthenospermia
Morphology (%)	4-44	<4: Teratospermia

be present: Sertoli cell-only syndrome (SCOS), spermatogenic arrest at various stages of germ cell maturation (spermatogonia, spermatocyte, and spermatid), and hypospermatogenesis. Also, men with quantitative defects of sperm production have different follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels, testis volume, and degree of androgenization (9).

Other classifications of azoospermia are OA and NOA, and both of them have very different etiologies and treatments (10).

According to Lee et al. (11), the causes of azoospermia were divided into two major categories, chromosomal and extrachromosomal. In the category of chromosomal defects, there were chromosomal inversions, translocations, micro/macrodeletions of the Y chromosome, and numerical chromosomal anomalies, also known as aneuploidies non-chromosomal defects; there were epigenetic changes in the genome and included defects of the sperm mitochondrial genome. There are also several monogenic causes. Although the information on monogenic grounds of NOA is limited, established, and recently reported NOA genes associated with transcriptional and endocrine regulators of reproduction, guardians of meiosis have been notified (12).

Herein we represent several syndromes associated with quantitative disorders of spermatogenesis.

Klinefelter Syndrome

Klinefelter syndrome is the most common form of male hypogonadism, with a prevalence of 0.2% healthy male population (13). Klinefelter syndrome is detected in 14% of azoospermic men. However, there are classical findings of eunuchoid body structure, sparse beard growth, less body hair, gynecomastia, small testicles, and azoospermia. Slender stature with relatively long legs is typical for Klinefelter syndrome. Half of the patients have nondisjunction in first paternal meiosis (due to lack of normal Xp-Yp recombination in the pseudoautosomal region). During the oogenesis, it can occur either in meiosis-1, which is affected by maternal age, or meiosis-2. Ninety-five percent of patients with Klinefelter Syndrome have a 47,XXY karyotype; 5% of the cases are mosaic (such as 46,XY/47,XXY) (14). In addition, endocrinology consultation (for hormone replacement in case of indication) is necessary for patients with Klinefelter syndrome. Spermatogenesis may defect to varying degrees, but males are usually sterile. Sperm can be obtained by performing micro-testicular sperm extraction (micro-TESE) with successful surgical intervention in 69% of the patients. Some recent studies emphasize that cryopreservation of prepubertal spermatogonial stem cells may be beneficial in these patients (15).

XX Male Syndrome

XX male is a rare sex chromosomal disorder in infertile men and occurs in about 1 in 20,000 newborn males. Approximately 80% of 46,XX testicular DSD cases are SRY-positive as a result of translocation from the Y chromosome to the X chromosome (16). Most patients with SRY-positive 46,XX DSD, have a male phenotype with small testes and may have cryptorchidism or hypospadias, azoospermia resulting in primary infertility, and gynecomastia with normal cognitive development. Postpubertal testicular histology of SRY-positive XX men shows atrophy and hyalinization of the seminiferous tubules, but the testicles are also devoid of germ cells. In men with XX-male syndrome, patients with reduced testosterone production should receive appropriate replacement therapy. Currently, there is no therapy for infertility of men with XX-male syndrome. Patients with reduced testosterone production have to obtain appropriate testosterone replacement therapy (17).

47,XYY Syndrome

In a report covering the chromosome analyses performed between 1961 and 2014 in Denmark in 2020, the total prevalence of males diagnosed with 47,XYY syndrome was 9 per 100,000 males (239 47,XYY males among a population of 2.79 million males, 9% of expected) (18,19). Nevertheless, the maximum average prevalence was 18 47,XYY males per 100,000 newborn males (18% of predicted); these patients have non-disjunction of the Y chromosome in meiosis 2. Spermatogenesis can be affected in a variable range from normal to severe failure. Although no obvious physical anomaly is observed in their phenotypes, half of the cases have an intelligence score of 10-15 points below the average, and attention deficit and hyperactivity are remarkable. In addition, individuals with this genotype have more educational and behavioral problems than 46,XY individuals (14). However, a review of 50 years of literature published in 2015 also concludes that there is no appreciable evidence that patients with XYY syndrome have an antisocial or aggressive behavior disorder (20).

Y Chromosome Deletions

Genes responsible for testicular development and initiation and maintenance of spermatogenesis in adulthood are located on the human Y chromosome. (13). In the Y chromosome, which contains 60 million bases, very few of them are expressed as functional genes. Although there are 27 genes identified, 9 of them are located in the short arm (p) of the chromosome and 18 in the long arm (q) arm of the chromosome. Azoospermia factor (AZF), one of the most studied regions related to male infertility, is located in Yq and consists of three independent regions as *AZFa*, *AZFb*, and *AZFc* located in Yq. *AZFa*, *AZFb*, and *AZFc* residues are primarily expressed in testes and play an essential role in spermatogenesis. Deletions in *AZF* loci cause spermatogenesis defects (oligospermia, azoospermia, etc.) with the deletion of one or more candidate genes in these regions. Some of the candidate genes in the *AZF* regions are *USP9*, *DBY*, *DAZ*, and *RBMY*. After 20 years from the first molecular definition of the *AZF*, Yq deletion screening has now become a routine test for infertile males in many countries to identify the cause of male infertility. It is known that this test reveals a serious spermatogenesis defect and helps to determine the success rates of sperm retrieval in the use of assisted reproductive techniques and to predict the success of these technics (14).

Other Chromosomal Abnormalities

Common autosomal chromosomal changes are Robertsonian type translocations, reciprocal translocations, paracentric inversions, and marker chromosomes are ten times more often in patients with oligozoospermia (4-8%) than in patients with normozoospermia (9). These patients should be informed about PGD in those who have undergone in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI).

In addition, fluorescence in situ hybridization (FISH) studies and investigation of aneuploidy in semen in patients with normal/abnormal chromosomal results are important to decide whether PGD should be performed before ICSI. The isodicentric Y chromosome, which is considered another structural chromosomal anomaly, is one of the most common structural anomalies of the Y chromosome and may lead to a major OA clinic. Most of these anomalies disappear during cell proliferation, and cells with dicentric Y chromosomes are found in mosaic. The phenotypic findings of these patients are affected by the structure of the dicentric Y chromosome, the fracture site, and the degree of mosaicism, resulting in a reasonably wide range ranging from Turner syndrome-like women to infertile men (14). It has been reported that men with Down syndrome have impaired spermatogenesis and loss of function in Sertoli and Leydig cells. Although these individuals seem to have normal puberty that starts at an average age, the decrease in testicular volumes after puberty and an increase in gonadotropin production are indicators of gonadal dysfunction, which decreases with age. In addition, other studies have shown that the sperm count is abnormally low in men with Trisomy 21, suggesting that the resulting azoospermia or oligospermia may be related to damage to the spermatogenesis process (21).

Other Single Gene Disorders

Some of the genes that will be evaluated in this section lead to hormonal dysregulation and some of them cause spermatogenesis defects and cause male infertility. Kallmann syndrome is a disease with hypogonadotropic hypogonadism (HH) that can be evaluated in this group, and the genes responsible for some of the cases are; *KAL-1*, *FGFR-1*, *PROK-2*, *PROKR-2*, *CHD-7*, and *FGF-8* (1). Furthermore, many genes can cause non-syndromic male infertility, which can progress with ambiguous genitalia and cause sex chromosome phenotype mismatch, and a group of these genes is associated with hormone biosynthesis defects. In addition to these, LH receptor defects, Androgen receptor defects (*AR* gene), hemizygous mutation of the *SRY* gene, hemizygous "duplication" in the *NR0B1* (*DAX1*) gene, a homozygous mutation in the *DHH* gene, heterozygous mutations in the *MAP3K1* and *NR5A1* genes are among the causes of monogenic fertility. Although cystic fibrosis (CF) is a multisystem disease characterized by abnormal mucus production, it is one of the major causes of obstructive infertility in men. Ninety-eight percent of men diagnosed with CF are infertile. Insufficient development of seminal vesicles or congenital bilateral absence of the vas deferens (CBAVD), accounts for 1.2-1.7% of all male infertility can be seen in these patients. Testicular functions and spermatogenesis are expected to be intact in these patients and assisted reproductive techniques are used to approach infertility problems. In addition, the expected phenotype in infertile individuals differs depending on the types of variants detected in CF patients (6).

QUALITATIVE SPERM DISEASES

Structural defects of sperm can be detected with a simple spermiogram and divided into subtypes. In general, it is thought that environmental, epigenetic, and genetic factors affect the morphology of sperm together. However, the effect of genetic causes is powerful in patients with OAT or with specific morphology. We will examine the qualitative sperm diseases under four subtitles globozoospermia, macrozoospermia, acephalic sperm syndrome (ASS), and multiple morphological abnormalities of the flagella (MMAF) (22).

Globozoospermia

Globozoosperm is briefly sperm with a round head without an acrosome. Since it does not have an acrosome, it cannot penetrate the zona pellucida and so causes infertility. If all of the sperm counts are globozoospermia, it is called type 1; if 20-90% of the sperm count is globozoospermia, it is called type 2 globozoospermia. Globozoospermia accounts for 0.1% of infertile men. The most common cause is intragenic deletions and point mutations of the *DPY19L2* gene, explaining 60-80% of the cases (9).

The *DPY19L2* gene stabilizes the acrosome, supporting the nuclear membrane of the acroplaxoma, elongation of the sperm head in the development of normal sperm morphology, and acrosome formation. In recent studies, point mutations in the *SPATA16* and *PICK1* genes have been detected in cases of unexplained globozoospermia. The *SPATA16* and *PICK1* genes are involved in vesicle transfer from the Golgi body to the acrosome and acrosome biogenesis (23). In addition, *ZPBP1*, *SPINK2*, and *CCDC62* are candidate genes involved in the pathogenesis of globozoospermia; mutations of their genes have been reported in some cases, sperm structures mimicking globozoospermia have been shown in mutant mouse studies, and they have been shown to play an essential role in acrosome biogenesis (24). The *GOPC* gene, which is associated with these genes, is one of the candidate genes that may have an effect on the etiology of infertility (25). In type 1 globozoospermia patients, artificial oocyte activation is provided by ICSI, and in type 2 globozoospermia, sperm with a solid structure is selected, and fertility is provided by ICSI (23).

Macrozoospermia

Macrozoospermia is defined as detecting nearly 100% sperm with macrocephalic multiflagella in semen analysis. The most common cause of macrozoospermia is the homozygous c.144delC variant in exon 3 of the *AURKC* gene (26). Although this variant explains 85% of the cases, the remaining etiology is elucidated as homozygous or compound heterozygous variants with nonsense mutations such as p.Y248* seen in exon 6 (27). The *AURKC* gene is necessary for accurate chromosomal segregation and cytokinesis, and its mutations result in tetraploid sperms as a result of errors during meiotic division. In patients with macrozoospermia, the *AURKC* mutation must be analyzed before performing assisted reproduction tests. ICSI is not recommended since all sperm will be polyploid if a mutation is detected. If the mutation is negative, sperm FISH should be performed to evaluate the euploid sperm ratio, and PGD is recommended in cases with a moderate aneuploid sperm ratio (26).

Acephalic Sperm Syndrome

Observation of a high rate of headless sperm in the spermiogram is called ASS, and it is a rare severe type of teratozoospermia. Headless flagellar structures and only the head structure of the sperm can be observed during the examinations. In addition, the presence of isolated flagella

or the presence of tailless head structures with flagella can give an idea about the origin of the pathology (28). Since Sertoli cells will phagocytize head structures in testicular pathologies, the rate of headless sperm is significantly increased in semen analysis, whereas in epididymal pathologies, almost the same number of flagella and head will be seen since flagella and head structure will separate later. Improper attachment of the implantation pit and basal layer, defective or incomplete connection structures due to the absence of pericentriolar granular material, migration of flagella to the caudal end, and defective regulation of flagella formation play a role in the pathogenesis of acephalic sperm (29,30). ASS was first described in 1981 (31), and the factors in its genetic etiology could not be clarified until 2016. In 2016, the AR inheritance pattern was determined by familial segregation analyses in men with more than 50% acephalic sperm in semen analysis, and then 16 pathogenic variants were detected in the *SUN5* gene by whole-exome sequencing. The *SUN5* protein is an essential protein that provides the head and tail connection of the sperm. Homozygous or fused heterozygous frameshift, missense, nonsense mutations, and deletions of sequences encoding the transmembrane, SUN, and conserved coil-coil region of this protein have been shown to cause infertility. Today, *BRDT*, *PMFBP1*, *TSGA10*, *DNAH6*, *HOOK1*, *CEP112*, and *ACTRT1* (32) genes have also been associated with ASS. Biallelic mutations that lose function in these genes cause infertility through the mechanisms mentioned in the pathogenesis of ASS. Successful pregnancy and healthy delivery have been achieved in patients with mutations in the *SUN5*, *PMFBP1*, *TSGA10*, *HOOK1*, and *CEP112* genes with ICSIs performed by selecting sperm without flagella (33).

Multiple Morphological Abnormalities of the Flagella

In human sperm, the flagellum is the organelle that provides the necessary motility to successfully reach the oocyte and fertilize in the female genital tract. It is generally examined in three parts: the midpiece consisting of flagella, mitochondrial sheath, and outer dense fiber, the principal piece covered with fibrous sheath, and the terminal piece containing only axonemes. The most important structure of the flagella is the microtubular complex, which is organized in the form of 9+2, and this structure provides the main movement of the sperm. The microtubular complex consists of a pair of central microtubular nuclei and nine pairs of type A and type B microtubules around it, and these pairs slide over each other with the help of arms made of dynein protein. In addition, peripheral pairs are held together by nexin-dynein complexes, while peripheral microtubules are connected to the central nucleus by proteins called radial rays. Dynein is a multiprotein ATPase complex divided into the inner dynein arm (IDA) and the outer dynein arm (ODA) (34,35). Any mutation affecting these structures can result in faulty head-tail or midpiece, principal piece connectivity, abnormal mitochondrial sheath structure, excessive folding of the flagellar structure, and residual cytoplasmic residues. As a result of mutations, tailless, short-tailed, and bizarre morphology sperms can be detected in the spermiogram; these disorders are called MMAF. The genetic etiology of MMAF was not clarified until 2014, and currently, 18 genes account for the cause of 30-60% of patients (35). In the study in which the first genetic etiology was revealed, the homozygous p.G3930Afs* variant and three different homozygous variants were detected in the *DNAH1* gene in 4 patients with strong consanguinity in 20 patients in North Africa, and these mutations were shown to cause MMAF in mice (36). In subsequent studies, mutations of *DNAH1*, *DNAH2*, *DNAH6*, and *DNAH17* genes that impair the function of IDA and ODA, mutations of *CFAP43*, *CFAP44*, and *CFAP65* genes that affect the IDA-related T/TH complex, mutations of the *CFAP70* gene that cause errors in the role of the ODA-related *CFAP70* complex in intraflagellar transport, mutations of the *WDR66* gene that cause defects in peripheral and central microtubule transduction of the radial beam complex, mutations of the *FSIP2* gene that disrupt the organization of periaxonemal structures; Mutations of the *CEP135* gene, which causes defects in the biogenesis of the centrosome and centrioles, mutations of the *TTC21A*, *TTC29* and *CFAP69* genes that cause intraflagellar transport errors, and mutations of the *QRICH2* gene, which is located in the ubiquitin-proteasome pathway and causes protein degradation, have been shown to be clinically associated with MMAF. In addition, it should be kept in mind that *AK7* and *ARMC2* genes, whose functions are not clearly known but shown by whole-exome sequencing in segregation studies, may also play a role in the pathogenesis of MMAF (37). It has been demonstrated that frameshift, missense, and nonsense mutations and deletions of 18 genes causing loss of biallelic function lead to clinical trials. In the literature, it has been shown that successful pregnancy is achieved by the ICSI method in couples with *DNAH2*, *DNAH6*, *DNAH17*, *CFAP43*, *CFAP44*, *CFAP65*, *CFAP70*, *CEP135*, *TTC29*, and *SPEF2* mutations. Successful pregnancy could not be achieved in patients with mutations in the *DNAH17* gene, and in patients with *CFAP65* mutation, although successful pregnancy was achieved, all of them resulted in abortion (35).

OTHER CAUSES OF INFERTILITY

Hypothalamo-pituitary-gonadal axis dysfunction is one of the important causes of male infertility. Developmental anomalies in this axis are called hypogonadism and are basically classified as hypergonadotropic (primary) and hypogonadotropic (secondary). Since the primary problem of hypergonadotropic hypogonadism is in the gonads, it progresses with high LH, FSH, and low testosterone levels. Its etiology includes infection, radiotherapy, cryptorchidism, autoimmune, liver and kidney diseases, trauma, and genetic causes. The most common genetic cause of hypergonadotropic hypogonadism is Klinefelter syndrome in men.

HH, on the other hand, is associated with the low synthesis of gonadotropes and low testosterone levels because of developmental and synthesis deficiency at the hypothalamo-pituitary level. HH is a rare cause of infertility and appears below 1% (38). If we do not consider invasive, infiltrative, ischemic, and traumatic causes, 50% of CHH is idiopathic. CHH may occur with pubertal tarda, absence of body hair, gynecomastia, cryptorchidism, and small testicular volume (39). In the early embryonic development stages, the gonadotrophin releasing hormone (GnRH) neurons migrate from the inner

part of the nasal epithelium to the forebrain, and in the following period, they move to their final location in the hypothalamus. Olfactory neurons also develop from the nasal plate close to the neurons that secrete GnRH, and as they migrate via similar pathways, anosmia or hyposmia is also observed in some of the CHH cases (38). CHH is divided into two major categories: Kallmann syndrome and normosmic hypogonadotropic hypogonadism (nHH). CHH may also be observed in association with Gordon Holmes, CHARGE, Waardenburg, Bardet-Biedl, and Prader Willi syndromes, such as Xp21 and Xp22.3 deletion. There have been identified more than 25 different genes related to Kallmann syndrome, 35 genes about CHH in the literature, and according to some of the studies, more than 60 genes of CHH. CHH shows AD, AR, X linked recessive (XLR), and oligogenic inheritance patterns. The genes involved in HH-related pathways were roughly classified as follows and then listed in Table 2 (9,38-43):

i. Genes associated with GnRH neuronal embryonic differentiation and migration: *CHD7*, *FGF8*, *FGFR1a*, *HESX1*, *WD11*, *SOX10*, *ANOS1* (*KAL*), *FGF17*, *IL17RD*, *DUSP6*, *SPRY4*, *FLRT3*, *KLB*, *PROK2*, *PROKR2*, *HS6ST1*, *SEMA3A*, *SEMA3AE*, *DCC*, *AMH*, *AMHR*, *IGSF10*, *SMC*, *NDNF*, and *FEZF1* genes. CHARGE, Kallmann syndrome with anosmia, combined pituitary hormone deficiency, and Waardenburg syndrome are in this group.
ii. Genes associated with GnRH neuron regulation: *DMXL2* (polyendocrinopathy polyneuropathy syndrome), *TAC3*, *TACR3*, *KISS1*, *KISS1R*, *GNRH1* genes (GnRH neuron pulsatile release), *GNRH1*, *GNRHR*, *FSHB*, *LHB* genes (pituitary gonadotropin secretion).
iii. Genes associated with HH and obesity-*LEP*, *LEPR* genes.
iv. X-linked adrenal hypoplasia and CHH-*NROB1* (*DAX1*) genes.
v. Genes related to gonadotropin secretion and activation-*PCSK1*, *LHB*, *FSHB*, *LHR*, and *FSHR* genes.
vi. HH associated genes with cerebellar ataxia-*POLR3A*, *POL3RB*, *OTUD4*, *RNF216*, *PNPLA6*, and *STUB1* genes.

Kallman syndrome is a rare (1/30000) clinical entity and shows wide phenotypic and genotypic variability. The disease presents with CHH findings and hyposmia/anosmia. In addition to these major findings, renal developmental disorders, finger anomalies (polydactyly, syndactyly, camptodactyly), cleft or lip palate, abnormal eye movements, synkinesis, dental agenesis can also be seen. More than 20 genes have been identified in the etiology of this disease. *ANOS1* (*KAL1*), *FGFR1*, *FGF8*, *FGF17*, *IL17RD*, *DUSP6*, *SPRY4*, *FLRT3*, *KLB*, *PROK2*, *PROKR2*, *HS6ST1*, *CHD7*, *WDR11*, *SEMA3A*, *SEMA3E*, *IGSF10*, *SMCHD1*, *CCDC141*, *FEZF1* are the most common genes. These genes are related to neuronal migration pathways. The *ANOS1* (*KAL1*) gene is localized in Xp22.3 and encodes a protein called anosmin-1. This gene is attached to the cell membrane with heparan sulfate and is responsible for GnRH neuron adhesion and axonal migration (44). Anosmin is colocalized with the *FGFR1* gene. The *ANOS1* gene strengthens the FGF signal by interacting with the FGFR-FGF-HEPARAN sulfate proteoglycan complex on the cell surface, and this also provides GnRH neuronal migration. These genes, which are involved in different molecular pathways, ultimately affect the GnRH neuronal migration and cause anosmia/hyposmia and CHH clinic (39,44).

CHARGE syndrome is a rare genetic syndrome that affects many parts of the body (1-8500/10000). The features of this syndrome are coloboma, heart defects, atresia choanae, genital, growth, and ear abnormalities. These patients may also present with HH findings, lip, cleft palate, and rarely tracheoesophageal fistula. Rarely do patients have scoliosis kyphosis, poly-oligodactyly. The *CHD7* gene is the genetic cause of this syndrome, responsible for chromatin remodeling. Point mutations, deletions, and rarely translocations are the main reasons for the patient's symptoms in different systems. Unlike anosmic HH, this syndrome is not accompanied by a loss of smell (45).

Waardenburg syndrome is a rare genetic disease that is the most common syndromic cause of hearing loss and progresses with loss of pigmentation in the hair, skin, and eyes (1/40000). The eyes have iris heterochromia and hypopigmentation. *EDN3*, *EDNRB*, *MITF*, *PAX3*, *SNAI2*, and *SOX10* genes play a role in its etiology. *SOX10* genes are essential for infertility. *SOX10* is the gene transcription factor responsible for neuronal migration during early embryonic developmental stages. Rarely, *SOX10* mutations can also cause anosmic HH (46).

Gordon Holmes syndrome is a rare syndrome characterized by neurological symptoms and HH (prevalence is unknown). Patients present with delayed puberty findings, cerebellar findings such as dysarthria, and balance problems. *RNF216*, *OTUD4*, *PNPLA6*, and *STUB1* gene mutations are involved in the etiology of the disease (47).

GENETIC COUNSELING IN MALE INFERTILITY

Genetic counseling; for the individual who comes to receive counseling to understand the effect of genetics on diseases, it is an interactive process that helps students understand and adapt to the medical, psychological, and familial impact of the current medical condition. In this process, the individuals' personal medical history and family histories should be examined by removing their detailed pedigrees. Individuals should be told about the possible causes of their current situation, the tests that can be done to determine these causes, and what kind of a period awaits them on the way to diagnosis. While informing about the current situation or results of the patient, the results and options should be presented with full transparency, and the individual should be allowed to make their own choices. A genetic counseling process should proceed in confidence and should never be directive. In addition to all these, recent developments in assisted reproductive technologies and their widespread use have been useful for couples with infertility. Using IVF and ICSI technologies, men with infertility can have their biological children. Clinicians following the patient should be aware of this, and necessary guidance

Table 2. Genes, function, and clinical phenotypes related to hypogonadism

Genes	OMIM	Inheritance	Clinical Phenotype	Gene Function
ANOS1(KAL)	300836	XLR	HH 1 with or without anosmia	Growth and migration of GnRH neurons
FGF8	600483	AD	HH 6 with or without anosmia	Formation and migration of GnRH neurons
FGFR1	136350	AD	HH 2 with or without anosmia	Formation and migration of GnRH neurons
HESX1	60182	AD, AR	GH deficiency, pituitary dysplasia	Interacting other genes, product TF, development of the forebrain
SOX10	602229	AD	Waardenburg syndrome type 2E, 4C	Cell migrations from the spinal cord to specific regions
CHD7	608892	AD	CHARGE, HH 5 with or without anosmia	Regulating of chromatin remodeling and GnRH neuron specification
WDR11	606417	AD	HH 14 with or without anosmia	GnRH neuron specification and regulating GnRH production
FGF17	603725	AD	HH 14 with or without anosmia	GnRH neuron and gonadotroph differentiation and fate specifications
IL17RD	606807	AD, AR, DD	HH 18 with or without anosmia	GnRH neuron and gonadotroph differentiation and fate specifications
SEMA3AE	608166	AD	CHARGE syndrome	Development of the olfactory system and the migration of GnRH neurons
SEMA3A	603961	AD	HH 16 with or without anosmia	Development of the olfactory system and the migration of GnRH neurons
PROK2	607002	AD	HH 4 with or without anosmia	Migration and development of both GnRH and olfactory neurons
PROKR2	607123	AD	HH 3 with or without anosmia	Migration and development of both GnRH and olfactory neurons
FLRT3	604808	AD	HH 21 with or without anosmia	GnRH neuron and gonadotroph differentiation and fate specifications
DUSP6	602748	AD	HH 19 with or without anosmia	GnRH neuron and gonadotroph differentiation and fate specifications
SPRY4	607984	AD	HH 17 with or without anosmia	GnRH neuron and gonadotroph differentiation and fate specifications
HS6ST1	604846	AD	HH 15 with or without anosmia	Guiding cell communications during neural development and
NDNF	616506	AD	HH 25 with or without anosmia	GnRH neuron migration and development of the olfactory scaffold
FEZF1	616030	AR	HH 22 with or without anosmia	Transcription repressor and guidance GnRH neurons and olfactory axons
DMXL2	616113	AR	PNEPS with HH	Regulation exocytosis vesicles of GnRH neurons and gonadotrophs
TAC3	162330	AR	HH 10 with or without anosmia	Expressed in the hypothalamus, GnRH neuron activation, and networking
TACR3	162332	AR	HH 11 with or without anosmia	Expressed in the hypothalamus, GnRH neuron activation, and networking
KISS1	603286	AR	HH 13 with or without anosmia	Regulation of GnRH neuron activation and hormone secretion
KISS1R	604161	AR	HH 8 with or without anosmia	Regulation of GnRH neuron activation and hormone secretion
GNRH1	152760	AR	HH 12 with or without anosmia	Direct secretion and action of GnRH
GNRHR	138850	AR	HH 7 with or without anosmia	Direct secretion and action of GnRH
LEP	164160	AR	Obesity with HH	Afferent to GnRH neurons
LEPR	614963	AR	Obesity with HH	Afferent to GnRH neurons
NR0B1(DAX1)	300473	XLR	Adrenal hypoplasia with HH	GnRH neuron and gonadotroph differentiation and fate specifications
PCSK1	162150	AR	Obesity with HH	Gonadotropin secretion and action
LHB	152780	AR	HH 23 with or without anosmia	Gonadotropin secretion and action
FSHB	136530	AR	HH 24 with or without anosmia	Gonadotropin secretion and action
LHCGR	152790	AR	Leydig cell hypoplasia with HHG	The receptor of luteinizing hormone and chorionic gonadotropin
POLR3A	614258	AR	4H syndrome 7 with HH	Encoding RNA polymerase catalytic subunits
POLR3B	614366	AR	4H syndrome 8 with HH	Encoding RNA polymerase catalytic subunits
OTUD4	611744	AR	Gordon Holmes and HH	Playing a role in the ubiquitin-proteasome system
RNF216	609948	AR	Gordon Holmes and HH	Playing a role in the ubiquitin-proteasome system
PNPLA6	603197	AR	Boucher-Neuhauser, Gordon Holmes	Coding NTE protein, contributes to membrane stabilization, and release of hormones from PG
STUB1	607207	AR	Spinocerebellar ataxia 16 with HH in some families	Playing a role in the ubiquitin-proteasome system

XLR: X linked recessive, AD: autosomal dominant, AR: autosomal recessive, DD: developmental delay, HH: hypogonadotropic hypogonadism, GH: growth hormone, CHARGE: coloboma, heart defects, atresia of choanae, retardation of growth or development, genital or urinary defects, PNEPS: polyendocrinopathy polyneuropathy syndrome, HHG: hypergonadotropic hypogonadism, GnRH: Gonadotropin releasing hormone, TF: transcription factors, NTE: neuropathy target esterase, PG: pituitary gland

and planning should be provided for patients within appropriate indications. If we go into detail about some of the causes of male infertility that we have explained above;

i. Men with Klinefelter syndrome (including mosaic (47,XXY/46,XY) individuals) have hypogonadism, which affects the patient's spermatogenesis process and testosterone production. This situation causes individuals to encounter masculinization deficiency and azoospermia in their spermiograms. When the sperm samples obtained from individuals with Klinefelter syndrome are examined, the picture we usually encounter is haploid sperm morphology. Still, the use of PGD and FISH methods increases the chances of these individuals obtaining a healthy embryo in Klinefelter syndrome. In addition, the literature has reported that natural pregnancy can be achieved in these individuals, and most of the known cases consist of mosaic cases (47,XXY/46,XY) or non-mosaic (47,XXY) cases with younger age. In addition, micro-TESE, one of the assisted reproductive techniques used in these patients, offers more successful results in obtaining sperm than the conventional TESE method.

ii. Mutations in the *CFTR* gene cause congenital bilateral absence/dysfunction as mentioned above and are detected in 1-2% of infertile men. The majority of the mutations of this gene are point mutations, but there are rare cases where deletion and duplication are detected. In the light of this information, necessary studies should be planned by using appropriate molecular tests in individuals with clinical symptoms suggesting a mutation in the *CFTR* gene. In addition, the carrier status of the partner for *CFTR* mutations should be checked. Possible scenarios and options should be explained with the results, and if necessary, the couple should be directed to the relevant centers for PGD.

iii. Autosomal chromosomal abnormalities such as translocations can be detected, especially in cases with oligoasthenoteratozoospermia, and since this may cause unbalanced translocations in the fetus, it is recommended to use PGD in addition to the use of assisted reproductive techniques in these individuals. If there is an imbalance between non-homologous chromosomes in Robertson translocation, one of the translocation types, this can lead to monosomy and trisomies. If this imbalance is between homologous chromosomes, it is not expected that the pregnancy will continue healthy.

iv. The genetic etiology of the patient with infertility problems due to Y microdeletion and monogenic causes should be determined well. According to the mutation detected, joint councils with endocrinology and urology departments should be formed, and the treatment process of the patient should be carried out correctly. For example, it is possible to achieve fertility in a male patient with Kallman's syndrome with appropriate hormone replacement therapy at the right time and using assisted reproductive techniques. However, having a child with assisted reproductive techniques alone is insufficient for the family's treatment. The sons of patients with Y microdeletion may have the same deletion as their fathers and may encounter the same problem, or the child may inherit monogenic mutations and experience infertility or issues affecting other systems in isolation. Therefore, proper genetic counseling should be given to the family before pregnancy, and PGD options should be mentioned.

CONCLUSION

When all these data and research are evaluated, infertility is one of the main issues negatively affecting public health. Although technology is developing extremely fast on the way to genetic diagnosis, there are still many undiagnosed infertility cases. In the approach to the infertility problem, after a detailed and careful clinical evaluation, appropriate genetic tests should be planned by choosing the proper methods, and appropriate genetic counseling should be given to the couples to achieve a healthy pregnancy in line with the test results.

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Nonsurgical Treatment of Male Infertility: Specific Therapy

Erkek İnfertilitesinin Cerrahi Dışı Tedavisi: Spesifik Tedavi

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ABSTRACT

Approximately 15% of married couples undergo a fertility examination. Approximately half of the infertility cases have a male factor. Numerous advances have been made in reproductive medicine in the last few years. Infertile couples who previously were considered untreatable now have a chance at genetic paternity. Although it is possible to solve the problem with assisted reproductive techniques in cases with severe male factor, applying these treatment methods to all infertile partners is extra treatment, the cost increases, and rare but serious risks such as ovarian hyperstimulation may occur in the female partner. Prior to any andrological treatment, a serious diagnostic work-up should be performed and treatment should be individualized, providing adequate treatment options in selected cases. Primarily, specific therapeutic therapy directed against the etiological cause of infertility should be attempted. Specific medical treatment of infertility is based on identifying the causes of reversible infertility and treating it with appropriate drugs. Infertility causes such as hormonal disorders, i.e. congenital hypogonadotropic hypogonadism, hyperprolactinemia, genital tract infections, and sexual dysfunctions can be easily diagnosed and successfully treated with medical methods that do not require surgery. In this review, reversible causes of male infertility and nonsurgical specific medical treatment methods are discussed in the light of the current literature.
Keywords: Male infertility; nonsurgical; specific therapy.

ÖZ

Evli çiftlerin yaklaşık %15'i fertilite incelemesine maruz kalmaktadır. İnfertilite vakalarının yaklaşık olarak yarısında erkek faktörü bulunmaktadır. Son yıllarda üreme tıbbında çok sayıda ilerleme kaydedilmiştir. Daha önce tedavi edilemez olduğu düşünülen infertil çiftlerin artık genetik olarak babalık şansı olmaktadır. Her ne kadar şiddetli erkek faktörü bulunan vakalarda yardımcı üreme teknikleri ile sorunu çözmek mümkün ise de, bütün infertil eşlere bu tedavi yöntemlerini uygulamak fazladan tedavi olur, maliyet yükselir ve kadın partnerde ovaryen hiperstimülasyon gibi nadir ancak ciddi riskler meydana gelebilir. Herhangi bir androlojik tedaviden önce ciddi bir tanısal çalışma yapılmalı ve seçilmiş vakalarda yeterli tedavi opsiyonları sunularak, tedavi kişiselleştirilmelidir. Öncelikle infertilitenin etiyolojik nedenine yönelik spesifik terapötik tedavi denenmelidir. İnfertilitenin spesifik medikal tedavisi, geriye dönüşlü infertilite nedenlerinin ortaya çıkarılması ve uygun ilaçlar ile tedavi edilmesi esasına dayanmaktadır. Konjenital hipogonadotropik hipogonadizm gibi hormonal bozukluklar, hiperprolaktinemi, genital sistem enfeksiyonları ve cinsel fonksiyon bozuklukları gibi infertilite nedenlerinin tanısı kolaylıkla konulabilmekte ve cerrahi gerektirmeyen medikal yöntemlerle başarılı bir şekilde tedavi edilebilmektedir. Bu derlemede, erkek infertilitesinin geriye dönüşlü nedenleri ve cerrahi dışı spesifik medikal tedavi yöntemleri mevcut literatür ışığında tartışılmıştır.
Anahtar kelimeler: Erkek infertilitesi; cerrahi dışı; spesifik tedavi.

INTRODUCTION

Infertility is defined as the inability of a sexually active, non-contraceptive couple to achieve spontaneous pregnancy within one year (1). Primary infertility refers to couples that have never had a child and cannot achieve pregnancy after at least 12 consecutive months of having sex without using birth control methods. Secondary infertility refers to infertile couples who have been able to achieve pregnancy at least once before (with the same or different sexual partner).

About 15% of the couples do not achieve pregnancy within one year and seek medical treatment for infertility. One in eight couples encounters problems when attempting to conceive a first child and one in six when attempting to conceive a subsequent child (2). In 50% of involuntarily childless couples, a male-infertility-associated factor is found, usually together with abnormal semen parameters (1). For this reason, all male patients belonging to infertile couples should undergo a medical evaluation by a urologist trained in male reproduction.

Infertility services are increasingly being utilized due to the later age of first pregnancy and associated reduction in female fertility. Assisted reproductive technology (ART) remains an effective option for infertile couples. However, the cost is considerable, and there is a small but definite risk of ovarian hyperstimulation as well as fetal and maternal consequences associated with multiple gestation and developmental defects. In half of the infertility cases, a male factor is involved. Thus, identifying the pathology and treating the male may allow couples to regain fertility and conceive through natural intercourse. The goal of specific medical management of infertility is to diagnose reversible causes of infertility and treat them with appropriate medications to achieve seminal improvement and pregnancy (3).

Management of male infertility is always a difficult task, and the pathologic process is often poorly understood. Even though modern ART can help overcome severe male factor infertility, the application of these methods in all infertile couples would definitely represent over-treatment. Several conditions can interfere with spermatogenesis and reduce sperm quality and production. A careful diagnostic work-up is necessary before any andrological treatment can be initiated so that adequate treatment options can be selected for individual patients. Most hormonal imbalances can be readily identified and successfully treated nonsurgically. However, the treatment of men with unexplained idiopathic infertility remains a challenge. In the absence of a correctable etiology, patients are managed with either empirical medical therapy or ART. Empiric medical therapy continues as a viable option. However, physicians and patients must understand that the success rates with any of the pharmacological therapies remain suboptimal (4).

In this review, we discussed the specific nonsurgical treatment of male infertility including congenital hypogonadotropic hypogonadism (CHH), hyperprolactinemia, genital tract infection, and male sexual dysfunction in light of current literature.

CONGENITAL HYPOGONADOTROPIC HYPOGONADISM (CHH)

It is possible to classify hypogonadism in two or three ways; i) primary hypogonadism, it is of testicular origin, may be due to congenital or acquired causes; ii) secondary hypogonadism, it originates from the pituitary gland, it may be due to congenital or acquired causes; iii) tertiary hypogonadism, it is hypothalamic, may be due to congenital and acquired causes (5).

In some sources, secondary and tertiary hypogonadism together are defined as only secondary hypogonadism. Since the pathologies leading to hypogonadism are numerous, in this review, isolated hypogonadotropic hypogonadism and male infertility, which only cause secondary and tertiary hypogonadism and are the most common endocrine problems in the clinic, will be discussed. Two important spectrums of diseases that cause isolated hypogonadotropic hypogonadism are Kallmann syndrome and idiopathic hypogonadotropic hypogonadism (IHH). The main problem in these two diseases is gonadotropin releasing hormone (GnRH) deficiency or GnRH receptor resistance, which has been reported in very few cases. Other than that, other hypothalamo-pituitary axis functions are usually normal. While only hypogonadism is seen in IHH, Kallmann syndrome can be seen with anosmia or hyposmia, hearing loss, color blindness, congenital heart diseases, adrenal diseases, nervous system diseases, psychiatric diseases, and many other system pathologies (6).

Hypogonadotropic hypogonadism cases are seen in 0.5-1% of all infertile men, in addition to impaired spermatogenesis at varying rates, androgen deficiency may also have systemic effects such as mild anemia, deterioration in coagulation parameters, decrease in bone density, muscular atrophy, and regression in sexual and cognitive functions. Diagnosis and treatment are extremely important (4).

In planning the treatment of hypogonadism; it is important whether the onset time of hypogonadism is congenital, pre-pubertal, or post-pubertal, and whether there is a desire for fertility after puberty. These must be taken into account in the treatment. Since fertility is not possible in hypergonadotropic hypogonadism, the mainstay of treatment is testosterone replacement therapy (TRT). Unlike hypergonadotropic hypogonadism in IHH; since fertility is possible, the aim is not only to develop secondary sex characteristics but also to provide fertility if there is a desire for children. For this purpose, GnRH or gonadotropin therapy (conventional or unconventional) is applied, taking into account the level of gonadotropin secretion disorder. After fertility is achieved, gonadotropin treatment is discontinued and treatment is continued with TRT (7,8).

Pulsatile GnRH Treatment

It is effective in providing fertility in IHH due to hypothalamic insufficiency. It is done by means of a pump placed under the skin and injecting GnRH rhythmically. Quite successful results can be obtained in selected cases (9). It is not effective in pituitary-induced hypogonadism and GnRH receptor resistance.

Gonadotropin Treatment

It is used to provide fertility in IHH due to both hypothalamic and pituitary insufficiency. There are two types of gonadotropin therapy, conventional and unconventional (4,7).

Conventional Gonadotropin Therapy

It is done by using human chorionic gonadotropin (hCG) and human menopausal gonadotropin (hMG). Both of these hormones are obtained from human urine by

purification. hCG is similar in structure to luteinizing hormone (LH), and just like LH, it binds to Leydig cells and causes testosterone secretion (10). There are studies reporting that hCG, albeit to a lesser extent, binds to tubule cells and has an intrinsically follicle stimulating hormone (FSH)-like effect (9). hMG, on the other hand, has a similar structure to FSH and can stimulate spermatogenesis like FSH. hMG has a slightly LH-like effect, but the dose used to induce spermatogenesis is not sufficient to stimulate Leydig cells. Therefore, hCG should be used to ensure adequate testosterone production (11-13). Conventional gonadotropin therapy is first started with hCG therapy. hCG is administered by intramuscular or subcutaneous injection. Usually, 1000-2500 IU is given 2-3 times a week. The dose is adjusted to keep the testosterone level within the normal range. Spermatogenesis may begin during this time, sometimes due to residual FSH secretion. Treatment continues in this way for at least 8-12 weeks. Then, hMG or recombinant FSH, which can be administered intramuscularly or subcutaneously, is added to the treatment. It is recommended to apply 75-150 IU 2-3 times a week (4,7-9,11). Antibodies to gonadotropins may occur sometimes form during gonadotropin therapy. This may result in treatment failure. In some patients, gynecomastia may develop as a result of increased testosterone level and aromatization into estradiol during hCG treatment (14,15).

