



Male infertility, azoospermia and cryptozoospermia incidence among three infertility clinics in Turkey

Türkiye'deki üç infertilite kliniğinin erkek infertilitesi, azoospermi ve kriptozoospermi insidansı

Seda Karabulut¹ , İlknur Keskin² , Pelin Kutlu³ , Nuri Delikara⁴ , Özhan Atvar⁵ , Metin İ. Öztürk⁶ 

Cite this article as: Karabulut S, Keskin İ, Kutlu P, Delikara N, Atvar Ö, Öztürk Mİ. Male infertility, azoospermia and cryptozoospermia incidence among three infertility clinics in Turkey. Turk J Urol 2018; 44(2): 109-13.

ABSTRACT

Objective: Semen parameters are directly correlated with the infertility of the male. Incidence rates of male factor infertility, azoospermia and cryptozoospermia differ according to many factors such as geographic region, age, occupation and body weight. The aim of the present study is to determine the incidence of male factor infertility, azoospermia and cryptozoospermia among patients who have been admitted to three separate infertility clinics in Turkey for infertility investigation and analyze the outcomes of these patients.

Material and methods: A total of 9733 men, who have been admitted to 3 infertility clinics in Turkey due to infertility between March 2011 and October 2016, were included in the study. Male infertility, azoospermia and cryptozoospermia incidence were calculated according to WHO criteria.

Results: Male factor infertility was determined in 3114 (32%) of the patients including cases with azoospermia and cryptozoospermia. Azoospermia cases were observed in 570 (5.85%) and cryptozoospermia in 850 (8.73%) men. Azoospermic, and cryptozoospermic patients constitute 18.3%, and 27.2% of the male infertility cases. Sperm retrieval rates in diagnostic or oocyte pick-up plus testicular sperm extraction groups were found to be comparable (16.39%, and 41.3%, respectively).

Conclusion: The data obtained may help to estimate the number of *in vitro* fertilization cycles and testicular sperm extraction cases, to determine social security policies, and reproductive potential, and in the light of these data to establish social insurance policies. These data will help patients to decide on treatment alternatives, and guide the urologists about the issue.

Keywords: Azoospermia; cryptozoospermia; infertility.

ÖZ

Amaç: Semen parametreleri erkek infertilitesi ile direkt ilişkilidir. Erkek faktörlü infertilite, azoospermi ve kriptozoospermi insidansı coğrafi bölge, yaş, meslek ve vücut ağırlığı gibi birçok faktöre göre değişmektedir. Bu çalışmanın amacı, Türkiye'de 3 farklı infertilite kliniğine infertilite nedeniyle başvuran hastalar arasında erkek faktörlü infertilite, azoospermi ve kriptozoospermi oranlarını belirlemek ve bu hastaların sonuçlarını analiz etmektir.

Gereç ve yöntemler: Mart 2011 ve Ekim 2016 tarihleri arasında, Türkiye'de 3 farklı infertilite kliniğine infertilite nedeniyle başvuran 9733 erkek çalışmaya alınmıştır.

Bulgular: Hastaların 3114'ünde (%32) erkek faktörlü infertilite (azoospermi ve kriptospermi vakaları dahil) belirlenmiştir. Hastaların 570'inde (%5,85) azoospermi, 850'sinde (%8,73) kriptozoospermi belirlenmiştir. Azoospermi vakaları erkek infertilite vakalarının %18,3'ünü, kriptozoospermi vakaları ise %27,2'sini oluşturmaktadır. Azoospermik ve kriptozoospermik hastalarda tanısal ya da oosit toplama işlemi ile eşlenik testiküler sperm ekstraksiyonu gruplarının sperm bulma oranları benzer (%16,39-%41,3 sırasıyla) bulunmuştur.

Sonuç: Elde edilen sonuçlar *in vitro* fertilizasyon siklusu ve TESE vakalarını tahmin etmede ve bu veriler ışığında sosyal güvenlik politikalarının belirlenmesine, üreme potansiyelinin belirlenmesi ve bu veriler ışığında ulusal sağlık politikaları belirlenmesine yardımcı olacaktır. Veriler aynı zamanda hastalara tedavi seçenekleri arasında karar vermelerine ve ürologlara konu ile ilgili yönlendirme yaparken fayda sağlayacaktır.

Anahtar Kelimeler: Azoospermi; kriptozoospermi; infertilite.

