Male Infertility: Causes and Current Developments in Diagnostic Work-Up

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Male factor infertility gained importance during the last two decades in the infertility work-up and necessitates the revision of basal semen analysis parameters. Diagnostic evaluation of the male begins with a detailed history, physical examination and a semen analysis. Hormonal and genetic evaluation is done if indicated. The expeditious progress in the assisted reproductive techniques, highlight the importance of genetic testing in the evaluation of the male patient and in cases like azoospermia in which the prognosis may change the treatment protocol.

Key Words: Male infertility, Semen analysis, Genetic evaluation

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Infertility is defined as failure to conceive with 1 year of regular unprotected sexual intercourse. 50% of infertile couples conceive in 3 months, while 72% and 85% conceive in 6 months and 1 year respectively. Although many factors that belong to either women and/or men cause infertility, causes of male infertility gain importance during the last two decades.[1] Male factor as the single cause of subfertility is present in at least 20 % of couples, and in 20-40% of cases both a male and female factor is present.[2]

Male gonadal development begins with the migration of endodermal cells of yolk sac to the genital ridge at 5th week of gestation. There are approximately 300.000 spermatogonia in each gonad which increases to 600.000 million by puberty. Different from the female germ cell development, proliferation of germ cells continue during the adult life which reaches to almost 100-200 million sperm production daily.

There are two stages during the development spermatozoa from spermatogonia. The developmental stages from spermatogonia to primary and secondary spermatocytes and then to spermatids is called spermatogenesis. The maturational process of spermatozoa from spermatids is called spermiogenesis.[3] The growth in testes takes approximately 70 days and additional 12-21 is needed for the transport of sperms from testes to epididymis and ejaculatory ducts.

There are two important structures in testes; 1) seminiferous tubules that consist of germ cells and sertoli cells, in which spermatogenesis takes place; 2) leydig cells which are hormonally active and located between the seminiferous tubules. Tight- junctions between the sertoli cells protect the germ cells of different maturational stages from the environmental toxins and antibodies; serve to form the blood-testes barrier.

Causes of male infertility can be classified in 4 groups:
Hypothalamic-pituitary causes,
Testicular causes (Primary testicular failure),
Disorders of sperm transport (Post testicular),
Idiopathic

1) Hypothalamic - Pituitary Disorders

Hypothalamic hypogonadism is a rare cause of male infertility (1-2%) which is characterized by the congenital or acquired deficiency in Gonadotrophin Releasing Hormone (GnRH) or gonadotropins. Isolated gonadotrophin deficiency caused by absent or defective GnRH secretion is the most common congenital cause characterized by sexual infantilism.[4] When this condition is accompanied by anosmia, color blindness, hearing loss or renal malformations, the disorder is known as Kallman syndrome. KAL1, FGFR1, PROK2 and PROKR-2 mutations were found to be related with this syndrome.[5-7] There is also limited evidence in the literature about the single nucleotide polymorphism at the promotor region of FSH gene as a cause hypogonadotropic hypogonadism[8-10] Hypogonadism may also be presented as a part of the genetic syndromes like Laurence-Moon-Biedl syndrome, Prider-Willi syndrome or familial cerebellar ataxia.[11] Other causes are listed in table 1.

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Table 1: Causes of male infertility

1. Hypothalamic-Pituitary Disorders
a) Congenital Disorders
Congenital GnRH deficiency (Kallmann syndrome)
Multiorgan genetic disorders (Prader-Willi Syndrome, Laurence-Moon-Beidl syndrome)
b) Acquired Disorders
Hypothalamic or pituitary tumors (macroadenoma, craniopharngioma)
Infiltrative diseases (sarcoidosis, tuberculosis)
Trauma, surgery, radiation
Drugs
c) Systemic Disorders
2. Primary gonadal disorders
a) Congenital
Klinefelter syndrome
Cryptorchidism
Myotonic dystrophy
Varicocele
Androgen insensitivity syndromes
Deletions of Y chromosomes
b) Acquired
Viral orchitis (mumps, echovirus)
Granulomatous orchitis (tuberculosis, lepra)
Epididymo-orchitis (chlamydia, gonorrhea)
Drugs
Radiation
Environmental toxins
Hyperthermia
Trauma
Torsion
Systemic diseases (renal failure, hepatic disease, cancer, sickle cell anemia)
3. Post-testicular disorders
Epididymal dysfunction
Anomalies of vas deferens (congenital absence, vasectomy, infection, Young syndrome)
Ejaculatuar dysfunction
4. Idiopathic