Unconventional Gonadotropin Therapy

It is performed with highly purified urinary human follicle stimulating hormone (u-hFSH) and recombinant human follicle stimulating hormone (r-hFSH). 95% of hMG preparations currently in use contain coprified protein. This protein both reduces the FSH-like effect of hMG and is responsible for allergic events. To overcome these drawbacks and increase specific activity, u-hFSH and r-hFSH have been developed. u-hFSH is obtained from human urine by advanced purification techniques. r-hFSH is obtained by administering genes isolated from human fetal liver cells to Chinese mice. Its specific activity is more and allergic events are less frequent. There is no intrinsic LH activity (11,14-16). Antibodies to hCG may occur and allergic events may occur during the use of hCG preparations that are still in use. Therefore, efforts to develop recombinant human LH (r-hLH) have yielded results, but doses for use in men have not yet been reached. Promising results have been reported in treatments with recombinant hCG in recent years (17). Although there are few studies comparing the clinical efficacy of conventional and unconventional gonadotropin therapy, there is no consensus on this issue yet (16-19). New studies are needed.

Testosterone Replacement Therapy (TRT)

The TRT is used in the treatment of hypergonadotropic hypogonadism and in the maintenance treatment of IHH after spermatogenesis is achieved. The aim of testosterone therapy is to keep the serum testosterone level within physiological limits as much as possible and to maintain the secondary sex characteristics and sexual functions.

In men with IHH, hCG therapy has advantages over testosterone therapy. One of them is testicular enlargement, and hCG promotes testicular growth. This is particularly evident in cases in the prepubertal period. With hCG therapy, better libido and less fluctuating testosterone levels are achieved. In addition, hCG therapy stimulates intratesticular testosterone production and initiates spermatogenesis. The disadvantages of hCG therapy are that it is more expensive than testosterone therapy and requires more frequent injections. In some patients, serum estradiol levels may be elevated with hCG therapy. These patients may experience decreased libido and gynecomastia. In some patients, antibodies to hCG may develop and the effect of hCG is reduced (20). In cases with a testicular volume of less than 4 ml, who have not completed pubertal development, both LH and FSH treatments are required to initiate spermatogenesis. After the initiation of spermatogenesis with this combination therapy, the treatment is continued with hCG alone. Spermatogenesis can be achieved with hCG therapy alone in patients with partial gonadotropin deficiency in the prepubertal period with a testicular volume of more than 4 mL. If this treatment is not sufficient and fertility cannot be achieved, hMG can be added to the treatment. A semen analysis can be done every three months. If adequate sperm count and sperm parameters are not achieved in six months, the dose can be doubled (20). Patients with IHH usually have a deficiency in pulsatile GnRH secretion. Even if, these patients respond to gonadotropin treatments, low-dose pulsatile GnRH therapy is a more physiological treatment for these patients. The LH-FSH combination is an effective therapy for restoring spermatogenesis in male patients with gonadotrophin deficiency. Ortac et al. (21), reported in a retrospective study that; investigated the efficacy and safety of gonadotropin therapy for the restoration of spermatogenesis in 112 CHH patients. In addition, the potential effect of baseline factors on treatment outcomes was also explored. The authors reported, approximately 85.7% (96/112) of the patients had detectable sperm in ejaculates during treatment. Dwyer et al. (22) and the European Metrodin HP Study Group (23) reported that FSH/hCG treatment induced spermatogenesis in 84% and 89.3% of patients with hypogonadotropic hypogonadism, respectively. A meta-analysis of 30 studies by Rastrelli et al. (24), reported the overall success rate of gonadotropin therapy on gonadotrophin-deficient male patients was 75% (range, 69-81%). Treatment of hypogonadotropic hypogonadism may take a long time to induce successful spermatogenesis. Dwyer et al. (22), reported that the time to develop spermatogenesis ranges from 3 to 19 months and 9 to 12 months for mono and combined gonadotrophin treatments, respectively. Different time periods for stimulation of sperm production may reflect different population groups and treatment regimens (22). In some studies, the mean time to achieve spermatogenesis was 9.4 months, consistent with other studies reporting the median time of the first appearance of sperm appearance reported as 9-12 months (23-28). Matsumoto et al. (27), found that the median time of first sperm appearance was 12.9 (range, 6.1-17.1) months. However, Liu et al. (25) reported a longer median time of 15 months to obtain sperm in the ejaculate. They speculated that this difference may relate to longer treatment sessions with lower dose hCG treatment and longer follow-up time. Several studies have focused on the predictive factors for successful spermatogenesis in patients with hypogonadotropic hypogonadism (29,30). Ortac et al. (21), reported that larger basal testicular

volume, older age, and a lower incidence rate of undescended testis were positive predictive factors for achieving sperm in the ejaculate. Testicular size increased significantly after gonadotrophin therapy in patients with induced spermatogenesis. The authors concluded that these findings support previous studies in which basal testicular volume and the rate of undescended testis were reported as the main prognostic factors for the restoration of spermatogenesis. The relationship between testicular development and sperm restoration is expected since it is known that normal testicular function is an essential part of a functional hypothalamic-pituitary-gonadal (HPG) axis in healthy adult men (31). It was proposed that mean testicular volumes >4 mL defined an important threshold for the success of sperm restoration (32). Spratt et al. (33), reported that patients with partial/normal nocturnal LH pulses had larger testicular volume (4 mL) compared with those without LH pulses.

We have reported in a currently published study (34); there are several treatment methods for patients with CHH.

1. The patients do not wish to have children: TRT in oral, intramuscular, or transdermal form is commonly used due to its low cost. The oral form is avoided if possible due to the toxic effects on the liver. Transdermal administration has two forms of application, to the scrotum or other body areas. Due to their wide availability in our country, we mostly used the transdermal and intramuscular forms.

2. The patients who wish to have children: These cases were treated using three different protocols.

a. Pulsatile gonadotropin releasing hormone (GnRH) therapy: This treatment is applied with a mini-pump at 5-20 μg/120 min for 12-24 weeks. This is an expensive method with no commercial preparation available in Turkey; therefore, we did not use it in any of our cases.

b. Conventional LH and FSH therapy: We first applied 1500 IU hCG (LH-effective) twice a week for 3-6 months, followed by hCG+hMG or recombinant FSH (150 IU three times a week) for a minimum of 18 months.

c. LH and FSH therapy with an incremental dose: Based on the idea that each patient's hormone requirement is different, some health centers prefer to use this treatment protocol and report favorable outcomes in recent years. In this protocol, serum hormone levels are monitored, and the dose of hCG is increased to 5000 IU and the dose of FSH up to 225-450 IU. In the examination of the patient files, the majority of our patients were seen to have been treated with this method. The patients who did not wish to have children immediately received TRT and those who wanted to have children were treated with hCG+FSH. Initially, only hCG therapy was applied, and when the serum T level was above 400 ng/dL, FSH therapy was added. The initial hCG treatment was planned as 1500 IU twice a week, but this dose was not sufficient in approximately 80% of patients (52 patients); that is, the serum T levels were observed to be below 400 ng/dL; thus, the dose of hCG was increased to 2500 IU twice a week. The time to achieve serum T levels greater than 400 ng/dl was 3 to 6 months. However, during this period, no sperm was observed in the ejaculate of any patient. When the serum T levels were above the threshold, the hCG therapy was continued at the same dose with the addition of FSH. Recombinant FSH preparations were not available at the beginning; thus, urinary FSH was used (15 patients); then, recombinant FSH was added to the treatment (40 patients). The initial FSH dose was 150 IU two to three times a week. In cases where sperm was not detected in the ejaculate within 6 months, the dose of FSH was increased to 450 IU twice a week. At the end of the second year, the mean testicular volume reached 8±4 mL, with the lowest volume being 8 mL and the highest being 14 mL. It was observed that the testicular volume increased more in patients with initial testicular volumes of >4 mL. However, the testicular volume did not reach healthy adult levels in any of the patients. Starting from the sixth month of the treatment, the semen volume of the patients was observed to gradually increase from 0.2 to 2±0.5 mL. At the beginning, all cases were azoospermic, with their semen containing no sperm for the first 6 months. The sperm was detected in the ejaculate only after the 9th month. When all cases were evaluated, five (7.7%) patients did not have any sperm in the semen despite the long-term treatment (>2 years), and in 10 (15.3%) cases, sperm was seen at the 9th month. The ejaculate contained sperm at the 12th month in 35 (53.8%) patients, the 15th month in 42 (64.6%) patients, the 18th month in 52 (80%) patients, and the 24th month in 60 (92.3%) patients. At the end of the second year, the sperm count of the patients with sperm-containing ejaculate ranged from 2×106/mL to 82×106/mL. Interestingly, the rate of progressive motility was >80%.

In the literature, spontaneous recovery has been reported in 10% of IHH cases, which were attributed to the constitutional delay of puberty (35). Although IHH is usually diagnosed easily, its treatment continues to be challenging since it presents as a wide spectrum of diseases, rather than a single form. There is no clear treatment protocol because the doses of medication and the duration of use required for the treatment vary from one patient to another. In addition, the necessity of lifetime treatment may be wearisome for the patient, his spouse, and the treating physician. For this reason, the patient and spouse should be convinced by the physician that the treatment will eventually be successful, but will last a lifetime and may need occasional modifications. Testosterone, GnRH, and gonadotropins are used in the treatment. Although TRT is sufficient for secondary sexual development and general body health, this treatment cannot achieve fertility. The intratesticular testosterone required for spermatogenesis should be at least 50-150 times more than that in the peripheral blood, and it is not possible to reach this amount using TRT. Therefore, treatments that increase intratesticular testosterone, for example, GnRH and hCG/LH, should be undertaken. TRT is easily applicable and cost-effective, and thus it is widely applied in cases where fertility is not of any concern at the time. However, patients wishing to have children should stop taking TRT approximately 2 years before the desired time of conception, and GnRH or hCG/LH+FSH treatment should be initiated (11,18,36,37). Gonadotropins therapy depends on whether the patients who wanted to have children. The success rate generally accepted for fertility is an increase in sperm count to 1.5×106/mL. At this limit, approximately 80% of patients achieve success at the end of the 18th month. Long-term results regarding pregnancy outcomes have not been reported in these series (38,39). In our currently published study, the spouses of 42 (64.6%) of the 65 patients had spontaneous pregnancy, while 8 (12.3%) achieved pregnancy

through intrauterine insemination (IUI), and 10 (15.3%) through in vitro fertilization (IVF) using the sperm ejaculate. The remaining five (7.6%) patients did not have any sperm in the ejaculate; thus, microsurgical testicular sperm extraction (micro-TESE) + intracytoplasmic sperm injection (ICSI) was attempted to achieve pregnancy. However, in only 3 of the 5 patients, sperm could be retrieved, and the remaining 2 patients did not respond to a repeat micro-TESE. Interestingly, there was no statistically significant difference between the total motile sperm count and pregnancy rates. Compared to the literature, our series has the longest follow-up, and highest pregnancy and success rates. This can be explained by our approach that included persuading the patients to undergo long-term treatments, and increasing the dose of medicine without hesitation (34).

HYPERPROLACTINEMIA

Prolactin inhibits the aging of spermatozoa by affecting spermatozoa in an intrinsic way and plays a direct role in the functioning of sperm functions. Prolactinemia is seen at a rate of 1% in andrology practice (40). In a study in which 30 patients with hyperprolactinemia were examined, although the prolactin level decreased with bromocriptine administration, there was no improvement in semen parameters (41). Hyperprolactinemia, defined as an excess of the hormone prolactin, is among the major endocrinopathies related to male infertility (41). The diagnosis is rather straightforward, as hyperprolactinemia may be found with routine serum tests; however, determining the origin of the condition can be difficult. Hyperprolactinemia can arise as a result of hypothyroidism, liver illness, stress, and the use of certain drugs (such as phenothiazines and tricyclic antidepressants), as well as in the presence of functional pituitary adenomas (41,42). The symptoms of excess prolactin may be asymptomatic in some cases or lead to a hypoandrogenic state or galactorrhea, while reduced libido and erectile dysfunction are reported in other cases (43). Hyperprolactinemia can cause male infertility due to its inhibitory effects on the hypothalamus (44). As a result, the hypothalamus is unable to secrete gonadotropins, which in turn affects testosterone production and spermatogenesis. Prolactin levels that are too high are associated with a decreased ability to produce testosterone (44). Because of the numerous impacts on the HPG axis, a patient may present with a variety of symptoms, including diminished sexual desire, erectile dysfunctions, and reduced semen quality (41). Once hyperprolactinemia has been diagnosed, the practitioner should order a magnetic resonance imaging (MRI) scan of the pituitary gland to rule out any other potential causes. If a prolactinoma is discovered, it can be classified according to its dimension and form. The most important distinction is between microadenomas, which are lesions smaller than 10 mm in diameter, and macroadenomas, which are lesions larger than 10 mm in diameter.

The medical treatment for prolactinoma is focused on inhibiting the release of prolactin with the use of dopamine agonists if the tumor is found to be present, which include, pergolide, cabergoline, bromocriptine, and quinagolide, with cabergoline and bromocriptine being the most well-characterized agents (45). Bromocriptine is an ergo derivative. The plasma half-life is 3.3 hours. Due to its short duration of action, it should be used 2-3 times a day. The initial dose is 0.625-1.25 mg daily. The dose is gradually increased to 5-7.5 mg/day once a week. Short-acting forms to slow-release forms are preferred. Side effects of bromocriptine are nausea, vomiting, dizziness, headache, nasal congestion, dry mouth, constipation, dizziness due to orthostatic hypotension, and syncope.

Cabergoline is an ergo derivative. Its half-life is 65 hours. Therefore, taking it 1-2 times a week is usually sufficient. Cabergoline is the first choice because it has more tumor-reducing effect and less side effects. Usually, 0.5-1 mg/week dose is sufficient, while increasing the maximum dose (7 mg/week) in rare cases may be required.

Quinagolide is not an ergo derivative. It binds to the dopamine receptor. It starts with 25 μg per day for the next three days and continues with 50 μg per day for the next three days. Recommended from the seventh day the dose is 75 μg per day. The maintenance dose is usually 75-150 μg per day.

These agonists take advantage of dopamine's inherent suppression of prolactin release to achieve their effects. In certain cases, this can really result in the tumor shrinking, albeit the process usually takes months to complete. Nausea, vomiting, and postural hypotension are among the side effects that can occur after using dopamine agonists. However, despite the fact that inhibition of excess secretion of prolactin prevents disruption of the HPG axis, few studies have examined the effects of dopamine agonists on reproductive functions. Bromocriptine was used in a 1974 trial to treat men with functional prolactinomas and hypogonadism, and the results revealed no increase in sperm motility (46). De Rosa et al. (47) treated 43 patients with hyperprolactinemia and impaired semen parameters by administering cabergoline for 24 months and compared the results with 60 healthy men at the end of the treatment. Significant improvements in semen quality were achieved in patients with normal prolactin levels. The seminal fluid characteristic values were found close to the control group. However, viable spermatozoa count, sperm membrane function, sperm kinetic index, and sperm nuclear DNA integrity remained abnormal in 9.3-53% of patients. On the other hand, a study compared cabergoline and bromocriptine in the same cohort of patients, and both treatment regimens showed significant improvements in sperm quantity, motility, rapid advancement, and morphology in a period of six months (47). In a later study conducted at the same institution, seminal fluid parameters were compared between men who had prolactinomas and men who did not. According to a study conducted on healthy control males, after 2 years of treatment with cabergoline (starting dose 0.5 mg weekly, gradually titrated to prolactin levels), the majority of men had regained testicular functions in comparison to the healthy control. When bromocriptine and cabergoline are compared, it is observed that cabergoline has higher effectiveness at normalizing levels of prolactin and regressing tumor load (48). Furthermore, when comparing cabergoline to bromocriptine, a higher percentage of individuals demonstrate a clinical response to cabergoline. Finally, compared to bromocriptine, cabergoline has a considerably better rate permanent remission rate and fewer side effects (49). All things

considered, cabergoline is frequently the initial treatment option for males with prolactinomas after other options are exhausted. In many situations, treatment of prolactinomas with dopamine agonists is beneficial; nonetheless, a considerable proportion of men may still remain persistently hypogonadotropic despite receiving treatment. The use of clomiphene citrate, according to a study, may be a successful therapy option for these men. Hypogonadal men treated with clomiphene (50 mg per day for 3 months) had increased levels of testosterone as well as improved sperm motility (49). Prolactinomas can be treated with ablative therapies such as radiation therapy or transsphenoidal excision, which are both effective. Ablative therapy is usually reserved for patients who have failed to respond to medical treatment. Ablative treatments work by removing the prolactin source and, as a result, the suppression of GnRH secretion that is occurring. It is still vital to monitor the patient's gonadotropin levels after treatment since additional intervention with exogenous gonadotropins may be required to maximize therapeutic benefit (50).

GENITAL TRACT INFECTIONS

Infection of the male urogenital tract is a potentially curable cause of male infertility (51). The World Health Organization (WHO) considers urethritis, prostatitis, orchitis, and epididymitis to be male accessory gland infections (MAGIs) (52). A systematic review of the relationship between sexually transmitted infections, such as those caused by *Chlamydia trachomatis*, genital mycoplasmas, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, and viruses, and infertility was unable to draw a strong association between sexually transmitted infections and male infertility due to the limited quality of reported data (53).

The clinical significance of an increased concentration of leukocytes in the ejaculate is controversial (54). Although leukocytospermia is a sign of inflammation, it is not necessarily associated with bacterial or viral infections, and therefore cannot be considered a reliable indicator (55). The deleterious effects of chronic prostatitis (CP/CPPS) on sperm density, motility, and morphology have been demonstrated in a recent systematic review based on case-controlled studies (56). Both *C. trachomatis* and *Ureaplasma* spp. can cause decreased sperm density, motility, altered morphology, and increased DNA damage. Treatment of CP/CPPS is usually targeted at relieving symptoms. The indications and aims of therapy are the reduction or eradication of micro-organisms in prostatic secretions and semen, normalization of inflammatory (e.g., leukocytes) and secretory parameters, and improvement of sperm parameters associated with fertility impairment. Only antibiotic therapy of chronic bacterial prostatitis (NIH II according to the classification) has provided symptomatic relief, eradication of micro-organisms, and a decrease in cellular and humoral inflammatory parameters in urogenital secretions. Although antibiotics might improve sperm quality, there is no evidence that treatment of CP/CPPS increases the probability of natural conception (51-56).

Asymptomatic presence of *C. trachomatis* and *M. hominis* in the semen can be correlated with impaired sperm quality, which recovers after antibiotic treatment. However further research is required to confirm these findings (57). The aim of treatment of epididymitis is as follows; microbiological cure of infection, improvement of clinical signs and symptoms, prevention of potential testicular damage, prevention of transmission, and decrease of potential complications such as infertility.

Considering that male genital tract infections are in their majority caused by bacterial pathogens, such infections can be treated with antibiotics and anti-inflammatories and are therefore potentially correctable causes of male infertility. Yet, it is to be noticed that many of these pathogens are sexually transmitted. Therefore, both partners have to be treated after proper diagnosis and administration of a suitable antibiotic after semen culture. Whereas guidelines for the treatment of acute bacterial epididymitis, epididymo-orchitis, and specific granulomatous orchitis have been published, this is not the case for chronic infections and inflammations. Hence, treatment of these diseases rather relies on empirical and a small number of uncontrolled studies.

On the other hand, for viral infections such as mumps orchitis, a systemic treatment with 2β-interferon was used to prevent testicular atrophy (58,59). Lesions due to the inflammatory processes can be alleviated with an antiphlogistic therapy, with both corticosteroids and non-steroids, and have shown significant positive effects on various semen parameters. In addition, antioxidant therapies to counteract oxidative stress may be considered, but are still debated (60). A new study reported that MAGIs are not clearly associated with impaired natural conception, antibiotic treatment often only eradicates micro-organisms; it has no positive effect on inflammatory alterations and cannot reverse functional deficits and anatomical abnormalities. Although antibiotic treatment for MAGIs may result in an improvement in sperm quality, it does not enhance the probability of conception (61).

Treating MAGIs may improve sperm quality, although it does not necessarily improve the probability of increasing conception. Weak data are insufficient to conclude whether antibiotics and antioxidants for the treatment of infertile men with leukocytospermia improve fertility outcomes. Weak refer sexual partners of patients with accessory sex gland infections that are known or suspected to be caused by sexually transmitted diseases for evaluation and treatment.

MALE SEXUAL DYSFUNCTION

Sexual dysfunction is more common in infertile men than in the normal population. Sexual dysfunctions may be the result of infertility as well as the cause of infertility. Therefore, every infertile man should be evaluated for sexual dysfunction. While erectile dysfunction, decreased libido, and premature ejaculation are mostly the result of infertility, retrograde ejaculation and unejaculation are more common causes of infertility. Erectile dysfunction can be easily treated with PDE5 inhibitors. If there is no response to PDE5 inhibitors and there is a decrease in libido, serum total testosterone level should be checked. These patients should not be given exogenous testosterone to preserve fertility but should be given selective estrogen receptor modulators, aromatase inhibitors, or hCG, which increase endogenous testosterone production. If the patients had anejaculation and used antipsychotics or

alpha-blockers, these drugs should be temporarily discontinued until pregnancy occurs. Retrograde ejaculation is the most important sexual dysfunction that causes male infertility. If retrograde ejaculation is due to anatomical reasons such as prostatectomy or bladder neck resection, medical treatment cannot be achieved and ART should be used in these patients. If retrograde ejaculation is not anatomical but neurogenic, medical treatment should be attempted (62).

Retrograde ejaculation occurs due to many reasons such as diabetes mellitus, spinal cord pathologies, intra-abdominal surgical interventions, and surgical interventions for the urinary system, and may cause infertility (63-65).

The traditional medical treatment of patients who are infertile due to retrograde ejaculation is the use of sympathomimetic agents. Pseudoephedrine is widely used in the treatment of these patients and the rate of obtaining sperm in the ejaculate reaches up to 58% (66).

Some studies reported successful pregnancies in patients with retrograde ejaculation by using midodrine, the alpha agonist methoxamine, or the antihypotensive amazinium and theophylline (66-69).

In their recent study, Hu et al. (70) investigated the effect of amoxapine, a norepinephrine reuptake inhibitor, in patients with retrograde ejaculation. They administered vitamin B12 to 13 patients and amoxapine to 13 patients, and found the rate of sperm retrieval in the groups to be 16% and 80%, respectively (70).

In another study, patients with retrograde ejaculation due to diabetes mellitus were divided into three groups, and the groups received 25 mg of imipramine twice a day, 120 mg of pseudoephedrine twice a day, and the combined form of these two agents twice a day. The sperm retrieval rate in the groups was 38.5%, 47.8%, and 61.5%, respectively (71).

CONCLUSION

Numerous advances have been made in reproductive medicine in the last few years. Infertile couples who previously were considered untreatable now have a chance at genetic paternity. ART provides a great opportunity to families with infertility. The increasing use of ICSI as an efficient therapy for cases of male infertility has become an applicable means to overcome multiple sperm deficiencies. Even men with potentially treatable causes of infertility can be treated with ART instead of a specific therapy. However, the potential medical risks such as those of multiple-gestation pregnancies and the associated costs cannot be ignored. Primarily, specific therapeutic therapy directed against the etiological cause of infertility should be attempted. Specific medical management of infertility is based on identifying reversible causes of infertility and treating them with appropriate medications. However, if no specific etiology can be identified, empiric therapy can be introduced in an attempt to improve semen parameters and subsequent fertility potential through natural intercourse. It is mandatory that a treatment timeline and endpoints be established prior to the initiation of empiric therapy. Many of the empiric therapies do hold a potential benefit, and as greater understanding is gained of what is now considered idiopathic infertility, a more specific application of these therapies may yield more successful results.

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Nonsurgical Treatment of Male Infertility: Non-Specific Therapy

Erkek İnfertilitesinin Cerrahi Dışı Tedavisi: Spesifik Olmayan Tedavi

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ABSTRACT

In male infertility, all possible known causes should be carefully evaluated and if detected, targeted treatment options for the cause should be implemented. The known causes of male infertility such as hypogonadotropic hypogonadism, hyperprolactinemia, genital tract infections, disorders of ejaculation, thyroid hormone disorders and varicocele can be treated efficiently by targeted therapies or surgical corrections. Unfortunately, these known causes cover about 20% of male infertility and the rest remains idiopathic. On the other hand, management of idiopathic, unexplained male infertility, in which no etiological factors can be found, is a challenge for both the clinician and couples seeking solutions. In the era of assisted reproductive technology, few medical options in this regard are still available with limited benefits and low scientific foundation based on theoretical concepts but empirical medical therapy continues as a mostly off-label option for obtaining a natural pregnancy. Comprehending the hypothalamic-pituitary-gonadal axis and the regulation of hormones is crucial in this regard. Empirical therapies have the potential to overcome overtreatment with assisted reproductive technology yet clinicians and couples must be aware of the limitations of empirical therapies and should be counseled in this direction. In this review, non-specific medical treatment options for idiopathic male infertility were covered.
Keywords: Male infertility; medical treatment; empiric therapy.

ÖZ

Erkek infertilitesinde bilinen tüm olası nedenler dikkatlice değerlendirilmeli ve tespit edilirse o neden için hedefe yönelik tedavi seçenekleri uygulanmalıdır. Erkek infertilitesinin hipogonadotropik hipogonadizm, hiperprolaktinemi, genital sistem enfeksiyonları, boşalma bozuklukları, tiroid hormon bozuklukları ve varikosel gibi bilinen nedenleri, hedefe yönelik tedaviler veya cerrahi düzeltmeler ile etkili bir şekilde tedavi edilebilir. Ne yazık ki, bu bilinen nedenler erkek infertilitesinin yaklaşık %20'sini kapsamaktadır ve geri kalanı idiyopatik olarak kalmaktadır. Öte yandan, etiyolojik faktörlerin bulunamadığı, idiyopatik, açıklanamayan erkek infertilitesinin yönetimi hem klinisyen hem de çözüm arayan çiftler için zorlu bir süreçtir. Yardımcı üreme teknolojisi çağında, teorik kavramlara dayanan sınırlı yararları ve düşük bilimsel temeli olan bu konuda hala birkaç tıbbi seçenek mevcuttur, ancak spesifik olmayan tıbbi tedavi, doğal bir hamilelik elde etmek için çoğunlukla endikasyon dışı bir seçenek olarak devam etmektedir. Hipotalamus-hipofiz-gonad aksını kavramak ve hormonların düzenlenmesi bu konuda çok önemlidir. Ampirik tedaviler, yardımcı üreme teknikleriyle yapılacak olan aşırı tedavisinin üstesinden gelme potansiyeline sahiptir, ancak klinisyenler ve çiftler, ampirik terapilerin sınırlamalarının farkında olmalı ve bu yönde danışmanlık almalıdır. Bu derlemede, idiyopatik erkek infertilitesi için spesifik olmayan tıbbi tedavi seçenekleri ele alınmıştır.
Anahtar kelimeler: Erkek infertilitesi; medikal tedavi; ampirik tedavi.

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INTRODUCTION

Infertility is defined as the failure of obtaining pregnancy after 12 months or more of regular unprotected sexual intercourse. Of all couples, about 15% are infertile. Male factor accounts for 50% of all infertility. The known causes of male infertility such as hypogonadotropic hypogonadism, hyperprolactinemia, genital tract infections, disorders of ejaculation, thyroid hormone disorders and varicocele can be treated efficiently by targeted therapies or surgical corrections. Unfortunately, these known causes cover about 20% of male infertility and the rest remains idiopathic (1).

In idiopathic male infertility, empirical therapies have been used for many years which are based on hypotheses and theories. Clinicians have to consider the treatment duration as at least 3-6 months based on the coverage of a full 74-day spermatogenetic cycle. Couples should be aware that these treatments have a low to moderate success chance when compared with assisted reproductive technology (ART). Clinicians should not insist on giving empirical therapy options to couples that lack improvement in seminal parameters and fail to have pregnancy after at least two spermatogenetic cycles. In this review, current empirical, non-specific therapy options for the nonsurgical treatment of male infertility have been discussed and summarized.

Lifestyle Changes

Environmental and lifestyle factors have the potential to contribute to idiopathic infertility (2). Therefore, simple changes in these factors can affect the sperm parameters in a positive manner. Firstly, few studies suggest that weight loss has a positive impact on sperm parameters (3,4). However, a meta-analysis of 28 cohorts and 1022 patients suggested that after bariatric surgery, sperm quality and function do not change in morbidly obese men (5). Weight loss has a clear advantage of the elimination of excess fat in the body which causes secondary hypogonadism. Similarly, physical activity has positive effects on the hormonal profiles of men, and also a recent meta-analysis has documented that moderate and high-intensity training can result in better seminal parameters (6). Cessation of smoking and heavy chronic alcohol consumption has proven positive effects on seminal parameters as well (7,8).

Gonadotropin Releasing Hormone Treatment

As known, the hypothalamus plays a crucial role by secreting pulsatile gonadotropin releasing hormone (GnRH) which induces the secretion of gonadotropins (follicle stimulating hormone, FSH, and luteinizing hormone, LH) from the anterior pituitary gland. These hormones are responsible for providing intratesticular testosterone levels and the normal process of spermatogenesis. This axis is a well-regulated system with multiple feedback mechanisms. Although exogenous GnRH administration is mostly used for a known etiology, hypogonadotropic hypogonadism, it may also be hypothesized to be used for empirical therapy with its beneficial effects on spermatogenesis. The underlying pathophysiology of the empirical use of GnRH is due to a subclinical endocrinopathy (9).

Despite being very effective in hypogonadotropic hypogonadism, administrating exogenous GnRH in idiopathic infertility has major drawbacks. Firstly, in the current literature, there is no evidence of the efficacy of this therapy when used empirically. Up-to-date, there are two randomized controlled studies which both fail to show a benefit of pulsatile GnRH in idiopathic male infertility (10,11). The seminal parameters were comparable and the pregnancy rates did not differ in both studies. In addition to the lack of scientific evidence, the high economic cost of this treatment, limited availability, and the difficulty of giving it in a pulsatile fashion with pump systems deter its clinical use. The use of pulsatile GnRH in idiopathic infertility is not recommended in the current European Association of Urology (EAU) guidelines (12).

Gonadotropins

Two major gonadotropins, FSH and LH are secreted from the anterior pituitary gland for the induction of spermatogenesis and steroidogenesis. Also, human chorionic gonadotropin (hCG), as an analog of LH, activates Leydig cells for the production of sex steroids, and human menopausal gonadotropin (hMG), having both LH and FSH activity, can be used for gonadotropin replacement. Usage of gonadotropins has failed to show a satisfying benefit and efficacy in numerous studies in terms of seminal parameters and pregnancy rates (13-15). In a review published in 2006, it is suggested that the seminal parameters were better with gonadotropin usage but not significantly different in pregnancy rates (16). On the other hand, a Cochrane database analysis of six randomized placebo-controlled studies showed better pregnancy rates in the treatment group (17). A more recent review by Jung and Seo (18) in 2014 concluded that seminal structure and pregnancy rates are better with gonadotropin therapy but the literature still lacks satisfactory evidence of empirical use of gonadotropins and combination with ART. Since in idiopathic infertility, most men have a testicular failure, resulting in azoospermia or severe oligoasthenoteratozoospermia. The feedback mechanism in testicular failure results in mostly elevated FSH and LH levels and the rationale to give more gonadotropins to these men is unclear (19). It may be stated that larger sample-sized placebo-controlled randomized studies are needed in this regard. It must be noted that the use of exogenous testosterone replacement to treat male infertility is contraindicated as it inhibits spermatogenesis (12).

Aromatase Inhibitors

Anastrozole, letrozole, exemestane, and testolactone are labeled aromatase inhibitors that inhibit the conversion of testosterone to estrogen. Also, these agents block the negative feedback of testosterone on the hypothalamic pituitary axis by lowering the converted estrogen which is a more potent inhibitory signal. Conversion of testosterone to estrogen takes place mostly in fat cells and therefore, the underlying mechanism of idiopathic infertility in obese men with high estrogen levels may be due to aromatase activity. These oral drugs are relatively safe and well-tolerated in men.

Even though its tolerability and convenience, indecisive results about aromatase inhibitors are attained when the literature is scanned. A double-blinded, placebo-controlled, cross-over study by Clark and Sherins (20) did not show an improvement in seminal parameters and pregnancy rates after 8 months of treatment with testolactone. Another study evaluated 27 oligospermic patients and concluded that letrozole 2.5 mg per day for over 6 months

increased the sperm counts and hormonal profile but failed to achieve normal levels (21). Similar results were achieved in an older study by Raman and Schlegel (22) in 2002 with anastrozole in infertile men with low serum testosterone/estrogen ratio. Overview of the studies conducted with aromatase inhibitors lead to the conclusion that these agents have potential in men with abnormal hormonal levels but the optimal testosterone/estrogen ratio for normal spermatogenesis remains vague and studies are still needed for this purpose. The latest systematic review published in 2020 about aromatase inhibitors on idiopathic male infertility documented 8 trials and reported that all agents have significantly improved the hormonal and seminal outcomes (23). While the evidence is promising, the lack of prospective studies makes the empirical use of aromatase inhibitors for idiopathic infertility off-label and debatable, especially in normogonadal men.

Antiestrogens / Selective Estrogen Receptor Modulators

These agents block the inhibitory feedback pathway of estrogen to the hypothalamus and anterior pituitary gland, resulting in an increase in GnRH and stimulating FSH and LH (24). For idiopathic infertility, antiestrogens are the most commonly used treatment for many years. Clomiphene citrate, tamoxifen citrate, toremifene, and raloxifene with various dosages have been used in this regard. The key point of this treatment is to monitor the serum gonadotropins and testosterone levels as higher levels may influence spermatogenesis negatively. The side effects cover weight gain, gynecomastia, libido changes, dizziness, headache, and nausea which are seen below 5% of patients and considered acceptable.

When the literature is scanned for efficacy, controversial results are seen. A randomized, double-blind study conducted by the World Health Organization evaluated the efficacy of clomiphene 25 mg for 6 months in 1308 men with idiopathic infertility. Semen quality and pregnancy rates did not differ from control after treatment in this study (25). A review by Cocuzza and Agarwal (26) in 2007 indicates that there are only two studies that reveal better semen quality and pregnancy rates with clomiphene citrate, with many studies suggesting the opposite. The same argument is present with tamoxifen citrate. As many trials suggest different results in this subject, meta-analyses have been conducted to clear the doubtful conclusions. In the meta-analysis by Kamischke and Nieschlag (27), antiestrogens showed no significant influence on pregnancy rates. Contrarily, a meta-analysis of 11 studies indicated that antiestrogens improve the pregnancy rate by 2.4 fold in total. In subgroup analysis, clomiphene citrate 50 mg improved the pregnancy rate by 5 fold and tamoxifen 20-30 mg by 2.8 fold. Clomiphene citrate 25 mg did not improve the pregnancy rate (28). Similar results were confirmed by a more recent meta-analysis of 16 studies (24). Antiestrogen use for normogonadal men with idiopathic infertility is still off-label and is still debated.

Miscellaneous Treatments

Besides the treatment modalities which focus on hormonal parameters, nutritional supplements, vitamins, anti-inflammatory, and antioxidant agents need to be referred. Mostly, these treatments target the improvement of sperm quality by a variety of mechanisms such as reactive oxygen species (ROS), production of prostaglandins, and enhancing the kallikrein-kinin system. The main goal of supplementary treatments is to ensure the integrity of sperm DNA and intracellular energy and membrane metabolism.

The enzymatic antioxidant mechanism in semen consists of superoxide dismutase, catalase, and glutathione peroxidase. On the other hand, glutathione, pantothenic acid, coenzyme Q10, l-carnitine, vitamin A, E, C, and minerals such as zinc, selenium, and copper are some of the non-enzymatic mechanisms of antioxidation. The imbalance between ROS and antioxidant agents leads to a low fertile potential (29).

The clinical studies that evaluate the effect of antioxidant supplements on sperm quality and function are mostly not randomized controlled studies which have a low scientific value. In a systematic review of 17 randomized studies that included 1665 infertile men evaluating the antioxidant agents' power, 14 studies showed an improvement in either semen quality or pregnancy rates after treatment. In terms of seminal parameters, motility was improved by 63%, sperm count was improved by 33% and morphology improved by 17% (29). Although these encouraging results, this review had its own limitations, studies were not controlled and their methodic designs were different. A very recent randomized controlled trial by Steiner et al. (30) in 2020 concluded that antioxidants do not improve semen parameters or DNA integrity and pregnancy and live birth rates. In a Cochrane database meta-analysis including 61 studies with 6264 subfertile men, although antioxidant treatment positively affected seminal parameters, pregnancy, and live birth rates, there is a high risk of bias in the studies, and the level of evidence was concluded as "very low" (31). Up-do-date, there are no specific recommendations on the use of antioxidant agents in the treatment of idiopathic male infertility and these agents remain completely empirical in terms of both dosage and duration of treatment.

CONCLUSION

For all possible known causes of male infertility, the goal is to target the treatment in order to get satisfactory outcomes. ART has become a major opportunity for couples with infertility and its use has become a routine practice. In cases of idiopathic male infertility, empirical treatment options can be discussed with couples in order to enhance the fertility potential with "natural" intercourse. Clinicians should be aware of the time and should set a deadline for this therapy. Over years, a better understanding of the "idiopathic" male infertility will lead to more specific directions of empirical therapies, and therefore better results.

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Surgical Treatment of Male Infertility

Erkek İnfertilitesinin Cerrahi Tedavisi

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ABSTRACT

Male infertility may occur due to obstructive and non-obstructive reasons, and some pathologies may be corrected with surgical and medical treatment. Such treatment may increase the possibility of spontaneous pregnancy, the success of assisted reproductive technology, and also testicular sperm retrieval rate. This review will focus on surgical treatment alternatives in infertile males. Although treatment options for varicocele in infertile men include open surgical, radiologic, and laparoscopic approaches; microsurgical varicocele repair has the highest improvement in postoperative sperm parameters with lower complication rates. Recent advances in microsurgical anastomosis techniques have increased the patency rate for proximal epididymal obstruction. Although treatment options for distal ejaculatory duct obstruction include endoscopic resection, balloon dilatation, and laser incision/excision, transurethral resection of the ejaculatory duct (TURED), is still the primary gold standard treatment of distal ejaculatory duct obstruction. The testicular sperm retrieval rate has increased with the management of correctable pathologies in men with non-obstructive azoospermia. In case of treatment failure of correctable or uncorrectable pathologies of male factor infertility, surgical sperm obtained from the urogenital tract may necessary for assisted reproductive technology. Surgical success rates for male infertility and the success of surgical sperm obtained procedures have increased dramatically over the last decades attributable to the development of microsurgical techniques and endoscopic equipment, instrumentation, and techniques.
Keywords: Male infertility; varicocele; obstructive azoospermia; surgical treatment.

ÖZ

Erkek infertilitesi obstrüktif ve obstrüktif olmayan sebeplerle oluşabilir ve bunların bazıları cerrahi ve medikal tedavi ile düzeltilebilir. Bu tedavi yöntemleri çiftlerde spontan gebelik şansını artırabildiği gibi bu tedaviler ile üremeye yardımcı tedavi yöntemleri başarısı ve testiküler sperm bulma şansı artabilir. Bu derlemede infertil erkeklerde cerrahi tedavi yöntemleri irdelenecektir. Varikoselli infertil erkeklerde tedavi opsiyonları açık ve laparoskopik cerrahi ve radyolojik tedavi olmakla birlikte, mikrocerrahi varikoselektomi postoperatif sperm parametrelerinde en yüksek artış oranı ve en düşük komplikasyon oranlarına sahiptir. Proksimal epididimal obstrüksiyonların cerrahi tedavisinde mikrocerrahi anastomoz yöntemlerindeki ilerlemeler kanalın açılma başarısını artırmaktadır. Distal ejakülatör kanal tıkanıklığının cerrahi tedavisinde endoskopik rezeksiyon, balon dilatasyon, lazerle insizyon/eksizyon yöntemleri bulunmakla beraber, transüretral ejakülatör kanal rezeksiyonu (transurethral resection of the ejaculatory duct, TURED) hala altın standart tedavi yöntemidir. Nonobstrüktif azospermide düzeltilebilir patolojilerin giderilmesiyle testiküler sperm elde etme oranlarında artış gösterilmiştir. Düzeltilebilir veya düzeltilemez patolojilere bağlı erkek infertilitesinde tedavi başarısızlığı durumunda yardımcı üreme teknolojisi için ürogenital sistemden cerrahi olarak sperm elde etmek gerekli olabilir. Erkek infertilitesi cerrahi tedavilerinin başarı oranları ve cerrahi olarak sperm elde etme başarı oranları, mikro cerrahi tekniklerinin, endoskopik ekipman, enstrümantasyon ve tekniklerin gelişmesine bağlı olarak son dekatlarda çarpıcı bir şekilde artmıştır.
Anahtar kelimeler: Erkek infertilitesi; varikosel; obstrüktif azospermi; cerrahi tedavi.