¹Medipol University, International School of Medicine, REMER (Regenerative And Restorative Medicine Research Center), İstanbul, Turkey

²Medipol University School of Medicine, REMER (Regenerative And Restorative Medicine Research Center), İstanbul, Turkey

³Medicana Çamlica Hospital, IVF Center, İstanbul, Turkey

⁴Florence Nightingale Hospital, IVF Unit, İstanbul, Turkey

⁵Reyap Hospital, IVF Unit, Tekirdağ, Turkey

⁶Department of Urology, University of Health Sciences, Haydarpaşa Numune Training and Research Hospital, İstanbul, Turkey

Submitted:
01.06.2017

Accepted:
02.10.2017

Correspondence:
Seda Karabulut
E-mail:
sedakarabulut@medipol.edu.tr

©Copyright 2018 by Turkish Association of Urology

Available online at
www.turkishjournalofurology.com

Introduction

According to the glossary of World Health Organization (WHO), infertility is defined as 'a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after attempts of regular unprotected sexual intercourse during at least a period of 12 months.'^[1] Infertility is seen in about 15% of the married couples and about 30-50% of the cases are due to male factors.^[2] Semen parameters are directly correlated with male infertility. Azoospermia is defined as 'absence of spermatozoa in the sediment of a centrifuged semen sample of a man' and cryptozoospermia is as 'very low spermatozoa concentration (≤ 1 million/mL) in the ejaculate of a man' according to WHO. These situations are generally diagnosed during a routine male infertility investigation.

Azoospermia is seen approximately in 1% of the male population^[3] and may be as high as 20% among male infertility cases.^[3,4] Incidence rates of azoospermia and cryptozoospermia differ according to genetic differences, geographic region, age, occupation and body weight of the male partner.^[5] Causes of male infertility include genetic alterations (Klinefelter syndrome, Y chromosome abnormalities, single gene disorders), hormonal abnormalities (hypogonadotropic hypogonadism), anatomic reasons or infections (orchitis), surgeries (trauma or cancer), and cancer treatments (chemotherapy, radiation).^[6-8]

Regardless of the cause and incidence, the situation affects many couples facing infertility problems. Assisted reproductive techniques, especially intracytoplasmic sperm injection (ICSI), is the only treatment option for these couples. Testicular sperm extraction (TESE) methods are usually the only option in cases of azoospermia and in most cases of cryptozoospermia, in which sperm cells are searched in the surgical biopsy materials obtained from testicles.

We aimed to analyze incidence rates of the male infertility, azoospermia, cryptozoospermia detected in three infertility clinics in Turkey to see the prevalence of male infertility subgroups. Also we aimed to analyze sperm retrieval rates of diagnostic and oocyte pick-up (OPU) accompanying TESE/TESA cases to help informing patients and experts about these two options and to plan treatment strategies according to these data.

Material and methods

Study design

A total of 9733 men, who were admitted into three separate infertility clinics in Turkey for investigation of infertility between March 2011 and October 2016, were retrospectively analysed regardless of the etiology of infertility, age, job or body weight of the male partners. Written consent forms were

taken from the patients. The local ethical committee of İstanbul Medipol University (10840098-604.01.01-E.8463), approved this study on 22.03.2017.

Semen analysis

Semen analysis was performed according to WHO criteria (WHO, 2010). Specimens were collected by masturbation after 2-7 days of sexual abstinence. At least two semen analyses were performed for each infertile patient. Semen analysis was performed with a phase contrast microscope (Olympus, CX40) after 10-30 min of liquefaction. If the sample was not liquified then the sample was pipetted in order to achieve liquefaction. All the samples were mixed thoroughly before semen analysis to achieve a homogeneous sample. All parameters were replicated by the same technician and taken into account if the difference between the parameters was not more than 10%. Liquefaction time (min), appearance, volume (mL), pH, viscosity, sperm concentration (mil/mL), total motility rate (%), A, B, C, D motility rates (%), forward progressive sperm motility (%), and percentage of spermatozoa with normal morphology (%) were determined. All the semen samples, (except those with $< 10^6$ /mL sperm concentration.) were prepared by density gradient semen preparation technique. Briefly, two solutions (PureSperm, PureSperm Wash Nidacon) with different densities (90% and 45%) were prepared and 1 mL from each solution was layered in a conical based tube (Falcon 2095) to make an unmixed transition layer. One milliliter of the neat semen sample was put onto them and the tube was centrifuged at 600 x g for 20 min. The pellet (0.4 μ L) was taken and put in another tube and washed two times by adding 1 mL of PureSperm Wash solution and again centrifuged at 1000 x g for 6 minutes. Finally, pellet was taken out and sperm parameters (concentration, total motility rate, forward progressive sperm motility rate, normal morphology rate) were analyzed. Samples with $< 10^6$ /mL sperm concentration. were centrifuged at 3000 x g for 15 minutes. The supernatant was discarded and all the pellet was examined to detect azoospermia and at least 100 microscopic fields were examined to detect cryptozoospermia. Patients were diagnosed as cryptozoospermic in cases with ≤ 1 million/mL sperm concentration. and as azoospermic if no spermatozoa was detected in the whole ejaculate. Internal and external quality controls and quality assurances of andrology laboratories in the three centers were conducted at 3-month-intervals by Medek Co, Turkey).