2) Testicular Disorders

Primary gonadal failure (hypergonadotropic hypogonadism) is one of the major causes of male infertility (30-40%) and can result from acquired or congenital disorders. The disorders in this group are one of the most important causes of azoospermia and oligospermia.

a) Chromosomal abnormalities

Chromosomal abnormalities are seen more frequently in infertile male population than the fertile counterparts. The incidence is inversely correlated with abnormalities in sperm count. The overall incidence of male chromosome abnormalities are less than 1% in men with normal sperm concentration while the incidence rise to 5% in severe oligospermic patients (<5 million/mL) and 10-15% in azoospermic patients.[12]

Klinefelter's Syndrome (KS) is the most common chromosomal abnormality seen in severe cases of male infertility. Almost two thirds of the chromosomal abnormalities seen in infertile male patients are the KS.[13] In the classic form (47, XXY) almost all the patients have azoospermia while in the mosaic (47, XXY /46, XY) forms have a limited sperm production.[14,15] Testicular exploration can reveal sperm in 50% of azoospermic KS patients and pregnancy rates can reach up to 30-50% with intracytoplasmic sperm injection (ICSI).[16] 46, XX karyotype KS may be encountered due to translocation testes determining gene (SRY) to X chromosome. Although the classic forms have typical features of the syndrome which is long stature, gynecomastia, small and atrophic testes, non-mosaic patients with normal stature and masculinization were also reported.[14]

b) Y chromosome microdeletions

Y chromosome microdeletions are recognized as a common genetic cause of severe oligospermia and azoospermia which are diagnosed by polymerase chain reaction (PCR).[17,18] Microdeletions are found in 2% of males with normal fertility, 7% with severe spermatogenesis defects and 16% with severe oligospermia and azoospermia.[19] Microdeletions at long arm Y chromosome especially Yq11 region which is called "Azoospermia Factor (AZF)" have been identified in 20% of infertile males. AZFa (proximal), AZFb (central) and AZFc (distal) regions carry the genes that have important function at spermatogenesis. Microdeletions at AZFa and AZFb regions have the worse prognosis and the probability to find sperm at testes biopsy is very low. The histology shows sertoli cell only pattern in deletions of entire AZFa region[14] and spermatogenic maturation arrest in almost half of the AZFb deletions.[19] Microdeletions of AZFc region may cause infertility of varying severity and sperm can be detected in ejaculates of 50% of these cases. Even in azoospermic males with AZFc deletion, TESE produce sufficient outcome.[14] Most important determinant of outcome of sperm retrieval at diagnostic testis biopsy is the maturational stage that sperm could achieve rather than the predominant pattern at biopsy specimen. If diagnostic biopsy result reveals sertoli cell only, the chance of sperm retrieval is only 24%, 42% if biopsy reveals maturation arrest and rises to 81% if it revels hypospermatogenesis.[20]

In a study conducted in our clinic, 44 patients with AZF deletions were studied. When 27 of them with successful sperm retrieval was analyzed, it was demonstrated that 3 of them had AZFa, 3 had AZFb, 3 had both AZFa and AZFb, 6 had AZFa+b+c deletions and 12 had AZFc deletions. Pregnancy occurred only in 2 of the patients with AZFc deletions.[21]

Because Y chromosome deletions will be transmitted to the sons of affected male, genetic counseling and genetic testing should be offered to men before the procedure.

c) Single Gene Mutations and Polymorphisms

Normal sexual development and spermatogenesis requires normal androgen synthesis and functional androgen receptors. The studies revealed a reverse correlation between transcriptional activity of androgen receptor and CAG trinucleotide repeat at exon 1 region of androgen receptor.[22] In fertile male population it's reported that the men with short repeat sequences had the highest sperm count,[23-26] but in men with idiopathic infertility there is still conflicting results. In a meta-analysis including 33 studies, men with idiopathic infertility were found to have longer CAG repeats than the fertile controls.[27] Kennedy Disease, characterized by the muscle weakness, testicular atrophy and oligo/azoospermia is caused by long CAG repeats at androgen receptor transactivation region.[28]