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INTRODUCTION

Male infertility may occur due to obstructive (ejaculatory duct, vas deferens, and epididymal obstruction) and non-obstructive (varicocele, secondary hypogonadism, gonadotoxins exposure) reasons as correctable pathologies, and may also occur due to genetic disorders and testicular atrophy after mumps orchitis and undescended testis as uncorrectable pathologies (1-11). The aim of the evaluation of men for infertility is to point out to diagnose correctable pathologies, to detect genetic diseases, and also to diagnose life-threatening diseases (2,3,12-14). In addition, to increase the probability of spontaneous pregnancy, medical and surgical treatment may increase assisted reproductive technology (ART) success, and also testicular sperm retrieval rate (15-20). This review will focus on surgical treatment alternatives in infertile males.

Surgical Treatment of Varicocele

Varicocele is an excessive dilation of the pampiniform plexus. It is also the most commonly seen and correctable cause of male factor infertility (1,21). Varicocele is among the cost-effective treatments for infertility. The pampiniform plexus consists of the internal spermatic veins, external spermatic vein, deferential vein, and gubernacular vein. The left internal spermatic vein drains into the left renal vein at a straight angle, whereas the right internal spermatic vein drains directly into the inferior vena cava at an oblique angle. Therefore, left-sided varicocele is more common than right-sided. However, there are some variations, such as the number of gonadal veins, localization of drainage, and termination angle in some cases that could explain a higher incidence of bilateral varicoceles and also causes varicocele recurrences after the surgery or intervention (22). These variations were found more frequently on the left side (30%), as compared to the right side (10%). In some cases (10%), the variations were present bilaterally. Venous reflux is likely to be induced via collateral pathways, whereas in adolescents congenital venous abnormalities (renospermatic bypass, Nutcracker phenomenon, and valve abnormalities) are predominantly present (23). In addition, obesity is another risk factor for varicocele recurrence. Increased body mass index in men with varicocele is associated with larger spermatic vein diameters when supine (24).

Physical examination is the reference standard to diagnose varicoceles in subfertile men. Additional radiologic imaging is not necessary to diagnose subclinical varicocele, because only a varicocele detected by physical examination should be considered potentially significant (6).

When clinical palpable varicocele coexists with impaired semen quality, surgical repair may potentially restore spermatogenesis and fertility. Recent meta-analyses suggested that varicocele repair has a beneficial effect on fertility status in infertile men with palpable varicocele. Ficarra et al. (25) reviewed randomized clinical trials for varicocele repair and found a significant increase in pregnancy rate in patients who underwent varicocele treatment (36.4%) compared with patients having no treatment (20%). Marmar et al. (26) reported a 33% pregnancy rate in patients who underwent surgical varicocelectomy and a 15.5% pregnancy in the controls receiving no varicocelectomy.

Indications for the treatment of varicocele are the presence of clinical palpable varicocele with infertility history and abnormal semen parameters, and pain, if medical conservative treatment such as analgesics/anti-inflammatory drugs fails (25).

The aim of the treatment of infertile men with varicocele is to improve semen parameters and also to achieve pregnancy with or without the use of ART (2,4,20,27,28). Treatment options for varicocele in infertile men include open surgical, radiologic, and laparoscopic approaches (29,30). However, anatomic variations of testicular veins affect outcomes of surgical and radiologic treatment of varicocele. In a venographic study, the most anatomic reason for surgical failure was gonadal vein duplication (66% of the cases) (31). In this series, most cases had laparoscopic or open surgery at the suprainguinal level. Therefore, other veins at the lower level could not be identified.

The best treatment modality for varicocele in infertile men should include higher seminal improvement and spontaneous pregnancy rates with lower complication rates such as recurrence or persistence, hydrocele formation, and testicular atrophy. Even if we do our best to treat varicocele, only 35-50% of the patients will have a positive response to varicocele treatment, and 50% will not respond to varicocele treatment due to recurrence, genetic abnormality, or technical failure. Therefore, the best method should have the lowest complication rates, and the ideal technique should aim for ligation of all internal and external spermatic veins with preservation of spermatic arteries and lymphatics (19,29,30).

Radiologic treatment of varicocele seems to have some advantages including a shorter recovery period, no anesthesia, and lower cost, however, has some disadvantages such as operation failure, higher recurrence rate, thrombosis, and contrast agent allergy. Patients with bilateral grade 3 varicocele should not be considered for embolization because of significantly higher technical failure rates for right-sided varicocele. Patients who present for treatment of varicocele due to infertility should be recommended for surgery rather than embolization, due to evidence-based data that suggests pregnancy rates are improved following surgery but not with embolization (32).

Laparoscopic varicocelectomy can be performed either transperitoneally or extraperitoneally and seems to have a postoperative recovery period. However, it is not possible to ligate external spermatic veins and other veins at the lower level which might cause recurrence.

High ligation has a less arterial injury at the proximal level, however, it is unable to ligate external spermatic, gubernacular veins, and other internal spermatic vein branches originating from the duplicated gonadal vein at the lower level.

Microsurgical varicocele repair can be performed via an inguinal or subinguinal approach. Although the subinguinal approach to microsurgical varicocelectomy obviates the need to open the aponeurosis of the external oblique, it is associated with a greater number of internal spermatic veins and arteries compared with the inguinal

approach. Subinguinal microscopic varicocelectomy has disadvantages, needing more skills because of the higher number of internal spermatic vein channels, and a higher risk for arterial injury due to smaller artery in diameter at the level of the external inguinal ring (33). In a study, conducted with 102 consecutive men who underwent subinguinal microsurgical varicocelectomy, a mean number of 12.9 internal spermatic veins, 0.9 external spermatic veins, 1.8 internal spermatic arteries, and 2.9 lymphatics were identified per cord. In addition, 88.2% of the internal spermatic arteries were surrounded by a dense complex of adherent veins, and the incidence of dilated external spermatic veins was 49.4% (34).

Open microsurgical inguinal or subinguinal varicocelectomy techniques have been shown to result in higher spontaneous pregnancy rates and fewer recurrences and postoperative complications than conventional varicocelectomy techniques in infertile men. The use of higher magnification allows surgeons to preserve the internal spermatic artery and lymphatics and also to visualize and ligate all spermatic veins (33).

We published a review/meta-analysis to compare all techniques (29). Overall spontaneous pregnancy rates were 37.69% in the Palomo technique series, 41.97% in the microsurgical varicocelectomy techniques, 30.07% in the laparoscopic varicocelectomy techniques, 33.2% in the radiologic embolization, and 36% in the macroscopic inguinal (Ivanissevich) varicocelectomy series, revealing significant difference among the techniques. Overall recurrence rates were 14.97% in the Palomo technique series, 1.05% in the microsurgical varicocelectomy techniques, 4.3% in the laparoscopic varicocelectomy techniques, 12.7% in the radiologic embolization, and 2.63% in the macroscopic inguinal (Ivanissevich) or subinguinal varicocelectomy series, revealing significant difference among the techniques. Overall hydrocele formation rates were 8.24% in the Palomo technique series, 0.44% in the microsurgical varicocelectomy techniques, 2.84% in the laparoscopic varicocelectomy, and 7.3% in the macroscopic inguinal (Ivanissevich) or subinguinal varicocelectomy series revealing significant difference among the techniques. We conclude that the microsurgical varicocelectomy technique has higher spontaneous pregnancy rates and lower postoperative recurrence and hydrocele formation than conventional varicocelectomy techniques in infertile men.

Postoperative pregnancies occur with a mean duration of 7 months (3-11 months) after surgery (2). The current treatment modality is microsurgical inguinal or subinguinal varicocelectomy with high improvement in postoperative semen parameters (50% at least 50% increase in total motile sperm count) and pregnancy rates (36-43%) and highly low complication and recurrence rates (0-1%) (29,30).

Microsurgical varicocele repair has a significant potential not only to obviate the need for ART but also to downstage the level of ART needed to bypass male factor infertility (2). After varicocelectomy, intrauterine insemination (IUI) may be tried again for men who had not achieved pregnancy by natural intercourse. Following varicocelectomy, the results with IUI seem to improve or 11-21% pregnancy rates per cycle (34). The initial sperm concentration is predictive of unassisted pregnancy outcome in this population (13,35). Varicocelectomy may also enhance spermatogenesis within the testis, potentially increasing the chance of successful testicular sperm extraction surgery in patients with previously failed in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) (2). Varicocele repair may also increase IVF success in men who have had a varicocele. Agarwal and Esteves (36) reviewed 4 retrospective randomized studies, including 870 cycles with regard to varicocele presence and ICSI. They concluded that performing varicocelectomy in patients with clinical varicocele prior to ICSI is associated with improved pregnancy outcomes.

Surgical Treatment of Ejaculatory Duct Obstruction

Ejaculatory duct obstruction is detected in 1-5% of azoospermic men (1). Ejaculatory duct obstruction, although rare, is a surgically correctable cause of male infertility (6,9,18). The etiology is congenital (ejaculatory duct atresia, stenosis, and cyst) and acquired (trauma, infection, inflammatory, stone, dysfunction of seminal vesicle, and prostate cancer) (9,18,19). Calculus formation secondary to infection may also cause obstruction (15). Cyst formation from prior instrumentation or infection may also occur (15). In many cases, patients with ejaculatory duct obstruction have no significant antecedent history.

It is diagnosed with low ejaculate volume and seminal fructose level, acidic pH, and dilated seminal vesicle on transrectal ultrasound.

Although treatment options for distal ejaculatory duct obstruction include endoscopic resection, balloon dilatation, and laser incision/excision, transurethral resection of the ejaculatory duct (TURED), first described by Farley and Barnes in 1973, is the primary gold standard treatment of distal ejaculatory duct obstruction (15). A 24 French resectoscope is placed into the urethra, and resection is carried out at the level of the verumontanum. Overall results from surgical correction of ejaculatory duct obstruction show a 60-70% improvement in semen parameters and a 20-30% pregnancy rate (37). In cases who did not achieve spontaneous pregnancy, TURED may increase the chance for ART and also downstage the level of ART from ICSI to IUI (9).

A common complication of TURED is the reflux of urine into the ejaculatory ducts and subsequently into the seminal vesicles, vas deferens, or even the epididymis. This reflux into the epididymis can lead to acute or chronic epididymitis. Other complications include retrograde ejaculation secondary to a bladder neck injury, incontinence secondary to external sphincter injury, and, although rare, rectourethral fistula secondary to rectal injury (6,9,13,15). Postoperative bleeding, bladder neck contractures, and erectile dysfunction are also known complications. Large defects within the prostate can allow the mixing of semen and urine, which can further impair sperm quality.

Surgical Treatment of Obstructive Azoospermia

Treatment includes vasovasostomy (VV) or vasoepididymostomy (VE) in proximal obstruction and TURED in distal obstruction. Sperm for ART may be achieved from vas deferens, epididymis, testis, and seminal vesicle in cases who failed treatment or unpossible treatment due to localization of obstruction. Vasectomy

reversal can be done with VV and VE, using microsurgery. After a vasectomy reversal, spontaneous pregnancy is possible. Patency and pregnancy rates are 70-90% and 50-70%, respectively although the success rate varies with the duration of vasectomy, pathology of obstruction, and experience of the surgeon (38). In cases who failed surgery, IVF/ICSI can be done with epididymal sperm.

Surgical Treatment of Proximal Obstructions

Epididymal obstruction can be secondary to vasectomy, congenital, inflammatory, or idiopathic (39). In an azoospermic man with normal semen volume, normal testicular size, bilateral palpable vas deferens and a normal testicular biopsy demonstrating sufficient spermatogenesis, the most likely site of obstruction is the epididymis. Prior to performing VE, vasography should be performed to document vasal patency. Vasography should only be performed at the time of a planned surgical reconstructive procedure. If vasography is performed as a separate procedure, then an additional site of obstruction can be created. Vasography can be performed with either an open or a puncture technique. The puncture technique eliminates the need for closure of the vas deferens. Radiographic contrast can be injected distally toward the abdomen, and a plain x-ray is taken to define the anatomy of the vas deferens. The patency of the vas deferens can also be verified by simply injecting saline distally. If it flows easily, then the vas deferens is assumed to be patent. Injection should not be performed toward the epididymis, because this could cause injury.

Recent modifications address one of the main technical difficulties encountered in VE, that is, suture placement in an open collapsed epididymal tubule. These newer intussusception techniques involve the placement of sutures in a distended epididymal tubule before it is opened. The technique reported by Berger uses three 10-0 sutures and that by Marmar uses 2. The main theoretical advantage of these newer intussusception techniques is that the resultant invagination of the epididymis may reduce leakage from the anastomosis. Whether these modifications will translate into improved pregnancy rates is not known (40,41).

Vasal obstruction can occur secondary to vasectomy and other scrotal surgery besides vasectomy, or lower abdominal or inguinal surgery, such as renal transplantation or herniorrhaphy. Reconstruction after renal transplantation is usually not feasible, as the abdominal end of the vas deferens retracts proximally in the retroperitoneum. Obstruction caused by hernia repair may be correctable, though these repairs are technically challenging. Crossover transseptal procedures (VV or VE) is possible when a normal testis with unreconstructable obstruction is present on one side and an atrophic testis and patent ductal system are present on the contralateral side (42).

Vasal obstruction secondary vasectomy can be corrected with microsurgical VV. When a secondary epididymal obstruction occurs after vasectomy, VE is required. Patency and pregnancy rates for VV range from 75% to 93% and from 46% to 82%, respectively (42). VE is more technically demanding and less successful than VV. Patency rates range from 67% to 85%, and pregnancy rates range from 27% to 49%. Patency can take as long as 6 months for VV and as long as 1 year following VE. The average time to achieve pregnancy was 1 year for VV.

Several investigators have attempted to use fewer sutures, augmented by fibrin glue or laser soldering for both VV and VE procedures, allowing for a shorter operative time. In addition, robotics have been used for both VVs and VEs, with the hope that it may help with microsurgical technical issues, including eliminating tremor and improving dexterity with microsurgical instruments (43). While these techniques are not the current clinical standard, they appear to yield similar patency rates and may represent alternatives for the surgeon who performs only an occasional vasectomy reversal.

Surgical Sperm Retrieval Methods

In cases surgical treatment options fail or are not possible due to localization of obstruction or the presence of older female age, sperm for the use of ART can be obtained from the urogenital tract including vas deferens, epididymis, testis, and seminal vesicles. Sperm can be obtained by microsurgical testicular sperm extraction (micro-TESE) from the testis with a success rate of 36 to 78% in non-obstructive cases, and by microsurgical epididymal sperm aspiration (MESA) from the epididymis with a success rate of 60 to 98% in the obstructive cases (44).

Men with non-obstructive azoospermia (NOA) can now be treated by using intra-oocyte round spermatid injection (ROSI) or elongated spermatid injection (ELSI) in cases for which no mature sperm are available, sperm precursors, obtained from either the ejaculate or the testis. But the rates of fertilization and pregnancy with spermatids have been disappointing (45).

CONCLUSION

Varicocele is the most commonly seen and surgically correctable cause of male factor infertility. The other reasons of male factor infertility that are surgically correctable are congenital or acquired obstructive pathologies. Treatment strategies and surgical success rates for male infertility have increased dramatically over the three decades attributable to the development of microsurgical techniques and endoscopic equipment, instrumentation, and techniques. In case of treatment failure of correctable or uncorrectable pathologies of male factor infertility, surgical sperm obtained from the urogenital tract may necessary for ART. The success of surgical sperm obtained procedures has increased dramatically over the last decades attribute to technological progress similar to surgical techniques.

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Management of the Infertile Male with Azoospermia

Azospermisi olan İnfertil Erkeğe Yaklaşım

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ABSTRACT

Azoospermia means the complete absence of spermatozoa upon examination of the semen. Azoospermia may result from a lack of spermatozoa production in the testicles, or from an inability of produced spermatozoa to reach the emitted semen. Azoospermia is generally examined in two groups as obstructive and non-obstructive. Pretesticular causes usually include endocrine disorders that affect spermatogenesis, testicular causes include primary testicular pathologies, and posttesticular causes include obstructive pathologies. In order to make the exact diagnosis, a good urological, hormonal and genetic evaluation is required. The underlying etiologic cause determines the treatment strategy in azoospermic cases. If it is possible to see sperm in the ejaculate, pregnancy is tried to be achieved with ejaculate sperm, if not, with the spermatozoa obtained by interventional methods, by using assisted reproductive techniques. As the etiology of azoospermia is understood and treatment methods are improved, it is thought the rate of obtaining sperm both in the ejaculate and with interventional methods will increase, and as a result, higher pregnancy rates should be achieved. It is thought that there will be important developments in the treatment of azoospermia in the near future, as there have been significant advances in gene therapies and mesenchymal stem cell studies started on humans.
Keywords: Azoospermia; infertility; diagnosis; treatment.

ÖZ

Azospermi ejakülat incelemesinde sperm bulunmaması hali olarak tanımlanmaktadır. Azospermi testiste spermatozoa üretiminin olmamasından ya da üretilen spermatozoaların emisyon ile dışarı atılan ejakülata ulaşamamalarından kaynaklanabilir. Azospermi genel olarak obstrüktif ve non-obstrüktif olmak üzere iki grupta değerlendirilmektedir. Pretestiküler nedenler genellikle spermatogenezi etkileyen endokrin bozuklukları içerir, testiküler nedenler primer testis patolojilerini içerir ve posttestiküler nedenler ise obstrüktif patolojileri içerir. Doğru ve kesin bir tanıya ulaşabilmek için, iyi bir ürolojik, hormonal ve genetik değerlendirme yapılmalıdır. Azospermik olgularda tedavi stratejisini altta yatan etiyolojik neden belirler. Eğer ejakülatta sperm görülmesi mümkün ise bu spermlerle, ya da ejakülatta sperm görülmeyen olgularda girişimsel sperm elde etme teknikleri ile elde edilen spermlerle, yardımcı üreme teknikleri kullanılarak gebelik elde edilmesi amaçlanmaktadır. Azospermi etiyolojisi iyi anlaşılıp, tedavi yöntemleri geliştikçe, hem ejakülatta sperm görülme oranları hem de girişimsel metotlarla sperm elde edilme oranları artacak, bu sayede gebelik elde edebilme olasılığının da daha yüksek olacağı düşünülmektedir. İnsan üzerinde başlayan gen terapilerinde ve mezenkimal kök hücre çalışmalarında önemli gelişmeler kaydedilmesi nedeniyle yakın gelecekte azospermi tedavisinde de önemli gelişmelerin olabileceği düşünülmektedir.
Anahtar kelimeler: Azospermi; infertilite; tanı; tedavi.

INTRODUCTION

Azoospermia is defined as the absence of spermatozoa in the ejaculate. For the diagnosis of azoospermia, no spermatozoa should be seen at least in two microscopic seminal examinations. In order to make the differential diagnosis of azoospermia and severe oligospermia, semen analysis must also include pellet analysis in which the ejaculate fluid is evaluated after at least at a speed of 3000 G centrifugation. While the incidence of azoospermia is 1% in the normal population, this rate is reported to be 15% in men presenting with infertility (1). Azoospermia occurs as a result of the lack of adequate hormonal stimulation, spermatogenesis disorders, or obstructive pathologies. Differential diagnosis is very important in the treatment of men represented with azoospermia. In order to make the differential diagnosis in etiology, a good detailed physical examination and some basic laboratory tests are needed.

MEDICAL HISTORY

It is very important to know the medical and surgical history of the patient well, before the initial evaluation of the azoospermic patient. The story of the patient should include past fertility status, the frequency and timing of the sexual intercourse, duration of infertility, viral orchitis in childhood, and history of cryptorchidism. Also, the presence of genital trauma or pelvic surgery, history of testis torsion, epididymitis or urethritis-like infections, presence of gonadotoxic radiotherapy or chemotherapy, and family history of infertility should be questioned (2).

PHYSICAL EXAMINATION

Before proceeding to genital examination a well-detailed systemic examination may give some clues about the etiology of azoospermia. Thyroid examination, and lung, heart, and abdominal examination should be performed. For example; the detection of gynecomastia on examination may be a sign of an estrogen-secreting adrenal or testicular tumor. The patient should be evaluated for signs of Kallman and Klinefelter syndromes. A patient with Klinefelter syndrome presents with many phenotypes from complete hypogonadism to normal virilized type. It should also be kept in mind that short stature may be observed in some severe Y chromosome anomalies. After systemic evaluation, palpation of testicles and evaluation of their volumes should be performed. The expected testis volume in a normal fertile male is about 18-20 ml and the volume is directly related to spermatogenesis. Seminiferous tubules constitute a significant part of the testicular volume. When spermatogenesis is disrupted or reduced, a decrease in testis size is observed due to the loss of seminiferous tubules. In patients with non-obstructive azoospermia (NOA), testicular volume is often less than 15 ml and the diameter of the epididymis is thin (3). Epididymis and vas deferens should be palpated and the continuity should be checked. Also, the presence of varicocele should be carefully evaluated.

DIAGNOSTIC METHODS

Semen Analyses

Semen analysis should be evaluated in accordance with World Health Organization (WHO) 2010 guidelines. In an azoospermic patient at least two semen analyses should be performed, and also a two-week interval should be given between the first and the second test. Ejaculate volume takes an important place in the evaluation of the azoospermic patient. Secretion of the seminal vesicle accounts for most of the ejaculate volume. In cases with normal testicular volume and low semen volume (<1.5 ml), ejaculation disorder or ejaculatory duct obstruction should be considered. Retrograde ejaculation should be evaluated in a patient with unejaculation, or low ejaculation volume. Therefore in these cases, it is necessary to look for sperm in the urine sample after ejaculation. When azoospermia is detected in a patient, the semen sample should be centrifuged at 3000 G for 15 minutes, and then examined under the microscope at 400x magnification. In a study, the sperm detection rate was reported 35%, after centrifugation of the semen samples of the men with NOA (4).

Seminal plasma is a good source of markers that can detect many disorders in the male reproductive system. In the future, more information about the causes of azoospermia should be obtained by measuring and evaluating the different proteins in seminal plasma.

Endocrinological Evaluation

Hormonal tests have an important role in the evaluation of azoospermic cases, especially helpful in the differential diagnosis of primary and secondary testicular failure. Assessment of follicle stimulating hormone (FSH) and testosterone levels is sufficient to reveal most of the endocrine pathologies. FSH levels are regulated by inhibin B secreted by Sertoli cells (5). Inhibin B levels decrease and FSH level increase in cases where spermatogenesis is damaged such as Sertoli cell only (SCO) or testicular irradiation. The diagnostic accuracy of FSH is limited, and FSH level rises only in the final stage of the absence of spermatogenesis. FSH level may be normal in focal SCO or hypospermatogenesis (6). Serum FSH level is associated with the total spermatogonium count, not with the number of mature spermatids and the number of spermatozoa in the ejaculate. FSH levels have no clinical value in the selection of patients who are candidates for testicular sperm extraction (TESE) (7). Serum testosterone and luteinizing hormone (LH) levels may also aid in diagnosis. Low testosterone levels accompanying high LH levels may indicate hypergonadotropic hypogonadism (8).

Diagnostic Testicular Biopsy

Testicular biopsy is the definitive diagnostic test in the diagnosis of azoospermia. In selected patients with normal testicular volume, palpable vas deferens, and normal serum FSH levels, a testicular biopsy should be performed in order to differentiate obstructive azoospermia (OA) and spermatogenesis disorders. Detection of normal spermatogenesis in such a patient suggests an obstructive pathology. Testicular biopsy can be done open or percutaneously and should be done bilaterally. For an adequate assessment, each cross-sectional area should contain at least 20 seminiferous tubules. In the evaluation of the biopsy, the number of spermatids per tubule can be used to predict the expected number of spermatozoa in the ejaculate (9). These values are helpful in the differential diagnosis of obstructive and NOA.

ETIOLOGY OF AZOOSPERMIA

In order to understand the etiology of azoospermia easily, it is useful to make 3 classifications as pretesticular, testicular, and posttesticular azoospermia. Pretesticular

causes usually include endocrine disorders that affect spermatogenesis, testicular causes include primary testicular pathologies, and posttesticular causes include obstructive pathologies. While pretesticular and posttesticular causes are usually treatable, treatment success is very low in testicular azoospermia. In clinical practice, azoospermia is classified into two groups as OA and NOA. While pretesticular and testicular causes are evaluated in the NOA group, posttesticular causes are classified in the OA group.

NON-OBSTRUCTIVE AZOOSPERMIA

Pretesticular Causes

It is a condition encountered in 3% of infertile men and is due to endocrine-related problems.

Hypogonadotropic Hypogonadism (HH)

Hypogonadotropic hypogonadism (HH) is a rare and correctable cause of male infertility and it can occur from congenital or acquired causes. The most common cause include Kallmann syndrome, pituitary trauma or tumor, and the use of anabolic steroids. Kallman syndrome is a congenital malformation resulting from midline cranial structures. It is the most common cause of congenital HH (10). The pathophysiology of this syndrome is the absence of gonadotrophin releasing hormone (GnRH) release due to the non-migrating of GnRH-secreting neurons to the olfactory lobe during embryological development. The most common findings are the absence of a sense of smell, cleft palate, small testis, and in severe cases congenital deafness, skull and facial asymmetry, and cryptorchidism. Clinical findings in Kallmann syndrome depend on the degree of hypogonadism and the majority of patients represent with delayed puberty. Patients with a milder defect may have a moderate spermatogenesis disorder with an abnormal phenotype.

The use of exogenous testosterone may cause hypogonadism and azoospermia by suppressing the hypothalamohypophysial axis. Exogenous androgens impair spermatogenesis by suppressing FSH levels and cause a decrease in testicular testosterone levels (11).

When HH is detected in an azoospermic case the first step is imaging the pituitary gland with magnetic resonance imaging (MRI). Serum prolactin levels should be measured and hyperprolactinemia should be treated before GnRH replacement therapy in a case with elevated prolactin level. Because excessive prolactin secretion suppresses GnRH secretion from the hypothalamus with negative feedback. The use of some antihypertensive drugs and tricyclic antidepressants, stress, and pituitary adenomas may cause hyperprolactinemia or the cause may be idiopathic. Normal prolactin level is under 25 ng/dl and especially over 100 ng/dl suggests pituitary adenomas (12).

Androgen Resistance

The androgen receptor gene is located on the X chromosome and androgen resistance is seen at a ratio of 1:60000. There are more than 300 mutations in the androgen receptor gene and can be found in different forms, ranging from a female phenotype to an azoospermic man with normal virilization. The androgen receptor gene has 8 exons and the critical region is the region of CAG nucleotide repeats. It has been reported that men with normal virilized idiopathic azoospermia have more frequent CAG repeats than normal fertile men (13).

Testicular Causes

Also known as primer testicular failure, this deficiency of spermatogenesis includes testicular damage due to varicocele, undescended testis, testicular torsion, mumps orchitis, gonadotoxic effects of the drugs, genetic disorders, and idiopathic causes. Microsurgical testicular sperm extraction (micro-TESE) is applied to obtain sperm in this patient group which is clinically called NOA. NOA is divided into three histopathologic groups; hypospermatogenesis, maturation arrest, and SCO. In hypospermatogenesis, all stages of spermatogenesis are observed in the testis but the amount is low (14). In maturation arrest, germ cells cannot complete their maturation and remain in a certain phase. While spermatogonia and spermatocyte are seen in early-stage maturation arrest, spermatids are found in late-stage maturation arrest, but no mature spermatozoa cells are found in any stage (15). In SCO there are no germ cells (14). According to histological diagnosis, the rate of sperm finding in micro-TESE has been reported 73-100% in hypospermatogenesis, 27-86% in the maturation arrest group, and 22-41% in the group thought to have SCO, respectively (16).

Varicocele

Varicocele is detected in 4.3-13.3% of azoospermic men. For the first time, Tulloch reported in 1955 that spermatogenesis resumed and pregnancy was achieved after varicocelectomy in men with azoospermia. Spermatozoa are found in the ejaculate at a rate of 21-55% when varicocelectomy is performed in an azoospermic patient. Although spontaneous pregnancy rates are low in these cases, obtaining sperm from the ejaculate eliminates the need for an invasive procedure such as TESE (17).

While recommending varicocelectomy to the azoospermic patient, it should be informed that TESE and intracytoplasmic sperm injection (ICSI) may be required later on. Especially in patients with SCO or early-stage maturation arrest, spermatogenesis may not start after varicocelectomy. Therefore these patients should be aware of this possibility and should consider a direct alternative to micro-TESE instead of varicocelectomy (18). In addition, before deciding on varicocelectomy in an azoospermic patient with varicocele, genetic disorders such as Y chromosome microdeletions should be investigated. Varicocelectomy may contribute to healthier sperm being obtained by interventional methods, even if there is no sperm in the ejaculate after the surgery.

Undescended Testis

Undescended testis is the most common (2.7%) genital malformation in newborn boys and it decreases to 0.8% by the age of one year (19). There are 4 different theories about how cryptorchidism causes infertility; testicular dysgenesis, endocrine axis disorder, endocrinological damage, and obstruction. If the testicles have not descended spontaneously within the first year, genetic research is recommended for Klinefelter syndrome (20). Early treatment of undescended testis reduces the risk of infertility. Fertility is usually normal in unilateral undescended testicles, the age at the surgery and the testicular volume are the most important factors determining fertility. Azoospermia is found at a rate of 13% in unilateral patients and 31% in bilateral patients who have undergone undescended testis surgery (21).

Testicular Torsion

Testicular torsion is detected at a rate of 1:4000 in men under the age of 25. If surgery is not performed within the first 6 hours, the most important complication is testicular loss which can negatively affect fertility. It has been reported that spermatogenesis is impaired in both testicles after torsion. Although the reason is not certain, it is thought that anti-sperm antibodies appearing secondary to the deterioration of the blood-testicular barrier may be the cause (22).

Mumps Orchitis

The incidence of mumps orchitis decreased considerably with vaccination, 67% of the cases are unilateral and 33% are bilateral. The main cause of the pathology is thought to be necrosis developing in the seminiferous tubules due to congestion, edema, and increased pressure. The probability of infertility in mumps orchitis developing before puberty is very low (23).

Gonadotoxins and Drugs

In a patient presenting with infertility, it should be questioned whether he use any drugs or food supplement. Exposure to exogenous androgens, antiandrogens, chemotherapy drugs, radiotherapy, or toxins such as pesticides and insecticides may adversely affect spermatogenesis. Drugs and toxins may affect spermatogenesis with 4 mechanisms; direct gonadotoxic effect, by affecting the hypothalamo-pituitary axis, ejaculation disorder, and decrease in libido.

The testis consists of two parts: the germ cells, the seminiferous tubules containing Sertoli cells, and the interstitial area where the Leydig cells responsible for testosterone production are located. A continuous cell division takes place and about 100 million spermatozoa are produced daily in seminiferous tubules, therefore drug use mostly affects this region. Since the production of mature spermatozoa takes an average of 74 days, the effect of a drug on spermatogenesis occurs 3 months later (24). Cyclophosphamide, which is used for autoimmune diseases, may cause permanent azoospermia, especially when used at doses exceeding 7.5 mg/m^2 (25). Sulfasalazine also causes transient azoospermia, and spermatogenesis is expected to return to normal 3 months after the drug is discontinued.

Genetic Causes

Genetic anomalies are found in 15% of azoospermic patients and 5% of oligospermic men (26). The 2 most common genetic tests used in the diagnosis of NOA are; the analysis of microdeletions on the long arm (Yq) of the Y chromosome and the karyotype.

Klinefelter Syndrome

The most common karyotype anomaly in infertile men is Klinefelter syndrome (47,XXY), and 90% of patients have non-mosaic X chromosome polysomy. Advanced maternal and paternal ages increased the risk of Klinefelter syndrome (27). The phenotypic characteristics of Klinefelter syndrome are; tall, euicoid structure, micropenis, small and hard testicles, decreased facial hair, and female-type genital hair. Patients are often azoospermic but in a recent study, it is reported that spermatozoa could be found in 69% of cases with micro-TESE. These patients are more likely to have sperm in the ejaculate or by the micro-TESE method just after puberty. In advancing ages testicles become fibrotic and germ cells may disappear, so it is very important to diagnose at an early age and to obtain and freeze spermatozoa (20).

47,XYY Syndrome

This syndrome occurs as a result of a problem in the separation of the Y chromosome during the meiosis stage of the formation of the spermatozoa. These patients are tall, and have low intelligence and antisocial personality disorder characteristics. SCO or maturation arrest is detected in testicular biopsies of these cases (28).

Mixt Gonadal Dysgenesis

They have 45,XO and 46,XY mixed genotypes and anatomically they have a gonad with testis on one side and a line on the other side. The testis is usually undescended and does not contain germ cells. Since the risk of developing gonadoblastoma and seminoma is high in the streak-shaped gonad, this gonad should be surgically removed (29). These patients also have various degrees of ambiguous genitalia.

Y chromosome Microdeletions

Y chromosome microdeletion analysis should be performed in patients with severe oligoasthenospermia or azoospermia. It was determined that microdeletions from proximal to distal in the azoospermia factor (*AZF*) region on the q arm of the Y chromosome (Yq, *AZFa*, *AZFb*, *AZFc*) cause azoospermia (30). The frequency of Y microdeletion was reported as 7.3% in the infertile population and this rate was reported as 9.7% in men with azoospermia. Microdeletions on Y chromosome composed of 60% *AZFc*, 16% *AZFb*, 8% *AZFb+c*, 5% *AZFa*, 4% *AZFa+b+c*, and 6% non *AZF* deletions. *AZFc* is the most common Y chromosome microdeletion and is seen at a ratio of 1:4000. Its incidence in azoospermic patients has been reported as 13%.

AZF deletion determination in male infertility has prognostic importance for couples who want ICSI treatment. Depending on the complete or partial deletion of Y chromosomes, severe sperm disorders may be seen in men, ranging from mild sperm disorders to azoospermia. *AZFa* microdeletion is detected in 1% of NOA patients. It has been reported that complete *AZFa* deletion is associated with type 1 SCO syndrome and azoospermia (30). The American Society of Reproductive Medicine (ASRM) reported that sperm cannot be found with micro-TESE in those with *AZFa*, *AZFb*, and *AZFb+c* microdeletions.

Relationship between Azoospermia and microRNA

MicroRNAs (miRNA) are regulatory molecules that participate in important processes such as embryological development, cell differentiation, and apoptosis in the cell (31). In a study, it was shown that four different miRNA types (hsa-miR-34b, hsa-miR-34c, hsa-miR34c-5p, and hsa-miR-122) decreased while one miRNA (hsa-miR-429) increased in men with NOA. In another study, it was reported that before TESE is performed, it can be predicted whether there will be spermatozoa in the testis by measuring two different miRNA types (miR-539-5p and miR-941) (32). In spermatogonia, pachytene spermatocytes, and round spermatids, miRNA levels were found to be different between patients with NOA and OA. Therefore, it has been claimed that spermatogenesis can be initiated in azoospermic patients by using miRNA inhibitors and miRNA copies (33). If this theory is realized, it will be an important milestone in the treatment of azoospermia.

TREATMENT OF NON-OBSTRUCTIVE AZOOSPERMIA

In the treatment of NOA cause-oriented treatment modality should be preferred. Especially with the advances in assisted reproductive techniques and obtaining spermatozoa from the testis is used as an effective method in solving this problem. For this purpose, empirical treatment and interventional procedures are used in the treatment of NOA.

Empirical Hormonal Treatments

One of the most commonly used agents in the treatment of NOA is clomiphene citrate. In a study patients diagnosed with NOA and having no SCO were given 50 mg clomiphene citrate every other day, with a testosterone level of 600-800 ng/dl. At the end of the 9th month, 64% of patients have spermatozoa in their ejaculate while spermatozoa were found in testicular biopsy in 36% of the patients (34). In another study involving 1054 patients, clomiphene citrate, human chorionic gonadotropin (hCG), and aromatase inhibitors were given and it was reported that these treatments did not contribute to either spermatozoa findings or pregnancy and birth rates (35).

Methods for Finding Spermatozoa in Non-Obstructive Azoospermia

Methods for spermatozoa finding in NOA include testicular sperm aspiration (TESA), TESE, and micro-TESE. Percutaneous epididymal sperm aspiration (PESA), which is used in OA, is not preferred in NOA because of the lower success rates.

Testicular Sperm Aspiration (TESA)

It is a method of aspirating spermatozoa percutaneously by a needle through the skin of the scrotum. Although it is not the preferred method for NOA patients due to the low success rates, it is the cheapest method if success can be achieved. Another disadvantage of this method is that hematoma may develop due to vascular injuries (36).

Testicular Sperm Extraction (TESE)

First, the testis is exposed by the incision into the scrotum, and then an incision is made into the tunica albuginea. Since more tissue is removed from the testis in large pieces, this method may cause a problem, especially in patients with a small testicular volume. The rate of obtaining spermatozoa with TESE is reported to be two times higher than with TESA (36).

Microsurgical Testicular Sperm Extraction (micro-TESE)

Unlike TESE, in micro-TESE, after the testis is exposed, the entire testicular tissue is reviewed under the microscope at 15-20 magnification, and samples are taken from the most opaque and plump tubules. It is the most effective method to obtain spermatozoa from the testis. Predictive factors for finding spermatozoa in micro-TESE are; serum FSH and testosterone level, testicular volume, age, and obesity. For men with low testosterone levels, it is recommended that testosterone levels should be increased to normal levels before micro-TESE is performed (35). Although some studies have reported that high FSH levels decrease the rate of finding spermatozoa, it has been reported that the predictive value of FSH is very low, in a meta-analysis (37). In a meta-analysis evaluating 1764 patients, it is reported that there was no correlation between testis volume and spermatozoa finding rates (38). If testicular volumes are normal in an azoospermic patient, obstructive pathologies are more likely to be considered. NOA is more common in obese patients and the rate of finding spermatozoa is below the average (39).

OBSTRUCTIVE AZOOSPERMIA

After the testis the reproductive tract continues to the ductuli efferentes, then the epididymis, vas deferens, vesicula seminalis, and opens into the verumontanum through to the ejaculatory canal. The embryological origin of the posttesticular duct is the wolf duct (mesonephric duct).

Posttesticular Cause

Posttesticular azoospermia is seen due to obstruction or ejaculation disorders. Causes of OA are; the absence of vas deferens, vasal obstruction, and epididymal or ejaculatory duct obstruction. Among the findings to support OA; are the inability to palpate vas deferens, testicular volumes of 16 ml or more, presence of dilated caput epididymis, and normal serum FSH levels. Detection of quantitatively normal spermatogenesis in testicular biopsy performed for diagnostic purposes is also in favor of OA. Finding the etiology of azoospermia is very important in the selection of the sperm retrieval technique and in determining the probability of detecting spermatozoa. In cases without obstruction, sperm motility increases as you move away from the testis and caput epididymis. On the other hand, if there is an obstruction at the level of vas deferens, degenerated spermatozoa and macrophages increase in the most distal level of the epididymis while the best motility can be achieved in the caput epididymis or efferent channels (40).

Congenital Agenesis of Vas Deferens

This pathology is seen in 1% of the infertile population and 6% of those with azoospermia (41). There are two causes; cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation and the anomalies in the development of mesonephric duct. Cystic fibrosis (CF) is the most common autosomal recessive disease in Caucasians. CFTR gene mutation is found in 80% of those with bilateral vas deferens agenesis and 43% of those with unilateral agenesis (42). Testicular volumes and spermatogenesis are normal in patients with congenital absence of vas deferens but their ejaculates are acidic and their volumes are 1 ml or less. If the development of the wolf channel is affected before the 7th day of the gestational period, the development of the reproductive system is also affected and epididymal agenesis, seminal vesicle agenesis, or hypoplasia may occur resulting in low ejaculate volume (43). In this patient group, spermatozoa can be obtained from the head of the epididymis by microsurgical or percutaneous methods for assisted reproductive techniques.

Vasal Obstruction

The most common cause is cord injuries during the inguinal hernia repair. It can be caused by direct injury or developed due to the inflammation caused by the mesh placed during surgery. It should be considered when normal testicular volumes and full epididymis are palpated on physical examination. Another common cause in the USA is a vasectomy performed for contraceptive purposes. If fertility is desired again in these patients vasovasostomy can be performed. Vasectomy reversal which is the microsurgical method, first described by Siber and Owen is accepted as a gold standard treatment method today (44). In a study examining the long-term result of 1303 patients, the incidence of finding spermatozoa was 89% and the rate of spontaneous pregnancy was reported as 59% (45).

During the vasovasostomy operation, when the proximal vas incision is made the fluid coming from the lumen is thick and there are no spermatozoa in the microscopic

examination, and also there is no other stenosis at the distal part, epididymovasostomi technique is performed. Many studies have reported that, even in cases where the epididymis was too short after epididymovasostomi, the intact epididymis can allow some spermatozoa to gain motility and fertilization ability (46,47).

Epididymal Obstruction

Epididymal obstruction is the most common cause seen in 30-67% of patients with OA (48). Previous epididymal surgeries and infections may cause epididymal obstruction. Although its pathology is not known exactly, chronic sinusitis and respiratory dysfunctions are seen together with OA, in Young's syndrome which is characterized by a defect in ciliary activity and mucus structure. Since spermatogenesis is normal in these patients, it is possible to have children with assisted reproductive methods.