Urologic examination

Medical data of patients from one of the centers (Florence Nightingale Hospital) were included in the present study. Urology consultation was offered for the azoospermic and cryptozoospermic patients Urologic, anatomic, genetic, and general physical examinations and hormonal (total testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin, estradiol (E2) tests were performed. According to these results

men that are thought to have a lower chance to have adequate number of sperms for fertility (in cases of Klinefelter syndrome, partial AZF deletions, reduced testis volume, decreased testosterone levels) were offered diagnostic TESE in order to avoid high treatment costs and drug use. The men that thought to have a higher chance to find sperm were offered TESE accompanying oocyte pick-up for ICSI. The couples and the specialists decided the treatment strategy based on the data obtained.

Statistical analysis

Statistical Package for the Social Sciences (SPSS Inc.; Chicago, IL, USA) for Windows 16.0 software package was used for the statistical analysis. Mann-Whitney U test was used to evaluate the differences between the groups. The results were evaluated within 95% confidence interval and the statistical significance was defined as $p < 0.05$.

Results

Among 9733 men, male factor infertility was seen in 3114 (32%), azoospermia in 570 (5.85%) and cryptozoospermia in 850 (8.73%) cases. Other male factor infertility cases (17.42%) included men with poor sperm motility (asthenozoospermia) and/or lower sperm concentration (oligozoospermia) and/or abnormal sperm morphology (teratozoospermia) according to WHO criteria (Figure 1). In the present study azoospermia, and cryptozoospermia were seen in 18.3%, and 27.2% of male infertility cases, respectively.

Urology consultation was offered to the azoospermic and cryptozoospermic patients. The patients with lower chance to find sperm were offered diagnostic TESE in order to avoid high costs and drug use for the couples, the men who had a higher chance to find sperm is offered TESE accompanying oocyte stimulation for ICSI.

One hundred and four patients underwent diagnostic TESE and spermatozoa were found in 72 of them (69.2%) and these sper-

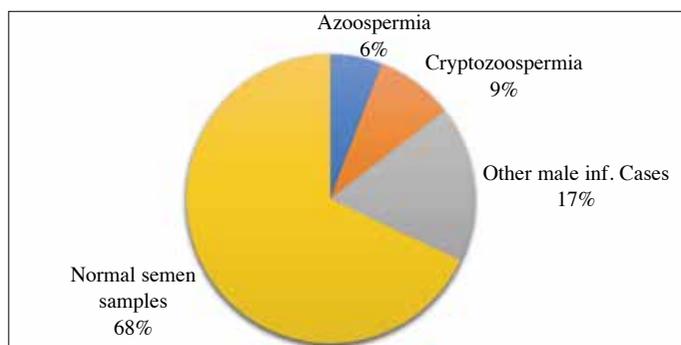


Figure 1. Distribution of patients according to their semen characteristics among three IVF centers

Table 1. Patient characteristics and ICSI outcome parameters in the simultaneous TESE/TESA cases and patient characteristics and sperm (+) case rates in the diagnostic TESE group of one of the centers

	TESA/TESE	Diagnostic TESE	p
Patient, n	65 (61 azo+4 crypto)	104	
Age (Mean)	32.57	33.1	0.3
Number of MII oocytes (mean)	6.6		
Fertilization rate (%)	50.9		
Sperm (+) rate (%)	81.5	69.2	0.1
Number of no ET	15		
Number of transferred embryos (mean)	1.69		
Clin. preg. Rate (%)	22.2		