Inactivating mutations at estrogen receptor alpha was shown to have normal sperm count but decreased motility in a patient[29] and the results were supported by animal studies.[30-32]

Gene mutations in various autosomal and X-linked diseases were shown to play important roles during spermatogenesis. Men with myotonic dystrophy, characterized by mental motor retardation, cataract and hypogonadism, have long repeat sequence of CTG trinucleotide at DMPK gene region and 60-80% develops testicular atrophy and oligoasthenoteratospermia.[33]

d) Cryptorchidism

It is defined as the failure of descent of the testes into the scrotum during fetal development. As the descent is androgen dependent, it's more common in disorders of androgen secretion or action.[34,35] Both unilateral and bilateral cryptorchidism is associated with impaired spermatogenesis and an increased risk of testicular tumors. Germ cell dysfunction is correlated with the duration of suprascrotal location of the testes. During the first year of life due to the hyperactivity of cremasteric reflex, testes are transiently located outside the scrotum.[36] Infertility is not a problem if they're located in normal scrotal localization at the end of one year. Usually, in adult males with cryptorchidism FSH is elevated and LH is in normal range. FSH and Inhibin B elevation after repair of cryptorchidism carry the risk of increased infertility.

e) Varicoceles

Varicoceles are the dilatations of the panpiniform plexus in the scrotum. They are more common at the left side because the left spermatic vein joins to the high pressure left renal vein with a right angle.[37] They are more prevalent in infertile males (40%) than in normal male population (15%).[38] It's believed that venous reflux and the testicular temperature rise are the probable mechanisms responsible for the testicular dysfunction due to the varicoceles. Only the palpable varicoceles were found to be related to infertility.

Besides these causes, infections like mumps, gonorrhea and tuberculosis, alkylating agents (cyclophosphamide, chlorambucil), antiandrogens (flutamide, cyproterone), radiation (temporary damage till 15 rad, irreversible damage above 600 rad), smoking, hyperthermia, environmental toxins and different systemic diseases (renal failure, hepatic cirrhosis, sickle cell anemia) can cause testicular damage.[39]

3) Disorders of Sperm Transport

Maturation of sperms continues in epididymis. They are carried to the urethra via vas deferens which than diluted with the secretions of the seminal vesicles and prostate. Most important cause of obstruction in this pathway is the bilateral congenital absence of the vas deferens (CBAVD). It's seen in 1-2% of infertile men and 6-10% of patients with obstructive azoospermia.[40] 38-71% patients with CABVD are related to cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation.[41] It should always be kept in mind that beside this mutation there may be other mutations that could not be detected with present technology, so testing should be offered to his partner before the treatment to exclude possibility that she may be a carrier too.

Evaluation of Male Infertility

The evaluation of infertile male should begin with a detailed history and physical examination. The history should include childhood diseases, history of cryptorchidism, previous surgery, systemic medical illnesses, alcohol use, smoking, exposure to environmental toxins and gonadotoxic drugs, sexual history, sexually transmitted infections, coital frequency and timing. During physical examination particular attention should be directed to examination of the body habitus, hair distribution and breast development, palpation and measurement of the testes, the presence and consistency of the vasa and epididymides, the presence or absence of a varicocele. The next step in the evaluation of the infertile male should be the semen analysis and endocrine tests.

Semen Analysis

Semen analysis is the cornerstone in the evaluation of the male infertility. The semen sample should be collected after 2-5 days of sexual abstinence.[12] Longer abstinence intervals increase volume and proportion of immotile and morphologically abnormal sperm while the shorter interval decrease semen volume but has no effect motility and morphology. If the results are abnormal, second sample should be collected 2-4 weeks later. The specimen should be examined within an hour after the collection.

The World Health Organization (WHO) has published re-

vised lower reference limits for semen analyses in 2010.[42,43] Both the 1999 and 2010 reference values are shown in table 2.