Ejaculatory Duct Obstruction

Ejaculatory duct obstruction in the literature; is defined by different names such as Mullerian duct cyst, urogenital sinus cyst, or midline cyst (48,49). It is detected in 1-3% of azoospermic patients and is characterized by acquired or congenital unilateral or bilateral ejaculatory duct obstruction. The most common congenital causes are ejaculatory duct atresia or stenosis and midline cysts. Among the acquired reasons; are previous trauma and pelvic interventions, genitourinary infections, inflammatory process, vesicoseminal dysfunction, and prostate cancer (50).

A gel-like fructose-rich secretion secreted by seminal vesicles constitutes 80% of the ejaculate volume. Other 5-10% of the ejaculate volume is composed of fluid from the testicles and epididymis that contains spermatozoa. Therefore vas deferens obstruction does not affect semen volume. If low ejaculate volume, low seminal fructose level, and acidic pH are detected in semen analysis, pathologies that may cause distal ejaculatory duct obstruction should be considered. Despite normal testis volume and hormone profile, azoospermia with low ejaculate volume and the detection of dilated seminal vesicles in transrectal ultrasound (TRUS), indicate ejaculatory duct obstruction (51).

If ejaculatory duct obstruction is caused by a midline cyst, transurethral resection of the ejaculatory duct (TURED) is performed with the aim of decorticating the cyst wall. This treatment is firstly described by Farley and Barnes in 1973. Ejaculate volume usually returns to normal after TURED, and the probability of spontaneous pregnancy is reported to be 13-30% (52,53).

Ejaculation Disorder

Ejaculation disorder should be considered in patients with low (<1 ml) ejaculate volume. Retrograde ejaculation is the most common ejaculation disorder and is characterized by the flow of seminal fluid back to the bladder. Causes include anatomical, neurogenic, pharmacological (alpha-blockers, neuroleptics, tricyclic antidepressants), or idiopathic reasons (54). The diagnosis is achieved by showing spermatozoa in a urine sample taken after ejaculation. The presence of more than 10-15 spermatozoa in each high magnification field in the urine sample is considered to be diagnostic. Conversely, in patients with emission disorders, no spermatozoa are detected in the urine sample.

CONCLUSION

There are many different causes in the etiology of azoospermia. Diagnostic and treatment approaches for these different etiologies are quite diverse. In recent years, especially with the development of technology and understanding of the subgroups of azoospermia, the rate of obtaining pregnancy get increased. Recent studies involving miRNAs that play a regulatory role in embryological development, emphasize that miRNA inhibitors and miRNA transcripts can initiate spermatogenesis in azoospermic patients. If this theory is realized, it would be an important milestone in the treatment of azoospermia. In order to achieve promising developments in the azoospermic patients to have children, more studies are needed.

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Surgical Sperm Retrieval Techniques for Assisted Reproductive Technology

Yardımcı Üreme Teknolojisi için Cerrahi Sperm Elde Etme Teknikleri

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ABSTRACT

Intracytoplasmic sperm injection provides in vitro fertilization for many infertility conditions. It is also an important treatment option for fertilization in azoospermic men. Sperm production in azoospermic male testicles is possible. In these patients, sperm extraction from the epididymis or testis is required before intracytoplasmic sperm injection. Numerous sperm retrieval procedures for obtaining sperm from the epididymis or testicles have been developed. The spermatozoa obtained by these methods are used in intracytoplasmic sperm injection for in vitro fertilization. The most important factor when determining the sperm retrieval method is whether the azoospermia is obstructive or non-obstructive. In addition, the experience of the surgeon performing the procedure is also effective in determining the sperm retrieval technique. In this review, sperm retrieval methods are presented, and current developments in these sperm retrieval methods are also mentioned. The indications of surgical sperm retrieval methods in both obstructive and non-obstructive azoospermic patients, the technical aspects of each method, possible complications, advantages and disadvantages of these methods are presented, and their superiority to each other are also discussed. In addition, evidence for the fertilization rates after intracytoplasmic sperm injection using sperm obtained by these methods and continuation of pregnancy is presented in a comparative manner and critically discussed.
Keywords: Sperm retrieval; assisted reproductive techniques; male infertility; azoospermia.

ÖZ

İntrasitoplazmik sperm enjeksiyonu, birçok infertilite koşulu için in vitro fertilizasyon sağlamaktadır. Azospermik erkeklerde de fertilizasyon için önemli bir tedavi seçeneğidir. Azospermik erkek testislerinde sperm üretimi mümkündür. Bu hastalarda intrasitoplazmik sperm enjeksiyonundan önce epididim veya testisten sperm alınması gerekir. Epididim veya testislerden sperm elde etmek için çok sayıda sperm alma prosedürü geliştirilmiştir. Bu yöntemlerle elde edilen spermler, in vitro fertilizasyon için intrasitoplazmik sperm enjeksiyonunda kullanılmaktadır. Sperm alma yöntemini belirlerken en önemli faktör azosperminin obstrüktif veya non-obstrüktif olup olmadığıdır. Ayrıca işlemi yapan cerrahın tecrübesi de sperm alma tekniğinin belirlenmesinde etkilidir. Bu derlemede sperm elde etme yöntemleri anlatılmış ve ayrıca bu sperm elde etme yöntemleri ile ilgili güncel gelişmeler de ele alınmıştır. Hem obstrüktif hem de non-obstrüktif azospermik hastalarda cerrahi sperm elde etme yöntemlerinin endikasyonları, her bir yöntemin teknik yönleri, olası komplikasyonları, bu yöntemlerin avantaj ve dezavantajları anlatılmış ve birbirlerine göre üstünlükleri de tartışılmıştır. Ayrıca, bu yöntemlerle elde edilmiş olan sperm kullanılan intrasitoplazmik sperm enjeksiyonu sonrası fertilizasyon oranları ve gebeliğin devamına ilişkin kanıtlar karşılaştırmalı bir şekilde sunulmuş ve eleştirel bir şekilde tartışılmıştır.
Anahtar kelimeler: Sperm toplama; yardımcı üreme teknikleri; erkek infertilitesi; azospermi.

INTRODUCTION

Two significant discoveries in male infertility have occurred recently (1-3). The first was the definition of intracytoplasmic sperm injection (ICSI) in males with significant spermiogram abnormalities for in vitro fertilization (1). The second was proof of ICSI can be used in azoospermic cases and that sperm from the epididymis or testicles can be used for normal fertilization and pregnancy (2,3). Azoospermia is the absence of spermatozoa in the ejaculate, which affects 1-3 percent of the male population. This percentage is around 10% among infertile males. Although azoospermia is the medical term for infertility, sperm production in azoospermic male testicles is possible (4). As a result, numerous sperm retrieval procedures for obtaining sperm from the epididymis or testicles have been developed. The spermatozoa obtained by these methods are used in ICSI for in vitro fertilization (1-6).

The most important factor when determining the sperm retrieval method is whether the azoospermia is obstructive or nonobstructive. In addition, the experience of the surgeon performing the procedure is also effective in determining the sperm retrieval technique. In obstructive azoospermia (OA), there is no defect in sperm production of the testicles. The produced sperm cannot be ejaculated to the obstruction of the seminal ducts (4,7). OA can occur for many reasons. It is defined as congenital or acquired. In rare cases of acquired OA such as vasectomy, there is a chance of normal fertilization by treating the obstruction with surgical methods (8). Although it is an effective treatment, recanalization may not be possible in some cases. In the majority of congenital or acquired OA cases, sperm can be obtained from the epididymis or testis.

Non-obstructive azoospermia (NOA) occurs due to defects in sperm production (4). NOA can also occur due to congenital and acquired causes. In NOA cases, the only method to obtain sperm before ICSI is to search for sperm in the testis. There is a possibility of spermatogenesis in different areas of the testis in men with NOA. Therefore, spermatozoa can be found in the testicles in 30-60% of cases (6). Testicular sperm extraction (TESE) is a sperm retrieval technique applied in NOA (6,9), and the sperm retrieval rate increases if microsurgery is applied (6,10). Table 1 summarizes the advantages and disadvantages of various sperm retrieval techniques.

PERCUTANEOUS SPERM RETRIEVAL METHODS

Percutaneous sperm retrieval methods are minimally invasive procedures. Its important advantages are that it can be applied in a short time under local anesthesia and can be repeated. It is easier to learn and less costly than surgical methods. The procedure can be performed by aspirating sperm from the testis, epididymis, or vas deferens (11).

Percutaneous Epididymal Sperm Aspiration (PESA)

The success rate of sperm retrieval in patients who underwent percutaneous epididymal sperm aspiration (PESA) for OA is around 51-100% (12). It has been reported that the motility of sperm obtained with PESA is between 62% and 94% (13). PESA is a useful sperm retrieval method in men with OA due to vasectomy. Collins et al. (14) reported a study comparing the success of sperm retrieval. They investigated men after vasectomy who were known to have no previous infertility problems. They performed microsurgical epididymal sperm aspiration (MESA) and

Table 1. Advantages and disadvantages of different sperm retrieval techniques

	Advantages	Disadvantages
PESA	Quick and inexpensive There's no need for microsurgical experience Repeatable minimal morbidity There will be no open surgery Instruments and materials are limited	Few sperm retrieved Fibrosis and obstruction at the aspiration site Risk of hematoma/spermatocele Limited number of sperm for cryopreservation
MESA	Large number of sperm retrieved High number of sperm for cryopreservation Reduced risk of hematoma Reconstruction possible[1]	Open surgical exploration required Increased cost and time-demanding Operating microscope required Microsurgical instruments and expertise required Postoperative discomfort
TESA	Fast and low cost; Repeatable No open surgical exploration No microsurgical expertise required Few instruments and materials Minimal/mild postoperative discomfort	Relatively low success rate in NOA cases Few sperm retrieved in NOA cases Limited number of sperm for cryopreservation Risk of hematoma/testicular atrophy
TESE	No microsurgical expertise required Repeatable	Costlier and more time-consuming Open surgical exploration is needed In NOA cases, few sperm are retrieved Risk of testicular atrophy[3] Risk of testicular androgen production may be impaired[3] Postoperative discomfort
Micro-TESE	Higher success rates in NOA cases[2] Larger number of sperm retrieved[2] Low risk of complications Relatively higher chance of sperm cryopreservation[2]	Surgical exploration required Operating microscope required Increased cost and time-demanding Postoperative discomfort Microsurgical instruments and expertise required

PESA: percutaneous epididymal sperm aspiration, MESA: microsurgical epididymal sperm aspiration, TESA: testicular sperm aspiration; TESE: testicular sperm extraction; micro-TESE: microsurgical testicular sperm extraction, NOA: non-obstructive azoospermia, [1]: in cases of post-vasectomy obstructions, [2]: compared with TESA and TESE in NOA cases, [3]: multiple biopsy-TESE

PESA on both testicles. Both procedures yielded the same percentage of effective sperm retrieval. As a result of this study, it was emphasized that PESA should be performed in men with OA after vasectomy. In the study conducted by Yafi and Zini (13), 255 men with OA for different reasons who underwent PESA were investigated. In this study, OA cases with many causes such as vasectomy and congenital bilateral absence of the vas deferens (CBAVD) were investigated. It was reported that 75.3% of motile sperm were found with PESA. In addition, it was determined that the probability of finding motile sperm was increased in young men and those with high testicular volume. If PESA was repeated on the ipsilateral testis, lower sperm retrieval rates (26.3%) were observed (15). It has been reported that 25% cannot find sperm with PESA (13). Therefore, additional procedures such as testicular sperm aspiration (TESA) or TESE may be required.

Testicular Sperm Aspiration (TESA)

TESA can be done in a variety of ways. Percutaneous aspiration of the testicular parenchyma is performed by introducing a fine or large-diameter needle into the testis through the scrotal skin. During needle insertion, the main branches of the testicular artery should not be injured. The needle is frequently inserted at an oblique angle towards the medium and lower testicular poles, at the anterolateral or anteromedial part of the superior testicular pole. Vascular structures are least likely to be found in these regions. To avoid vascular injury, loupe magnification might be utilized. Negative pressure is established by pulling the syringe plunger to aspirate the sperm in the seminiferous tubules. Furthermore, the needle's tip is pushed in and out of the testis on an oblique plane to reach different areas. A tube holding a hot sperm medium is filled with the sample. The sperm is taken to a laboratory and examined under a microscope. In case of unsuccessful results, TESE or TESA can be performed on the contralateral testis (16).

Percutaneous Vasal Sperm Aspiration (PVSA)

Vasal sperm aspiration can be applied to infertile men who develop due to obstruction at the prostate or distal vas deferens level. It is also an option for men with ejaculatory dysfunction. Percutaneous vasal sperm aspiration (PVSA) is a successful technique with a high rate of achieving pregnancy. Qiu et al. (17) reported the results of sperm retrieval with PVSA. This study consisted of infertile men with anejaculation. There was a pregnancy rate of 73.1% and a 100% retrieval rate after intrauterine insemination (IUI). Sperm in the vas deference are mature and in high volume. Therefore, sperm obtained after PVSA are of high quality for assisted reproductive technology (18). PVSA localization should be determined according to the level of obstruction. In cases of distal obstruction such as inguinal or ejaculatory duct obstruction, the scrotal vas deferens may be preferred.

SURGICAL SPERM RETRIEVAL METHODS

Microsurgical techniques or conventional methods are used in surgical sperm retrieval. These procedures are more painful than percutaneous sperm retrieval techniques. Therefore, it can be performed under general anesthesia. Local anesthesia can be preferred with intravenous sedation or epidural anesthesia (16).

Microsurgical Epididymal Sperm Aspiration (MESA)

Temple-Smith et al. (19) described MESA for the first time and reported it in 1985. During the procedure, a 2-3 cm transverse incision is made into the scrotum. Its tunic opens and a large-looking tubule is found. The fluid leaking from the tube is aspirated and poured into a tube containing the hot medium. It is transferred to the laboratory through these tubes and the sample is examined. Sperm count or quality may not be sufficient. Re-aspiration is performed from a different part of the epididymis (from cauda to hood) and/or contralaterally. If motile sperm cannot be collected after the procedure is repeated, simultaneous TESE or TESA can be used (16).

Conventional Testicular Sperm Extraction (TESE)

Taking samples from the testicular parenchyma for sperm research and the use of found sperm in in vitro fertilization were first described by Devroey et al. (3) in 1995. It is a standard open surgery method and no optical magnification is performed. It can be performed under general anesthesia. Local anesthesia can be preferred with intravenous sedation or epidural anesthesia. The "window" method is used in the operation. An approximately 2 cm long transverse incision is made in the scrotal skin. If there is a possibility of sperm extraction from both testicles, a vertical incision can also be made from the scrotal raphe. The testis is reached by passing the subcutaneous layers, dartos, and tunica vaginalis. The testicular parenchyma is exposed by making an incision of approximately 1 cm on the tunica albuginea. On removing the testicular parenchyma, little pressure is given to the testicle. A small piece of testicular parenchyma is cut with scissors and a sample is taken. After that, the sample is placed in sperm culture media. Multiple samples can be taken from the same incision if necessary. Additional albugineal incisions may be made in the upper, middle, and lower testicular poles to obtain multiple biopsy specimens. Samples are sent to the laboratory for sperm analysis. After an adequate sample is taken, the non-absorbable suture closes the tunica albuginea (16).

Microsurgical Testicular Sperm Extraction (micro-TESE)

TESE with a microscope in azoospermic patients was first reported by Schlegel (20) in 1999. Delivery of testis is performed as described in MESA. A single, large, mid-section incision is then made under 6-8x magnification in an avascular region of the tunica albuginea, exposing the testicular parenchyma extensively. Large seminiferous tubules are searched with the microscope. Dissection of testicular parenchyma is performed with 16-25x magnification. Superficial and deep areas of the testicular parenchyma can be examined under the microscope. Multiple biopsies can be taken during this procedure. Enlarged tubules may not be seen during the procedure. In this case, any tubule that is different is excised. If all tubules look the same, random micro-biopsies are done from different areas. The sperm cells in the samples should not lose their vitality. Therefore, the tissues are transferred with a Petri dish containing the sperm medium. Blood clots are cleaned from the samples before they are examined. Tunica albuginea is closed with non-absorbable sutures. The process is terminated by closing the scrotal layers with absorbable sutures (16). The microsurgical testicular sperm extraction (micro-TESE) surgical method is shown step by step in Figure 1.

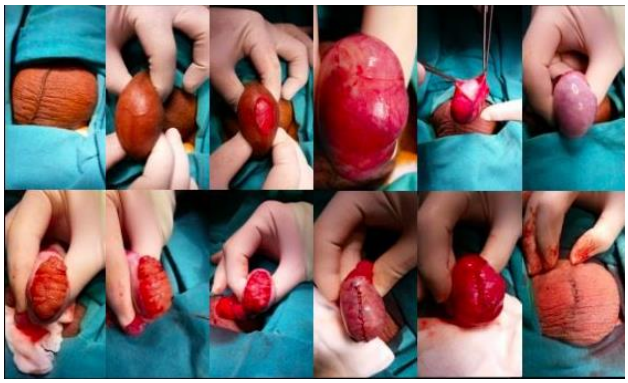

Figure 1. Microsurgical testicular sperm extraction surgical method (photos from Dr. Tahsin Turunç's personal archive)

Sperm Retrieval Postoperative Care and Complications

Percutaneous sperm retrieval methods are minimally invasive methods. The procedure is applied without hospitalization to the patient. Patients usually return to their normal activities the next day. Although open surgery (micro-surgery or conventional) is more invasive than percutaneous methods, they return to their normal activities after 2-3 days. Scrotal cold application and elevation are recommended in patients to reduce edema and relieve pain. For about a week, patients are recommended to avoid ejaculation and strenuous physical activity. Oral analgesics and anti-inflammatory drugs should be used for complaints of pain and scrotal swelling (6).

Complications such as persistent pain, infection, swelling, hematoma, and hydrocele may occur after sperm retrieval. The incidence of complications has been reported to vary between 0-70% (21-24). PESA complications generally have minimal morbidity compared to open surgery. Complication rates vary according to the type of procedure. Rarely, it varies according to the type of azoospermia. Fibrosis at the aspiration site is common in percutaneous methods, but serious complications are rare (16). Intratesticular hematoma often occurs in patients undergoing TESE due to single or multiple biopsies. However, it usually resorbs spontaneously without damaging the testicular function (23). Devascularization may develop in the testicular parenchyma after conventional TESE. Therefore, a temporary or permanent decrease in serum testosterone level may be observed (9,22). The complication rate is higher in conventional TESE compared to micro-TESE (9,20,22,24). During micro-TESE, before the tunica albuginea incision is made, the underlying testicular vessels are detected. Thus, intratesticular blood flow is preserved. It also causes minimal parenchymal damage by identifying tubules that are likely to produce sperm (22). Since androgen production is low in Klinefelter syndrome (KS) patients, a decrease in serum testosterone has been reported after micro-TESE (21). However, in most KS patients, testosterone levels return to preoperative values during the 1-year follow-up period. Sperm retrieval methods should be performed by surgeons with adequate training to reduce the risk of postoperative complications (25).

Comparative Outcomes and Expectations

Cochrane review has been reported that randomized controlled studies comparing sperm retrieval techniques are not sufficient and that the simplest and least invasive technique should be chosen (26). There is a meta-analysis study comparing ICSI results (n=1,103 cycles) of sperm from men with OA and NOA. In this study, it was determined that the success of ICSI was lower in NOA cases. OA cases had higher natural fertilization rates (relative risk, RR: 1.18; 95% confidence interval, CI: 1.13-1.23) and clinical pregnancy rates (RR: 1.36; 95% CI: 1.10-1.69) when compared to NOA cases. In these groups, the rise in ongoing pregnancy rates was not significant (RR: 1.19; 95% CI: 0.87-1.61). There was no statistically significant difference in implantation (RR 1.01; 95% CI: 0.87-1.61) or abortus rates (RR: 0.84; 95% CI: 0.48-1.48) between the groups (27). The findings of this meta-analysis matched Cochrane's recommendations.

For the treatment to be successful, the laboratory effort and expectations around surgical sperm retrieval operations techniques are equally critical. Partners must be prepared for ICSI simultaneously time to enhance treatment success. In NOA, the experience of the andrology laboratory increases the success of the procedure. It is recommended to use the "recommended minimum search time" scale for adequate effort during sperm collection. Laboratory technicians are more equipped for the work at hand when it is considered that the effort is aligned with procedural complexity. Furthermore, since testicular sperm motility is often stable after incubation in vitro for at least 24 hours following retrieval, the use of testicular sperm obtained prior to ICSI is now regularly done to ease the procedure schedule for both couples (28).

CONCLUSION

In spite of the fact that there is a wide range of careful sperm retrieval procedures, there is no proof that it is an ideal method for both OA and NOA. Generally, sperm production may be insufficient or focal in NOA, and it may be very difficult to find sperm compared to OA. There are not any randomized controlled studies in the literature that are contrasting the viability of sperm retrieval strategies for NOA. The patient's clinical circumstance ought to be founded on, as every technique has its strengths and restrictions. Since ICSI has not generally success, urologists need to perform dependable techniques that have lesser morbidity and acquire adequate sperm for different ICSI techniques. With a superior comprehension of the cryobiological behavior of sperm and the overall reproductive capacity of sperm from anatomical physical sources, sperm retrieval procedures can be optimized and individualized.

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Evaluation and Interpretation of Female Infertility

Kadın İnfertilitesinin Değerlendirilmesi ve Yorumlanması

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ABSTRACT

Infertility is a problem that affects one out of seven couples and is defined as the inability to achieve pregnancy despite unprotected intercourse for 1 year. One of the most important enemies of infertile patients is time. In order to reveal the possible causes of infertility, importance should be given to the systematic, rapid, and cost-effective evaluation, and the evaluation should be started with the least invasive examinations. In the evaluation of an infertile woman, a detailed medical history, reproductive history, family history, and physical examination are required to reveal the anatomical and physiological causes of infertility. This evaluation process is also the most suitable period for giving pre-pregnancy counseling, providing necessary counseling for preventive medicine practices, and conducting genetic research and consultations if necessary. In the evaluation of infertility, the presence of ovulation, the structure and function of the female genital system, and semen analysis in the male partner should also be evaluated. During all these evaluation processes, the psychological morbidity caused by the inability to have children should also be taken into consideration and the couples should also be supported in this respect. In this review, it was aimed to present the steps to be followed in the evaluation of an infertile woman in the light of current literature.
Keywords: Biochemical tests; genital system; imaging; infertility; ovulation.

ÖZ

İnfertilite, her yedi çiftten birisini etkileyen bir sorun olup 1 yıl boyunca korunmasız cinsel ilişkiye rağmen gebelik elde edilememesi olarak tanımlanır. İnfertil hastaların en önemli düşmanlarından birisi zamandır. İnfertilitenin olası nedenlerini ortaya çıkarmak için yapılacak değerlendirmenin sistematik, hızlı ve maliyet-etkin olmasına önem gösterilmeli ve değerlendirmeye en az invaziv olan tetkiklerle başlanmalıdır. İnfertil bir kadının değerlendirilmesinde, infertilitenin anatomik ve fizyolojik nedenlerini ortaya çıkarmak için ayrıntılı bir tıbbi öykü, üreme öyküsü, aile öyküsü ve fizik muayene gereklidir. Bu değerlendirme süreci aynı zamanda gebelik öncesi danışmanlık verilmesi, koruyucu hekimlik uygulamaları için gerekli danışmanlığın verilmesi, gerektiğinde genetik araştırma ve konsültasyonların yapılması için de en uygun dönemdir. İnfertilitenin değerlendirilmesinde ovülasyonun varlığı, kadın genital sisteminin yapısı ve işlevi ve aynı zamanda erkek partnerde semen analizi de değerlendirilmelidir. Tüm bu değerlendirme süreçleri boyunca çiftlerde çocuk sahibi olamamanın neden olduğu psikolojik morbidite de göz önüne alınmalı ve aynı zamanda çiftler bu açıdan da desteklenmelidir. Bu derlemede, infertil bir kadının değerlendirilmesinde izlenecek olan adımların güncel literatür bilgileri ışığında sunulması amaçlanmıştır.
Anahtar kelimeler: Biyokimyasal testler; genital sistem; görüntüleme; infertilite; ovulasyon.

INTRODUCTION

If pregnancy cannot be achieved despite regular sexual intercourse for 1 year in women younger than 35 years of age, and for 6 months in women over 35 years of age, infertility evaluations should be started. This period can be kept shorter than 6 months in women aged 40 and over, women with menstrual irregularities, or women with known risk factors for infertility, for example, those with endometriosis, those with genital system anomalies, those with a history of chemotherapy or radiotherapy, and those with a known male factor in their partner (1,2).

In the evaluation of an infertile woman, a detailed medical, reproductive history, family history, and physical examination are required to reveal the anatomical and physiological causes of infertility. This evaluation process is also the most suitable period for giving pre-pregnancy counseling, providing necessary counseling for preventive medicine practices, and conducting genetic research and consultations if necessary.

Inability to have children causes significant psychological morbidity for couples. The process, which includes making the diagnosis of infertility, and making the necessary evaluations and treatments, is also a very stressful process for infertile couples from a psychological point of view. When evaluating an infertile couple, clinicians should also consider the possibility that they may have mood states such as depression, anger, anxiety, and marital conflicts (2).

As with all diseases, the evaluation of an infertile woman begins as soon as the patient enters the examination room. The physician can obtain many clues from the patient's external appearance (short stature, extreme thinness, obesity, male pattern baldness, presence of acne, moon face, etc.), even from her speech (voice thickening due to virilization). Afterward, the evaluation continues with taking a detailed anamnesis of the patient and her family, performing a physical examination, and performing the necessary laboratory tests and imaging.

Anamnesis

As with all patients, a detailed anamnesis is one of the most basic parts of the examination in the evaluation of an infertile woman. The patient's age, how long the pregnancy is desired, the frequency of coitus, the drugs used (including those taken without a prescription), alcohol, smoking, history of illegal substance use, occupation, presence of stress, weight/diet changes, previous pregnancies, if any, should be questioned in detail. Low body weight, heavy exercise, presence of psychological stress may cause hypothalamic amenorrhea (3).

Menarche age, number of menstruation in a year, average menstrual cycle length, and whether menstrual cycles are painful should be questioned in the menstrual history of the patients. Regular cycles occurring at intervals of 21-35 days accompanied by moliminal symptoms (breast tenderness, ovulatory pain, a feeling of bloating in the body, etc.) show that the woman is ovulatory with a probability of 99.5% (4). The probability of sporadic anovulatory cycles in a regularly menstruating woman is very low (1-14%). Even in an ovulatory woman, slight shifts in cycle duration are normal (5). In a study involving more than 1000 cycles, 56% of women had a shift in menstrual cycle duration of >5 days over a 6-month period and 75% of women at 1-year follow-up (6).

A history of oligomenorrhea or amenorrhea is sufficient to clinically diagnose anovulation. The etiology of anovulation should be investigated in these patients, but tests to confirm ovulation are not required. In a meta-analysis of more than 12000 women, normal (28-31 days) or long (32-35 days) and short (21-27 days) menstrual cycle duration was shown to be associated with reduced ovarian reserve (7).

The presence of intermittent bleeding suggests problems such as polyps and chronic endometritis. Cervicitis, cervical polyps, and cervical dysplasias should be considered in postcoital bleeding. In heavy and/or prolonged menstrual bleeding, myomas, bleeding diathesis (Von Willebrand's disease, etc.), and adenomyosis are among the probable diagnoses. In the gynecological history, sexually transmitted infections, previous surgeries, history of pelvic inflammatory disease (PID), presence of abnormal pap smear, dyspareunia (pain during sexual intercourse), and pelvic/abdominal pain should be questioned. In the presence of severe dysmenorrhea, dyspareunia, and pelvic pain, endometriosis should be kept in mind. Ovarian reserve usually does not decrease in young women who have undergone a unilateral oophorectomy because there are many primordial follicles in the other ovary. However, unilateral oophorectomy in older women may cause a decrease in ovarian reserve (8).

The contraceptive methods they used before should be questioned in detail. For example, patients should be told that there may be a fertility delay of up to 18 months in the use of intramuscular depo medroxyprogesterone (9). It should be kept in mind that in infertile women with a history of using an intrauterine device (IUD) as a contraceptive method, IUD fragments may remain in the uterine cavity or that the patient may be misremembering that the IUD was removed although it had not been removed (10).

Symptoms of hyperandrogenism (hirsutism, male pattern hair loss, acne, virilization) should be questioned. These symptoms suggest polycystic ovary syndrome (PCOS). Weight gain and the presence of PCOS in the family should also be evaluated in favor of PCOS (11). It should be questioned how long hirsutism has existed. In the presence of severe hirsutism of sudden onset, androgen-producing tumors should be investigated. Congenital adrenal hyperplasia (CAH) may be partial and diagnosis may be delayed in patients with hyperandrogenism since CAH is not considered among the preliminary diagnoses (11).

Symptoms of thyroid diseases (weight gain, constipation, palpitations, heat/cold intolerance, etc.), the existence of galactorrhea, sexual dysfunctions, frequency, and timing of coitus should be questioned.

Family history should be questioned in detail, including individuals with infertility, birth defects, genetic mutations, and intellectual limitations. Premature ovarian insufficiency (POI) may develop in women with fragile X premutation, while male relatives of these women may have learning problems, developmental delay, or autism. Menarche history of family members and whether they have menstrual disorders should be questioned (3).

Physical Examination

Secondary sex characteristics of both partners should be evaluated. Insufficient development or absence of secondary sexual characteristics in women with primary

amenorrhoea suggests hypogonadotropic or hypergonadotropic hypogonadism. In patients with primary amenorrhea, physical examination findings such as short stature, square-shaped rib cage, and low hairline suggest Turner syndrome (3).

Visual field examination should be performed in cases with symptoms suggestive of headache, galactorrhea, or pituitary dysfunction. In the presence of hirsutism, modified Ferriman-Gallwey scoring should be done (3).

In the gynecological examination, adnexal tenderness, presence of mass, uterine mobility, shape, and size should be evaluated. Tenderness in the adnexal region or pouch of Douglas should suggest the presence of endometriosis or PID. The presence of fibroids can cause an increase in the size of the uterus and irregularity in its contours. The absence of uterine mobility should suggest the presence of pelvic adhesions (1).

The presence of vaginal and/or cervical anomalies in gynecological examination suggests Müllerian anomalies. Abnormal vaginal/cervical discharge may indicate infection. Chlamydia trochomatis and gonorrhea infections are among the common causes of tubal subfertility. Each acute episode of PID causes subfertility in 10-15% of cases (12).

The body mass index (BMI) of the patients should be calculated. Abnormalities in BMI may accompany problems such as hypothalamic amenorrhea, and PCOS hypogonadism. Obesity causes insulin resistance, a decrease in serum sex hormone binding globulin (SHBG) levels, and thus an increase in free androgen levels.

Ovulation

15% of all infertility causes and 40% of female infertility are due to ovulatory disorders. Although ovulation disorders mostly cause menstrual disorders such as oligomenorrhea/amenorrhea, they may rarely be present in women with regular menstruation. The underlying cause of the ovulation disorder should be investigated and treated if a specific cause is found. The most common causes of ovulation disorder include PCOS, obesity, perimenopause, weight changes, excessive exercise, thyroid dysfunction, and hyperprolactinemia (1).

Almost all women with regular cycles are ovulatory. About 60% of women with hyperandrogenism and regular cycles are ovulatory. The midluteal (1 week after ovulation or 7 days before the expected menstruation) serum progesterone value can be measured to indicate ovulation. Although a single serum progesterone measurement >30 nmol/L (>3 ng/ml) is generally used as an ovulation finding, ovulation may occur at lower values. A value of ≥15.9 nmol/L for serum progesterone has been shown to have 89.6% sensitivity and 98.8% specificity in detecting ovulation (13). Since serum progesterone levels fluctuate up to 7 times within hours, a single progesterone measurement should not be used to indicate the quality of the luteal phase, although it is used to indicate ovulation (14).

There are urinary luteinizing hormone (LH) detection kits produced to detect the mid-cycle LH peak (surge) occurring 1-2 days before ovulation and thus to indicate ovulation. Detection of urinary LH is an indirect indicator of ovulation (15). Urinary kit results show a good correlation with serum LH peak, especially when performed in the middle of the day or in the evening. In PCOS cases, the basal LH level is tonically high, which may cause false positive results in urinary kits. Ease of use, reliability, and the rate of false positive and false negative results may vary according to the kit brand used (16). The rate of false positivity and negativity in urinary kits is around 5-10% on average (1). In cases where no ovulation can be detected using a urinary kit at home, it may be useful to measure serum LH levels.

There is usually a biphasic body temperature pattern in ovulatory cycles and a monophasic temperature pattern in anovulatory cycles. Because the thermogenic effect of progesterone released from the corpus luteum with ovulation on the hypothalamus causes an increase in body temperature. Therefore, if the body temperature is regularly measured sublingually every morning from the first days of the menstrual cycle (while the body is in a basal state, not getting out of bed yet, not eating or drinking anything), an approximate increase in body temperature is detected during the luteal period (17). In a normal cycle, body temperature increases 1-2 days after the LH surge and this increase continues to be detected for at least 10 days. The 7 days before this increase is detected is the period when fertility is highest. However, since the increase in body temperature shows ovulation retrospectively, it is useless to determine the coitus time in women who want to become pregnant. In addition, sometimes the biphasic temperature pattern may not be detected in ovulatory cycles. The body temperature monitoring test is not a routinely recommended test for detecting ovulation (1).

Daily ultrasonographic follow-up (showing the disappearance of the growing follicle) or endometrial biopsy (demonstrating the secretory endometrium) to detect ovulation is not part of the routine evaluation because they are too expensive or invasive (18). In addition to detecting ovulation, endometrial biopsy has also been used in the past to demonstrate a luteal phase defect. However, it is no longer used for this purpose as it is an expensive, invasive procedure and is useless in evaluating endometrial receptivity (19).

Biochemical Tests

In all women with amenorrhea, pregnancy should be excluded by the serum beta human chorionic gonadotropin (beta-hCG) analysis. PCOS is usually associated with increased gonadotrophin releasing hormone (GnRH) pulsatility (increased serum LH level), while hypothalamic amenorrhea or hyperprolactinemia is associated with decreased GnRH pulsatility (decreased serum LH level) (11). While serum estradiol level is usually normal in PCOS cases, it may be decreased in hypothalamic amenorrhea, POI, and hyperprolactinemia cases. High serum androgen levels can be detected in association with PCOS or CAH (3).

If the total testosterone level is >200 ng/ml, computed tomography imaging of the ovaries and adrenal glands is recommended to exclude androgen-producing tumors (1).

In cases with hyperandrogenism, the level of 17-hydroxy progesterone should be measured in the early follicular phase of the menstrual cycle, and if it is found to be high (>200 ng/dl), the patient should be consulted with endocrinology for stimulation tests to exclude the diagnosis of non-classical 21 hydroxylase deficiency (3).

Serum prolactin level measurement is not routinely recommended in infertile patients. Serum prolactin level

should be measured in those with galactorrhea, amenorrhea, or oligomenorrhea. Serum prolactin level shows diurnal variation and reaches its highest level at night during sleep. Prolactin level can be measured at any time during the day. Slightly elevated prolactin levels (500-1000 mIU/L) may be due to reasons such as exercise, nipple stimulation, coitus, and stress related to venipuncture. Dopamine antagonist drugs (antiemetics, antipsychotics), dysfunctional pituitary adenomas causing disconnection hyperprolactinemia, and pituitary adenomas producing prolactin cause high prolactin levels. If the prolactin level is >1000 mIU/L in repeated measurements and if there are complaints of accompanying amenorrhea, galactorrhea, visual field disorders, and headache, patients should be consulted with endocrinology (20).

In cases with hyperprolactinemia, the elevation of macroprolactin (large aggregates of prolactin molecules in complex with antibodies), a type of prolactin with low bioactivity, should also be considered.

Measuring follicle stimulating hormone (FSH) and estradiol levels in women presenting with amenorrhea is useful in the differential diagnosis of women with ovarian failure (high FSH, low estradiol) from women with hypothalamic amenorrhea. Women mostly go through menopause between the ages of 45-55 (21). However, 1% of women enter menopause before the age of 40, and this is defined by the term 'POI'. POI is diagnosed in a woman younger than 40 years of age who presents with amenorrhoea, if the FSH level measured twice, at least 4 weeks apart, is >25 IU/L. The clinical course may be variable in POI cases according to natural menopause, and spontaneous pregnancies may occur in approximately 20% of cases. Therefore, it is more appropriate to use the term POI instead of early menopause when describing these cases. It is important to investigate the etiology in POI cases, including possible causes such as autoimmune causes, chromosomal abnormalities, and fragile x syndrome premutation. Oocyte donation can be offered as an option to women diagnosed with POI and wanting a child (22).

Cushing's syndrome may cause menstrual irregularities accompanied by weight gain, and hyperandrogenism. Therefore, if there are clinical signs suggesting Cushing's syndrome (red-purple striae, plethora, proximal muscle weakness, ecchymosis without trauma, unexplained osteoporosis, etc.) in patients presenting with menstrual cycle disorders, patients should be referred to endocrinology for further evaluation.

Overt thyroid dysfunctions can cause menstrual and ovulatory disorders and thus subfertility. However, The National Institute for Health and Care Excellence (NICE) recommends that thyroid function tests be performed only in women with symptoms of thyroid disease and not in all menstrual irregularities (23).

Ovarian Reserve Tests

A woman's age is one of the factors that most affect the risk of subfertility and the response to fertility treatments. Ovarian reserve reflects the number of oocytes in the ovary and is an indicator of a woman's fertility potential. Decreased ovarian reserve indicates that a woman who is in the reproductive period and who has regular menstruation will have a lower response to ovarian stimulation compared to her peers. However, female age is the single most important determinant of fecundity and is more valuable than ovarian reserve markers in predicting fertility-related clinical outcomes such as 'time to conception' and 'at what age to enter menopause'. Therefore, ovarian reserve markers should not be used to determine a patient's likelihood of spontaneous conception (24).

If a decreased ovarian reserve is detected in the evaluations, this does not mean that the woman cannot conceive or is subfertile. Ovarian reserve markers are useful in estimating the number of follicles that will respond to gonadotropin stimulation and the number of oocytes to be collected in in vitro fertilization (IVF) treatments. For this reason, evaluation of ovarian reserve markers before IVF treatments may be useful in adjusting the gonadotropin dose in patients who are predicted to have an excessive or low response, and in counseling patients to have realistic expectations from treatment (25). While interpreting the results of all ovarian reserve tests, the patient's age, risk factors, previous treatments, and the patient's response to reproductive treatments should also be considered. Ovarian reserve tests are not helpful in fertile women and should not be used for the purpose of routine screening.

Ovarian reserve tests include both biochemical analyzes and ultrasonographic imaging of the ovary. Total antral follicle count (AFC), 2-10 mm in size, in the ovaries in ultrasonography (USG) should be done in the follicular phase. Although it is not a definitive criterion accepted all over the world, AFC <4 may be useful in predicting low response and AFC >16 in predicting excessive response.

Anti-mullerian hormone (AMH) is a glycoprotein that is a member of the TGF-beta family and is secreted by small antral (<8 mm) and preantral follicles. During the menstrual cycle, there is minimal fluctuation in serum AMH level. Therefore, serum AMH level can be measured on any day of the menstrual cycle (24). When different kits are used in AMH measurements, different results can be obtained (26). Thanks to the developments in the kits used for measurement over the years, serum AMH level measurements have become more reliable.

In IVF treatments, AMH level correlates with the number of eggs to be collected and is the biomarker that best predicts poor or excessive response. However, because of its low diagnostic sensitivity in predicting live birth, low AMH values should not be used as a criterion for not accepting patients for IVF treatment (27). Although there is no definitively accepted international threshold value, NICE states that <5.4 pmol/L (Beckman Coulter generation II assay) can predict low ovarian response, and a value >25 pmol/L can predict excessive response (23).

In some patient groups, such as infertile patients, cancer patients, and patients with significant ovarian damage due to radiotherapy or surgery, serum AMH levels may be useful to detect a decrease in the ovarian follicle pool (28). However, in women without infertility problems, serum AMH levels are not useful in predicting future fertility potential, time to conception, or age at menopause (29).

The serum AMH level is increased in PCOS cases and has the potential to be used as a diagnostic criterion for PCOS in the future, although not yet (30).

Although measurement errors may occur for technical reasons in both serum AMH level and total AFC measurement, these two indicators correlate well with each

other and can be used separately or in combination for ovarian reserve (24).

In the guideline of the American Society for Reproductive Medicine (ASRM), AMH, AFC, basal FSH, and estradiol measurements are recommended as ovarian reserve markers. Increased serum FSH (>8.9 IU/L) level in the early follicular phase indicates decreased ovarian reserve (1). NICE guidelines do not recommend using markers such as ovarian volume, ovarian blood flow, serum estradiol level, serum inhibin B level, or clomiphene citrate challenge test to predict fertility treatment outcomes (23).

Imaging

USG is useful in the evaluation of uterine/ovarian anatomy and morphology and AFC. Uterine abnormalities can be detected in 16.2% (13% polyps, 2.8% submucous fibroids, 0.3% adhesions) of patients who applied for infertility evaluation (31). The incidence of these uterine abnormalities increases to 39.6% in those with abnormal uterine bleeding. Therefore, it is important to evaluate infertile cases with USG. Transvaginal USG (TVUSG) is valuable in the diagnosis of pathologies such as fibroids, polyps, and adenomyosis that play a role in infertility. Since intramural fibroids cannot be seen in hysteroscopy (HS) or hysterosalpingography (HSG), USG is used for diagnosis.

