



Spermiogram Test Results of Patients Presenting to our IVF Center Due to Infertility

İnfertilite Nedeni İle IVF Merkezimize Başvuran Hastaların Spermiyogram Sonuçları

Male Infertility is Increasing

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Özet

Amaç: Bu çalışmanın amacı Androloji laboratuvarına spermiyogram analizi için başvuran olguların semen numunelerini WHO 2010 kriterlerine göre değerlendirmektir. **Gereç ve Yöntem:** Ocak 2010 ile Aralık 2015 tarihleri arasında spermiyogram testi için Androloji laboratuvarına başvuran 10,153 semen numunesi retrospektif olarak taranmıştır. **Olgular;** normozoospermi, oligozoospermi, şiddetli oligozoospermi, teratozoospermi, astenozoospermi, oligoastenoteratozoospermi ve azoospermi olarak sınıflandırılmıştır. Ayrıca başvuruların yaş, body mass index (BMI), medeni durum, sigara içme durumu, alkol tüketimi, uyuşturucu kullanımı, hazır gıda tüketimi, cep telefonu kullanımı ve varikosel ameliyatı geçirip geçirmediği de değerlendirilmiştir. **Bulgular:** Yıllara göre 17-65 yaş aralığındaki 10,153 başvurunun dağılımı; 2010 yılında 574, 2011 yılında 1,118, 2012 yılında 1,583, 2013 yılında 2,008, 2014 yılında 2,346 ve 2015 yılında 2,524 başvuru olup, 9,219 (%90.80) olgu evli, yaş ortalaması 37.73±14.04, BMI'i 25.79±4.03 idi. Başvuranların 4,715'i (%46.44) sigara içmekte, 67'si (%0.66) alkol kullanmakta olup 693 (%6.82) olgu ise hem alkol hem de sigara kullanmaktayken sadece 14 (%0.13) başvuruda uyuşturucu madde tüketimi tespit edildi. Hazır gıda tüketen 3,298 (%32.48) kişi varken, 9,997 (%98.46) olgu ise cep telefonu kullanmakta idi. Varikosel ameliyatı geçiren 1,827 (%17.99) olgu mevcuttu. WHO 2010 kriterine göre 4,330 (%42.56) normozoospermi, 1,952 (%19.22) teratozoospermi, 1,238 (%12.20) oligoastenoteratozoospermi, 807 (%7.94) severe oligozoospermi, 759 (%7.48) astenozoospermi, 717 (%7.06) azoospermi ve 351 (%3.45) oligozoospermi olgusu tespit edildi. **Tartışma:** Verilerimiz erkek infertilitesinde bir artışın yaşandığını göstermekte olup, bu artışın nedenlerinin tespit edilmesine ve önlenilebilir faktörlerin azaltılmasına yönelik çalışmalar gerekmektedir.

Anahtar Kelimeler

Erkek İnfertilitesi; Spermiyogram; WHO 2010

Abstract

Aim: The aim of this study was to evaluate the semen samples of patients who had presented to our andrology laboratory for spermiogram analysis, according to the WHO 2010 criteria. **Material and Method:** Spermiogram tests of 10,153 patients who had presented to our andrology laboratory between January 2010 and December 2015 were retrospectively analyzed. The cases were classified as normozoospermia, oligozoospermia, severe oligozoospermia, teratozoospermia, astenozoospermia, oligoastenoteratozoospermia, and azoospermia. Age, body mass index (BMI), marital status, smoking habit, alcohol consumption, drug abuse, fast food consumption, cellular phone use, and history of previous varicocele surgery were evaluated as well. **Results:** The distribution of 10,153 admissions across years was as follows: 2010: 574; 2011: 1,118; 2012: 1,583; 2013: 2,008; 2014: 2,346; and 2015: 2,524. 9,219 of the participants (90.80%) were married. All were between 17-65 years of age; the mean age was 37.73±14.04. The mean BMI was 25.79±4.03. 4,715 (46.44%) of the presenting individuals were smokers, 67 (0.66%) used alcohol, and 693 (6.82%) were smokers and alcohol users. Only 14 (0.13%) had drug abuse. 3,298 (32.48%) consumed fast food and 9,997 (98.46%) used cellular phones. 1,827 (17.99%) had a history of varicocele surgery. According to the WHO 2010 criteria, 4,330 (42.56%) had normozoospermia, 1,952 (19.22%) had teratozoospermia, 1,238 (12.20%) had oligoastenoteratozoospermia, 807 (7.94%) had severe oligozoospermia, 759 (7.48%) had astenozoospermia, 717 (7.06%) had azoospermia, and 351 (3.45%) had oligozoospermia. **Discussion:** Our data demonstrate an increase in male infertility over the period of the study, and further studies are needed to illuminate the causes of this increase and the preventable factors.