The most vital parameters in the evaluation infertile male are the concentration, motility and morphology[12] and the reference values are important to classify fertile and subfertile population.[42,43] Sperm parameters that are used to predict male fertility are concentration >48 million/mL, motility >63% and morphology >12% while concentration <13.5 million/mL, motility < % 32and morphology <%9 are the values to predict male subfertility.[12] But it should be kept in mind that normal reference values for semen parameters do not reflect normal sperm concentration in the general population and these values are not the minimum values required for conception. Men with normal reference values may be still be infertile and conversely, men having values outside the reference ranges may be fertile.

Although each parameter of the semen analysis must be considered in the context of whole, especially three parameters –concentration, motility and morphology- deserve special attention. If one of these 3 parameters is in abnormal range the probability of male infertility is 2-3 times higher, 5-7 times higher when two are abnormal and 16 times higher when all parameters are in abnormal range.[44]

The criteria for normal morphology based on shape, length, width, area occupied by the acrosome, and neck and tail defects are called "strict" criteria and have good predictive value in terms of fertilization in vitro and pregnancy rates after in vitro fertilization (IVF).[45,46]

If the progressive motility is below the reference limit, 32%, probability of male infertility is 5 times higher. Generally, decreased motility is related to presence of sperm autoantibodies, genital tractus infections, testicular/epididymal dysfunction or partial obstruction in ejaculatory ducts. Due to the structural abnormality of sperm tails, large number of viable but immotile sperms can be encountered in Kartagener's syndrome. If all the sperms are immotile, EosinY or Trypan Blue staining or hypo-osmotic sperm swelling test (HOS) are used to detect the viable sperms. Viable sperms that are detected by HOS can be used for ICSI, but the sperms stained with EosinY or Trypan Blue could not be used.[47] Viable sperms gain motility when they are incubated with pentoxifylline.[48]

According to WHO 2010 reference limits, the lower reference limit for sperm concentration is 15 million/mL. If no spermatozoa are seen, the diagnosis of azoospermia is given. It's seen in 1% of male population and 10-15% of infertile males. The diagnosis of azoospermia can be established only after the specimen is centrifuged at 3000 g for 15 minutes and the pellet is examined. The absence of sperm should be documented at least in two different specimens collected at separate occasions.[18] Azoospermia is caused by obstructive reasons (obstructive azoospermia, OA) in 40% of the cases whereas it's nonobstructive (NOA) in 60% of the cases.[49] The most common causes of OA are CBAVD, scrotal and inguinal surgeries and obstruction of ejaculator ducts due to serious infections. NOA is usually caused by primary or secondary testicular failure or endocrinopathies. The distinction between OA and NOA is mainly done by history, physical examination and hormonal evaluation. Patients with NOA have small testes (<15 mL), high serum FSH and low serum testosterone values.[13] Testes length < 4.6 cm and FSH >7.6 IU/L predicts NOA with 89 %sensitivity, while testes length >4.6 cm and FSH<7.6 IU/L predicts OA with 98%.[50]

Specialized Clinical Tests on Semen

These are not the routine tests but done in special circum-

Table 2: World Health Organisation 1990 and 2010 reference values of semen parameters

Parameters	WHO 2010	WHO 1999
Semen volume (ml)	1.5 (1.4-1.7)	≥2.0 ml
Total sperm number (10^6)	39 (33-46)	≥40x10^6
Sperm concentration (10^6/ml)	15 (12-16)	≥20x10^6 /mL
Total motility (PR+NP,%)	40 (38-42)	Grade a+b+c > %50
Progressive motility (PR,%)	32 (31-34)	≥%50 motile (grade a+b) or ≥%25 grade a
Vitality (vital sperm,%)	58 (55-63)	≥%75
Sperm morphology (normal, %)	4 (3.0- 4.0)	>%14
pH	≥7.2	7.2-8.0
Peroxidase-positive leukocyte (10^6 per ml)	<1.0	<1.0
MAR test (%)	<50	<50
Immunobead test (%)	<50	<50
Seminal zinc (µmol/ejaculate)	≥2.4	≥2.4
Seminal fructose (µmol/ejaculate)	≥13	≥13

stances to determine the infertility causes accurately. These are anti-sperm antibodies,[42,51], zona-free hamster oocyte penetration test ((HOPT) and hemi zona binding assay.[52,53]