HSG or hystero contrast sonography (HyCoSy), sonohysterography (SHG), and laparoscopic chromopertubation (gold standard) can be used to evaluate tubal patency.

HSG is valuable in terms of showing whether the occlusion in the tuba is proximal or distal, giving an idea not only about the patency of the tuba but also its structure. When performing HSG, the delayed transmission of contrast medium suggests fimbrial phimosis or loculation of contrast medium at the tip of the tubal suggests peritubal adhesions (1). HSG has a sensitivity and specificity of 65% and 83%, respectively, in demonstrating tubal patency. HSG is more successful in demonstrating distal tubal occlusion than proximal occlusion (32). If a proximal occlusion is seen in both tubes, the possibility of transient tubal/myometrial contraction or displacements in catheter position should be considered rather than a true occlusion, and additional tests (eg, repeat HSG withdrawal, fluoroscopic/hysteroscopic selective tubal perfusion, laparoscopic chromopertubation) should be considered to see if there is a true occlusion (1).

HSG may also be useful in the diagnosis of congenital uterine anomalies as it shows the size and shape of the uterine cavity. However, HSG is not sufficient in the differential diagnosis of the septate uterus and bicornuate uterus. Magnetic resonance imaging or three-dimensional USG can be used for this distinction. Although HSG is useful in the evaluation of polyps and submucous fibroids extending into the cavity, it has low sensitivity (50%) and a low (30%) positive predictive value (33). The fact that the patient is given X-rays is among the disadvantages of HSG.

Diagnostic HSG also has a therapeutic effect. In a systematic review including 12 randomized studies, it was shown that the pregnancy rate in subfertile cases who underwent HSG with oil-based media was 3.3 times higher than in those who did not have HSG. That increase in pregnancy rates was found to be similar if the medium used was water-based or oil-based (odds ratio, OR: 1.21, 95% confidence interval, CI: 0.95-1.54) (34).

HyCoSy is based on the principle of imaging the contrast agent (often a contrast agent containing air bubbles) introduced into the uterine cavity with a transcervical catheter, accompanied by USG. Compared to the standard HSG, the experience of the person performing the HyCoSy is more important. HyCoSy has a sensitivity of 76-96% and a specificity of 67-100% in demonstrating tubal patency and has >%90 accuracy for evaluation of uterine cavity when compared with HS. (35). In a systematic review published in 2014, HSG and HyCoSy were shown to have similar diagnostic values in demonstrating tubal occlusion in subfertile women (35).

Demonstrating tubal patency with HS is a newly used method. In a meta-analysis including 6 studies, the sensitivity and specificity of HS in evaluating tubal patency were reported as 88% and 85%, respectively (1).

In SHG, saline is administered into the uterine cavity with the help of a catheter. SHG is useful in demonstrating the shape and size of the uterine cavity and shows that at least one tuba is open (not which tuba is open) by monitoring fluid collected in the Douglas pouch. SHG has a high (>90%) positive and negative predictive value in the detection of intrauterine pathologies, synechia, polyps, and submucous myomas (33).

Although laparoscopy is the gold standard for the evaluation of tubal patency, it is not a routinely used method in infertility evaluation. However, if laparoscopy is to be performed for another indication, the patency of the tuba can be shown by chromopertubation performed by giving diluted methylene blue or indigo carmine dye from the cervical canal, or if there is tubal obstruction, the location of this obstruction (proximal or distal segment) can be determined. Laparoscopy also allows the diagnosis and treatment of fimbrial phimosis or peritubal adhesions because these problems may not be detected by methods such as HSG or SHG. If HSG or laparoscopy findings suggest proximal tubal occlusion, fluoroscopic/hysteroscopic elective tubal cannulation is performed to confirm the diagnosis and provide tubal recanalization (1).

Some clinics request a chlamydia antibody test (CAT) in the first step when evaluating tubal patency. If the antibody is positive, it suggests a tubal pathology but does not provide a prediction of whether the tuba is open or not. For the diagnosis of distal tubal disease, CAT has an average sensitivity (40-50%) and positive predictive value (60%), while its negative predictive value is quite high. Therefore, a positive CAT requires additional evaluation for tubal evaluation, while a negative CAT suggests no tubal damage (1).

HS is the gold standard method for the diagnosis and treatment of intrauterine pathologies. However, when performed alone, HS does not provide any information about the myometrium, fallopian tubes, and adnexal structures. Operative HS is a more expensive and invasive method than HSG or SHG. The use of office HS, which has a smaller diameter compared to operative HS, is more comfortable for the patient, has a lower cost, and allows the treatment of some intrauterine pathologies. If imaging of the tuba is not required, SHG or office HS will be sufficient for imaging the cavity only (1).

Evaluation of Cervical Factor

Evaluation of the cervical canal in a gynecological examination is important in detecting pathologies such as cervical stenosis and chronic cervicitis. Infectious cervicitis should be treated. It is very unlikely that abnormalities in cervical mucus production alone are a cause of infertility. A sperm mucus penetration test or postcoital test is not recommended for infertility evaluation. The postcoital test is a historical test used in the diagnosis of cervical-factor infertility, based on the principle of microscopic examination of the cervical mucus sample and detection of motile sperm in the first hours after coitus that occurs before the expected ovulation. Postcoital testing is no longer recommended in the evaluation of an infertile woman since the postcoital test is subjective, has low reproducibility, low patient compliance, does not change our clinical management, and does not predict the potential for conception (36).

Evaluation of Peritoneal Factors

Peritoneal factors due to endometriosis, and pelvic/adnexal adhesions may cause or contribute to infertility. The patient's history and/or physical examination findings may give an idea of peritoneal factors, but they are not sufficient for diagnosis. If no reason was found to explain infertility in the evaluation of women, peritoneal factors should be considered. The definitive diagnosis of peritoneal factors can be made by laparoscopy. Mild or minimal endometriosis has little effect on fertility. Most women with severe adnexal adhesions have data suggesting the presence of these adhesions either in the history (pelvic pain, presence of endometrioma on ultrasound, dyspareunia, previous pelvic surgery or infection), or in HSG findings.

As a result, laparoscopy is not routinely recommended in infertile women unless there are findings suggestive of pelvic pathology (37).

Karyotyping

Karyotyping should be requested in women with primary amenorrhea due to hypergonadotropic hypogonorrhea and/or with the phenotypic features of Turner syndrome, in women with ambiguous external genitalia, and in women with a presumptive diagnosis of androgen insensitivity syndrome. Karyotyping and fragile X premutation tests should be requested in women with a personal or family history of early (<40 years) ovarian failure.

If the male partner has severe oligospermia or azoospermia, karyotyping and additional Y chromosome microdeletion tests should be recommended.

If there is a history of recurrent pregnancy loss, karyotyping of both partners should be performed (3).

Antibody Tests

Antiphospholipid, antisperm, antinuclear, and antithyroid antibodies are not required in routine infertility evaluation. It is recommended to evaluate women with recurrent pregnancy loss in terms of antiphospholipid syndrome (38).

Mycoplasma Culture

Routine culture of ureaplasma urealiticum and mycoplasma hominis is not recommended in the evaluation of infertile women because these organisms have not been shown to have a significant role in female infertility (39).

CONCLUSION

Female fertility declines with advancing age, and the single most important predictor of fecundity is age. A detailed medical, reproductive anamnesis, family history, and physical examination are necessary to reveal the anatomical and physiological causes of infertility. Infertility evaluations should be started after 12 months in women <35 years old, after 6 months in women aged ≥35 years, and immediately in women aged >40 years. In the evaluation of infertility, the presence of ovulation, the structure and function of the female genital system, and semen analysis in the male partner should be evaluated. HSG or SHG may be preferred for the evaluation of tubal patency. In cases without hirsutism, the presence of regular menstrual cycles at intervals of 21-35 days is sufficient to indicate ovulation. Ovarian reserve tests should not be used for screening in non-infertile women. Laparoscopy, advanced sperm function testing, postcoital testing, thrombophilia testing, immunologic testing, karyotype, endometrial biopsy, and serum prolactin have no place in the routine evaluation of infertile women.

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Current Approach to Genetic Causes of Female Infertility and Genetic Counseling

Kadın İnfertilitesinin Genetik Nedenlerine Güncel Yaklaşım ve Genetik Danışmanlık

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ABSTRACT

Infertility is a disease of the male or female reproductive system and is defined as the inability to achieve pregnancy after 12 months or more of regular and unprotected sexual intercourse. Data shows that more than 186 million people worldwide are infertile. About 10% of the women of reproductive age are unable to conceive or maintain a pregnancy. In this study, the causes of female infertility were reviewed under several headings and the importance of genetic counseling in infertility was also mentioned. There are many different causes of female infertility, including both genetic and non-genetic causes. In this review, current developments and approaches in the genetic etiology of female infertility were reviewed under six main headings, chromosomal abnormalities, female genital system disorders, hypogonadotropic hypogonadism, primary ovarian failure, polycystic ovary syndrome, and gonadal dysgenesis. Also, the role of genetic counseling in these diseases was discussed. The aim of genetic counseling is to inform people with a hereditary disease or at high risk of carrying it about the course of the disease and treatment methods, and also to guide future generations and family members about their risks. After all tests and examinations, genetic counseling has a very important place in reproductive health.
Keywords: Female; genetics; infertility; genetic counseling.

ÖZ

İnfertilite, erkek veya kadın üreme sisteminin bir hastalığı olup, 12 ay veya daha uzun süre düzenli ve korunmasız cinsel ilişkiden sonra gebelik elde edilememesi olarak tanımlanır. Veriler, dünya çapında 186 milyondan fazla insanın infertil olduğunu göstermektedir. Üreme çağındaki kadınların yaklaşık %10'u gebe kalamaz veya hamileliğini sürdüremez. Bu çalışmada kadın infertilitesinin nedenleri çeşitli başlıklar altında incelenmiş ve ayrıca infertilitede genetik danışmanlığın önemine de değinilmiştir. Kadın infertilitesinin hem genetik ve hem de genetik olmayan nedenler de dahil olmak üzere birçok farklı nedeni vardır. Bu derlemede kadın infertilitesinin genetik etiyolojisindeki güncel gelişmeler ve yaklaşımlar, kromozom anomalileri, kadın genital sistem bozuklukları, hipogonadotropik hipogonadizm, primer over yetmezliği, polikistik over sendromu ve gonadal disgenezi olmak üzere altı ana başlık altında incelenmiştir. Ayrıca bu hastalıklarda genetik danışmanlığın rolü de tartışılmıştır. Genetik danışmanlığın amacı, kalıtsal bir hastalığı olan veya taşıyıcı olma riski yüksek olan kişileri hastalığın seyri ve tedavi yöntemleri hakkında bilgilendirmek, aynı zamanda gelecek nesillere ve aile bireylerine de riskleri konusunda rehberlik etmektir. Tüm test ve tetkiklerden sonra, üreme sağlığında genetik danışmanlık çok önemli bir yere sahiptir.
Anahtar kelimeler: Kadın; genetik; infertilite; genetik danışmanlık.

INTRODUCTION

Infertility is a disease of the male or female reproductive system and is defined as the inability to achieve pregnancy after 12 months or more of regular and unprotected sexual intercourse (1). Infertility has been an important medical, religious, political, and social problem since the existence of humanity. Data show that 48 million couples and 186 million people worldwide have infertility. About 10% of women of reproductive age are unable to conceive or maintain a pregnancy to term (2). Although infertility is a disease of both gender, most fertility tests and treatments have been applied to females due to infertility has been almost synonymous with women for centuries (3). Only women were blamed for not being able to have children and especially in societies where children are emotionally valued, this has always created a feeling of shame or guilt in women due to reproductive failure and it has been very rare for males to be cited as the reason. Therefore, significant efforts have been made to achieve pregnancy, and the female body has always been the center of attention (4).

Genetic counseling is an educational process that aims to provide patients and their relatives at risk of development with recent genomic information about the consequences of the disease, the risk of developing the disease, the preventability of this risk, and the methods of prevention or treatment (5).

In this study, we gathered the causes of female infertility under several headings and mentioned the importance of genetic counseling in infertility.

The causes of infertility have been investigated for centuries by learning the physiology and pathology of the female reproductive system. Embryology of the female reproductive system and oogenesis should be reviewed in order to understand the cause of infertility.

EMBRYOLOGY OF THE FEMALE REPRODUCTIVE SYSTEM DEVELOPMENT

The chromosome effect on the developing gonad is uncertain, regardless of whether it is XX or XY up to the sixth week of gonadal development; this stage is also known as the indifferent stage. By the sixth week of development, primordial germ cells develop into the epiblast migrate from their previous extraembryonic position to the lateral part of the genital ridges, and are surrounded by sex cords to form a pair of primitive gonads there. Since females do not have a Y chromosome, the gonad begins to develop in the direction of the ovary in a process that starts in the eighth week of pregnancy and continues for a few weeks. During this period, the cortex develops, the medullary structure shows regression, and the oogonia begin to develop into the follicular cell. About the third month of intrauterine development, oogonia enter meiosis I and remains in this stage until puberty.

The absence of testis and Y chromosome in the female embryo results in the absence of Anti-Müllerian hormone (AMH) and thus, the Müllerian duct develops from the nephrogenic ridge lateral to the genital protrusions. This canal gives the beginning of the fallopian tubes, the uterus, and the upper third of the vagina. The lower 2/3 of the vagina develops from synovaginal bulbs. In the absence of a testicle (or more specifically, in the absence of androgens), the female external genitalia (clitoris, labia major, and labia minora) develop from the genital tubercle regardless of the presence or absence of an ovary (6,7).

Oogenesis

Gametes originate from primitive germ cells that form in the epiblast during the second week of pregnancy and migrate to the yolk sac. Oogenesis is the process of transforming oogonia into mature oocytes, which can be examined in two parts, prenatal and postnatal (8).

Prenatal Period

Oogonia are reproduced continuously by mitosis in the gonadal prominences. Meanwhile, some of them enter the 1st meiotic division; remain in the prophase, and develop into primary oocytes. The oocytes are surrounded by squamous epithelial cells and are defined as primordial follicles. A newborn female has primordial follicles and primary oocytes. All of these oocytes remain in the prophase of the first meiosis and do not complete the first meiosis until puberty. They remain at this stage due to the oocyte maturation inhibitory (OMI) factor, which is secreted from the follicle epithelial cells. By puberty, some of these follicles undergo atresia and only 400,000 of them reach puberty.

Postnatal Period

Approximately 15-20 of the primordial follicles that reach puberty begin to mature in each ovarian cycle under the influence of follicle stimulating hormone (FSH) and form the primary follicle. Usually, only one of the primary follicles develops into a mature follicle. In the process of maturation, it goes through the secondary and tertiary follicle stages, respectively. The oocyte is expelled by ovulation as a tertiary follicle (Graaf's follicle). The secondary oocyte which completes the first meiosis just before ovulation, begins the second meiotic division and remains at the metaphase. The oocyte completes the second meiosis only if fertilization occurs. A menstrual cycle is crucial for human reproduction as it is required for oocyte selection, maturation, and ovulation in preparation for fertilization and subsequent pregnancy. The median menstrual cycle has two distinct ovarian phases; the follicular and luteal phases that are separated by ovulation. After these consecutive stages, fertilization usually takes place in the ampulla of the fallopian tubes (9).

CAUSES OF FEMALE INFERTILITY

There are many distinct causes of female infertility, including both genetic and non-genetic reasons. In this review, current developments and approaches in the genetic etiology of female infertility are reviewed in six main headings, which are: chromosomal abnormalities, female genital system disorders, hypogonadotropic hypogonadism, primary ovarian failure, polycystic ovary syndrome (PCOS), and gonadal dysgenesis. Chromosomal abnormalities are the most common disorders among the genetic causes that cause infertility.

Chromosomal Abnormalities

The frequency of chromosomal abnormalities, including chromosomal polymorphisms, is relatively high, which has been found in 1.3-15.0% of couples failing to conceive. The incidence of chromosomal anomalies in women is 10.0% (10). Since ova are prone to genetic changes due to aging, the risk of chromosomal abnormality

increases with maternal age. Besides aging, environmental factors and lifestyle can also affect the pathogenesis of chromosomal abnormalities (11).

Chromosomal abnormalities are classified as numerical and structural, and numerical abnormalities are the most common chromosomal abnormalities which are divided into two groups, aneuploidy, and polyploidy. Chromosomal aneuploidy is the most common identified cause of spontaneous abortion and developmental errors in humans. Aneuploidies generally occur during oogenesis and are generally subdivided into two groups, trisomy, and monosomy (11).

Triple X syndrome (47,XXX) is a sex chromosome aneuploidy which is the most common chromosomal abnormality in females with an additional X chromosome. It is usually not inherited and is mainly caused by the non-segregation of chromosomes during maternal meiosis. However, an additional X chromosome, seen in almost 20% of cases and caused by post-zygotic segregation, is seen in only some cells of the affected individuals, resulting in 46,XX/47,XXX mosaicism. Therefore, mosaic cases are usually fertile with milder clinical manifestations and have offspring with normal chromosome numbers. Females with triple X syndrome have a rapid increase in height until puberty due to the extra *SHOX* gene. In some cases, primary infertility is accompanied by tall stature, congenital urogenital anomalies, premature ovarian insufficiency (POI), amenorrhea, and early menopause (11).

Turner syndrome (TS), also known as monosomy X, occurs when the X chromosome is partially or completely missed in females. Its main clinical manifestations include growth disorders, reproductive system abnormalities, cardiovascular abnormalities, and autoimmune diseases (12). Females with TS have an extremely high risk for POI and infertility. Although approximately 70-80% of affected individuals do not have spontaneous pubertal development and 90% have primary amenorrhea, the remaining individuals may have a small remnant of ovarian follicles at birth or in early childhood (13). Various karyotype findings are seen in cytogenetic analyzes performed in TS cases. About 40-50% of affected females have the karyotype 45,X; 15-25% have mosaicism (45,X/46,XX); 20% have isochromosomes, and a small percentage of them have ring X chromosomes. In addition, 10-12% of women also have varying amounts of Y chromosome material (14).

Structural chromosomal abnormalities are classified into two groups: balanced and unbalanced abnormalities. Since there is no segment loss in balanced chromosomal abnormalities, it does not cause a phenotypical change or disease at the balanced abnormality carrier. However, individuals with this balanced chromosomal abnormality can form unstable gametes due to segmental loss or gains and cause disease in subsequent generations (14).

Chromosome deletion is an abnormality in which a segment of the chromosome is deleted. The length of the deleted region affects the number of genes deleted and the severity of the predicted phenotype. Chromosomal deletions affecting the sex chromosomes will most likely impair reproductive development. Deletion in X chromosomes can cause defective chromosomal synapses, meiotic arrest, as well as POI, gonadal dysgenesis, and infertility (14).

Chromosomal duplications occur when a region of the chromosome is duplicated. As the gene dose increases in the affected area, the amount of protein also produced increases, and the increased protein dose may cause toxic effects. Because embryogenesis is controlled by balanced protein levels, additional gene copies can impair gametogenesis and fetal development (15).

The ring chromosome is formed by breaking both ends of the chromosomes and joining these broken ends. It is rarely inherited and is usually lost during cell division. However, if passed on to the next generation, it can form new rings that coexist with the normal cell line in the offspring. This can result in a mosaic karyotype. Women with ring chromosome may have subfertility and low ovarian reserve (15).

Female Genital System Disorders

The mammalian female reproductive system; fallopian tubes, uterus, cervix, and upper part of the vagina develop from the Mullerian ducts. Defects during the development of Mullerian structures can lead to varying severity of congenital defects in the female genital tract (2).

Abnormalities in the female reproductive system can be classified as agenesis, atresia and embryologically abnormal septation of the fallopian tubes, uterus, cervix, or vagina originating from the Mullerian duct. A few genes are well defined, which affect the development of the Mullerian duct (2).

Heterozygous sequence variants, deletions and expansions in the polyadenosine tail of the *HOXA13* gene are well-known causes of the hand-foot-genital syndrome, which is characterized by a fusion of Mullerian structure and uterine malformations with extremity anomalies (16).

Abnormal expression of HOXA11-AS1 antisense RNA (a long non-coding RNA) and rare variants in other Homeobox A family genes (*HOXA10* and *HOXA11*) have been identified in individuals with sporadic uterine malformations, and in infertile women with endometriosis (2).

Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome, also called Mullerian agenesis, is the second most common cause of primary amenorrhea. It is characterized by congenital absence of the uterus, cervix, and upper part of the vagina in phenotypically normal 46,XX females. The incidence of MRKH syndrome is approximately 1 in 4,500-5,000 newborn females and is generally divided into two subtypes: MRKH type 1, in which only the upper vagina, cervix, and uterus are affected and MRKH type 2, which is associated with comorbidities. MRKH type 2 usually includes Mullerian renal cervical somite (MURCS), characterized by cervix-thoracic defects as well as malformations affecting the kidney and skeletal systems. The association of abnormalities in Mullerian duct development with defects in other organs indicates disruption of pathways involved in the embryonic development of structures derived from the intermediate mesoderm (17).

The etiology of MRKH syndrome is unclear and conflicting; most cases are sporadic, however, several reports of familial clustering suggest a genetic cause (17). These cases of familial clustering appear to occur by autosomal dominant inheritance with incomplete penetrance and variable expressivity. Investigations based

on these cases have subsequently been directed to certain candidate genes (18). Defects in the *LHX1*, *HNF1β*, *TBX6*, *HOX* (Hoxa9-13 and Hoxb9-13), *SHOX*, *WNT* (*WNT4*, *WNT9B*, and *WNT7*), *MMP14*, and *LRP10* genes are some of the possible causes (17). *WNT4* mutations are associated with Mullerian aplasia, hyperandrogenism, and renal malformations due to failure to suppress androgens in the ovary (18). Mutations in *HNF1β* (also known as *TCF2*) have been associated with maturity-onset diabetes of the young renal dysfunction and Mullerian aplasia (18). *WNT7A* mutations have recently been recognized as causing Al-Awadi/Raas-Rothschild/Schinzel phocomelia syndrome; which is characterized by several limb deformities beside the uterine hypoplasias/aplasia (18).

Nik-Zainal et al. (19) identified three microdeletions at 16p11.2, 17q12, and 22q11.2 that were significantly more frequent in the syndromic Mullerian aplasia case population compared to a healthy control group. *TBX6*, an important gene in paraxial mesoderm development, is located on 16p11.2, while 17q12 covers the genes *HNF1β* and *LHX1*.

Endometriosis is another anatomical cause of female infertility. In a study conducted in Japan, it was shown that the rs10965235 SNP of the *CDKN2B-AS* gene may be associated with the development of endometriosis. Hypermethylation of the promoter region of the gene may also be associated with endometriosis (20). In addition *WNT4*, *NFE2L3*, *HOXA10*, growth-regulating estrogen receptor binding 1 (*GREB1*), *ID4*, and *VEZT* genes are associated with endometriosis (20-22). In addition, *AMH* gene polymorphism has been associated with endometriosis-associated infertility (23).

Hypogonadotropic Hypogonadism

Hypogonadotropic hypogonadism is a rare disease characterized by a decrease in the release of gonadotrophin releasing hormone (GnRH) or disruption of its function (24,25). This disease is often manifested by underdevelopment of puberty and infertility. Hypogonadotropic hypogonadism is a genetic heterogeneous disease with more than 25 identified genes, which has both sporadic and familial cases. The most commonly affected genes are *ANOS1* (*KAL1*), *SOX10*, *IL17RD*, *FGFR1*, *CHD7*, *PROKR2*, *GHRHR*, *FGF8*, and *WDR11* (24). Kallmann syndrome includes a significant part of patients with hypogonadotropic hypogonadism with anosmia, a characteristic feature (24,26). The presence of anosmia should alert clinicians to possible congenital hypogonadotropic hypogonadism (CHH), and molecular genetic testing in affected patients may facilitate diagnosis (24).

Primary Ovarian Failure

Primary ovarian failure, known as early ovarian failure or early menopause, is defined as the termination of menstruation before the expected age of menopause. The expected age of menopause is defined as before the age of 40, and the diagnosis is confirmed by a high level of FSH. About 1% of women are affected by POI before the age of 40, and 0.1% before the age of 30 (27,28). Necessary tests should be performed for TS, triple X, and Fragile X premutation carriage, which are the genetic conditions that most often cause this condition in a patient with primary ovarian failure (29).

FMR1 is an mRNA-binding protein that regulates translation. CGG premutation at 5'UTR of the *FMR1* gene increases mRNA expression in females, and the *FMR1* protein is overexpressed in neuron and granulosa cells, which leads to premature ovarian failure and infertility. *FMR1* premutation carriage frequency is 1/150-300 (24), while *FMR1* premutation carriage is detected in 2% to 15% of women with isolated POI, and in 14% to 20% of women with familial POI (29).

Triple X, the other sex chromosome aneuploidy that causes POI, is a chromosomal anomaly that occurs in about one of 1,000 females due to maternal meiosis nondisjunction error, which advanced maternal age is an important risk factor (29). Although there are no structural and functional pathologies in the reproductive system, POI is frequently observed in triple X patients (30). It is thought that genes that escape from X inactivation in patients with triple X syndrome may cause POI (31). Although most patients with triple X syndrome can achieve pregnancy, the prevalence of sex chromosome anomaly, genitourinary system anomaly, neural tube defects, and cardiac malformation is high compared to the healthy population (31,32).

There are many examples of other syndromic diseases that occur with POI, such as galactosemia, Perrault syndrome, ataxia telangiectasia, and McKusick-Kaufman syndrome. Also, many studies have shown that DNA repair genes are associated with follicle maturation-follicle quality, reproductive aging, and the age of onset of menopause. Fanconi anemia, Warsaw fracture syndrome, ataxia telangiectasia, and xeroderma pigmentosum are examples (24,33,34).

Polycystic Ovary Syndrome

PCOS is an endocrinopathy characterized by increased ovarian androgen biosynthesis, anovulation, and infertility (34,35). It is the most common form of female infertility, affecting about 10% of women of reproductive age. It is associated with high pregnancy loss rates despite low pregnancy rates (33). PCOS has a significant hereditary component based on familial clustering and twin studies. Studies have shown that the risk of developing PCOS in daughters of women with PCOS is five times higher than in a healthy society (36). Similarly, it has been observed that PCOS disease develops in approximately 60-70% of the daughters of women with PCOS (37). PCOS-related candidate genes (*DENND1A*, *LHCGR*, *FSHR*, *ZNF217*, *YAP1*, *INSR*, *RAB5B*, *C9orf3*) have been identified via genome-wide association studies. Some recent studies about the expression of miRNAs have shown that there is a difference between women whit PCOS and healthy women, and the plasma miRNA may play an important role in the formation and development of PCOS (34).

Gonadal Dysgenesis

Gonadal dysgenesis is a genetic defect that causes a complete or partial loss of gonadal development during fertilization or the early embryonic development period (34). 46,XY complete gonadal dysgenesis (Swyer's syndrome) is a sexual differentiation disorder with a female phenotype. The vagina, uterus, and fallopian tube are developed in the female gender in a hypoplaztic manner. The gonads consist of fibrous stroma, also usually there is no breast development (37,38). The phenotypic

difference between complete and incomplete gonadal dysgenesis depends on the level of differentiation of the testicular tissue and the production of testosterone and AMH by the fetal testicle (37).

The *SRY* gene encodes the testis specific testis determining factor (TDF), which plays an important role in sexual differentiation and development in males. About 15% of Swyer's syndrome has an *SRY* gene mutation associated with the high mobility group (HMG) box on the Y chromosome (34,37). It is thought that Y chromosome structural changes and *DHH*, *MAP3*, *DEC1*, *SOX9*, *GATA4*, *AR*, *DMRT1*, *DMRT2*, *NROB1*, *FOG2*, *WT1*, *NR5A1* genes may be associated with other causes of the syndrome (37,38).

WHAT IS THE ROLE OF GENETIC COUNSELING IN FEMALE INFERTILITY?

The aim of genetic counseling is to provide information about the progress of the disease and treatment methods for people with a hereditary disease or at high risk of carrying it, as well as to guide the next generation and family members about the risks of recurrence through to anamnesis and pedigree analysis. The purpose of a medical geneticist is to ensure the understanding of the information about the disease by the family accurately and completely and to offer solutions. In reproductive health, the most important indications for genetic counseling are infertility and recurrent pregnancy losses such as stillbirths, miscarriages, and premature infant deaths. Advanced maternal age due to a higher risk of aneuploidy, neural tube defects, the presence of ultrasound-identified soft markers, and elevated risk in maternal serum screening are the other main indication for genetic testing for reproductive genetics. Also, being a carrier of a certain genetic condition (for example, balanced chromosomal rearrangements), an affected first child or family history of mental retardation, chromosomal abnormalities, congenital malformations such as cleft palate, neural tube defects, or congenital heart defects; single-gene disorders, subfertility or a wide range of different genetic disorders should be considered as important indications for genetic counseling. Premarital counseling or preconception counseling is recommended for couples with a higher probability of genetic disorders, especially for consanguineous couples (39).

Karyotype analysis is always recommended as a first step genetic test when considering that chromosomal disorders significantly affect fertility and miscarriage risk. Patients with reciprocal translocations or any other structural chromosomal abnormality have a significantly increased risk of infertility, including primary or secondary amenorrhoea or hypogonadotropic hypogonadism with oligomenorrhea. Balanced rearrangements do not cause health problems for their carriers as they do not cause loss or duplication of genetic material, but can lead to gametes in which genetic information is unbalanced, thus causing infertility or multiple miscarriages (39). Some abnormalities, such as the triple X karyotype, could not be clearly associated with infertility. The primary cause of fertility loss in individuals with TS is thought to be accelerated germ cell loss and impaired folliculogenesis in fetal life.

However, depending on the percentage of cells with 46,XX karyotype remaining in the ovaries, although spontaneous puberty and pregnancy may occur in some individuals, pregnancy cannot be completed in these individuals. This situation is due to the insufficiency of the endometrium and uterus (40).

Unlike male infertility, little is known about the genetic basis of female infertility. Therefore, first tier tests are recommended to determine chromosomal disorders; and specific or single-gene tests are less approved in infertile women. Isolated infertility due to genetic causes is rare; more commonly, syndromic diseases cause female infertility. Currently, genetic tests are mainly used for patients with POI, chromosomal abnormalities, and *FMR1* premutation carriers (41).

Females with a normal karyotype produce a variable percentage of oocytes with chromosomal abnormalities because of crossing-over and/or meiotic nondisjunction errors. The three main groups of abnormalities are 45X, trisomy, and polyploidy. These abnormalities are associated with advanced maternal age. While applying assisted reproductive technology (ART), gametes or embryos are analyzed with preimplantation genetic testing (PGT), and healthy gametes or embryos can be obtained. Aneuploid embryos need to be screened, and only euploid embryos are transferred to increase the chances of healthy pregnancies (41).

MRKH syndrome is considered a multifactorial condition caused by both genetic and environmental factors during embryonic development, resulting in a range of phenotypes and severities. Most studies have been conducted in small groups without analyzing unaffected relatives. Ultimately the etiology of MRKH syndrome has not been clearly elucidated, and it is difficult to demonstrate the roles of identified candidate gene variants in impairing urogenital development or differentiation. For the individual affected by MRKH syndrome and their relatives, a multidisciplinary approach is required. Recent discoveries, including whole genome/exome sequencing and genome editing, have contributed to the identification of molecular factors regulating the development of Mullerian ducts, characterizing their roles, and facilitating the clinical diagnosis of MRKH syndrome (42).

CHH has a heterogeneous clinical phenotype and genetic background. Genetic data on hypogonadotropic hypogonadism is increasing with the increasing use of next-generation sequencing (NGS), but these results must be interpreted correctly in clinical practice. According to recent data, in more than 50% of cases, the disease-causing genetic changes can be found via NGS. In analyses with NGS-based methods, variants of unknown significance can be difficult to interpret. Proving the disease-causing effects of potential candidate genes and variants may be possible through extensive clinical genotype-phenotype studies and in vitro or in vivo animal experiments. Another major challenge in CHH is to distinguish accurate oligogenic inheritance from incidental findings unrelated to CHH. It can be difficult to determine the inheritance pattern due to non-complete penetrance and variable expressivity besides the oligogenicity. This could be a struggling issue for genetic counselors. However, over time, with increasing genetic data associated with clinical information, CHH will be deciphered, and variant interpretation will be easier (43).

FMR1 gene is one of the well-known genes related to POI. Therefore, it is necessary to analyze the *FMR1* gene in women presenting signs of ovarian dysfunction of unknown cause. This would also be useful for detecting intermediate and premutation alleles that lead to a mutation and thus fragile X syndrome. In addition, detection of these alleles among young women allows for genetic counseling necessary for planning their reproductive lives, taking into account possible ovarian dysfunction (44).

CONCLUSION

Genomic medicine applications require multidisciplinary applications in current approaches At Medical Genetics High-Risk Polyclinics, each patient is evaluated with detailed family history as well as clinical findings. The necessary genetic tests are selected with effective genetic counseling, and preventive medicine is applied by determining other family members who are at risk besides the patients.

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Optimizing Female Infertility in Premature Ovarian Insufficiency

Erken Yumurtalık Yetersizliğinde Kadın İnfertilitesinin Optimizasyonu

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ABSTRACT

Premature ovarian failure is a complex pathology with numerous etiologies and multiple system sequelae resulting for early deprivation of ovarian sex hormones. In the studies to determine the etiology, it is seen that many patients are in the unexplained group. On the other hand, genetic predisposition and autoimmune cause of premature ovarian insufficiency (POI) are the most common known etiologic causes. Early diagnosis and energetic treatment are important in order to prevent symptoms related to estrogen deficiency. Initiating hormone replacement therapy as soon as possible will prevent menopausal symptoms and reduce long-term complications in women. Another important problem in these patients is infertility, which occurs due to ovarian failure. In fact, fertility, which begins to decrease before menstrual irregularity, turns into infertility with a rapid decline in the number of follicles in the following period. Here, the detection of patients at risk and appropriately timed fertility preservation treatments (such as freezing of oocyte, embryo, or ovarian tissue) or assisted reproductive techniques can be offered. Besides this, studies like stem cell therapy, platelet-rich plasma (PRP), and in vitro activation of ovarian tissue in patients with POI are recent and still investigational but may be promising in the future. In the present review, the current pathophysiology and treatment options of premature ovarian failure were discussed.
Keywords: Premature ovarian insufficiency; infertility; diagnosis; treatment.

ÖZ

Erken yumurtalık yetmezliği, az bilinen etiyolojisi ile neden olduğu yumurtalık hormonlarının azalmasıyla saptanan, kadınlarda birçok sistemi etkileyen patolojilere yol açar. Etiyolojisini belirlemeye yönelik çalışmalarda pek çok hastanın açıklanamayan grupta olduğu görülmektedir. Diğer yandan genetik yatkınlık ve otoimmün nedenli erken yumurtalık yetersizliği (premature ovarian insufficiency, POI) bilinen en sık etiyolojik nedenlerdir. Temelde östrojen eksikliği ile ilgili semptomların önlenmesi amacı ile erken tanı ve tedavi önemlidir. Hormon replasman tedavisinin bir an önce başlanması, kadınlarda menopozal semptomların önlenmesini ve uzun dönemli komplikasyonların azalmasını sağlayacaktır. Bu hastalarda diğer önemli sorun ise over yetmezliği ile ortaya çıkan infertilitedir. Aslında adet düzensizliği ortaya çıkmadan önce azalmaya başlayan fertilite, ilerleyen dönemde folikül sayısının daha hızlı azalması ile ciddi bir infertilite sorunu haline gelmektedir. Burada özellikle risk altındaki hastaların tespiti ile uygun zamanda fertilite koruyucu tedaviler (oosit, embryo veya over dokusunun dondurulması gibi) ve yardımla üreme teknikleri önerilebilir. Bununla birlikte, infertilite yakınması olan POI tanısı alan hastalarda; kök hücre tedavisi, trombositten zengin plazma (platelet-rich plasma, PRP) ve over dokusuna in vitro aktivasyon gibi uygulamalar yeni ve deneysel aşamada olsa da gelecekte umut verici olabilir. Sunulan bu derlemede erken yumurtalık yetmezliğinin güncel patofizyolojisi ve tedavi seçenekleri tartışılmıştır.
Anahtar kelimeler: Erken yumurtalık yetersizliği; infertilite; tanı; tedavi.

INTRODUCTION

Premature ovarian insufficiency (POI) is the depletion of ovarian reserve in patients before the age of 40. The diagnosis of POI is necessary for both menstrual irregularities (amenorrhea or oligomenorrhea) for more than 4 months and for follicle stimulating hormone (FSH) to be higher than >25 mIU/ml in two different measurements. Its incidence is about 1% of the female population and can be as high as 3.7% (1,2). Before the age of forty, POI is an unexpected condition in which women have to deal with its chronic consequences, such as reproductive, psychological, and sexual health, osteoporosis, and cognitive and cardiovascular defects. Infertility is one of the consequences of POI, which has genetic, autoimmune, chromosomal, and gonadotoxic treatment and often has idiopathic causes. Although follicles are detected in the ovary in 73% of POI patients, the probability of spontaneous pregnancy is very low (about 4.8%), which is unfortunately very low for their peers (3,4). In many countries, oocyte donation or adoption is recommended due to the very low pregnancy rates in these patients. Donations can give the POI population a chance of a pregnancy rate of up to 40-50% (5). In places where donations are prohibited, as in our country, there is little or no chance of having a baby for adoption. In this section, we address the fertility difficulties of patients diagnosed with POI rather than other disorders caused by POI.

Pathophysiology

Most of the etiology of POI is unknown, it is defined as idiopathic, in descending order, iatrogenic (secondary to ovarian surgery or chemotherapy or radiotherapy), genetic, autoimmune, and infection. The primary POI is acquired spontaneously, but the secondary POI depends on the pelvic region after ovarian surgery, chemotherapy, or radiotherapy. Ethnicity has a role because in some ethnic populations, such as Caucasian, African-American, and Hispanic, POI is observed at much higher rates than in Japanese or Chinese women (6). Smoking is a risk factor that increases POI (odds ratio, OR: 1.82, 95% confidence interval, CI: 1.03-3.23), late menstruation, longer breastfeeding may reduce the POI risk factor (7).

Genetic

Although most of the etiology of POI is idiopathic, genetic causes account for 10-15 cases of POI (1). In genetic causes, X chromosome defects are seen as monosomy X (Turner syndrome 45XO), trisomy X, or deletions in the X chromosome. There are many more chromosomal defects in the POI population in patients with primary amenorrhea than in secondary amenorrhea. FMR premutations for familial reasons may also play a role in etiology. The European Society of Human Reproduction and Embryology (ESHRE) POI guideline recommended chromosomal analysis and fragile X mutation test analysis in all non-iatrogenic POI patients. Autosomal mutations have not yet been noted, unless clinically suspected (such as blepharophimosis, ptosis, and epicanthus inversus syndrome, BPES). If the Y chromosome is detected, gonadectomy is recommended for malignancy (1). There are new studies investigating rare new variants that cause POI by whole exome sequence analysis (8). In the future, the idiopathic norms of POI can be explained by genetic studies with whole exome sequence analysis.

Autoimmunity

In patients with autoimmunity, the ovarian reserve decreased by 20%. Adrenal insufficiency or Hashimoto's disease can be seen mostly in this population. Therefore, in patients with POI, 21-hydroxylase (21-OH) autoantibodies or adrenal cortical antibodies should be checked during the diagnosis of POI. The anti-TPO antibody should also be screened annually for autoimmune thyroiditis (1).

Iatrogenic

Iatrogenic causes are the second most common etiology after idiopathic. In bone marrow malignancies, radiation therapy after autologous stem cell transfer, especially pelvic radiation therapy, can cause 90% POI. After chemotherapy with alkylating agents, the ovarian reserve is also irreversibly reduced. In addition, benign surgery for the ovary also leads to a decrease in the ovarian reserve. Viral infections that can invade the ovary also cause premature ovarian failure. Ovarian surgeries should not be recommended in POI patients unless there is a risk of malignancy.

Fertility and Reproduction

Patients with POI have 25% ovulation during their reproductive years, that is, they still have follicles in their ovaries that give them a chance of pregnancy with their own germ cells. Although they have a very low spontaneous pregnancy rate, 5-10%, the highest pregnancy rate is 1-2 years after the diagnosis of POI (9). This period is the time to bring patients to the doctor in search of a remedy for menstrual irregularity or infertility, and just before the depletion of all oocytes. Therefore, this period may be the precious period when the probability of pregnancy with autologous oocytes is the highest. After the diagnosis of POI has been made, patients should be informed about infertility and menopause for fertility treatments or fertility preservation as soon as possible. This is an unexpected pathology. Most patients are young, and fertility is not their priority. Suddenly they face this early menopause and its burden can upset them and increase their anxiety. Accurate guidance and information play an important role in referencing this population.