Keywords

Male Infertility; Spermiogram; WHO 2010

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Introduction

Infertility is the failure to conceive for one year despite a regular sexual relations within a relationship, and affects approximately 15% of married couples who are within the reproductive period. Since male infertility accounts for 40% of infertility, seminal analysis is the most important laboratory test that presents clinical information on the male reproductive status [1]. As a result of the rapid developments observed in reproductive medicine at the end of the 20th century, the methods of seminal analyses have continuously changed [2]. The World Health Organization (WHO) has published normal parameters of a semen sample for standardization of the analysis procedure in 1987, 1992, 2002 and 2010 [3-6].

Male infertility is known to have increased in recent years [7]. Studies have shown that the semen quality is worsened due to increased rates of testicular cancer, cryptorchidism, and hypospadias over the past 50 years [8]. Furthermore, environmental chemical exposure negatively affects male fertility and the semen quality. Altered dietary habits and the modern life style lead to increased infertility for both men and women by negatively affecting the reproductive systems [9]. Moreover, cellular phone use, while becoming an important element of individuals' contact with the world, has been identified as impairing semen quality and increasing male infertility [10].

The aim of this study was to analyze the semen samples of patients who had presented to the andrology laboratory of our in-vitro fertilization (IVF) laboratory for spermogram analysis, according to the WHO 2010 criteria.

Material and Method

The spermogram tests of 10,153 patients who had presented to the andrology laboratory of Konya Education and Training Hospital IVF unit between January 2010 and December 2015 were retrospectively investigated. This study was approved by the institutional review board of the hospital (2016-10-05). The semen samples were evaluated by certified and experienced embryologists. Multiple spermogram tests of each patient were evaluated and those with the lowest count of sperm were included in the study. Patients with ejaculation problems, improperly given samples, azoospermia samples, and those with retrograde ejaculation were excluded from the study.

For semen analysis, the samples were collected in a sterile specimen container via masturbation in a special room after a minimum of 48-hour sexual abstinence. The samples were analyzed under the microscope according to the criteria of WHO 2010 (Table 1), and classified as normozoospermia, oligozoospermia, severe oligozoospermia, teratozoospermia, asthenozoospermia, oligoasthenoteratozoospermia, or azoospermia, as displayed in Table 2. Microscopic evaluation was performed on a Makler counting chamber; 10 different squares were counted 4 times and the mean count was calculated as millions/ml sperms. For sperm motility, the sperm cells were evaluated by a phase contrast microscope under 200X magnification, and were grouped as progressive motile, non-progressive motile, or immotile. For sperm morphology, the semen smear slide was air-dried and stained via Diff-Quick sperm staining kit. Immersion oil was dropped on and the slide was examined under 10x100 magnification. At least 200 sperm samples were investigated and

Table 1. Normal spermogram parameters according to WHO 2010

Variables	Normal parameter
Semen volume (ml)	1.5
Total sperm count	39 (million)
Sperm concentration	15 (million/mL)
Total motility	40 (%)
Progressive motility	32 (%)
Vitality	58 (vital sperm, %)
Sperm morphology	4 (normal forms, %)
pH	>7.2
Peroxidase-positve leucocyte	<1.0 (million/ml)