Leucocytes in Semen

White blood cells in semen have been associated with abnormalities in sperm motility and function. White blood cell count over 1 million/mL semen is called pyospermia. These patients should be evaluated for genital tract infections and inflammations.[12]

Sperm DNA Fragmentation Tests

In mammals, fertilization and embryonic development depends on the integrity of sperm DNA.[52] Sperm DNA fragmentation can be measured with direct (COMET assay, TUNEL assay) or indirect methods (Sperm Chromatin Structure Assay). The usefulness of these tests is controversial. A meta-analysis reported that there was no evidence to warrant their use in routine diagnostic work-up, but it is possible to define a subgroup of infertile men that may benefit from assessment of these tests.[55]

Endocrine Evaluation

Endocrine evaluation is not routine in the evaluation of infertile men but it's indicated in men with;

Sperm concentration below 10 million/mL,

Sexual dysfunction,

Clinical findings of specific endocrinopathy,[12]

The initial evaluation should include FSH and testosterone. When testosterone is below 300 ng/mL, free testosterone, LH and prolactine should also be measured. Hormone levels according to the specific pathologies are shown in table 2.

Inhibin B is recently emerged as a specific marker of spermatogenesis. It is reported that Inhibin B levels are higher in infertile men than their fertile counterparts and correlate better with sperm parameters than FSH.[56]

In infertile men with severe oligospermia (<5 million/mL), normal gonadotrophin but low testosterone levels (<300 ng/dL), the examination should include serum estrogen evaluation. The patients with Testosterone/Estrogen ratio below ten may benefit from aromatase inhibitors.[57]

Genetic Screening

Genetic abnormalities can cause infertility either by disturbing sperm production or sperm transport. Genetic screening should be considered in infertile men with risk factors. Risk factors may be listed as follows;

Severe oligoasthenoteratospermia (5 million/mL)

Obstructive azoospermia

Nonobstructive azoospermia

Oligoasthenoteratospermia and repeated implantation failure.[58]

Patients with OA should be screened for CFTR mutations while patients with NOA should be offered karyotype and Y chromosome analysis. Karyotyping should also be offered to patients with severe oligoasthenoteratospermia and repeated implantation failure.[58]

As a summary, diagnostic evaluation of male partner of an infertile couple should begin at the same time with the female partner. Initial evaluation should include semen analysis and hormonal evaluation together with a detailed history and physical examination. If indicated, additional hormone tests and genetic screening should be offered to the infertile patient.

Table 2: Hormone levels in specific endocrinopathies

	FSH	LH	Testosterone	PRL
Hypogonadotropic Hypogonadism	↓	↓	↓	Normal
Anormal Spermatogenesis	↑	Normal	Normal	Normal
Complet Testicular Failure	↑	↑	↓	Normal
PRL Secreting Tumor	Normal/↓	Normal/↓	↓	↑

FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, PRL: Prolactin

Erkek İnfertilitesi: Nedenler ve Tanısal Değerlendirmedeki Güncel Gelişmeler

İnfertilite nedenleri arasında erkeğe ait nedenler özellikle son iki dekadda önem kazanmış, yapılan bazal semen analizinde revizyona ihtiyaç kaçınılmaz hale gelmiştir. Erkek infertilitesinde temel değerlendirmede detaylı bir anamnez ve fizik muayene yanında uygun şartlarda alınmış semen analizi her hastada yapılması gereken temel tetkiklerdir. Gerekli görüldüğü taktirde yapılacak ek tetkikler ise hormonal değerlendirme ve genetik analizi içerir. Özellikle yardımla üreme tekniklerinde son yıllarda meydana gelen hızlı gelişme, erkeğe ait nedenlerin saptanması ve azospermik hasta grubu gibi prognozun tedavi planını etkileyebileceği olgularda genetik incelemenin önemini bir kat daha arttırmıştır.

Anahtar Kelimeler: Erkek infertilitesi, Semen analizi, Genetik değerlendirme

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