Fertility Treatments

In fertility clinics, when attending POI patients, the first reflex is to recommend in vitro fertilization (IVF) for these patients. Because of the limited time and low spontaneous pregnancy rates, clinicians recommend IVF, which has higher pregnancy rates, for the treatment of infertility, and also gives a chance to reduce the time to get pregnant. However, there is still not enough data on the ways of infertility treatment, such as expectation management, ovulation induction, or IVF. The spontaneous pregnancy rate in expectation management is very low, such as 4-5% (1). Since the follicular phase is shortened in POI patients, the luteal phase is not always followed after follicular growth and ovulation. Therefore, the endometrium is not ready for an ovulating euploid embryo. The low rate of spontaneous pregnancy may be a consequence of these cases in POI.

In some observational studies after ovulation stimulation (OS) with gonadotropin, the pregnancy rate was around 6.3% (4). In controlled studies in which

gonadotropin suppression was performed with gonadotropin releasing hormone (GnRH) agonist suppression cycles, the pregnancy rate did not differ from placebo-controlled cycles (4).

In a randomized controlled trial by Tartagni et al. (10), 25 POI patients were given ethinyl estradiol (EE) for two weeks before and during OS, and 25 POI patients did not receive EE, as a control group. Only eight of the study group with FSH levels below 15 mIU/ml in the early follicular phase ovulated, and four of them became pregnant. In another randomized controlled trial, 29 POI patients were given dexamethasone before OS, while 29 POI patients in the control group did not receive steroids. The study group had a higher pregnancy rate compared to the control group (11). These results may indicate that autoimmunity plays a role in the etiology of POI.

Check et al. (12), in their study, suggested that gonadotropin suppression with estrogen replacement and GnRH agonides with longer OS periods with recombinant FSH or human menopausal gonadotropin (hMG) may have better ovulation and pregnancy rates if patients have longer periods of amenorrhea.

In a comparative study by Ishuzuka et al. (13), they compared pregnancy rates in POI patients who underwent hormone replacement therapy (HRT) with or without ovarian stimulation between 2014 and 2020 in a clinic. OS was applied to 429 patients with 6891 cycles, 48.5% of patients had follicular growth, 5.8% had a live birth rate (LBR) in the IVF group, and 1.3% had an LBR in the intrauterine insemination group. The only group waiting for treatment without OS (37 patients with 117 cycles) observed follicular growth at a rate of 5.4% on HRT and no pregnancy was detected. In the same study, they reported that the pregnancy rate was higher in patients with an age of <35 years and an amenorrhea duration of less than 4 years before IVF treatment than in other POI patients in the same group (13).

Abnormal karyotype of patients may interfere with pregnancy rates in IVF in POI patients. In the study by Grin et al. (14), 49 POI patients with abnormal karyotype underwent IVF programs, with follicular growth in 57%, oocyte retrieval in 47%, and fertilization rate with 6.1% LBR in 70.7%. Patients with mosaic turner syndrome had longer amenorrhea intervals and a lower rate of follicular growth. In another study, by Jiao et al. (15), 955 POI patients were evaluated, 30% in the primary amenorrhea group had genetic etiology, and 11% in the secondary amenorrhea group had genetic etiology. In their studies, the pregnancy rate in the genetic group was 7.2%, the autoimmune group was 21.1%, the iatrogenic group was 34.8%, and the idiopathic group was 19.5%, so the lowest pregnancy expectancy was in patients with POI with genetic abnormalities. POI patients with a genetic etiology may interfere with pregnancy rates, but this is not sufficiently clear with current studies. Also, these patients are concerned about the penetration of pathology into their offspring. This is another issue that can be explored in the future.

DHEA and Testosterone

The ovarian reserve is irrevocably decreasing due to increasing age. The cause of the primordial follicular loss is not yet known, but this definite result has been tried to slow down with some adjuvant treatments such as dyhydroepiandrostenodione (DHEA) or testosterone. These molecules are synthesized by the ovary and the adrenal gland and enhance the effect of gonadotropin on follicle development through insulin-like growth factor 1 (IGF-1) (16,17). Some authors have concluded that the use of DHEA before IVF can increase ovarian reserve markers and ovarian response to ovarian stimulation (18-21). However, in many studies, the use of DHEA supplements in ovarian reserve or pregnancy rates before IVF treatment has not been observed (16,17). Qin et al. (17), in a meta-analysis of pretreatment with DHEA, have shown that the pregnancy rates could increase (OR: 1.47, 95% CI: 1.09-1.99), but decrease (OR: 1.08, 95% CI: 0.67-1.73) if only randomized controlled trials (RCTs) were included, which reveals that pretreatment with DHEA has no effect on pregnancy rates, number of eggs, fertilization, and miscarriage rates. These results suggested that we need more RCTs, not meta-analysis, to reach a more reliable conclusion about DHEA supplementation for POI patients.

Similarly, testosterone administration before IVF treatment has better oocyte count and pregnancy rates in those with a poor ovarian response, but there is limited data on testosterone supplementation in POI (22-24). However, these studies were heterogeneous and the study population was not uniform. Studies are underway on testosterone in weak responders (T-Transport group of Researchers), which may reveal better results that can guide us next year (NCT02418572).

In Vitro Activation

Kawamura et al. (25), suggested disruption of the hippo pathway and in vitro activation of AKT by laparoscopically derived oocyte cortex fragments. These parts of the ovarian cortex were made both mechanically and chemically to disrupt the hippo pathway and AKT activation, then laparoscopically translated into the ovary in 27 patients. A live birth can be performed. 37 patients were included in their second study, nine patients had follicular growth, four patients had embryo transfer, and two patients (%5.4) had an LBR (26). Hippo and AKT (protein kinase B) are signaling pathways that regulate the activation of primordial follicles. Activation without drugs can disrupt hippo signaling and cause activation of the primordial follicle and ovarian regeneration, follicle growth and ovulation may be possible. The breakdown of the ovarian cortex can now biomechanically activate the ovarian follicle pool. They removed the ovarian cortex, and a small sample was measured for stiffness and re-transplanted into the peritoneal fold. Activation of the ovarian cortex without drugs was performed in 19 patients, 52.6% of them continued ovarian function, and two of them gave live birth. Therefore, mechanical intervention can now also ensure the regeneration of the ovary (27).

Intra-Ovarian PRP Infusion

In recent case series, platelet-rich plasma (PRP) is a concentrated platelet plasma component of the autologous blood of patients injected into the ovary to regenerate folliculogenesis. The growth factors, cytokines, and chemoattractives of this plasma stimulated

angiogenesis and cell proliferation, enabling the primordial and primary follicles to transform into preantral follicles. Hosseini et al. (28), in an in vitro PRP injection study, was proposed to the primordial or primary follicles of the ovary at the preantral stage. Of the 311 patients diagnosed with POI, spontaneous pregnancy was present in more than 7.4% of the patients who received intraovarian PRP injection, and at least one cleavage embryo was present in 23.6% of them (29). Barad et al. (30) in recent studies, suggested that intrauterine PRP injection can give a 4.7% chance of pregnancy only in pregnancies with the option of oocyte donation. In the last study of Cakiroglu et al. (31), autologous PRP intrauterine injection of 510 patients with a poor ovarian response; spontaneous pregnancy was found to be 4.3%, the IVF program was 92.9%, the pregnancy rate was 20.5%, and the LBR was found to be 12.9%. These studies were before or after studies or observational studies, so we need randomized controlled trials to obtain more reliable results.

Stem Cell Therapy

Another experimental study is autologous stem cell transfer to the ovary. Bone marrow stem cell precursors were excreted through the colony-stimulating factor. Then the stem cells were collected through apheresis and injected into the ovarian artery through catheterization. Three out of 17 POI patients became pregnant spontaneously after autologous stem cell injection into the ovary (32). Mesenchymal stem cells can treat POI by performing a "home effect" for follicles. They can promote the growth and development of follicles, vascular formation, and immunomodulatory effects (33). In this study, animal preclinical and human clinical studies were reviewed. Follicular development, menstrual resumption, and spontaneous pregnancies have been reported after stem cell injection into most ovaries. With the very low success rate of having spontaneous pregnancy in POI patients, stem cells may offer a promising future for the health and reproduction of POI patients.

Fertility Preservation

Fertility preservation can be done by freezing oocytes, embryos, or ovarian tissue for future use. Embryos can be frozen to delay their development in couples who are not currently considering pregnancy. In single POI patients or menstruating adolescents, oocytes can be frozen. Limited follicle pools also make it difficult for mature oocytes to freeze. However, in adolescents who are expected to have POI after cancer treatments or ovarian surgeries in the future, ovarian tissue preservation should be recommended to preserve ovarian tissue. The preservation of ovarian tissue is no longer experimental. Since 2019, the American Society for Reproductive Medicine (ASRM) has established that patients treated with ovarian freezing gonadotoxic drugs preserve fertility (34), and in this way preserving future fertility may be their only chance for reproduction.

CONCLUSION

POI patients are still the most difficult infertility patients today. Although many treatment methods are proposed, there is no treatment algorithm. It is important for patients to individualize their treatment methods. However, the most important step in the treatment is the follow-up of the POI infertile patient by a specialist physician.

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Surgical Treatment Options for Female Infertility

Kadın İnfertilitesi için Cerrahi Tedavi Seçenekleri

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ABSTRACT

Infertility, defined as failure to achieve pregnancy within 12 months of unprotected intercourse or therapeutic donor insemination in women younger than 35 years or within 6 months in women older than 35 years, affects up to 15% of couples. An infertility evaluation may be offered to any patient who by definition has infertility or is at high risk of infertility. Hysterosalpingography, hysterosalpingo-contrast sonography, saline infusion sonohysterography, hysteroscopy, laparoscopy, and bacteriological and endocrinological examinations that will be made after these studies aim to focus more on the causes of infertility. With the development of assisted reproductive technology, the need for major reproductive surgery, which may be necessary for the primary treatment of infertility, has decreased over the years. Surgical methods are mainly considered as laparoscopic and hysteroscopic techniques. However, laparotomy is also rarely required and may be needed in cases such as adhesions, mass lesions, unsuccessful surgeries, or emergency surgery. When a surgical treatment is planned for infertile patients who required surgery, it is very crucial that the procedure should be performed by experienced surgeons. In this review, pathologies that require surgery for infertility treatment and surgical methods that can be applied to infertile patients were discussed, rather than medical treatments.
Keywords: Infertility; laparoscopy; hysteroscopy.

ÖZ

İnfertilite 35 yaşın altındaki kadınlarda 12 ay veya 35 yaşın üstündeki kadınlarda 6 ay korunmasız cinsel ilişkiye veya terapötik donör inseminasyonuna rağmen gebe kalamama hali olarak tanımlanıp çiftlerin %15'ini etkileyen durumdur. Tanım gereği infertilitesi olan veya infertilite riski yüksek olan herhangi bir hastaya infertilite değerlendirmesi önerilebilir. Histerosalpingografi, histerosalpingo-kontrast sonografi, salin infüzyon sonohisterografi, histereskopi, laparoskopi ve bu çalışmalardan sonra yapılacak bakteriyolojik ve endokrinolojik incelemeler ile infertilite nedenlerine daha fazla odaklanılması amaçlanmaktadır. Yardımcı üreme teknolojisinin gelişmesiyle birlikte infertilitenin birincil tedavisi için gerekli olabilecek majör üreme cerrahisine olan ihtiyaç yıllar içinde azalmıştır. Cerrahi yöntemler temel olarak laparoskopik ve histeroskopik teknikler olarak kabul edilmektedir. Ancak laparotomi de nadiren de olsa gereklidir ve adezyonlar, kitle lezyonları, başarısız ameliyatlar veya acil ameliyat gibi durumlar söz konusu olduğunda ihtiyaç duyulabilir. Ameliyat gerektiren infertil hastalarda cerrahi bir tedavi planlanırken işlemin deneyimli cerrahlar tarafından yapılması çok önemlidir. Bu derlemede, medikal tedavilerden ziyade infertilite tedavisi için cerrahi gerektiren patolojiler ve infertil hastalarda uygulanabilecek olan cerrahi yöntemler tartışılmıştır.
Anahtar kelimeler: İnfertilite; laparoskopi; histeroskopi.

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INTRODUCTION

Infertility, defined as failure to achieve pregnancy within 12 months of unprotected intercourse in women younger than 35 years or within 6 months in women older than 35 years, affects up to 15% of couples (1). Women older than 35 years should receive an expedited evaluation and undergo treatment after 6 months of failed attempts to become pregnant or earlier if clinically indicated. In women older than 40 years, more immediate evaluation and treatment are warranted (1,2).

In the evaluation of etiologies, female factors are responsible for 45% of all infertility causes. Male factor is a cause of infertility in 40-50% of couples (3). With the development of assisted reproductive technology (ART), the need for major reproductive surgery, which may be necessary for the primary treatment of infertility, has decreased over the years. In addition to medical history and physical examination, pelvic evaluation is completed with a gynecological examination, pelvic ultrasonography (USG), and transvaginal ultrasonography (TVUSG). It is possible to detect pathologies such as polyps and leiomyomas in the cervical canal by TVUSG. Many of the congenital uterine anomalies also can be detected at this stage.

Hysteroscopy and saline infusion sonohysterography (SIS) for endometrial polyps, fibroids, or adhesions provide information about the cavity. Magnetic resonance imaging (MRI) may be required in a virgin patient, vaginal agenesis, and complex cases. Hysterosalpingography (HSG) is a radiological method in which both uterine cavity, tubal patency, and peritoneal spread can be evaluated. Although it is accepted that the cavity is also evaluated, it is not preferred for cavity evaluation. Because in cavity evaluation, hysteroscopy is considered as a superior method. 3D hysterosonography has been found to be as valuable and successful as hysteroscopy in terms of intrauterine evaluation (4).

Congenital uterine anomalies can be recognized by 2D and 3D TVUSG, HSG, MRI, and sonohysterography. MRI is an expensive, but also an effective method. The gold standard in the diagnosis of congenital uterine anomalies is hysteroscopy and 3D USG. The need for L/S or MRI can be eliminated owing to 3D USG. 3D USG also allows distinguishing the septum-bicornuate uterus more clearly. Diagnostic laparoscopy is very valuable when the anomaly cannot be evaluated clearly.

The requirement of reproductive surgery can be evaluated in three categories (Table 1).

In this review, we will focus on pathologies that require surgery for infertility treatment and surgical methods that can be applied, rather than medical treatments.

HYSTEROSCOPY

Hysteroscopy is a very valuable method in confirming the diagnosis of intracavitary lesions detected by other imaging methods in infertile patients. It is an invasive diagnostic method and allows treatment during the procedure. It is considered as the gold standard in the evaluation of the uterine cavity. The disadvantage of this method is that hysteroscopy cannot distinguish the septum and bicornuate uterus and cannot evaluate the uterine wall. Complementary laparoscopy may be required. It is considered to be the best diagnostic method for endometrial polyps, uterine synechiae, and submucous myomas, owing to the direct visualization of the uterine cavity. However, it is not considered as the first diagnostic evaluation in infertile women due to its high cost and not being an easily accessible method. It is considered as an advanced examination since it is not routinely used and does not need to be done on every patient.

Hysteroscopy is not a method that can be performed at any time of the cycle. It should be done in the early proliferative phase, in the 2-3 days immediately after menstruation; when the endometrium layer is the thinnest, in order to show organic pathologies or anatomical disorders and to get the maximum benefit from the treatment opportunity it provides. Distention media used in hysteroscopy are saline, glycine, sorbitol, and mannitol. The choice of medium is decided according to the incision technique to be made and the energy source to be used (4). It is possible to benefit from a laparoscopy and abdominal USG to be performed simultaneously during the cavity correcting hysteroscopic incision procedure for the uterine septum. Abdominal USG is of course more preferred because it is not invasive. The uterine contour can be seen more clearly thanks to laparoscopy and USG. In this way, the risk of uterine perforation is reduced and it can be ensured that the septum is completely removed (5).

The indications for hysteroscopy are very diverse. These are infertility, abnormal uterine bleeding, endometrial polyps, leiomyomas, chronic endometritis, intrauterine synechiae, isthmocele, Mullerian anomalies, cervical stenosis, cervical polyps, cervical insufficiency, and retention of the pregnancy material. Abnormal findings on

Table 1. The requirement for reproductive surgery

Conventional Treatments	Enhancements of Outcomes	Preservation of Fertility
Tuboplasty	Hydrosalpinx surgeries	Ovarian transposition
Neosalpingostomy	Salpingectomy	Ovarian transplantation
Fimbrioplasty	Proximal tubal occlusion	Ovarian tissue cryopreservation
Tubal anastomosis	Hysteroscopic tubal occlusion	
Excision of endometrioma		
Hysteroscopy	Hysteroscopy	
Polypectomy	Polypectomy	
Myomectomy	Myomectomy	
Metroplasty	Metroplasty	
Adhesiolysis	Adhesiolysis	

USG, HSG, or SIS also require this procedure. This procedure is contraindicated in the presence of active bleeding, pregnancy, acute pelvic infection, and uterine malignancy.

Hysteroscopy is basically done in two ways. Office hysteroscopy is for diagnostic purposes as mentioned above. It is also known as diagnostic hysteroscopy. In operative hysteroscopy, it is aimed to treat uterine pathologies.

Diagnostic hysteroscopy has the advantage that it can be performed in the office or in clinical conditions. It is performed in the early stages of the postmenstrual cycle without the use of a speculum and tenaculum. The patient and the doctor can decide before the procedure whether to give anesthesia or not. After vaginal observation, the cervical external orifice is found. The hysteroscope goes forward in by monitoring the cervical canal. After passing the cervical internal orifice, the uterine cavity is viewed panoramically. The close neighborhood of the uterine cavity to the cervix, the corpus level, and the walls of the cavity in the fundus region are observed on the right/left, anterior/posterior positions. Since this process is done immediately after menstruation, it will be easier to observe possible pathologies in the cavity such as myoma, polyp, and isthmocele. Later, both tubal ostia are observed on both sides of the inner surface of the fundus.

In operative hysteroscopy, entry into the cavity is performed after the speculum is inserted and the anterior lip of the cervix is grasped with the tenaculum. It is preferred more to be done under general anesthesia. Dilatation can be done with dilatation plugs in patients with cervical stenosis. As in office hysteroscopy, the hysteroscope goes forward into the cavity. Afterward, the planned surgical procedure is completed.

LAPAROSCOPY

Laparoscopy is the surgical method for both the diagnosis and treatment of infertile patients. It is often preferred for hydrosalpinx, endometriosis, endometrioma, leiomyomas, and intra-abdominal adhesions. A shorter hospital stay and faster recovery can be achieved with laparoscopy.

Chlamydia trachomatis, *Mycobacteria tuberculosis*, and other pelvic inflammatory disease (PID) agents also cause damage and adhesions in the tuba uterina. The clinical value of the *Chlamydia trachomatis* serological screening test has been shown to be limited (6).

Another important aspect of laparoscopy is the benefit it provides to patients with a history of pelvic surgery. Since synechiae developed after surgery also affect the tuba-ovarian function, it is important to open the synechiae in terms of infertility treatment. Although the synechiae of these patients tend to stick again after opening, they also provide benefits in terms of pelvic pain and dyspareunia.

Since it is not possible to evaluate both the outer contour and the inner surface of the uterus together with laparoscopy. So hysteroscopy may be required as a complementary in the recognition of congenital uterine anomalies.

TUBAL CAUSES

It is important to have a complete, uninterrupted, and undamaged tubal anatomy and fimbrial tissue for the passage of both sperm, oocyte, and fertilized ovum in the formation of a healthy pregnancy. Tubal diseases account for nearly one-third of female infertility. These diseases can involve the entire tuba, or they can only be seen proximal or distal region. Medical treatment of tubal diseases that cause infertility is not possible. Surgery is required for treatment.

The causes of tubal diseases are mostly salpingitis (7). Most tubal disease is caused by an episode of tubal infection, PID, or endometriosis, which are both very common problems. Sexually transmitted diseases such as gonorrhea, chlamydia or appendicitis, and bowel infection may also be the result of PID (8). Fimbrial adhesions and hydrosalpingial tubal damages due to salpingitis isthmica nodosa (SIN) (diverticulum of the fallopian tube), tubal or cornual polyps, ascending infections, and pelvic tuberculosis are frequently seen.

The use of laparoscopy in tubal diseases varies according to the type of tubal disease. Proximal tubal occlusion may be due to obstruction resulting from plugs of mucus and amorphous debris, to spasm of the uterotubal ostium, or to occlusion, which is a true anatomic blockage from fibrosis due to SIN, PID, or endometriosis (9). Tubal ligation or segmental salpingectomies that cause midtubal occlusion are counted as iatrogenic tubal factors. Tubal ligation is one of the most preferred contraceptive methods worldwide and also one of the causes of tubal damage. The demand for reopening the tubes is quite common due to the decision to have more children or the desire to have a child from a second spouse. Although few women seek reversal for the procedure. The prevalence of post-sterilization regret ranges from 20% to 30% and varies by the length of time since the procedure (10). In such a case, two ways to have a child are tubal anastomosis or in vitro fertilization (IVF). Although the general approach is to apply IVF treatment to these patients, tubal anastomosis may be preferred to IVF due to certain beliefs or concerns. The purpose of tubal anastomosis is to reapproximate and recanalize the 2 separated tubal segments. It is also important to prevent the formation of adhesions. Tubal patency should then be confirmed with HSG.

Factors determining success in tubal anastomosis technique are the age of the woman, the sterilization technique, the residual tubal length, and the time elapsed after sterilization. For example, the pregnancy rates of women aged 15-29 years, 30-33 years, and 34-49 years old after tubal anastomosis were 73%, 64%, and 46%, respectively (11). In terms of sterilization technique, it was shown that pregnancy success according to the method of sterilization was 16 of 24 in which Fallope-ring was used, 14 of 15 with cauterization, and 8 of 10 in patients in whom the Pomeroy technique was used (12). Anastomosis is mostly performed according to the length of the residual tuba segment mostly as isthmic-isthmic, isthmic-ampullary ones. Pregnancy rates after anastomosis were 75% in women with a residual tubal length of 4 cm or more, whereas it was only 19% in those with a shorter tube (13). Based on their findings, it is considered that laparoscopic sterilization reversal will be a better option in women younger than 37 years who have ≥4 cm of a residual tube. For others, IVF is better (14).

Anastomosis operation can be performed with laparoscopy, conventional microsurgery, or robot-assisted laparoscopy. Pregnancy results of laparoscopic tubal

anastomosis and conventional microsurgery techniques are similar (15). In the robot-assisted technique, success is similar to these two methods. Also, the duration of the surgery, costs, and the experience of the surgeon are important factors. While the short recovery time is an advantage, more studies are needed to evaluate whether the use of the robot in this procedure provides additional benefits. Ectopic pregnancy rates were found to be similar in laparoscopic tubal anastomosis and microsurgery techniques, 2.5%, and 2.8% (15). However, the laparoscopic method was also found to be more costly than mini-laparotomy (16). As a result, laparoscopic tubal anastomosis is less invasive and may be an alternative to laparotomy to reverse tubal sterilization. It is useful to know that tubal anastomoses created by the V-notes technique, as a new method, are also possible.

When we look at anastomosis, single suture, 2 suture, and 4 suture techniques are suitable, and both techniques can be done by both laparoscopic and laparoscopy-guided robotic methods. The single suture technique is performed at the 12 o'clock position from the serosal aspect of the tuba, and the 2 suture technique is performed at the 6 and 12 o'clock positions. The 4 suture technique is the classical anastomosis technique. In this technique, sutures are passed from the mucosa-muscular layer border at 3, 6, 9, 12 o'clock positions. Then the serosa layer is sutured continuously. It is possible to perform all three of these techniques with laparoscopic or laparoscopy-guided robotic techniques in experienced hands. Suture techniques include micro-suturing using 6-0/10-0 sutures. There is also a suture-free technique, in which tubal recanalization is provided with micro clips using tissue bonding technology.

Hydrosalpinx is the condition of tubal dilatation and distension due to occlusion of the distal tuba uterina for any reason. Almost 30% of tubal damages are hydrosalpinxes (17). The cause of this distention is mostly PID. If it occurs the progression of the sperm in the endometrium and tube, caught of the oocyte by the fimbrial ends, the progression of the oocyte in the tubes, the formation of fertilization in the ampulla, and the progression of the embryo towards the endometrium may be affected. Even if it reaches the endometrium, its implantation into the endometrial layer cannot occur because receptivity will be affected. In addition, if it is implanted, the possibility of abortion increases. These conditions may also occur due to endometrial local inflammatory factors. Hydrosalpinx is a reason for blocking pregnancy formation from occurring normally or failure in IVF treatments. Embryos to be transferred may be dragged out of the cavity due to the reflux of fluid in the fallopian tubes. As a result, in the presence of hydrosalpinx, the success of implantation, pregnancy, and live birth decreases, and the risks of abortion and ectopic pregnancy increase. With the removal of hydrosalpinxes, the chances of IVF success increase.

In addition to all these, histories of ectopic pregnancies that were treated without salpingectomy with drugs such as methotrexate, with surgical reasons such as salpingotomy or total or segmental salpingectomy due to the treatment of tubal ectopic pregnancies are also important reasons that should not be ignored.

Before IVF treatment in patients with hydrosalpinx, laparoscopic salpingectomy or proximal tubal occlusion should be performed. Laparoscopic salpingoneostomy may also be an alternative method according to tubal score. The principle here is to preserve the integrity of the tuba and to make a distal tubal incision for the flow of the fluid in the tuba to the douglas instead of the endometrial cavity. For this procedure, the abdomen is entered with the laparoscopic technique, and scissors or electrocautery are used for the incision (18). An incision is made in an antimesenteric and avascular region of the distal tubal tip with a monopolar needle, scissors, or laser. Tubal mucosa is directly observed by entering with a thin hysteroscope. At this stage, apart from the laparoscopy equipment, a second telescope, additional light source, video camera, monitor, and irrigation are required for salpingoscopy. The tubal fimbriae are delicately grasped with laparoscopic tubal forceps. At this time, the hysteroscope is inserted through the 5 inch trocar and entered into the ampulla via fimbriae. The tubal mucosa is irrigated with saline and the ampulla area is observed for internal adhesion and damage to the tubal mucosa. The ostium is enlarged with fine forceps. In order to prevent the ostium from closing, the bulbous mucosa is everted and sutured to the serosa. Of course, for all these possibilities, the patient should not be old and the ovarian reserve should not be low. It would be more appropriate to perform salpingectomy or tubal blockage and apply IVF treatment for those with a tubal score of 3-4. An experienced surgeon is required in reconstructive tubal surgery. The patient should be informed that the risk of ectopic pregnancy is higher than that in the normal population.

Although it is preferred that salpingectomy to be laparoscopic, it can also be performed with open surgery technique. It is started from the closest border to the tuba by holding the fimbrial end. It is aimed to protect the ovarian vessels and not decreasing the reserve. Preferably scissors or bipolar energy powered ligasure are used. The tuba is removed from the closest place to the horn. The aim here is to reduce the risk of interstitial pregnancy. With salpingectomy, in which chronically infected tissue is excised, it is easier to reach the ovaries during oocyte collection for IVF. Risks such as abscess, torsion, and chronic pelvic pain are eliminated.

Proximal tubal occlusion is an alternative method to salpingectomy in patients with hydrosalpinx. The aim is to prevent the retrograde flow of tubal contents into the uterine cavity by creating a blockage in the cornual region between the tuba and the uterus. However, in patients with this blockage, fenestration to the distal tubal segment is required for fluid drainage in order to prevent a fluid increase in the tubal segment in between, and formation of cyst or abscess.

A proximal tubal occlusion is an option in excessive pelvic adhesions in cases where the pelvic anatomy is so distorted that the tuba cannot be released from the peritoneum, douglas, uterus, or ovaries; or in cases in which the ovarian reserve may be damaged during this procedure. Thus, ovarian blood flow is not impaired. It is less invasive than salpingectomy. In terms of implantation and pregnancy rates, is similar to salpingectomy, salpingostomy, and salpingoneostomy (19). Although it is theoretically possible to perform the blocking procedure hysteroscopically with the Essure method, the evidence for efficacy and safety is insufficient (20).

Tubal phimosis is the narrowing of the distal end of the fallopian tube which the tubal opening is still present. Fallopian tubes have a curled appearance. During laparoscopy, it is characteristic that the dyed fluid causes dilatation in the distal ampulla and gushes out from the narrow end (21). With laparoscopic tubal forceps, the tubal fimbriae is carefully grasped and lifted. A small incision is made from the antimesenteric side to the narrowed fimbrial end. The mucosa is everted to prevent reocclusion and closure of the ostium (22). This procedure in which phimosis is corrected is called laparoscopic fimbrioplasty. Fimbrial agglutination is the condition in which the adhesive brids of both tuba fimbrial ends are formed. It is mostly associated with serosal defects. In this case, it is unclear whether the fimbriae of the fallopian tubes can fully capture the ovum, and therefore their relationship with infertility. If seen, the brids can be cut (21). If these brids are cut, the fimbriae will also be opened.

UTERINE CAUSES

Uterine causes are not as common as tubal diseases. However, the uterus should be carefully evaluated. Because it can be one of the important causes of infertility that can be overlooked. When the fertilized ovum in the tuba reaches the uterine cavity, there should be no anatomical defect in the uterus, but an endometrium which ready for implantation is required for its receptivity. Acute or chronic endometritis, endometrial polyp, leiomyoma, congenital uterine pathologies, adenomyosis, Asherman syndrome, isthmocele, and previous uterine surgeries are uterine pathologies that may cause infertility. These pathologies are also important in terms of affecting the prognosis of pregnancies obtained by IVF. As a result, decreased endometrial receptivity or endometrial anatomical-mechanical disorders lie on the basis of uterine causes of infertility.

Mullerian anomalies cause infertility by preventing normal implantation, and are also a common cause of recurrent pregnancy loss. Among the different types of structural uterine anomalies, the septate uterus is the most common and associated with the poorest reproductive outcomes (23). The presence of a septum is also associated with poor obstetric outcomes such as malpresentation and preterm delivery (24). In the classification of anomalies, there are classifications of the European Society of Human Reproduction and Embryology-the European Society for Gynecological Endoscopy (ESHRE-ESGE), and the American Society for Reproductive Medicine (ASRM) (25). The issue of which anomalies will require treatment is still a discussion topic. Uterine septum, T-shaped uterus, and endometrial cavity presence types of uterine horns (endometriosis and due to adhesion risks) require treatment. Treatment for bicornuate uterus and uterus didelphis is controversial, but treatment may be required in cases of recurrent pregnancy loss.

It is recommended that uterine septums be treated in women who are infertile or have recurrent pregnancy loss (26). According to studies, pregnancy rate increases with septum incision; abortion and preterm birth rates decrease (27). The incision of the uterine septum is made under general anesthesia. It is important that the uterus is in the early proliferative phase so that the borders of the septum and how far it extends can be clearly determined. After entering the cavity or cavities with the hysteroscope, tubal ostia are seen. From the lowest level of the septum, it would be cut with scissors at equal distances to the anterior and posterior walls and going forward to the fundus. The process is continued until the fundal vascular structures or the pink myometrium layer are seen.

In cases where the uterine septum reaches the external os of the cervix, if the septum in the cervix is opened, the risk of cervical insufficiency will increase in case of a subsequent pregnancy. For this reason, the septum section between the internal and external os of the cervix is preserved. For this, the internal os level is determined with the help of a balloon that is inflated through one of the two cervixes. The process of cutting the septum, which starts at the level of the internal os, is going through the fundus. As an alternative to scissors, monopolar cautery, bipolar cautery, or laser can also be preferred.

Further termination of the septum incision is primarily associated with the risk of perforation. In addition, a large amount of incision carries the risk of rupture during pregnancy and delivery. The use of electrosurgery also has the potential to create new adhesions. Although it is accepted that a period of 8 weeks may be sufficient for healing after the cavity is brought into the shape it should be, this period is still controversial in terms of conceiving (28). All women with congenital uterine anomalies and be treated should be screened for cervical insufficiency after the 18th week of gestation.

Residual septum requiring re-incision after treatment of the uterine septum has also been reported. Internal fundal recess should be evaluated for the necessity for re-incision. According to ASRM, it is sufficient for this indentation to be over 1 cm. According to ESHRE-ESGE re-incision can be done if the residual septum is more than 50% of myometrium thickness (29).

A T-shaped uterus is a congenital uterine malformation with a normal appearance from the outside but a T-shaped cavity according to the definition of ESHRE-ESGE. It can be seen as a congenital anomaly, or it can take a T shape later on due to intrauterine adhesions. This anomaly is characterized by stenosis of the uterine cavity, and the lateral myometrial layer may also be thick. The use of diethylstilbestrol in its etiology was defined many years ago.

2D and 3D TVUSG, HSG, sonohysterography, hysteroscopy, and MRI are used in the diagnosis of T-shaped uterine anomalies. Diagnostic laparoscopy may be useful in excluding other pathologies. MRI is also useful in adolescents and when there are complicated anomalies. HSG and hysteroscopy alone are insufficient since they cannot show the thickness of the uterine side walls. 3D TVUSG is the most valuable method in the demonstration, measurement, and diagnosis of this thickness. Thickness is best evaluated in the mid-luteal phase (21).

A woman with a T-shaped uterine anomaly may be asymptomatic. She may also become pregnant and have term delivery. However, in women with infertility, recurrent implantation failures and habitual abortions are evaluated by hysteroscopy in the early follicular phase. After entering the cavity with a hysteroscope, a normal uterine cavity appearance is obtained by cutting both lateral walls from the tubal ostia to the cervical isthmic region with monopolar or bipolar electrodes. It should be

noted that there may be a risk of cervical insufficiency if the incision extends into the cervical canal. The depth of the incision is planned at least 10 mm of myometrial wall thickness has remained.

The use of antibiotics, hormone therapy, and adhesion barriers in the postoperative period after hysteroscopic metroplasty is controversial (21). There may be paid attention to cervical insufficiency, synechiae, and if a uterine rupture occurred. Metroplasty is not an indication of a cesarean section, and vaginal delivery is not contraindicated in every woman who has had metroplasty.

Endometrial polyps are pathologies that cause both infertility and excessive menstrual bleeding. Polyps vary in size, localization in the cavity, and whether their pedicles are thin or not. In terms of fertility, they may cause infertility by affecting embryo implantation and reducing uterine receptivity. It is clear that the removal of endometrial polyps in the cavity is associated with increased pregnancy rates (30,31). Localization may determine whether these polyps are the cause of infertility or not. For example, it is not clear whether very small polyps close to the tubal ostia cause infertility, but their removal is not recommended as they may cause postoperative stenosis and phimosis on or around the tubal os. Polyps are softer than fibroids. Some polyps may resemble pedunculated fibroids in appearance. Although the final decision will be made by pathological examination, it is essential for the infertile patient to be removed, even if there is a cavity-related myoma. In the presence of a polyp in the cavity, an observation is made in terms of the boundary line and vascularization by turning on the stem or base of the polyp. In the presence of significant vascularization, electrocoagulation is performed first. In terms of electrocoagulation preference, bipolar energy called the versa point can also be used. Thus, when the excision is made, precautions are taken in terms of bleeding, and the image is not distorted. Excision may be somewhat difficult in extremely mobile polyps. Dilated endometrial glands in the form of small white dots may also be exposed when the polyp is excised. After the stalks of the small polyps are cut from the place closest to the endometrium, the holder can be removed with forceps. If the polypoid mass is large, it can be taken out, but if it does not distort the image, it can remain inside to be removed at the end of the procedure. At the end of the resection, electrocoagulation can be performed at the base of the mass to prevent bleeding. One of the issues to be considered is to prevent the resectoscope from entering and exiting the uterine cavity too often. If the excised parts are more than one, they can be taken out of the cavity at the end of the procedure or intermittently. Thus, the risk of air embolism is not increased.

Leiomyomas are common benign smooth muscle tumors depending on their location in the uterus, the way they cause infertility and even their treatment may differ. Although not every fibroid is a cause of infertility. Pregnancy may be possible despite myoma. This is determined by the absence of submucosal or intracavitary component of the myoma, or the absence of pressure on the cavity despite being completely in the myometrium tissue.

It is clear that fibroids in the cavity and causing distortion in it, are associated with reduced pregnancy; but the effect of intramural myomas that do not affect the cavity on pregnancy is controversial (32). Type 0 and Type I fibroids can be resected by hysteroscopy. An experienced surgeon and good surgical equipment are essential for resecting Type II fibroids with hysteroscopy. Myomectomies should be performed intermittently in order to prevent post-procedural adhesions in myomas located on the 2 opposite walls of the cavity. 2 months is considered sufficient for these procedures. Thus, hysteroscopic resection of submucous fibroids increases pregnancy and live birth rates (33).

Type 0 fibroids can usually be excised in one step. Dilated endometrial glands in the form of small white dots seen when the polyp is excised are not seen in the fibroid excision. A white, fibrous, non-bleeding tissue is exposed. If the resected parts cause deterioration in the field of view, they can be taken out with the resectoscope. But if they do not distort the image, they can remain inside to be removed at the end of the procedure. After making sure that the base and stem of the fibroid are completely removed, bleeding must be controlled. At the end of the resection, electrocoagulation can be performed with bipolar cautery at the base of the mass to prevent bleeding.

Hysteroscopic resection for Type I and II fibroids are the same in principle. The difficulty here is to remove the fibroid tissue from the uterus. The localization of the fibroid and how deep the myometrium extends also determine the difficulty of resection. Resection of myomas that narrow the internal os of the cervix too much, occupy a lot of space in the cavity, and are close to the tubal ostia will of course not be easy. Moreover, even if the cavity seems to have healed, not only the endometrial part of the fibroid but also the intramural part of the fibroid must be completely removed. And this procedure may be surgically difficult. As the size and depth of fibroids increase, this excision process becomes more difficult. The risk of intramural residue and uterine perforation increases. It is important to know how the endometrium, myometrium, endometrial polyps, and myoma tissues are seen during the procedure in order to be sure that the fibroid tissue is completely removed. In addition, myometrium is more fibrous and has the appearance of connective tissue. At the beginning of the excision of the fibroid, when the cleavage is entered between the myoma capsule and the myometrium, the blunt dissection will facilitate the separation of the myoma from the myometrium tissue. Here, the hydrodistension that occurs in the uterine cavity during the procedure will also contribute to this separation. Thanks to blunt dissection, the risks of perforation and bowel injury will be reduced. Performing operations accompanied by abdominal USG can also contribute. Residual myometrial thickness can be measured. In the presence of a deep-seated intramural fibroid, the procedure may be interrupted and the cavity may need to be evacuated. The procedure is facilitated when the endometrial cavity is re-entered with the resectoscope, as the residual myoma protrudes into the cavity with uterine contractions that occur after the cavity is emptied. With the same principle, the intrauterine pressure is changed rapidly and the intramural part of the fibroid is delivered into the cavity. Even drugs such as methylergonovine or oxytocin can be given to create uterine contraction (21). Different techniques have been

proposed for hysteroscopic surgeries of type II fibroids. These techniques are 2 intermittent surgical procedures, the enucleation technique (34), and Bettocchi's (office preparation of partially intramural myomas, OPPIuM) technique (35). The primary goal in all these techniques is easy removal of the fibroid. This convenience is achieved by extruding the fibroid into the cavity. A laparoscopic or abdominal approach may be required in Type II fibroids that cannot be removed by hysteroscopy, rarely.

The most important concern when resecting Type II fibroids with a hysteroscope is whether remaining myometrial tissue will create a risk of uterine perforation after myoma excision. For this, it is preferable to measure the distance between the pseudocapsule of the myoma and the uterine serosa by TVUSG in the preoperative period. This distance must be at least 4-5 mm. In addition, the appearance of bloodless and white tissue during the procedure is an important indicator for reaching the uterine serosa, and for taking precautions or terminating the procedure. Gonadotropin releasing hormone (GnRH) analogues can also be used prior to the excision of myomas. They make the operation easier by shrinking the fibroids. However, it is important to know that new fibroids may appear after the operation.

Bipolar cautery is preferred for hysteroscopic resection of myoma and saline is preferred for bipolar cautery. In healthy women, saline loss of up to 2500 cc can be tolerated. In case of further losses, the process should be terminated. A bipolar resectoscope is more suitable for Type II fibroids in difficult cases that are expected to take a long time. It is not appropriate to use saline solution in monopolar cautery planned cases. Nonionic forms are used for monopolar processes. If the nonionic distention medium is resorbed in large amounts, it may cause hypervolemia, hyponatremia, glycine toxicity, neurogenic coma, and even death. These risks increase in case of loss of 1000 ml for nonionic forms. Therefore, the process should be terminated in losses of 1000 ml or above.

It is controversial how the delivery method will be in pregnancy cases after the excision of myomas. However, cesarean section is preferred more in terms of rupture risk in Type I and Type II fibroids.

In intrauterine adhesions, menstrual abnormalities, infertility, and recurrent pregnancy loss are seen as a result of partial or complete obliteration of the cervical canal and uterine cavity. These adhesions are also described as Asherman's syndrome. Its frequency increases in cases of postpartum curettage, difficult removal of the placenta, or curettage due to the rest placenta. In the postpartum period, the endometrium is more vulnerable to damage. Delayed endometrial regeneration due to hypoestrogenism is accepted as the reason for this. In this case, as the endometrium is sensitive, the basal layer is more easily damaged and adhesion development becomes easier. In addition, more aggressive postpartum curettage contributes to this situation more. The developing granulation tissue also causes dense adhesions.