Fertilization, sperm (+) and pregnancy rates are given as means (%).
 TESE: testicular sperm extraction; TESA: testicular sperm aspiration; azo: azoospermia; crpto: cryptozoospermia; ET: embryo transfer; Clin. Preg. Rate: clinical pregnancy rate

matozoa were kept frozen. Sixty-five cases with azoospermia underwent TESE/TESA with oocyte pick-up and sperm was found in 53 (81.5%) of them. Sperm-positive case rates were found similar in these two groups ($p=0.1$). Embryo transfer were performed in 50 of them. Results were known in 45 of them and pregnancy were assessed in 10 ICSI cases (22.2%). Patient characteristics and ICSI outcome parameters are given in Table 1.

Discussion

Incidence of azoospermia and cryptozoospermia differed among subgroups of patients with different genetic, etiologic and demographic characteristics as their resident geographic regions, jobs, life styles, feeding behaviours etc. Rates of male factor infertility, azoospermia and cryptozoospermia among patients attending three infertility clinics as part of routine infertility investigation were analysed. Our study group consisted of a selected group of patients facing infertility problems therefore it did not reflect the overall rates of male infertility, azoospermia and cryptozoospermia among Turkish population.

In the present study, we found incidence rates of azoospermia, and cryptozoospermia as 18.3 and 27.2%, respectively in cases with male infertility. Although the patient population and the genetic factors were different, our data for azoospermia were in accordance with the rate (20%) observed by different studies performed among cases with male infertility.^[3,4] Also, in their review of azoospermia Wosnitzer et al.^[10] found that about 10-20% of infertile men had presented with

azoospermia which is the most severe form of infertility. This may be partially explained by the theory that this prevalence are mostly because of individual in vivo mutations and genetic abnormalities were not genetically hereditated from the parents except the ICSI offspring. Cryptozoospermia rate is found to be 8.73% in the present study. These two situations occupies 14.58% of all male population and 45.56% of all male infertility cases. Testicular sperm extraction methods should be used for these cases.

Data obtained may be useful to estimate the number of patients that will undergo ICSI cycle and specifically TESE operations. ICSI is the only treatment option for azoospermic and most cryptozoospermic patients. In addition, testicular surgery or aspiration is necessary in azoospermic and some cryptoospermic cases in order to achieve conception via testicular sperm.

Urology consultation was offered for azoospermic and cryptozoospermic patients. One hundred and four patients underwent diagnostic TESE (n=104), and 65 patients TESE with oocyte stimulation for ICSI regardless of the presence of obstructive, and non-obstructive azoospermia. Sperm retrieval rates were found similar (69.2%-81.5% respectively) in these two groups with an overall sperm retrieval rate of 75.3% representing the sperm retrieval rate which was similar for all patients regardless of the etiology of the azoospermia and cryptozoospermia therefore the patient should decide whether to undergo a diagnostic or simultaneous TESE with OPU according to the couple's own dynamics. Both of the situations have its own advantages and disadvantages. Diagnostic TESE is more cost-effective because ovulation induction is not performed but the couples should consider the risks of frozen sperm because the success rates are lower when frozen sperm is used. Additionally sperm viability rates decrease especially in cases with low sperm count and quality. Simultaneous TESE with OPU is more expensive but the couples will have the opportunity to use fresh sperm as part of ICSI as soon as it is collected. But the couples should consider the risk of finding no sperm.

Our sperm retrieval rates were in accordance with the findings of Thornhill et al.^[11] in which they found 65.5% total sperm retrieval rates. Although sperm retrieval rates were within acceptable rates, pregnancy rates were observed to be lower when compared with treatment cycles where ejaculate sperm is used (22.2%-41.3%, respectively). Also our findings were in accordance with the results of van Wely et al.^[12] in which they also found a pregnancy rate of 24% in TESE-OPU cases. The decrease in the pregnancy rates can partly be explained by the fact that sperm maturation is not completed until ejaculation.

The results obtained may help to estimate the number of IVF cycles and TESE operations, and to plan social insurance policies. These data may also help us observe the reproductive status of male population at a specific interval, and take precautions as a part of the national health policy. These data are also informative for patients undergoing infertility investigation and treatment by providing them and also urology professionals counselling these patients with sperm retrieval rates of other patients facing these issues.