Table 2. Classification according to sperm concentration and morphology

Term	Meaning
Normozoospermia	Normal ejaculate defined the reference value
Aspermia	No ejaculate
Azoospermia	No spermatozoa in ejaculate
Oligozoospermia	Low sperm concentration than reference values (sperm count=million/ml)
Severe oligozoospermia	<1
Middle oligozoospermia	1-5
Mild oligozoospermia	5-20
Asthenozoospermia	Lower value than the reference value for mobility
Teratozoospermia	Lower value than the reference value for morphology
Oligoasthenoteratozoospermia	Disorder all three variables

evaluated according to the criteria of Kruger et al. The least reference value was accepted as 4% [11]. Age, body mass index (BMI), marital status, smoking habit, alcohol consumption, drug abuse, fast food consumption, cellular phone use, and history of previous varicocele surgery were also evaluated.

Statistical analyses were performed using SPSS 15.0 for Windows (SPSS, Chicago, IL, USA). We used the one-way ANOVA, Kruskal Wallis, and Pearson's Chi square or the Fisher's exact test for the analysis. Continuous variables with normal distribution were expressed as mean + standard deviation. The nominal data were expressed as the number of cases and percentages. Statistical significance was set at $p < 0.05$.

Results

Distribution of the participants according to year are presented in Table 3 and demographic characteristics are presented in Table 4. The number of admissions to our IVF center increased every year. Age, BMI, marital status, smoking habit, alcohol con-

Table 3. Distribution of the admissions according to years

Years	The number of applications	Rate (%)
2010	574	6.35
2011	1,118	12.37
2012	1,583	17.52
2013	2,018	22.22
2014	2,346	25.96
2015	2,524	27.93
Total	10,153	

sumption, drug abuse, fast food consumption, cellular phone use, and history of previous varicocele surgery rate were similar between the years.

It was observed that the normozoospermia rates according to the WHO 2010 criteria were decreased and abnormal semen analysis outcomes were increased over the years of the study period (Table 5).

Discussion

Increased male infertility was observed as the years progressed. In previous studies, the causes of infertility were identified as 40-50% female factor, 30-40% male factor, and 30% both male and female factor. However, recent studies have reported increased male infertility rates, similar to our study [7,12].

The first and basic test for the evaluation of the male factor is the spermogram test [4,13]. Anamnesis should be obtained prior to the test and physical examination should be carefully carried out. Examination of an infertile male should be performed by an experienced urologist with IVF certificate. History of infertility, sexual life, previous diseases, infections or operations, exposure to gonadal toxins or harmful substances, presence of a systemic disease, and drug use should be questioned carefully. The decision should be made according to a minimum of 2 tests with a 4 week interval, since a normal fertile male may present with different seminal results within time, and the values obtained may even be observed to be under normal values [2].

There are recent studies indicating a reduction in the semen both qualitatively and quantitatively. The mean seminal volume and sperm count have been reduced over the period from the 1940s to the 1990s [8]. Since male infertility is related to sperm

count, this decrease has directly led to an increase in male infertility. Likewise, parallel to the increase in male infertility, genitourinary abnormalities and male reproductive system infections have been shown to have increased [7]. We determined an increase in admission due to male infertility within the study years as well.

Exposure to harmful chemical substances has increased due to the rapid advances in technology and industry. This exposure is known to threaten public health, damage the reproductive systems of both males and females, increase the incidence of urogenital system abnormalities such as hypospadias and cryptorchidism, accelerate the progression of testicular cancer, and impair semen quality. Furthermore, in addition to their effects on the reproductive system, these chemical substances are believed to have negative effects on the developmental system and to increase the rate of infertility. Among those, polychlorinated biphenyls (PCB) and pesticides have been demonstrated to directly impair semen quality and to reduce sperm motility and fertility [14]. Another chemical agent, phthalate esters, which have similar estrogenic effects to PCB, have been found to be elevated in the seminal plasmas of infertile males compared to fertile males, and have been shown to negatively affect the semen quality and quantity and to reduce fertility [15]. Another factor known to cause an increase in male infertility is air pollution, which is a result of modern industrial life. It has been shown in studies investigating the correlation between air pollution and spermogram results that sperm morphology and motility reduce with an increase in air pollution, impairing the chromatin structure of sperm DNA [15]. However, further studies are needed to clearly describe this relationship.