A history of any trauma to the endometrial cavity such as abortion, postpartum curettage, and placental adhesion anomalies is very valuable in diagnosis. Especially postpartum amenorrhea and hypomenorrhea should also be questioned. Hysteroscopy is the gold standard. 2D and 3D TVUSG, HSG, and SIS can be used. In these examinations, endometrial irregularity, fibrosis, obliteration, differences in endometrial echogenicity, one or more linear echogenicities and abnormal shapes can be observed. Filling defects can be seen on HSG and SIS.

Besides being the gold standard in diagnosis, hysteroscopy is also very important in treatment. The aim of treatment is to restore the cavity, remove adhesions, regeneration of the impaired endometrium, integrity of the cavity, and continuity of the endometrium. Thus, menstrual irregularities and amenorrhea can be treated, as well as the implantation of the embryo to the endometrium and its progression to a healthy birth will be provided.

Hysteroscopy is performed under general anesthesia, preferably with a full bladder and abdominal USG guidance. After entering the cavity with the hysteroscope, avascular, simple, and thin adhesions are separated thanks to the pressure created in the cavity with saline. It is aimed to see bilateral tubal ostia and to observe the cavity integrity panoramically. The use of scissors is preferable to both monopolar and bipolar electrocoagulation. Because the use of electrosurgery has the potential to create new adhesions. In addition, endometrial damage is minimized with the use of scissors. Bipolar electrocoagulation is preferred if coagulation is required for bleeding that does not stop despite cutting with scissors.

One of the important complications is perforation. In the presence of advanced adhesion and obliterated cavity, hysteroscopy procedures should be performed by experienced surgeons. Preventing the recurrence of adhesions in the postoperative period is one of the important issues as well as complications. For this, some barriers are left in the cavity, and time is gained for the regeneration of the endometrium before new adhesions are formed. Even hormonal therapy containing estrogen is preferred by many surgeons to support this regeneration process. There is no standardization in hormonal therapy, duration, route of administration, and whether progesterone should be given together or not. Studies and evidence regarding the benefit and superiority of these postoperative treatments are not yet sufficient (21). Antibiotic treatment is also commonly preferred in the postoperative period.

Solid barriers such as foley catheters, intrauterine balloons, and intrauterine devices are preferred to reduce adhesion reformation. In addition, hyaluronic acid in gel form and combinations of carboxymethylcellulose and hyaluronic acid can also be used as semisolid barriers. Some surgeons also apply second look hysteroscopy within 2 months for control purposes. However, it should not be forgotten that approaches to prevent adhesions are also important rather than treating them. For example, in cases of abortion, hysteroscopic removal of residual pregnancy material instead of sharp curettage minimizes the damage to the endometrium (21).

Patients should be informed that pregnancy complications such as placenta accreta, increata, percreata, postpartum hysterectomy, and prematurity will increase in pregnancies after adhesiolysis. In addition, the follow-up and delivery of these pregnant women should be done in tertiary centers with experienced obstetricians (36).

Istmocele, also called diverticulum or niche, develops due to insufficient healing of the myometrium tissue on the

incision line of the uterus during a cesarean section. Istmocele generates menstrual blood and mucus accumulate in the pouch. This blood and mucus can cause abscess formation, affecting sperm motility and causing infertility. In addition, cervical mucus also causes a chronic inflammatory environment.

The patient should be evaluated after menses for the diagnosis. It can be detected incidentally on TVUSG. TVUSG, SIS, HSG, and MRI help in diagnosis. Hysteroscopy is the gold standard in diagnosis.

Treatment should be done for symptomatic patients. For secondary infertility, hysteroscopy-guided isthmocele resection, vaginal repair, laparoscopic, laparotomic, or combined approaches may be appropriate. Hysteroscopy and laparoscopy are used together in combined approaches. In the hysteroscopic approach, fibrotic tissue in the endomyometrial area at the inferior and superior edges of the scar line is resected. The thinnest base of the defect is also coagulated with the roll ball electrode because this area contains ectatic vessels covered with thin mucous membranes. Thus, the niche formed here will be expanded a little and the accumulation of menstrual blood will be prevented.

The most important risk in the repair is uterine perforation and bladder injury during the procedure. This risk increases if the myometrial residual thickness is less than 3 mm. Therefore, in case of residual myometrial tissue thinner than 3 mm, the laparoscopic approach is preferred because of the risk of perforation of hysteroscopy. In the laparoscopic approach, fibrotic scar tissue and the isthmocele area are resected from the healthy myometrial tissue line. 2 layers are sutured with 2-0 vicryl. The peritoneum can also be sutured as the third layer. It has been shown that myometrial thickness increases with this technique (37).

In the combined hysteroscopy laparoscopy technique, the localization of the isthmocele is detected with a hysteroscope, and excision is made by laparoscopy from the line where the light transilluminates. With the vaginal approach, the scar tissue is cut and opened. The fibrotic scar is excised. The incision is sutured in 2 layers. In minilaparotomy, the scar defect is excised. The residual myometrium tissue is repaired by suturing again. In procedures where scar tissue is excised and sutured by making an incision, a hysteroscopy can be performed to check the adequacy of the repair.

In general, hysteroscopic resection can be performed on those who do not want pregnancy. Hysteroscopy is not recommended in patients with residual myometrial thickness <3 mm at the defect site, due to the risk of bladder injury and uterine rupture. Incision, excision, and multi-layered suture are preferred for those who want pregnancy. The chance of pregnancy with isthmocele repair in secondary infertile patients has been reported to be 40-80%. Surgical excision of the defect may increase fertility since endometriosis can also be seen in the excision material (37). Hysterectomy can also be performed in symptomatic women who do not intend to become pregnant.

OVARIAN CAUSES

Ovulatory causes are the most common factors related to female infertility (3). Ovarian causes are rare causes of infertility that will require surgery. The most common cause of infertility due to anovulation is polycystic ovary syndrome (PCOS) (38). The ovulatory cause for which surgery may be most necessary is PCOS. Infertility treatment for women with PCOS includes lifestyle changes (diet, exercise, and changing habits), pharmacological treatments, surgical treatment (laparoscopic ovarian surgery), or IVF (39). Bariatric surgical treatment options are also on the agenda in the patient group where weight control cannot be achieved with diet and exercise.

The primary goal in patients with PCOS is monofollicular development. With the laparoscopic ovarian drilling (LOD) treatment method, monofollicular development can be achieved. Pregnancy can also be achieved without any additional treatment or follow-up. Concurrent LOD may also be appropriate in patients who have additional infertility factors and are currently scheduled for laparoscopy.

LOD may be advantageous in the group of patients who are difficult to follow up, who cannot be monitored for endocrine, who live far from the center, who have high luteinizing hormone (LH) values, and who are weak. Carbon dioxide, argon, or aluminum granite crystal laser, unipolar or bipolar electrocautery can be applied laparoscopically for the procedure. While a single ovary may be sufficient, both ovaries can be applied in the same session. There is no consensus on how many watts of energy will be used, how many holes will be drilled, and in how many seconds due to the risk of reducing the ovarian reserve for the procedure that can be applied to both ovaries in the same session. However, clinicians' preferences in this regard are accepted by using the watt X seconds X number formulation, not exceeding 600 watts. Accordingly, 4 or 5 holes, each of 30 watts, and drilling lasting 4 seconds are made.

CONCLUSION

Patient selection is important for laparoscopy and hysteroscopy procedures. Patients should be informed about the complications which can occur during the procedures and also during the pregnancy or giving birth if pregnancy happens. These operations must be done in tertiary centers by expert surgeons, preferably IVF specialists.

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Intrauterine Insemination, IVF/ICSI

Intrauterin Inseminasyon, IVF/ICSI

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ABSTRACT

Infertility is defined as the inability to conceive after one year despite regular intercourse. The need for treatment and treatment option are determined by the evaluation process that starts after this stage. Treatment mainly includes ovarian stimulation followed by timed intercourse, intrauterine insemination (IUI), and in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI). Choosing the appropriate patient and appropriate treatment method is critical here and is based on many factors. Maternal age and infertility etiology are the main determinants of treatment selection. As important as it is to think about IVF/ICSI selection quickly in patients over 38 years of age, it is equally important to switch to assisted reproductive techniques without waiting in the presence of very low sperm parameters. An inappropriate indication will fail even if the treatment is done in the best way. Appropriate patient selection and giving enough time to the patient in the treatment stages will increase success. In the presented article, IUI and IVF/ICSI patient selection criteria will be evaluated according to the causes of infertility. Here, the order in which the patient will be evaluated and the steps to be taken on the way to IVF/ICSI in the treatment of staged infertility will be evaluated in detail and descriptively.
Keywords: Infertility; intrauterine insemination; in vitro fertilization; treatment.

ÖZ

İnfertilite düzenli ilişkiye rağmen bir yılın sonunda gebe kalamama olarak tanımlanmaktadır. Bu aşamadan sonra başlayan değerlendirme süreci ile tedavi ihtiyacı ve tedavi seçeneği belirlenmektedir. Tedavi temel olarak over stimulasyonunu takiben zamanlı ilişki, intrauterin inseminasyon (IUI) ve in vitro fertilizasyon (IVF)/intrasitoplazmik sperm enjeksiyonunu (intracytoplasmic sperm injection, ICSI)'nu içermektedir. Burada uygun hasta ve uygun tedavi yönteminin seçilmesi kritiktir ve birçok faktöre bağlıdır. Anne yaşı ve infertilite etiyolojisi tedavi seçiminde ana belirteçtir. 38 yaş üzeri hastada hızla IVF/ICSI seçimi hakkında düşünmek ne kadar önemli ise çok düşük sperm parametreleri varlığında beklemeden yardımcı üreme tekniklerine geçmek de o kadar önemlidir. Uygun olmayan endikasyon tedavi en iyi şekilde bile yapılsa başarısız olacaktır. Uygun hasta seçimi ve tedavi aşamalarında hastaya yeterli süre verilmesi başarıyı artıracaktır. Sunulan makalede infertilite nedenlerine göre IUI ve IVF/ICSI hasta seçim kriterleri değerlendirilecektir. Burada hasta değerlendirmesinin hangi sıra ile yapılacağı ve basamaklı infertilite tedavisinde IVF/ICSI'ya giden yolda geçilmesi gereken basamaklar detaylı ve açıklayıcı olarak değerlendirilecektir.
Anahtar kelimeler: İnfertilite; intrauterin inseminasyon; in vitro fertilizasyon; tedavi.

INTRODUCTION

Infertility is defined as the inability to achieve pregnancy despite one year of regular and unprotected intercourse (1,2). It results from male factors in approximately 30-40%, female factors in 30-40%, unexplained in 10-15%, and multifactorial conditions in 10-15% of the patients. Treatment options for the active management of infertility include ovulation induction, intrauterine insemination (IUI), and in vitro fertilization (IVF). The choice of treatment option is exactly based on many factors such as the woman's age, the cause of infertility, ovarian reserve, and duration of infertility. However, several critical factors such as accessibility, effectiveness, cost, safety, and risks of treatment may also affect the choice of treatment option.

Intrauterine Insemination Today

The IUI is a simple, inexpensive, non-invasive, and safe treatment option for the management of infertility in eligible couples. The basic principle of IUI ensures that morphologically and genetically normal sperms with less DNA damage and more intact cell membrane are isolated with appropriate and simple sperm preparation techniques to reach the oocyte. IUI is mainly recommended for male factors, unexplained infertility, stage I-II endometriosis, and cervical factor (3). The National Institute for Health and Care Excellence (NICE) guideline in 2013 does not recommend IUI for male factor, unexplained infertility, and stage I-II endometriosis, which are the most common indications for IUI unless there are religious, cultural, and social prohibitions for IVF. They recommend direct IVF if not able to achieve pregnancy with expectant management after two years. However, this recommendation in the guideline for the more global use of IVF which is a more expensive, invasive, and difficult method is based on the results of only two studies (4,5). As a result, infertile couples with a good prognosis benefit from IUI would be clearly determinated to avoid unnecessary IVF applications.

Intrauterine Insemination for Whom? How to Decide?

Hunault et al. (6) first performed model validation in 2004 to select patients with a good prognosis for IUI. This model is based on female age, duration of infertility, primary/secondary infertility, and motile sperm count. It aims to determine the couple's chances of spontaneous pregnancy within one year. A new model system was also created by the collaborative effort for clinical evaluation in reproductive medicine (CECERM) group (https://www.freya.nl/probability.php) in 2007 and proposed as suitable for patients with both patent tubes, an infertility duration of >1 year, female age of <38, ovulatory females, and without severe male factors (7). The score for the chance of spontaneous pregnancy in a year according to this model is calculated by using the female age, infertility duration, primary/secondary infertility, and motile sperm count. Patients are eligible for IUI if the score is >30% in both models. In general, the literature suggests that the success of IUI is higher in patients with a young age, good ovarian reserve, short duration of infertility, and secondary infertility. A descriptive prospective validation study included 1,079 subfertile couples who underwent 4,244 cycles of IUI in seven fertility centers, was found that the ongoing pregnancy rate for IUI was 6.6% per cycle (8). According to the scoring system of this study, patient with a score <5 has the highest chance of pregnancy, and the pregnancy chance of this group is >12%. The scores based on female age were identified as 7 for 20-25 years, 9 for 26-31 years, 10 for 32-35 years, 11 for 36-39 years, and 12 for >40 years. The scores based on the duration of infertility were identified as 0 for 1-2 years, 1 for 2-5 years, 2 for 5-7 years, and 3 for 7-13 years. Scores based on the etiology of infertility were identified as 0 for unexplained, -3 for cervical, and 1 for male factors. Scores based on the stimulation protocol were 0 for none, -2 for clomiphene, and -2 for human menopausal gonadotropin (hMG) or follicle stimulating hormone (FSH). Lastly, scores based on the number of cycles were identified as 1 for 1, 2 for 2, 3 for 3, 4 for 4, and 5 for 5-13.

Natural or Stimulated Intrauterine Insemination?

A meta-analysis including 26 studies reporting on 5316 women compared the effectiveness and safety of IUI with clomiphene citrate (CC), letrozole, or gonadotrophins with each other and with IUI in a natural cycle (9). This meta-analysis indicated that gonadotropin-stimulated IUI cycles have higher pregnancy rates with high complication rates such as multiple pregnancies. Therefore, they recommended the use of strict cancellation criteria to avoid high multiple pregnancy rates. A Cochrane review in 2020 including 15 trials with 2068 women evaluated live birth rates in the unexplained infertile group by IUI treatment with or without ovarian stimulation compared to timed intercourse or expectant management with or without ovarian stimulation or IUI with ovarian stimulation compared to IUI in a natural cycle (10). Despite insufficient data, they showed that in couples with a low prediction score of natural conception, IUI in a natural cycle probably results in a higher cumulative live birth rate when compared to timed intercourse with ovarian stimulation. IUI with ovarian stimulation may result in a higher cumulative live birth rate compared to IUI in a natural cycle. IUI with ovarian stimulation probably results in a higher cumulative live birth rate compared to expectant management without ovarian stimulation. As a result, IUI with ovarian stimulation seems to have the highest cumulative live birth rate among these approaches. Three randomized controlled trials comparing IUI with ovarian stimulation vs IVF indicated similar live birth rates with 3-6 IUI with ovarian stimulation and 1-2 IVF treatments. The point highlighted in these studies was to increase the cancel rate against the high risk of multiple pregnancies (7-25%) in gonadotropin-stimulated IUI cycles when compared to IVF treatment with single embryo transfer (11-13). A Cochrane review including 27 randomized controlled trials (RCTs) with 4,349 couples evaluated the effectiveness and safety of different approaches (expectant management, ovarian stimulation, IUI, ovarian stimulation-IUI, and IVF/intracytoplasmic sperm injection, ICSI) in couples with unexplained infertility. It showed that IUI with ovarian stimulation improves pregnancy rates but it may also increase the incidence of multiple pregnancies. It highlighted that the use of strict cancellation criteria may prevent the increase in the incidence of multiple pregnancies. However, in this meta-analysis, the increase in the cost of IUI through the use of gonadotropins was not evaluated (14). IUI should

be canceled to prevent the possibility of increased multiple pregnancies in when >2 follicles with >15 mm or >5 follicles with >10 mm have occurred.

A retrospective observational study including 319 105 IVF and 30 669 IUI cycles compared to IUI vs IVF in terms of success rates, associated risks, and cost-effectiveness (15). They concluded that the success rate of IUI is almost similar to IVF and IUI is related to the lower risk of maternal and neonatal complications and lower cost. In 2015, a Cochrane review compared IVF to other treatment modalities (expectant management, IUI of ovulation induction-IUI) in terms of pregnancy rates in unexplained infertility (16). It found that there is no difference between IVF and ovulation induction-IUI in terms of live birth rate in patients who had not received any treatment before. In the light of literature, it still seems to be suitable for the use of IUI treatment for eligible patients before IVF treatment (17).

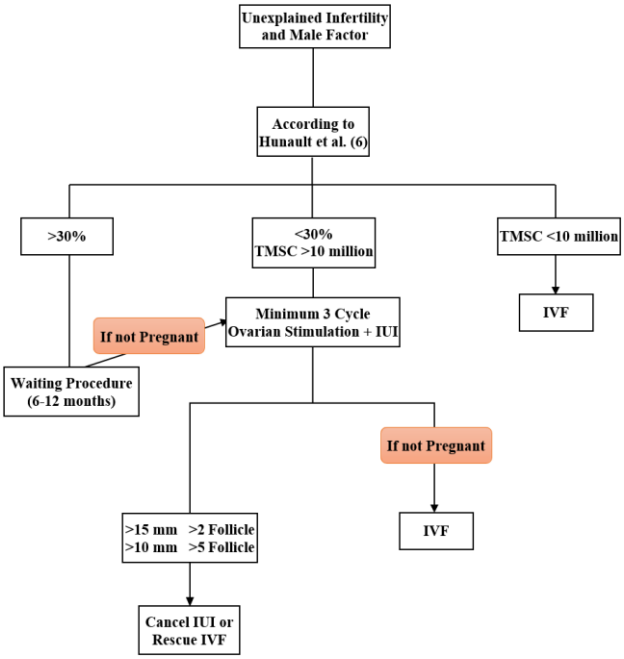

Figure 1. Decision making approach for IUI vs IVF
TMSC: total motile sperm count, IUI: intrauterine insemination, IVF: in vitro fertilization

What Do the Guides Say?

The NICE guideline in 2013 does not recommend IUI for male factor, unexplained infertility, and stage I-II endometriosis, which are the most common indications for IUI unless there are religious, cultural, and social prohibitions for IVF. They recommend direct IVF if it not being able to achieve pregnancy with expectant management after two years. The Canadian guideline in 2019 suggests that based on the female age and the duration of infertility, expectant management recommends in couples with unexplained infertility (18). Otherwise, IVF can be considered as either the first-line treatment choice or after 3 cycles of ovulation induction-IUI if not achieving pregnancy. It also highlighted the increase in the risk of multiple pregnancies in gonadotropin-stimulated IUIs. Likewise, the American Society for Reproductive Medicine (ASRM) guideline in 2020 recommends IVF treatment if not achieving pregnancy with 3-4 cycles of ovulation induction-IUI. ASRM does not recommend gonadotropin-stimulated IUIs because there is no superiority of gonadotropins to oral agents in terms of success and it is related to the increase in the risk of multiple pregnancies and higher cost (19). On the other hand, ASRM stated that IVF could be considered as a first-line treatment to decrease the time to achieve pregnancy if the woman's age is >38 because IVF has higher pregnancy rates in this age group.

When should In Vitro Fertilization be the First Choice?

Although a systematic review included eight RCTs compared the efficacy of ovarian stimulation-IUI and IVF in couples with unexplained infertility. They concluded that regardless of previous treatment history, there was no difference in live birth rates if the female age was <38 but it also demonstrated that the live birth rate in women over 38 age was two times higher in IVF (20). An RCT compared the results of immediate IVF and two cycles of ovulation induction-IUI (oral agents and gonadotropin) in women aged 38-42 years with unexplained infertility (21). The results of this study showed higher pregnancy rates with fewer treatment cycles in the immediate IVF group.

In the light of literature, IVF can be considered as the first-line treatment for older women because of the increase in the risk of aneuploidy related to advanced female age, higher pregnancy rates of IVF, and the decrease in time to achieve pregnancy. While total motile sperm count (TMSC) is one of the most important predictors to determine the success of IUI treatment, IVF can also be considered as first-line treatment in patients with TMSC <10 million and severe male factors although there is no clear threshold value determined in the studies (22).

CONCLUSION

Infertility treatment is a field of treatment that requires specialist training on the subject. Especially in IVF/ICSI applications, it is essential not to make mistakes in standard laboratory techniques as well as patient selection. The correlation between laboratory and clinical follow-up will increase success. Appropriate patient selection and giving enough time to the patient in the treatment stages will increase success.

Key facts:

- Couples with a good prognosis for IUI treatment can be determinated by using prognostic models.
- Ovarian stimulation-IUI would be suitable in couples with a good prognosis.
- There is no superiority of the use of gonadotropins to the use of oral agents for ovarian stimulation in terms of success rate during IUI treatment. However, the use of gonadotropins for ovarian stimulation during IUI treatment is associated with an increase in cost and risk of multiple pregnancies.
- The strict cancellation policy should be used to reduce the risk of multiple pregnancies related to ovarian stimulation-IUI cycles.
- IVF treatment can be considered if not achieving pregnancy with 2-3 cycles of ovarian stimulation-IUI.
- IVF can be considered as the first-line treatment in the following situations including women over 38 age, TMSC <10 million, and severe male factors.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

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Evaluation and Interpretation of AMH in Female Infertility

Kadın İnfertilitesinde AMH'nin Değerlendirilmesi ve Yorumlanması

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ABSTRACT

Anti-Müllerian hormone (AMH) is a glycoprotein that secreted by the granulosa cells of the pre-antral and antral ovarian follicles that have a diameter <8 mm. By inhibiting both initial recruitments of primordial follicles into primary follicles and also the sensitivity of antral follicles to follicle stimulating hormone (FSH) in cyclic recruitment AMH acts as a "follicular gatekeeper". AMH is recognized as an early marker of the decline in the follicular pool. Although AMH levels are accepted to be stable all through the menstrual cycle, inter- and intracycle variability are detected in the studies with the highly sensitive automated assays. Besides aging, body mass index, obesity, oral contraceptive use, previous ovarian surgery, chemotherapy, BRCA mutations, and ethnicity play a role on the AMH levels. Polycystic ovary syndrome (PCOS) is related with increased AMH level and thus proposed to be used as a diagnostic criterion. However, there is no universally accepted threshold value for AMH that can be used in the diagnosis of PCOS. AMH levels have also been used for designing an ideal treatment protocol in assisted reproduction. AMH measurements can be utilized for the prediction of poor or hyper ovarian response. The value of AMH levels in the prediction of pregnancy outcome remains controversial.
Keywords: Anti-Müllerian hormone; female; infertility.

ÖZ

Anti-Müllerien hormon (AMH), çapı 8 mm'nin altında olan pre-antral ve antral over foliküllerinin granüloza hücreleri tarafından salgılanan bir glikoproteindir. AMH, hem primordial folliküllerin primer folliküllere gelişimini hem de antral folliküllerin siklik recruitment aşamasında folikül uyarıcı hormona (follicle stimulating hormone, FSH) duyarlılığını inhibe ederek bir "folikül bekçisi" olarak görev yapar. AMH, folikül havuzundaki azalmanın erken bir belirteci olarak kabul edilmektedir. AMH düzeylerinin tüm menstrüel siklus boyunca sabit değerlerde olduğu kabul edilmekle birlikte, yüksek duyarlılıklı otomatize kitlerle yapılan çalışmalarda, hem aynı siklus içinde hem de farklı sikluslar arasında değişkenlik gösterdiği saptanmıştır. Yaşlanmanın yanı sıra, vücut kitle indeksi, obezite, oral kontraseptif kullanımı, geçirilmiş over cerrahisi, kemoterapi, BRCA mutasyonları ve etnik köken de AMH değerleri üzerinde etkiye sahiptir. Polikistik over sendromu (PKOS), artmış AMH düzeyi ile ilişkilidir ve bu nedenle tanı kriteri olarak kullanılması önerilmektedir. Bununla birlikte, AMH için PKOS tanısında kullanılacak evrensel olarak kabul edilmiş bir eşik değer belirlenmemiştir. AMH değerleri, yardımcı üreme tekniklerinde ideal bir tedavi protokolünün belirlenmesi için de kullanılmaktadır. AMH ölçümleri zayıf veya aşırı over cevabının öngörülmesi için kullanılabilir. AMH düzeylerinin gebelik sonuçlarının öngörülmesindeki yeri tartışmalıdır.
Anahtar kelimeler: Anti-Müllerian hormon; kadın; infertilite.

INTRODUCTION

Anti-Müllerian hormone (AMH) is a glycoprotein that belongs to the transforming growth factor β (TGF-β) family. In a male fetus, AMH is secreted by the immature Sertoli cells and predominantly is responsible for the regression of the fetal Müllerian ducts. In boys, AMH secretion continues until puberty and serves as a marker for differential diagnosis of sexual development disorders, hypogonadism, and cryptorchidism. In women, AMH is secreted by the granulosa cells of the pre-antral and antral ovarian follicles that have a diameter of <8 mm (1). Expression of AMH stops when the follicles reach a diameter of 8-10 mm. As AMH is expressed by the early growing follicles, its level is shown to be directly related to the primordial follicle pool (2). Moreover, researchers have demonstrated a positive correlation between the AMH levels and the antral follicle count (AFC) that represents the number of follicles with diameters of 2 mm to 9 mm (3). AMH is used as a biological marker for the evaluation of ovarian reserve as it reflects the follicular pool (4). Follicular pool assessment is designated to be used for the prediction of the number of growing follicles during ovulation induction that is related to the number of retrieved oocytes and the chance of achieving pregnancy during treatment cycles (5).

Regulation of AMH in Granulosa Cells

AMH is secreted by the granulosa cells of the growing follicle, and both AMH gene expression and AMH production is increased until the follicle reaches a diameter of 8 mm. Estradiol plays an important role in the regulation of AMH expression through estrogen receptor β (ER-β) that is present in the granulosa cells (6). Both AMH gene expression and AMH concentration are demonstrated to be negatively correlated with estradiol concentration (7) while being positively correlated with follicle stimulating hormone receptor (FSHR) and anti-Müllerian hormone receptor 2 (AMHR2) expression. Follicular fluid AMH concentration has a negative correlation with CYP19 mRNA expression, estradiol, progesterone, and inhibin-B (8). Before follicular recruitment, AMH is believed to play a role in the inhibition of aromatase (i.e., CYP19) expression while decreasing the conversion of androgens to estrogens. When the follicles reach a diameter of ≤8 mm, AMH gene and protein expression regress rapidly and a decrease in AMH gene promoter activity plays an important role in this process through ER-β. AMH has a limiting effect on follicular growth initiation in response to follicle stimulating hormone (FSH) stimulation prior to follicle selection. By inhibiting both the initial recruitment of primordial follicles into primary follicles and also the sensitivity of antral follicles to FSH in cyclic recruitment AMH acts as a "follicular gatekeeper". AMH is not shown at the cellular level after FSH-dependent follicular growth and dominant follicle selection begins. Atretic follicles and corpus luteum does not show AMH expression.

AMH has different isoforms with different molecular weights and even the same-sized follicles may contain different AMH isoforms (9).

AMH Levels during the Life-Span of Women

The AMH levels that are very low during birth in the female newborn rise gradually with increasing age until adolescence and then reach a plateau until 25 years of age (10). Serum AMH levels are negatively correlated with the age of adult women after age 25. Ethnicity is demonstrated to play a role in peak AMH levels at age 25 and the pace of age-related AMH decline in various studies (11). Due to the longitudinal decline of AMH after the mid-twenties, AMH is recognized as an early marker of the decline in the follicular pool when compared to FSH that increases only after age 35 due to the diminishing ovarian reserve (12). AMH levels become undetectable 5 years before menopause.

Is there an Intra- and Inter-Cycle Variability of AMH Levels?

Based on the preliminary studies, serum AMH levels were considered as stable all through the menstrual cycle (13), however, during the menstrual cycle variations to a degree were demonstrated in normally menstruating women and these variations were higher in younger women with higher AMH levels (14). New generation automated highly-sensitive assays have been developed for the precision of the test results as the old generation tests were manual. Besides the presence of different isoforms that can be detected differently by different assays various factors still have an impact on the measured values. Inter-cycle variability is also reported depending on the assay used and repeated measurements are performed in clinical practice in order to design an individualized treatment protocol for infertile patients.

What are the Factors Affecting Serum AMH Levels?

Body mass index (BMI), has been shown to have a negative impact on AMH levels (15), but the mechanism of this effect is not clear while leptin is believed to play a role. Some studies reported lower AMH levels in BRCA1/2 carriers and this is explained by the diminished oocyte pool related to the increased DNA damage in the oocytes in BRCA1/2 carriers although some studies showed controversial results (16). Similarly, conflicting results are published about the influence of vitamin D deficiency and vitamin D supplementation on AMH levels (16). Seasonal variations in vitamin D were reported to be parallel to the seasonal variations in AMH levels with a decreased AMH level in winter (17). Ovarian surgery especially in endometriomas and bilateral ovarian cysts is related to a decrease in AMH levels (16). Oral contraceptive use leads to a decrease in AMH levels that will recover 3-6 months after cessation of the contraceptive method (18). Chemotherapy also has a negative effect on AMH levels (16). Serum AMH levels were found to be lower in women with autoimmune diseases such as lupus erythematosus, rheumatoid arthritis, autoimmune thyroid disease, and also women with type 1 diabetes although some of the results are inconsistent (11). In women with polycystic ovary syndrome (PCOS), the number of small antral follicles that are producing AMH is increased, and thus the serum AMH level is 2 to 3 fold increase when compared to normo-ovulatory women (19). A serum AMH cut-off value of 11.4 ng/ml was given to predict the presence of amenorrhea in women with PCOS with fairly high sensitivity and specificity by Tal et al. (20). Besides the increased number of small antral follicles, higher production of AMH from granulosa cells induced by elevated LH levels and androgen-induced FSH-independent follicular development are also reported to contribute to the higher AMH levels detected in women

with PCOS (16,21). Leptin signaling pathway and insulin resistance may also play a role in the mechanisms that lead to the elevation of AMH levels in women with PCOS. AMH levels were proposed to be integrated into the Rotterdam classification as a substitute to the ultrasonographic definition of polycystic ovarian morphology (PCOM) as one of the three criteria used for the diagnosis of PCOS. Wongwananuruk et al. (22) proposed a threshold level of 4.7 ng/ml for diagnosis of PCOS, while Song et al. (23) reported 10 ng/ml as an optimal cut-off value for differentiation between PCOS and PCOM. At present, there is no universally accepted threshold value for AMH that can be used in the diagnosis of PCOS.

Can AMH be used for the Prediction of Ovarian Response during Treatment Cycles?

Currently controlled ovarian stimulation protocols are individualized in order to obtain the optimal response that will lead to an improved pregnancy rate. Besides, individual factors such as age, BMI, AFC, basal FSH level, and serum AMH levels have also been used in the decision tree for designing an ideal treatment protocol. Serum AMH levels are currently evaluated for prediction of poor response/cancellation or hyper response and thus the risk of ovarian hyperstimulation syndrome (OHSS) and even more prediction of total fertilization failure (TFF), pregnancy, and obstetric outcome.

A cut-off level of AMH for obtaining a positive ovarian response and prediction of pregnancy in clomiphene citrate (CC) cycles was evaluated. Coşkun et al. (24) reported a cut-off value of 2.78 ng/ml for the prediction of positive ovarian response to CC in patients with unexplained infertility. Xi et al. (25) recommended the use of AMH as a predictor of ovulation induction in patients with PCOS who received CC and gave a threshold value of 7.77 ng/ml.

AMH levels were reported to be valuable in the prediction of ovarian response in in vitro fertilization (IVF) cycles as well (26). An AMH level of below 1.1 ng/ml was found to be related with TFF (27). Dose adjustment of gonadotropins used for ovarian stimulation accompanied by either gonadotropin releasing hormone (GnRH) agonists or antagonists are important in the prevention of under or overstimulated cycles. The European Society of Human Reproduction and Embryology (ESHRE) strongly recommends the use of either AFC or AMH for predicting high and poor responses to ovarian stimulation. Although serum AMH measurement is recommended over other hormonal ovarian reserve tests, a cut-off value for the prediction of low or high ovarian response is not given (28). Toner et al. (29) summarized general guidelines on the evaluation of AMH levels in patients receiving infertility treatment: AMH concentration <0.5 ng/mL may be predictive of poor outcome in IVF treatment and treatment protocols for poor ovarian reserve such as microdose GnRH agonist flare-up protocol are recommended. Patients with an AMH level of <1.0 ng/mL may have a limited chance of pregnancy during treatment cycles. Mild stimulation is recommended in patients with an AMH level >3.5 ng/mL in order to avoid the risk of OHSS (29). The cut-off values given are not age specific so it is debatable whether there is a need for the definition of age-specific low AMH concentrations.

In clinical practice, the value of AMH as a marker for ovarian reserve is widely recognized however the patients should not be excluded from IVF treatment based on the low AMH levels. Patients with very low AMH values should be counseled about the possible outcome of the treatment cycle.

There are a remarkable number of studies evaluating the association between the AMH concentrations and implantation, clinical pregnancy, and live birth rates in IVF cycles, and a negative correlation was found with lower AMH levels, however, the results remain controversial (30).

CONCLUSION

AMH is an early predictor of ovarian aging and thus is widely used for designing treatment protocols and prediction of the ovarian response in treatment cycles. However, the patients should not be refrained from IVF treatment due to low AMH levels but should be counseled about the possibility of a poor outcome.

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Non-Specific Medical Treatment Methods in Female Infertility

Kadın İnfertilitesinde Spesifik Olmayan Medikal Tedavi Yöntemleri

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ABSTRACT

Infertility, which is defined as the inability to conceive despite one year of unprotected sexual intercourse, affects 15% of couples. Any patient with infertility by definition or at high risk of infertility may be offered an infertility evaluation. In women older than 35 years, this waiting period can be limited to 6 months, and then infertility evaluation can be started. In women older than 40 years, more urgent evaluation and initiation of treatment is the most important option. Evaluation of infertility must be done by experienced and trained physicians and necessary treatments must be followed by these physicians. Alternative treatment methods can be used in patients who do not respond after standard evaluation steps and generally accepted treatment options. Various supportive treatments come to the fore here. These options are used both to obtain better quality oocytes before treatment and to ensure that more follicles participate in stimulation. Antioxidants and metformin are the most commonly used agents before treatment in women who are thought to have insulin resistance, especially considering that oocyte mitochondrial DNA damage increases in advanced female age. On the other hand, agents such as growth hormone that should be used in a controlled manner by experienced specialists have been found effective in many publications. In the presented article, non-conventional treatment options for infertility are explained.
Keywords: Infertility; treatment; non-specific agents.

ÖZ

Korunmasız bir yıl cinsel ilişkiye rağmen gebe kalınamaması olarak tanımlanan infertilite, çiftlerin %15'ini etkilemektedir. Tanım gereği infertilitesi olan veya infertilite riski yüksek olan herhangi bir hastaya infertilite değerlendirmesi önerilebilir. 35 yaşından büyük kadınlarda bu bekleme süresi 6 ay ile sınırlandırılabilir ve sonrasında infertilite değerlendirmesine başlanabilir. 40 yaşından büyük kadınlarda daha acil değerlendirme ve tedaviye başlama en önemli seçenektir. İnfertilite değerlendirilmesi mutlaka deneyimli ve bu konuda eğitimli hekimler tarafından yapılmalı ve gerekli tedaviler bu hekimler tarafından takip edilmelidir. Standart değerlendirme basamakları ve genel olarak kabul edilen tedavi seçeneklerinden sonra cevap alınamayan hastalarda alternatif tedavi yöntemlerine geçilebilir. Burada çeşitli destek tedavileri öne çıkmaktadır. Bu seçenekler hem tedavi öncesi özellikle daha kaliteli oosit elde etmek için gerekse de hem de daha fazla folikülün stimülasyona katılmasını sağlamak için kullanılmaktadır. Özellikle ileri kadın yaşında oosit mitokondrisi DNA hasarı arttığı düşünüldüğünde, insülin direnci olduğu düşünülen kadınlarda antioksidanlar ve metformin tedavi öncesi en yaygın olarak kullanılan ajanlardır. Diğer yandan büyüme hormonu gibi deneyimli uzmanlar tarafından kontrollü bir şekilde kullanılması gereken ajanlar tedavide pek çok yayında etkin bulunmuştur. Sunulan makalede infertilite için konvansiyonel tedavi dışı seçenekleri anlatılmıştır.
Anahtar kelimeler: İnfertilite; tedavi; non-spesifik ajanlar.

INTRODUCTION

Despite the advances in infertility treatments, especially in the last two decades, additional treatments are still up-to-date, especially in patients with poor response. On the other hand, these options are frequently used in recurrent failures in in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles and in the presence of poor oocyte/embryo quality. While these options are often tablets, gels, or injectable preparations for ovulation induction, they also include improving laboratory conditions in IVF/ICSI applications.

Basically, all these auxiliary methods that are used both in laboratory and clinically in the infertility treatment process and ultimately aim to achieve a healthy pregnancy are called "adjuvant therapy", also termed as an adjunct therapy or add-on therapy (1). In the presented article, adjuvant agents used in ovulation induction regimens will be briefly mentioned.

Although the target in adjuvant treatments is oocyte, sperm, and endometrium, it is also included in methods that affect general body health. According to this, androgens, growth hormone, metformin, and antioxidants are used for ovarian response and oocyte quality, antioxidants and sperm selection practices are used for sperm count and quality, and estrogen, aspirin, heparin, immunity-oriented therapies (steroid, intravenous immunoglobulin (IVIG), anti-tumor necrosis factor (TNF) alpha, platelet rich plasma (PRP), general methods, and acupuncture are used to improve endometrial quality and implantation.

OVARIAN RESPONSE AND OOCYTE QUALITY

Androgens

Androgens play an important role in ovarian physiology. In particular, it acts as an estrogen precursor in the follicle within certain limits. Androgens in infertility are mostly used for women who respond poorly or are likely to do so in IVF/ICSI cycles. Dehydroepiandrostenedion (DHEA) is the most widely used androgen, especially in poor responders. It is also available without a prescription (2). It helps by increasing the amount of insulin-like growth factor-1 (IGF-1), which has a role in both providing an estrogen precursor and developing follicles. In this way, both the quality of the oocyte and the number of follicles that respond to the simulation increase. However, the fact that the patients were quite heterogeneous and included a limited number of cases in the studies questioned the reliability. In the Cochrane review, it was reported that a significant increase in the live birth rate was found with the use of DHEA (odds ratio, OR: 1.88, 95% confidence interval, CI: 1.30 to 2.71). However, it has also been suggested that there are not enough randomized controlled trials (RCTs) and therefore the data are insufficient. It is recommended to use 25 mg tablets 3x1 (75 mg/day) for at least 2-3 months before treatment. Androgenic side effects are negligible during use (3).

Testosterone is another agent used in clinical practice. It has a critical role in intra-ovarian follicle development and estrogen production. Since oral use is not active, it is recommended to use gel (12.5 mg/day) and patch (2.5 mg testosterone in the first 5 days of treatment). However, the use of this agent needs more research due to the lack of adequate RCTs (4).

Growth Hormone

It performs its main effect through the synthesis of IGF-1 in the follicle granulosa cells. It has a positive effect on both follicle growth and oocyte maturation (5). Although it is a subcutaneous use form, its use has been defined differently in different studies. Usages have been defined as 8 IU daily or 13 IU every other day or 2 IU daily. It is stated that it increases live birth in poor responders, especially at doses started before treatment (6). However, the small number of RCTs and the low number of cases in the presented studies prevent a definitive conclusion. On the other hand, there are articles stating that it only increases clinical pregnancies, does not increase the live birth rate, or there is no difference between clinical pregnancy and live birth rates. As a result, although it is frequently used in IVF (ICSI cycles), especially in poor responder patients, there are insufficient data to show that it especially increases live birth (7).

Metformin

It is an oral antidiabetic, insulin-sensitizing, agent that is frequently used against possible insulin resistance, especially in obese polycystic ovary syndrome (PCOS) patients. Blaming insulin resistance in the etymology of PCOS has led to the use of insulin sensitizers, especially for ovulation restoration (8). In the first studies on metformin, it was determined that 850 mg twice a day in IVF cycles did not make an additional contribution to the treatment. However, a decrease in the risk of ovarian hyperstimulation syndrome (OHSS) due to increased vascular endothelial growth factor (VEGF) with follicle development has been detected, especially in PCOS patients. Therefore, the main benefit of metformin in IVF cycles was stated in the Cochrane review as OHSS prevention (9). Although many studies have reported a decrease in abortion rates with the use of metformin, this has not been confirmed in large case series. Although spontaneous ovulation can be achieved with the use of metformin, its use as an ovulation agent is not recommended (10).