Further studies with larger groups are needed to investigate the incidence rates of male infertility subgroups in patients with different demographic characteristics (eg. geographic region, age, life style, job etc.) so as to take precautions according to the risks with the help of the data obtained.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul Medipol University (10840098-604.01.01-E.8463).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - S.K., İ.K.; Design - M.İ.Ö., S.K.; Supervision - S.K., N.D., Resources - N.D., Ö.A., P.K.; Materials - İ.K.; Data Collection and/or Processing - S.K., İ.K.; Analysis and/or Interpretation - S.K., İ.K., M.İ.Ö.; Literature Search - S.K., P.K.; Writing Manuscript - S.K., P.K.; Critical Review - M.İ.Ö., İ.K., N.D.; Other - Ö.A., P.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

Etik Komite Onayı: Bu çalışma için etik komite onayı İstanbul Medipol Üniversitesi'nden (10840098-604.01.01-E.8463) alınmıştır.

Hasta Onamı: Yazılı hasta onamı bu çalışmaya katılan hastalardan alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir - S.K., İ.K.; Tasarım - M.İ.Ö., S.K.; Denetleme - S.K., N.D., Kaynaklar - N.D., Ö.A., P.K.; Malzemeler - İ.K.; Veri Toplanması ve/veya İşlemesi - S.K., İ.K.; Analiz ve/veya Yorum - S.K., İ.K., M.İ.Ö.; Literatür Taraması - S.K., P.K.; Yazıyı Yazan - S.K., P.K.; Eleştirel İnceleme - M.İ.Ö., İ.K., N.D.; Diğer - Ö.A., P.K.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

References

1. Revised glossary on Assisted Reproductive Terminology (ART). The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, 2009.
2. Tournaye HJ, Cohlen BJ. Management of male factor infertility. *Best Pract Res Clin Obs Gyn* 2012;26:769-75. [\[CrossRef\]](#)
3. Jarvi K, Lo K, Fischer A, Grantmyre J, Zini A, Chow V, Mak V. CUA Guideline: The workup of azoospermic males. *Can Urol Ass J* 2010;4:163-7. [\[CrossRef\]](#)
4. Male Infertility Best Practice Policy Committee of the American Urological Association; Practice Committee of the American Society for Reproductive Medicine Infertility: Report on Evaluation of the Azoospermic Male (PDF). American Urological Association; American Society for Reproductive Medicine. I 2001; SBN 978-0-9649702-8-1.
5. Sermondade N, Faure C, Fezeu L, Shayeb AG, Bonde JP, Jensen TK, et al. BMI in relation to sperm count: An updated systematic review and collaborative meta-analysis. *Hum Reprod Update* 2012;19:221-31. [\[CrossRef\]](#)
6. Dohle, Gert R. Male infertility in cancer patients: Review of the literature. *Int J Urology* 2010;17:327-31. [\[CrossRef\]](#)
7. Menzies FM, Shepherd M. C, Nibbs RJ, Nelson, SM. The role of mast cells and their mediators in reproduction, pregnancy and labour. *Hum Reprod Update* 2010;17:383-96. [\[CrossRef\]](#)
8. Poongothai J, Gopenath TS, Manonayaki S. Genetics of human male infertility. *Singapore Med J* 2009;50:336-47.
9. Asadi F, Sadighi Gilani MA, Ghaheri A, Roodgar Saffari J, Zamanian M. The Prevalence of Y Chromosome Microdeletions in Iranian Infertile Men with Azoospermia and Severe Oligospermia. *Cell J* 2017;19:27-33.
10. Wosnitzer M, Goldstein M, Hardy MP. Review of Azoospermia. *Spermatogenesis* 2014;4:e28218. [\[CrossRef\]](#)
11. Thornhill JA, Fanning DM, Davis NF, Ward F, Shamoun O, Brinsden P. Testicular Sperm Extraction and Intracytoplasmic Sperm Injection: Outcomes in a specialist fertility centre. *Ir Med J* 2015;108:263-5.
12. van Wely M, Barbey N, Meissner A, Repping S, Silber SJ. Live birth rates after MESA or TESE in men with obstructive azoospermia: is there a difference? *Hum Reprod* 2015;30:761-6. [\[CrossRef\]](#)