Electromagnetic waves formed by cellular phones and the ra-

Table 4. Demographic characteristics of the participants

	2010 (n=574) (%)	2011 (n=1,118) (%)	2012 (n=1,583) (%)	2013 (n=2,008) (%)	2014 (n=2,346) (%)	2015 (n=2,524) (%)	Total (n=10,153) (%)
Age (years)	36.21+13.37	37.78+14.15	37.86+14.12	37.89+14.10	37.43+14.12	37.92+14.09	37.73+14.04
BMI (kg/m ²)	25.88+4.07	25.76+4.21	25.79+4.04	25.80+3.94	25.62+4.19	25.87+4.00	25.79+4.03
Marital Status	Married	1,011 (90.42)	1,425 (90.01)	1,809 (90.08)	2,113 (90.06)	2,345	9,219 (90.80)
	Single	58 (10.02)	107 (9.58)	158 (9.99)	199 (9.92)	233 (9.94)	179 (7.10)
Smoking status	268 (46.68)	521 (46.60)	730 (46.11)	928 (46.21)	1,097 (46.76)	1,171 (46.39)	4,715 (46.44)
Alcohol consumption	4 (0.69)	9 (0.80)	10 (0.63)	15 (0.74)	14 (0.59)	15 (0.60)	67 (0.66)
Drug abuse	1 (0.17)	2 (0.17)	2 (0.12)	2 (0.09)	3 (0.12)	4 (0.15)	14 (0.13)
Fast-food consumption	181 (31.53)	369 (33.00)	511 (32.28)	655 (32.61)	754 (32.13)	828 (32.80)	3,298 (32.48)
Cell phone usage	563 (98.08)	1,102 (98.56)	1,549 (97.85)	1,971 (98.15)	2,301 (98.08)	2,511 (99.48)	9,997 (98.46)
Varicocele operation	103 (17.94)	204 (18.24)	280 (17.68)	356 (17.72)	422 (17.97)	422 (17.97)	1,827 (17.99)

Table 5. Distribution of the spermogram results according to WHO 2010 criteria

Year (n=10,153)	Normozoospermia (4,330) (%42.65)	Teratozoospermia (1,952) (%19.22)	Oligoasthenoteratozoospermia (1,952) (%19.22)	Severe oligozoospermia (807) (%7.94)	Astenozoospermia (759) (%7.48)	Azoospermia (717) (%7.06)	Oligozoospermia (351) (%3.45)
2010 (574) (%)	306 (53.32)	93 (16.22)	35 (6.09)	37 (6.44)	46 (8.02)	24 (4.18)	33 (5.74)
2011 (1,118) (%)	579 (51.79)	186 (16.64)	60 (5.36)	68 (6.08)	91 (8.14)	50 (4.47)	84 (7.52)
2012 (1,583) (%)	687 (43.39)	326 (20.59)	211 (13.32)	135 (8.52)	82 (5.18)	114 (7.20)	28 (1.76)
2013 (2,008) (%)	840 (41.83)	387 (19.27)	268 (13.34)	158 (7.86)	153 (7.61)	150 (7.47)	52 (2.58)
2014 (2,346) (%)	939 (40.02)	461 (19.65)	318 (13.55)	193 (8.22)	184 (7.84)	183 (7.50)	68 (2.89)
2015 (2,524) (%)	979 (38.79)	499 (19.78)	346 (13.71)	216 (8.55)	203 (8.05)	196 (7.76)	85 (3.36)

dio frequency waves of transmitters are believed to increase male infertility via negative effects on sperm parameters [10]. It has been suggested in a study that the use and carrying of cellular phones reduced the motile sperm count. Likewise, the sperm count was demonstrated to be lower in societies with widespread use of cellular phones compared to societies with a lower rate of use [16]. The rate of cellular phone usage was observed to be high in our study. With regard to use of such technological devices that become a routine part of modern life but threaten public health, possible harmful effects should be declared and publicized, and these kinds of devices should be used less often.