Antioxidants

Oxidative stress is destructive pathological agents that can damage cell wall lipid structures and DNA in all cells. Oocyte mitochondria, which have had low metabolic activity in oocytes since the intrauterine period, are more sensitive to oxidative stress since they do not have sufficient DNA protective mechanisms (11). Mitochondrial DNA damage may cause oocyte division and maturation problems. However, the point to be considered in the use of antioxidants is that low level oxidation is necessary for cell division and saturation, and it can have a reverse effect in aggressive use.

Many antioxidants are used in clinical practice. The most common are: L-arginine, melatonin, vitamin E, vitamin D, Myo-inositol, d-chiro inositol, and CoQ10. A Cochrane review compared the effects of these antioxidants with each other and with placebo (12). As a result, although the sources are of low quality publications, there has been a slight increase in both live birth (OR: 2.13, 95% CI: 1.45 to 3.12, and clinical pregnancy (OR: 1.52, 95% CI: 1.31 to 1.76) rates. CoQ10 has a special place in IVF/ICSI cycles. As it is known, the energy required for the cell is supplied from the electron transport chain together with the Krebs

cycle in mitochondria. CoQ10 is frequently used to prevent or reverse mitochondrial dysfunction, especially in elderly and poor responder patients (12). This re-functioning mitochondrion will have an important role in cell saturation, division, and granulosa cell function (13). Although positive opinions about CoQ10 are common, it was found in a recent meta-analysis that it increased the clinical pregnancy rate, especially in poor responder patients, and had no effect on live birth and abortion rates. Therefore, more standardized RCTs are needed for both antioxidants and CoQ10 use.

SPERM COUNT AND QUALITY

In men, unlike women, it is an important advantage to be able to see the germ cells directly with a test. In this way, possible pathologies can be recognized with higher accuracy. Here, number, movement, and morphology are the most important parameters to be evaluated. However, since the subject is female infertility, adjuvant treatments in male factor infertility will not be mentioned.

TO IMPROVE ENDOMETRIAL QUALITY AND IMPLANTATION

Even if the best embryo is obtained, successful live birth cannot be expected unless the appropriate endometrium and immunity required for implantation and growth are provided. For this purpose, preparing the endometrium for implantation and preventing the overreaction of immunity to a newly developing organism are other female fertility treatment options.

Estrogen can be used in the form of estrogen-priming, which helps to stabilize the irregularly developing follicular cohort as well as make the endometrium suitable for implantation, especially during freezing/thawing cycles (8). However, estrogen is not considered as an adjuvant treatment option and will not be reviewed here (14,15).

Aspirin is a non-steroidal anti-inflammatory agent that acts by inhibiting the enzyme involved in prostaglandin synthesis. The first indication for aspirin use is to prevent thrombus formation and to maintain thromboxane-prostacyclin balance. In this way, there was the idea that blood supply and embryo growth would continue. For this purpose, it is recommended that patients be started before treatment (8). However, further studies have shown that aspirin has no effect on live birth and clinical pregnancy except for more specific indications (16). Antiphospholipid antibody syndrome is a serious autoimmune pathology with pregnancy complications such as recurrent pregnancy loss and preeclampsia. It is thought that the use of low molecular weight heparin rather than its use alone in this group of patients may play a role in the treatment of infertility and the prevention of pregnancy complications (17,18). In the evaluation made by Zhang et al. (19), it was stated that the use of low molecular weight heparin only in addition to aspirin can be recommended in IVF/ICSI cycles in anticardiolipin ab positive patients. Similarly, in the Cochrane review, it was stated that aspirin alone did not improve outcomes in IVF/ICSI cycles (19).

Heparin has been evaluated in numerous studies, both with and without aspirin. It is very effective on trophoblastic invasion, especially in antiphospholipid antibody syndrome (8). On the other hand, its antithrombotic activity through the inhibition of factor Xa and thrombin formation in patients with thrombophilia allowed its use in these patients as well. Although bleeding is an important complication, it is not of clinical importance. On the other hand, heparin increases the rates of endometrial decidualization and implantation. It does this by decreasing the amount of insulin-like growth factor binding protein (IGFBP), increasing the amount of heparin-binding epidermal growth factor (HB-EGF), and increasing the expression of adhesion molecules such as E-cadherin (20-22).

Corticosteroids have been used in female infertility due to their anti-inflammatory and immunosuppressive effects. It is especially useful in patient groups who are thought to have immunological infertility in recurrent implantation failure. Because there is the idea that changes in Th1/Th2 balance and increases in uterine NK cell count may play a role in infertility in these patient groups (23). On the other hand, combined immunosuppression may increase implantation, as immunological factors often include thyroid or ovarian auto-antibodies. A total of 1879 couples were included in a Cochrane review and it was determined empirically that steroid initiation had no effect on IVF/ICSI cycle success, the clinical pregnancy rate (OR: 1.16, 95% CI: 0.94 to 1.44) or live birth rate (OR: 1.21, 95% CI: 0.67 to 2.19) (24).

CONCLUSION

Despite the lack of sufficient data, adjuvant treatments are currently used intensively in infertile women. Many of these treatments are far from sufficient scientific data. Clinicians should be especially vigilant in terms of the content and naturalness of these preparations. The use of experimental methods without sufficient clinical data will cause both safety and efficacy problems. Until adequate RCTs are available, nonspecific adjuvant treatments for female infertility seem to continue to be discussed.

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Oral Agents for Ovulation Induction

Ovulasyon İndüksiyonunda Oral Ajanlar

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ABSTRACT

Infertility due to ovulation disorders is 25% of all infertility causes. The most common cause of ovulation disorders is patients with normogonadatropic normogonadism, which is group II according to the World Health Organization anovulation classification and mostly consists of patients with polycystic ovary syndrome which affects 6-20% of women of reproductive age. Oral ovulation induction agents are a suitable option only for patients in this group. The purpose of the ovulation induction is to stimulate the ovaries for monofollicular development. Oral agents used in ovulation induction can be divided into two groups, selective estrogen receptor modulators and aromatase inhibitors as first-line agents, and metformin and inositols as second-line agents. The aim of this review is to compare the use and efficacy of the primary oral ovulation induction agents, clomiphene citrate and letrozole, and also to reveal the contributions of the adjuvant drugs metformin and inositol. It is seen that letrozole is superior to clomiphene citrate in polycystic ovary syndrome and is currently preferred as the first-choice drug worldwide. Metformin alone increases the ovulation rate compared to placebo in women with polycystic ovary syndrome, but should not be used as first-line therapy for anovulation. Similarly, when inositol is used alone, it does not increase the pregnancy rate.
Keywords: Anovulation; polycystic ovary syndrome; clomiphene citrate; letrozole; metformin; inositol.

ÖZ

Ovulasyon bozukluklarına bağlı infertilite, tüm infertilite nedenlerinin %25'ini oluşturmaktadır. Ovulasyon bozukluklarının en sık nedeni, Dünya Sağlık Örgütü anovulasyon sınıflamasına göre grup II olan ve çoğunlukla üreme çağındaki kadınların %6-20'sini etkileyen polikistik over sendromlu hastalardan oluşan normogonadatropik normogonadizmli hastalardır. Oral ovulasyon indüksiyon ajanları sadece bu gruptaki hastalar için uygun bir seçenektir. Ovulasyon indüksiyonunun amacı, yumurtalıkları monofoliküler gelişim için uyarmaktır. Ovulasyon indüksiyonunda kullanılan oral ajanlar, birinci basamak ajanlar olarak selektif östrojen reseptör modülatörleri ve aromataz inhibitörleri ve ikinci basamak ajanlar olarak metformin ve inositoller olmak üzere iki gruba ayrılabilir. Bu derlemenin amacı, birincil oral ovulasyon indüksiyon ajanları olan klomifen sitrat ve letrozolün kullanım ve etkinliklerini karşılaştırmak ve ayrıca adjuvan ilaçlar olan metformin ve inositolün katkılarını ortaya koymaktır. Polikistik over sendromunda letrozolün klomifen sitrata göre daha üstün olduğu ve günümüzde dünya çapında ilk seçenek ilaç olarak tercih edildiği görülmektedir. Polikistik over sendromlu kadınlarda, tek başına metformin plaseboya kıyasla ovulasyon oranını arttırır, ancak anovulasyon için birinci basamak tedavi olarak kullanılmamalıdır. Benzer şekilde, inositol tek başına kullanıldığında gebelik oranını artırmamaktadır.
Anahtar kelimeler: Anovulasyon; polikistik over sendromu; klomifen sitrat; letrozol; metformin; inositol.

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INTRODUCTION

Ovulation disorders constitute 25% of infertility cases (1). In women during the ovulation period, menstruation occurs approximately once a month (24-38 days). Unpredictable menstrual cycles are observed in oligo-anovulatory women (from 39 days to six months). Even if ovulation occurs, this is not enough to ensure pregnancy. The basis of ovulation induction is to ovulate and the first step here is to find the cause of the ovulation problem. The World Health Organization (WHO) divided anovulation into three groups, in Table 1 (2). The only area where oral agents can be used for ovulation induction is anovulatory patients in WHO group II, the majority of whom are patients with polycystic ovary syndrome (PCOS). PCOS is the most common endocrine pathology in women of reproductive age. Depending on diagnostic criteria, this disorder affects 6-20% of women of reproductive age (3,4), and is the most common cause of oligo-anovulation (80%). It was first described by Stein and Leventhal in 1935. Multiple morbidities, including infertility, metabolic syndrome, obesity, impaired glucose tolerance, type 2 diabetes mellitus, cardiovascular risk, depression, obstructive sleep apnea, and endometrial cancer are associated with PCOS (5-7) Therefore, the first step in treatment is lifestyle changes. Lifestyle interventions consist of many components, including healthy diets, physical activity, reduced sedentary behavior, and behavioral strategies (8). In the treatment of infertility, oral ovulation induction agents, as well as lifestyle changes, constitute the first-line treatment. Women who fail to ovulate or fail to conceive after first-line treatment options are often referred to gonadotropin therapy. Laparoscopic ovarian puncture, evaluated in well-designed studies, may be an alternative to gonadotropins. In vitro fertilization is the last option for couples who cannot conceive after all these treatments. The purpose of the ovulation induction is to stimulate the ovaries for monofollicular development. Monofollicularity reduces the two main risks of induction of ovulation: ovarian hyperstimulation syndrome (OHSS) and multiple pregnancy (9). More than one follicle should not be targeted in ovulation induction (10).

The purpose of this review is to describe oral ovulation induction agents, mainly clomiphene citrate (CC), letrozole, and as adjuvant agents metformin and inositol, and also compare their efficacies.

Oral agents used in ovulation induction can be divided into two groups: i) First-line agents as a) selective estrogen receptor modulators (SERMs) and b) aromatase inhibitors; and ii) Second-line agents (insulin-sensitizing drugs) as a) metformin and b) inositols.

FIRST-LINE AGENTS

Selective Estrogen Receptor Modulators (SERMs)

SERMs are competitive inhibitors of estrogen binding to estrogen receptors (ERs) and all have mixed agonist and antagonist activity depending on the target tissue. There are three agents in this group; tamoxifen, raloxifene, and clomiphene (11). The antagonist effect of tamoxifen is particularly evident in breast cancer. Although its primary use is in the treatment of breast cancer, recent reviews suggest that tamoxifen and CC both have similar ovulation and pregnancy rates (12,13). Raloxifene is particularly used in the treatment of osteoporosis, it does not appear to have endometrial agonistic effects; unlike tamoxifen, it does not increase the risk of uterine cancer. CC is a nonsteroidal triphenylethylene derivative, most commonly used as an ovulation stimulant in fertility treatment. The commercially available form of clomiphene is the dihydrogen citrate salt, namely CC. It contains two stereoisomers: zu-clomiphene (38%, cis-isomer) and en-clomiphene (62%, trans-isomer), which is the more potent isomer with greater antiestrogenic activity and responsible for inducing follicular development (14). Clomiphene is cleared through the liver and excreted in the feces. More than half of the clomiphene is excreted after five days, but traces of radioactivity from labeled clomiphene are visible in the stool for up to six weeks after administration. Although this observation raises concerns about fetal clomiphene exposure, most studies suggest that the frequency of congenital malformations does not increase (15). On the other hand, there is some evidence for a possible relationship between CC exposure and fetal malformations, especially neural tube defects and hypospadias, which required to investigate further (16,17). Although CC has no progestational, corticotropic, androgenic, or antiandrogenic effects and does not affect adrenal or thyroid function, a meta-analysis of women taking CC (and other fertility drugs) has shown an increased risk of thyroid cancer (18).

Table 1. Anovulation classification according to World Health Organization (WHO)

WHO group I, Hypogonadotrophic hypogonadism
- Idiopathic
- Kallmann's syndrome (isolated gonadotrophin deficiency and anosmia)
- Functional hypothalamic dysfunction (e.g. excessive weight loss such as in anorexia nervosa, exercise, stress, drugs, iatrogenic)
- Pituitary tumor, pituitary infarct (e.g. Sheehan's syndrome)
WHO group II, Normogonadotrophic normogonadic ovarian dysfunction
- Polycystic ovary syndrome (PCOS)
WHO group III, Hypergonadotrophic hypogonadism (ovarian failure)
- Genetic (e.g. Turner's syndrome)
- Autoimmune causes
- Infection (e.g. mumps oophoritis)
- Iatrogenic (e.g. surgical menopause, post-radiotherapy, or chemotherapy)
- Idiopathic
- Other endocrinopathies, such as hyperprolactinemia, thyroid dysfunction, and other conditions of androgen excess such as congenital adrenal hyperplasia and androgen-secreting adrenal and ovarian tumors

CC acts primarily on the hypothalamus, which appears to bind to and deplete hypothalamic ERs, thereby blocking the negative feedback effect of circulating endogenous estradiol. This causes an increase in hypothalamic gonadotropin releasing hormone (GnRH) pulse frequency and an increase in serum concentrations of follicle stimulating hormone (FSH) and luteinizing hormone (LH). High FSH and LH stimulate follicular development in the ovaries (19). Antiestrogen effects are evident in the endometrium and cervix. The normal increase in uterine volume and endometrial thickening that occurs during spontaneous menstrual cycles are largely absent during clomiphene-induced cycles, despite higher estradiol levels (20,21). This may explain why pregnancy rates are relatively low, while ovulation rates are so high in women on a clomiphene cycle. A recent study showed that ovarian stimulation with CC delays endometrial maturity and may impair the implantation process, possibly due to mismatch (22). Data on the effect of clomiphene on cervical mucus are unclear. In a meta-analysis, a deleterious effect was seen only at doses ≥100 mg/day (23).

Since CC is competitively bound with estrogen, estrogen must be present in the environment for its effectiveness and the hypothalamic-pituitary axis must be intact.

The diagnosis of unexplained infertility (10-30%) and its etiology may remain unknown despite intensive evaluation of both male and female partners (24). The efficacy of human chorionic gonadotropin (hCG) trigger, timed intercourse and intrauterine insemination (IUI) approaches compared to the expectant approach in patients for whom CC is indicated are as follows.

- Anovulatory/oligo-ovulatory women:
  IUI has no benefit over timed intercourse
- Unexplained infertility:
  CC+timed intercourse = expectant approach
  CC+hCG trigger < expectant approach
  CC+IUI > expectant approach

Clomiphene Citrate Treatment Protocols

Before starting treatment, necessary evaluations should be made to rule out causes other than anovulatory infertility.

Standard Protocol: 50 mg/day orally for 5 days, typically on day 5 of the cycle (can be started on days 2-5). If there is no response, the dose is increased to 100 mg/day in the next cycle. Maximum recommended doses are 100 mg/day by the US Food and Drug Administration (FDA), and 150 mg/day by the American College of Obstetricians and Gynecologists (ACOG). Once ovulation has occurred, the same dose should be continued for four to six cycles. Lower doses (12.5-25 mg daily) may be used in women with clomiphene sensitivity or who develop persistently large ovarian cysts (25).

- The total daily dose should be taken once to maximize the effect.
- Sexual intercourse is recommended for one week, starting 5 days after the last dose.
- Pregnancy usually occurs in 3-6 cycles. Routine basal ultrasonography (USG) is not recommended.
- If an endogenous LH increase is detected for ovulation, routine hCG is not recommended, if any. Pregnancy rates are highest when the dominant follicle is 23-28 mm in diameter on USG for hCG trigger (26).
- No dosage adjustment is required in renal impairment, but should not be used in patients with liver disease.

Alternative Protocols

Longer Courses: Instead of the classic 5-day course, some CC-resistant anovulatory women may respond to longer CC courses (7 to 8 days). There is limited data on this practice (27).

Stair-step Protocol: CC can be initiated at any time during the stable follicular phase when there is no suspicion of pregnancy (predominant follicle, absence of the corpus luteum, and can be judged by three linear endometrial appearances on ultrasound). 50 mg CC is given for 5 days. 2-3 days after the last dose, it is checked whether a dominant follicle is formed by ultrasound examination. If there is no dominant follicle, 100 mg CC is started immediately after seven days of the last dose, without causing withdrawal bleeding with progestins (28). A summary of the main features and results of previous studies using the CC stair-step protocol is shown in the Table 2, derived from Horowitz and Weissman (29). According to the available data, it has been reported that the pregnancy and live birth rates in cycles starting with spontaneous bleeding or progesterone withdrawal bleeding are lower than in anovulatory cycles in which CC is initiated without progestin interruption bleeding (30).

Half of the patients ovulate with 50 mg CC, 20-25% with 100 mg, and 10% with 150 mg. As the age, body mass index (BMI), insulin resistance, and free androgen index increase, the ovulation rate decreases. There is no point in continuing as pregnancy rates per cycle remain unchanged after six months of treatment. Failure to conceive after six maximum ovulation therapy cycles indicates the need to further evaluate potential infertility factors or switch to another treatment strategy. The rate of multiple pregnancy with CC is 7-10%. CC does not increase the abort rate (31) and the risk of ectopic pregnancy.

The most common side effect of CC is ovarian enlargement. Less frequently, hot flashes may be associated with hypoestrogenism at the hypothalamic level. The side effects of CC are not dose dependent.

Table 2. Outcomes of the studies comparing the CC stair-step and traditional protocols (29*)

	Stair-step Protocol			Traditional Protocol		
Design	**n**	**Time to Ovulation**	**Ovulation Rate**	**n**	**Time to Ovulation**	**Ovulation Rate**
Retrospective	31	23-35 days	64% (100 mg) 74% (≤150 mg)	H	55-88 days	22% (100 mg) 35.5% (≤150 mg)
RCT	30	20.5±2 days	43%	30	48.6±4 days	33.3%
RCT	30	13.65±6.7 days	66.7%	30	32.8±20.4 days	50%
Retrospective	43	23±0.9 days	88%	66	47.5±6.3 days	39%

*: derived from Horowitz and Weissman (29), CC: clomiphene citrate, RCT: randomized controlled trial, H: historical control

Aromatase Inhibitors

Aromatase enzyme takes place in the last step of estrogen synthesis. It catalyzes the demethylation of carbon 19 of androgens to produce phenolic 18-carbon estrogens. Aromatase is the only member of the family 19 of P450 super enzymes called CYP19 (32), which is found in tissues such as the ovary, adipose tissue, breast, brain, liver, and muscle. Numerous aromatase inhibitors have been developed (Table 3). The third generation, nonsteroidal aromatase inhibitors commonly used today are letrozole and less commonly anastrozole (33).

Letrozole is currently the only registered indication for breast cancer (34). Letrozole potently inhibits aromatase activity (reduces endogenous estrogen synthesis by 97-99%) competitively and reversibly (35). Letrozole (2.5 mg once daily) has a plasma half-life of 41~48 hours. Letrozole metabolism may be markedly increased in patients with hepatic insufficiency (34). The aromatase inhibition effect starts after 2 days.

Letrozole's main mechanism of action for ovulation induction is that it reduces circulating estrogen levels significantly by decreasing the estrogen synthesis. This prevents negative feedback (central effect) in the hypothalamic-pituitary-gonadal axis (36). Also, because the conversion of androgen substrates to estrogen is inhibited, transient accumulation of intraovarian androgens can increase follicular sensitivity (peripheral effect) through amplification of FSH receptor gene expression (37). Typically letrozole is taken on a daily basis on the 3~7th day of the menstruation. During this period, follicle sizes are 6-8 mm and contain high levels of androgen receptors. Therefore, the increase in androgen levels during this period promotes granulosa cell mitosis and the induction of FSH receptors (38).

The negative effects of CC on the endometrium and possibly the cervix have made the use of multi-follicular growth aromatase inhibitors increasingly popular (39). Because letrozole does not inhibit negative feedback of estrogen to the hypothalamic-pituitary-gonadal axis, it generally induces single follicle development and prevents multiple pregnancies. It is also indicated for patients who cannot tolerate the side effects of CC and who cannot tolerate fertility-preserving treatment and assisted reproductive techniques in cancer patients.

Treatment Protocols of Letrozole

Prior to starting the treatment, thyroid stimulating hormone (TSH), prolactin, and FSH values should be checked to differentiate the patients who will not respond to letrozole, and hysterosalpingography (HSG) and spermiogram tests should be performed to exclude other factors causing infertility.

Standard Protocol: Typical treatment of letrozole consists of 2.5 mg daily taken during days 3~7 of menstrual for a 5-day course. If there is no response, dosage is increased to 7.5 mg/day in the next cycle.

Table 3. Aromatase inhibitors

1st generation	2nd generation	3rd generation
Aminoglutedimide	Fadrozole	Letrozole
	Formestane	Anastrazole
		Exemestane

Alternative Protocols

Single Dose: A single dose of 20 mg can be taken on the 3rd day of the cycle but related data is limited.

Stair-step Protocol: If ovulation does not occur, the sequential dose can be increased to 5 mg and 7.5 mg, respectively, without waiting for withdrawal bleeding. This protocol is widely used by many clinicians (40).

In a prospective randomized controlled trial comparing letrozole and CC for ovulation induction in women with PCOS in India in 2020, while there was no significant difference between the groups for mean endometrial thickness, a significant difference was found in favor of letrozole for monofollicular development. In addition, pregnancy rates were found to be 42% in letrozole and 20% in CC, with a significant difference. The gestation period was also found to be significantly shorter in the letrozole group (41).

In a meta-analysis by Tsiami et al. (42), they reviewed 26 randomized controlled trials over 13 years and found that those given letrozole were more likely to ovulate than those given CC.

Letrozole has been used off-label in the treatment of patients with anovulation. The reason it was not approved is related to its potential teratogenic effect. However, letrozole has a short half-life period (48 hours) in contrast to CC (2 weeks), so it is cleared before ovulation; therefore, it is unlikely to be teratogenic. In the study of Akbari Sene et al. (43), CC and letrozole were evaluated in terms of congenital fetal anomaly risk, and no difference was found between them.

For ovulation sufferers, letrozole generally increases their chances of getting pregnant. It provides monofollicular development in most cycles, thus reducing multiple pregnancies and OHSS may be the first treatment option for unexplained infertility (44).

In a randomized controlled trial in which the combination of letrozole and CC, and only letrozole was given for ovulation induction in patients with PCOS, researchers concluded that the combination of letrozole and CC was associated with a higher ovulation rate compared to letrozole alone (45).

SECOND-LINE AGENTS (INSULIN-SENSITIZING DRUGS)

Metformin

Metformin is an orally active, water-soluble biguanide used in type 2 diabetes mellitus, it is antihyperglycemic and does not cause hypoglycemia. It increases insulin sensitivity in peripheral tissue, where it inhibits hepatic glucose production and increases glucose uptake and use in muscle tissue. Reducing hyperinsulinemia in PCOS may normalize endocrine, metabolic, and reproductive functions, and leading to the resumption of ovulation (46). Practice committee of the American Society for Reproductive Medicine (ASRM) noted that metformin alone increased the ovulation rate in women with PCOS compared to placebo. However, it should not be used as a first-line ovulation induction agent due to the its lower efficacy than CC and letrozole (47).

Inositol

Inositols are a family of biomolecules that are important in regulating vital cellular functions, signal transduction, energy transmission, and ion channel

physiology, and serve as structural components of cell membranes (48). It mainly has two stereoisomers, Myo-inositol and D-chiro-inositol; they are incorporated intracellularly into insulin's second messengers, inositol phosphoglycans, and some effects of insulin are mediated by these inositol phosphoglycan mediators (49). D-chiro-inositol and its combination have been shown to improve metabolic, hormonal, and reproductive aspects of PCOS (50). In a meta-analysis evaluating the use of inositol in infertile PCOS patients, no clear information could be obtained that it increased pregnancy rates (51).

CONCLUSION

There are many studies and meta-analyses evaluating the effectiveness of CC and letrozole in the literature. In addition, it is also included in combinations with different adjuvant agents. There are many studies showing the effects of insulin-sensitizing drugs when given alone or as an adjuvant. As a result of the examination of all these studies, the following information is obtained:

- Letrozole appears to improve live birth and pregnancy rates compared to CC in subfertile women with anovulatory PCOS.
- There is high-quality evidence that rates of OHSS are comparable with letrozole or CC (52).
- Letrozole is a better alternative for ovulation induction in anovulatory women with PCOS due to the higher pregnancy rates and shorter gestational period (41).
- Fewer or a similar multiple pregnancy rates are obtained with letrozole compared to CC (41,52).
- There is no clear information about the negative effect of CC on cervical mucus. There is even a study showing that letrozole has a more negative effect (53).
- The letrozole stair-step protocol elicited a shorter ovulation time and higher ovulation, clinical pregnancy, and live birth rates than the CC stair-step protocol (54).
- Correction of hyperinsulinemia with metformin improves the spontaneous ovulation but not the live birth rates (55).

As a result, letrozole has proven superior over CC for WHO group II patients. Despite the advantage of letrozole in PCOS patients; in terms of mild oligoasthenospermia, early-stage endometriosis, and unexplained infertility conditions, letrozole and CC results were similar.

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AUTHOR GUIDELINES

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Main Text: The title of the article (English and Turkish), short title not exceeding 40 characters, Abstract (English and Turkish), Keywords (English and Turkish), Main Text (sectioned according to the type of article submitted), References, Tables and Figures should be included.

Ethics Committee Approval Document: Ethics Committee Approval Document should be uploaded as a separate file for all research articles.

Note: If there are figures, pictures or photographs in the article, each of them must be uploaded as separate files.

SECTIONS THAT SHOULD BE USED ACCORDING TO THE TYPE OF ARTICLE

Research Article

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, MATERIAL AND METHODS, RESULTS, DISCUSSION, CONCLUSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 200-250 words.
ABSTRACT should be structured as "Aim, Material and Methods, Results, Conclusion".
ÖZ, should be structured as "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç".

Review (Invited Only)

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, Subtitles Related to the Subject, CONCLUSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 150-200 words.

Case Report

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, CASE REPORT, DISCUSSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 100-150 words.

Other

The general writing rules are applied for the preparation of the writings (letter to the editor, editorial comment/discussion, etc.) except these three basic types of article. There is no title and abstract sections in these writings. The number of references is limited to 5. The dedicated article should be specified by giving the number and date. The name, institution and address of the author should be included at the end of writing. Answer to the letter is given by the editor, or authors of the dedicated article, by publishing again in the journal.

AUTHOR GUIDELINES

WRITING RULES

- Articles should be prepared as Microsoft Word® document.
- The required margins are 2.5 cm on all sides.
- Page numbers should be placed to bottom right corner of pages.
- All texts must be typed with double-space as left-aligned using 12 point Times New Roman font.

KEYWORDS

- Number of the keywords must be at least 2, words should be separated from each other by a semicolon (;).
- Keywords in Turkish must be given in accordance with Türkiye Bilim Terimleri (TBT) (http://www.bilimterimleri.com), and keywords in English must be given in accordance with Medical Subject Headings (MESH) (http://www.nlm.nih.gov/mesh/MBrowser.html).

STATISTICAL METHODS

- All research articles should be assessed in terms of biostatistics and indicated with appropriate plan, analysis and report. In these articles last subtitle of the MATERIAL and METHODS section should be the "Statistical Analysis".
- In this section, the statistical methods used in the study should be written by indicating the purpose of use, package programs and versions used for statistical analysis should be specified.
- p values should be given in three decimal digits (p=0.038; p=0.810 etc.).
- Further information to control the convenience of articles in terms of biostatistics, can obtained from www.icmje.org.

ABBREVIATIONS

- The term should be written in full words with the abbreviation in parenthesis where first mentioned, and the same abbreviation should be used throughout the entire text.
- Abbreviations used internationally should be used in accordance with the Scientific Writing Rules.

TABLES AND FIGURES

- Should be indicated at the end of the relevant sentence in the text as (Table 1) and/or (Figure 1).
- Tables (with headings) and figures (with captions) must be added after references at the end of the text as each to be on a separate page.
- The table headings should be written at top of the table (Table 1. Table heading) and the figure captions should be written below the figure (Figure 1. Figure caption) as their first letters being upper case.
- If any abbreviation or symbol is used in tables and figures, it should be explained as a footnote below.
- The figures and photographs should be upload as separate files in .png, .jpg, etc. format and at least 300 dpi resolution.
- Captions of figure and photograph should be given on a separate page respectively, after the page including last table.
- If figure, picture, table, graphic etc. which have been published before is used, written permission must be taken and it should be stated in the explanation of figures, pictures, tables, graphics. The legal responsibility in this regard belongs the authors.

ACKNOWLEDGEMENT

- If any conflict of interest, financial support, donation and other editorial (English/Turkish evaluation) and/or technical support, it must be stated in this section before the REFERENCES section.

REFERENCES

- References should be numbered according to the order of use and stated with numbers in parentheses as (1) or (1,2) or (3-5) at the end of the relevant sentence in the text.
- Reference list should be formed according to the reference order used in the text.
- If the number of authors are 6 or less, all authors should be specified, if there are 7 or more "et al." should be added after the first 6 authors are specified.
- The conference papers, personal experiences, unpublished papers, theses and internet addresses should not be used as references.
- DOI is the only acceptable online reference.

Article:

Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

Aho M, Irshad B, Ackerman SJ, Lewis M, Leddy R, Pope T, et al. Correlation of sonographic features of invasive ductal mammary carcinoma with age, tumor grade, and hormone-receptor status. J Clin Ultrasound. 2013;41(1):10-7.

Book:

Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Book Chapter:

Altobelli N. Airway management. In: Kacmarek R, Stoller JK, Heuer AJ, editors. Egan's fundamentals of respiratory care. 10th ed. St. Louis: Saunders Mosby; 2013. p.732-86.

YAZARLARA BİLGİLENDİRME

BİLİMSEL SORUMLULUK

Bilimsel yayıncılık standartları açısından, gönderilecek makaleler, Uluslararası Tıbbi Dergi Editörler Kurulu (ICMJE), Dünya Tıbbi Editörler Birliği (WAME) ve Yayın Etik Kurulu (COPE) kriterlerine uygun olarak hazırlanmalıdır.

- Gönderilecek makalelerde araştırma ve yayın etiğine uyulması zorunludur. Makalelerin sorumluluğu yazarlarına aittir.
- Makalelerin daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmaması gerekir.
- Değerlendirme sürecinin başlaması için makaleler, tüm yazarlar tarafından imzalanmış Telif Hakkı Devir Formu ile birlikte gönderilmelidir. Yazar sıralaması için Telif Hakkı Devir Formu'ndaki imza sırası dikkate alınır.
- Sorumlu yazar, tüm yazarlar adına makalenin son halinin sorumluluğunu taşır.

ETİK SORUMLULUK

- "İnsan" öğesini içeren tüm çalışmalarda Helsinki Deklerasyonu Prensipleri'ne (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) uygunluk aranır. Bu tip çalışmalarda yazarların, GEREÇ VE YÖNTEMLER bölümünde çalışmayı bu prensiplere uygun olarak yaptıklarını, kurumlarının etik kurullarından onay ve çalışmaya katılmış insanlardan "bilgilendirilmiş olur" (informed consent) aldıklarını belirtmeleri gerekmektedir.
- Çalışmada "Hayvan" öğesi kullanılmış ise yazarların, GEREÇ VE YÖNTEMLER bölümünde Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) prensipleri doğrultusunda çalışmalarında hayvan haklarını koruduklarını ve kurumlarının etik kurullarından onay aldıklarını belirtmeleri gerekmektedir.
- Olgu sunumlarında hastalardan "bilgilendirilmiş olur" (informed consent) alınmalıdır.
- Etik kurul onay bilgisi GEREÇ ve YÖNTEMLER bölümünde kurul adı, onay tarihi ve sayısı ile birlikte belirtilmelidir.
- Eğer çalışmada direkt-indirekt ticari bağlantı veya maddi destek veren kurum mevcut ise yazarlar; kullanılan ticari ürün, ilaç, firma vb. ile ticari hiçbir ilişkisinin olmadığını veya varsa nasıl bir ilişkisinin olduğunu (konsültan, diğer anlaşmalar), editöre sunum sayfasında belirtmelidirler.
- Yazarlar çalışma ile ilgili kişisel ve finansal tüm ilişkilerin bildirilmesinden sorumludur. Makalenin başvurusu ve/veya değerlendirmesi ile ilişkili herhangi bir çıkar çatışması olup olmadığının açıkça beyan edilmesi gerekmektedir.
- Makalelerin bilimsel ve etik kurallara uygunluğu yazarların sorumluluğundadır.

BAŞVURU DOSYALARI

Makaleler aşağıda belirtilen şekilde ayrı dosyalar halinde sisteme yüklenmelidir.

Telif Hakkı Devir Formu: Başvuru sırasında sistemden alınacak Telif Hakkı Devir Formu tüm yazarlar tarafından makaledeki yazar sıralamasına uygun şekilde imzalanmış olmalıdır.

Başvuru Mektubu: Makalenin türü, daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmadığı, varsa çalışmayı maddi olarak destekleyen kişi ve kuruluşlar ve bu kuruluşların yazarlarla olan ilişkileri (yoksa olmadığı) belirtilmelidir. Makalenin konusuyla ilgili olarak önerilen, yazarlarla ve kurumlarıyla ilgisi olmayan en az iki hakemin adları, akademik unvanları, kurumları, iletişim bilgileri ve e-posta adresleri yazılmalıdır. Editörlerin hakemleri seçme hakkı saklıdır.

Başlık Sayfası: Makalenin başlığını (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, tüm yazarların adlarını, akademik unvanlarını, ORCID® numaralarını, kurumlarını, e-posta adreslerini ve ayrıca sorumlu yazarın adını, yazışma adresini, telefon numarasını, e-posta adresini içermelidir. Makale daha önce bilimsel bir toplantıda sunulmuş ise toplantı adı, tarihi ve yeri (yoksa sunulmadığı) belirtilmelidir.

Ana Metin: Makalenin başlığı (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, Öz (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), Ana Metin (gönderilen makalenin türüne uygun olarak bölümlere ayrılmış), Kaynaklar, Tablolar ve Şekil açıklamaları yer almalıdır.

Etik Kurul Onay Belgesi: Tüm araştırma makaleleri için Etik Kurul Onay Belgesi ayrı bir dosya olarak yüklenmelidir.

Not: Makalede şekil, resim veya fotoğraf varsa bunların da her biri ayrı birer dosya olarak yüklenmelidir.

MAKALE TÜRÜNE GÖRE KULLANILMASI GEREKEN BÖLÜMLER

Araştırma Makalesi

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, GEREÇ VE YÖNTEMLER, BULGULAR, TARTIŞMA, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 200-250 kelime arasında olmalıdır.

ABSTRACT, "Aim, Material and Methods, Results, Conclusion" şeklinde yapılandırılmalıdır.

ÖZ, "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç" şeklinde yapılandırılmalıdır.

Derleme (Sadece Davetli)

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, Konu ile İlgili Alt Başlıklar, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 150-200 kelime arasında olmalıdır.

Olgu Sunumu

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, OLGU SUNUMU, TARTIŞMA, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 100-150 kelime arasında olmalıdır.

Diğer

Bu üç temel makale türü dışındaki (editöre mektup, editöryel yorum/tartışma vb.) yazıların hazırlanmasında da genel yazım kuralları geçerlidir. Bu tür yazılarda başlık ve öz bölümleri yoktur. Kaynak sayısı 5 ile sınırlıdır. İthaf olunan makale sayı ve tarih verilerek belirtilmelidir. Yazının sonunda yazarın ismi, kurumu ve adresi yer almalıdır. Mektuba cevap, editör veya makalenin yazarları tarafından, yine dergide yayınlanarak verilir.

YAZARLARA BİLGİLENDİRME

YAZIM KURALLARI
- Makaleler Microsoft Word® belgesi olarak hazırlanmalıdır.
- Sayfa kenarlarında 2,5 cm boşluk bırakılmalıdır.
- Sayfa numaraları sayfanın sağ alt köşesine yerleştirilmelidir.
- Tüm metinler 12 punto Times New Roman karakteri kullanılarak çift satır aralığı ile sola hizalanmış olarak yazılmalıdır.

ANAHTAR KELİMELER
- Anahtar kelime sayısı en az 2 olmalı, kelimeler birbirlerinden noktalı virgül (;) ile ayrılmalıdır.
- Türkçe anahtar kelimeler Türkiye Bilim Terimleri (TBT)'ne (http://www.bilimterimleri.com), İngilizce anahtar kelimeler Medical Subject Headings (MESH)'e (http://www.nlm.nih.gov/mesh/MBrowser.html) uygun olarak verilmelidir.

İSTATİSTİKSEL YÖNTEMLER
- Tüm araştırma makaleleri biyoistatistik açıdan değerlendirilmeli ve uygun plan, analiz ve raporlama ile belirtilmelidir. Bu makalelerde, GEREÇ VE YÖNTEMLER bölümünün son alt başlığı "İstatistiksel Analiz" olmalıdır.
- Bu bölümde çalışmada kullanılan istatistiksel yöntemler ne amaçla kullanıldığı belirtilerek yazılmalı, istatistiksel analiz için kullanılan paket programlar ve sürümleri belirtilmelidir.
- p değerleri ondalık üç basamaklı (p=0,038; p=0,810 vb.) olarak verilmelidir.
- Makalelerin biyoistatistik açıdan uygunluğunun kontrolü için ek bilgi www.icmje.org adresinden temin edilebilir.

KISALTMALAR
- Terim ilk kullanıldığında parantez içinde kısaltmayla birlikte açık olarak yazılmalı ve tüm metin boyunca aynı kısaltma kullanılmalıdır.
- Uluslararası kullanılan kısaltmalar Bilimsel Yazım Kurallarına uygun şekilde kullanılmalıdır.

TABLOLAR VE ŞEKİLLER
- Metinde ilgili cümlenin sonunda (Tablo 1) ve/veya (Şekil 1) şeklinde belirtilmelidir.
- Tablolar (başlıklarıyla birlikte) ve şekiller (açıklamalarıyla birlikte) kaynaklardan sonra ve her biri ayrı bir sayfada olacak şekilde metnin sonuna eklenmelidir.
- Tablo başlıkları tablo üstünde (Tablo 1. Tablo başlığı), şekil açıklamaları ise şeklin altında (Şekil 1. Şekil açıklaması), ilk harfleri büyük olacak şekilde yazılmalıdır.
- Tablolarda ve şekillerde kısaltma veya sembol kullanılmış ise altında dipnot olarak açıklanmalıdır.
- Şekiller ve fotoğraflar, .png, .jpg vb. formatta ve en az 300 dpi çözünürlükte ayrı dosyalar halinde yüklenmelidir.
- Şekil ve fotoğraf alt yazıları, son tablonun olduğu sayfadan sonra, ayrı bir sayfada sırasıyla verilmelidir.
- Daha önce basılmış şekil, resim, tablo, grafik vb. kullanılmış ise yazılı izin alınmalı ve açıklama olarak belirtilmelidir. Bu konudaki hukuki sorumluluk yazarlara aittir.

TEŞEKKÜR
- Eğer çıkar çatışması/çakışması, finansal destek, bağış ve diğer bütün editöryel (İngilizce/Türkçe değerlendirme) ve/veya teknik yardım varsa, bu bölümde, KAYNAKLAR bölümünden önce belirtilmelidir.

KAYNAKLAR
- Kaynaklar, kullanım sırasına göre numaralandırılmalı ve metin içinde ilgili cümlenin sonunda parantez içinde numaralarla (1) veya (1,2) veya (3-5) şeklinde verilmelidir.
- Kaynaklar dizini, metin içinde kaynakların kullanıldığı sıraya göre oluşturulmalıdır.
- Yazar sayısı 6 veya daha az ise tüm yazarlar belirtilmeli, 7 veya daha fazla ise ilk 6 yazar belirtildikten sonra "et al." eklenmelidir.
- Kongre bildirileri, kişisel deneyimler, basılmamış yayınlar, tezler ve internet adresleri kaynak olarak gösterilmemelidir.
- DOI tek kabul edilebilir online referanstır.

Makale:

Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

Aho M, Irshad B, Ackerman SJ, Lewis M, Leddy R, Pope T, et al. Correlation of sonographic features of invasive ductal mammary carcinoma with age, tumor grade, and hormone-receptor status. J Clin Ultrasound. 2013;41(1):10-7.

Kitap:

Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Kitap Bölümü:

Altobelli N. Airway management. In: Kacmarek R, Stoller JK, Heuer AJ, editors. Egan's fundamentals of respiratory care. 10th ed. St. Louis: Saunders Mosby; 2013. p.732-86.


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