Tobacco has been known to have harmful effects on spermatozoa and to have carcinogenic effects. The rate of smokers has progressively increased in societies, and the age of starting to smoke is continuously decreasing. 53% of males aged between 25 and 44 are smokers [17]. 43% of all participants in our study were smokers. The toxins in tobacco have been demonstrated to negatively affect semen quality, sperm motility and morphology, increase serum follicle-stimulating hormone (FSH) and luteinizing hormone levels, and reduce testosterone levels [18]. However, in another study [19], although the negative effect of tobacco on sperm parameters was not demonstrated, the negative effect on sperm metabolism was proven. The fertilization rates were observed to be decreased in smokers despite their having normal spermiogram parameters compared to non-smokers [20]. Similarly to tobacco, alcohol consumption has been shown to negatively affect semen quality, motility and morphology. Outcomes are even worse when tobacco and alcohol are used together [21]. In our study, the rate of alcohol consumption was quite low among those presenting to our IVF unit due to their religious and ethnic backgrounds; however, the smoker rate was slightly over the mean rate of Turkey (46.44%). Fast food consumption has increased worldwide due to the social lifestyles and changes in dietary habits of people. Foods with high carbohydrate and saturated fat levels, such as fast food, have become preferred to conventional and organic food. In addition to this consumption of fast and ready-to-eat foods, technological advances have resulted in a more sedentary lifestyle, with a resulting increase in BMI. Chemical and preservative substances used in these fast and ready-to-eat foods, along with essential fatty substances present at high levels within these foods, have been determined to have negative effects on semen quality. Additionally, milk in large amounts has been shown to impair semen quality. In contrast, fresh vegetable and fruits have been demonstrated to improve semen quality [22]. In other studies, dietary and social habits have been shown to directly affect semen quality and therefore fertility [9]. Sperm motility and morphology have been reported to be negatively affected by BMI, smoking, alcohol consumption, and excessive red-meat consumption, and positively affected by grain foods, fresh vegetables, and fruits. Likewise, BMI, tobacco, and alcohol negatively affect fertilization, while were retrospectively analyzed. The grain foods, fresh vegetables, and fruits positively affect it [22].

Normal semen parameters and normal fertility may be observed in the presence of varicocele; however, varicocele may lead to abnormal semen parameters, low sperm count, and abnormal

sperm morphology as well. The results of studies investigating the positive effects of varicocele surgery on fertility are complicated and routine varicocele repair is not recommended in subfertile couples. In the study of WHO on 9,000 infertile males, varicocele was detected in 11.7% of males with normal semen parameters, and in 25.4% of those with abnormal semen parameters [23]. Varicocele has been related to infertility by increasing the testicular temperature, delaying the removal of endogenous toxic metabolites, and causing hypoxia and stasis [24].

Currently, the routine surgery of varicocele ligation is not recommended, except for those with grade 3. However, in case of young age and short duration of infertility, surgery may be the primary approach [24]. It has been demonstrated that in cases of atrophic testes, high serum FSH levels, severe oligozoospermia, or azoospermia, the possibility of re-gaining fertility via varicocele surgery is low.

An alternative method to varicocele ligation is intra-cytoplasmic sperm injection (ICSI). However, conventional varicocele ligation is more cost-effective than ICSI [25].

The present data currently show a negative tendency in seminal quality and quantity and an increase in male infertility. Chemical substances harmful for the environment and public health, cellular phone use, use of instruments and devices that transmit electromagnetic waves, sedentary lifestyle, and increased BMI negatively affect semen quality and quantity.

In conclusion, the primary goal of preventive medicine should be developing a governmental program to determine and prevent the factors negatively affecting semen quality and quantity that impair public health. The society should be educated to gain healthier dietary habits, to quit sedentary lifestyles, and to increase their social lives. Fertility rates may thereby be elevated by reducing the preventable factors, and thus the cost of assisted reproduction will be reduced.

Competing interests

The authors declare that they have no competing interests.

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