A Clinical Research on Male Infertility in Arad County

D. Botezatu[1,3], A. Nesiu[1], C. Popescu[2], V Belengeanu[2], I. Ioiart[1,3]

[1] *Faculty of Medicine Pharmacy and Dentistry, "Vasile Goldis" Western University of Arad, Romania*

[2] *The Institute of Life Sciences, "Vasile Goldis" Western University of Arad, Romania*

[3] *Department of Urology, Arad County Emergency Hospital, Romania*

Abstract

Introduction and Objectives. Reproduction is a simple and natural experience for most couples who wish to start a family. The World Health Organization (WHO) and the American Society for Reproductive Medicine (ASRM) define infertility as a couple's inability to conceive a child after at least 12 months of unprotected sex. Data on the magnitude of the phenomenon of general infertility and male infertility in particular are very varied in the literature. In Romania, there are no conclusive studies indicating the frequency of infertility in general and male in particular. Male infertility is a complex multifactorial disease with a very heterogeneous phenotypic representation. In approximately 40% of primary testicular failure, etiology remains unknown and some of it is likely to be caused by genetic abnormalities that have not yet been identified. In the last 10 years, the search for "hidden" genetic factors has not been too successful in identifying recurrent genetic factors with potential clinical applications. We intend in our study to make a survey of the clinical conditions associated with infertility in our county.

Materials and Methods. The prospective study was conducted between 2013 and 2017. It was endorsed by the Ethics Committee of Arad County Hospital and the Scientific Commission for the Scientific Research of UVVG ("Vasile Goldis" Western University of Arad). Our study included male patients who agreed to the study and signed an informed consent after having received detailed information about the purpose of the study and how it was performed. We included in our study all patients of all ages, who addressed an andrological examination and also patients who addressed other urological problems but were diagnosed with male infertility or with modified spermograms. Our study did not apply selection criteria for age, ethnicity, socio-economic status etc.

Results. There is a clear need to understand the heterogeneous causes behind male infertility and the need to improve individualized treatment of the condition. Currently, there are only a few large-scale epidemiological studies addressing this subject and our research shows the results in our geographic area.

Conclusions. The incidence of male infertility cases was relatively constant (3%). Male infertility is characteristic of the 35-40 year old group. An important factor affecting male fertility is smoking. According to the data reported in the literature and also our data in 68% of the male infertility patients the cause has been identified. For 32% of investigated infertile men, the etiology of infertility could not be identified. Varicocele remains the main cause of male infertility, being present in over 30% of infertile men. Oligospermia or oligozoospermia is the most common form under which male infertility is manifested. The quality of sperm can be influenced by a number of factors. More than 20 factors associated with infertility in the study group, but without being a direct cause of infertility, were identified in the research.

Key-words: male infertility, Arad.

Correspondence to: Dr. Botezatu Dragos, M.D.
Arad County Emergency Hospital, Department of Urology
2-4 Andreny Karoly Str., code 310037, Arad, Romania
Tel: +40742903529
e-mail: dragosbotezatu@yahoo.com

Introduction and Objectives

Reproduction is a simple and natural experience for most couples who wish to start a family. However, for some couples it is very difficult to conceive, often facing a problem of infertility. Infertility affects approximately 15% of couples wanting a child, about half of cases involving infertility of the male factor. Male infertility can be caused by a number of factors and, therefore, even the most complex approach, including physical, serological and hormonal examinations, may not detect the etiology of the reproductive dysfunctions.

The World Health Organization (WHO) and the American Society for Reproductive Medicine (ASRM) define infertility as a couple's inability to conceive a child after at least 12 months of unprotected sex.[1] According to the European Urology Association's Guideline, infertility is defined as the inability of a sexually active couple not using contraceptive methods to achieve a pregnancy within 12 months[2]. Male infertility is a problem of the reproductive system, and infertility is often confused with sterility, which means that a man has completely lost his fertility. Men who are sterile can be considered infertile, but not all infertile men are necessarily sterile because an infertile male can become a father with medical help or a change in his lifestyle. For about one in five infertile couples, the problem lies exclusively with the male partner.

Data on the magnitude of the phenomenon of general infertility and male infertility in particular are very varied in the literature. Currently, we do not have conclusive data demonstrating the global prevalence of infertility[3]. According to WHO estimates, 60-80 million couples worldwide are currently suffering from infertility [4]. It is estimated that female infertility is between 8-12% globally. So this percentage does not necessarily reflect reality in certain countries or regions. Approximately 10% of couples in the United States are defined as infertile because of their inability to conceive. According to the National Center for Health Statistics, infertility in women increased from 4.56 million in 1982 to 7.26 million in 2002, then decreased slightly to 6.71 million in 2006-2010 [5-6]. In addition, the fertility rate in men younger than 30 has also declined by 15% worldwide[7].

Agarwal et al.[8] have recently reported data on the prevalence of global infertility where they also included data on male infertility disorders. In developed regions such as North America, Europe and Australia, infertility reporting is much more accurate than other regions of the globe. In North America 4.5 -6% of men are infertile [9] in Australia 8% of men are infertile and 9% of men over 40 years have visited an infertility clinic at one time [10] and in Europe 7.5% of men are infertile[11]. Olsen and colleagues found that infertility varies widely across Europe. After 12 months of not being able of conceiving a child, 51.1% of couples sought help for infertility in Denmark, 43.2% in Germany, 37.9% in Italy, 19.1% in Poland and 43.2% in Spain[12]. The rate of infertility in North Africa, Sub-Saharan Africa and Eastern Europe is approaching some of the highest percentages of male infertility estimated worldwide[8,13].

In 2010, the WHO changed the reference values for semen analysis in the case of male infertility diagnosis [14]. Changing them has led to the classification of several men as fertile. Thus, a man with reference values greater than 15 million spermatozoa, normal morphology greater than 4% and progressive motility of 40% is considered normal from the perspective of the changed reference values[15].

In conclusion, the prevalence of male infertility is not fully known due to data collection difficulties that are influenced by several factors including man's socio-cultural status, religion etc.

In Romania, there are no conclusive studies indicating the frequency of infertility in general and male in particular. According to the European Urology Association Guidelines, 15% of couples cannot have a pregnancy in the first year of unprotected sexual activity. This is the time when medical investigations are performed, and specific infertility treatment is recommended. Despite progress, especially in genetics, etiology is still unknown in approximately 40% of cases and is called idiopathic infertility. Male infertility is a complex multifactorial disease with a very heterogeneous phenotypic representation, and in at least 15% of cases, this condition is related to known genetic disorders, including both chromosomal changes and single gene alterations. In approximately 40% of primary testicular failure, etiology remains unknown and some of it is likely to be caused by genetic abnormalities that have not yet been identified. In the last 10 years, the search for "hidden" genetic factors has not been too successful in identifying recurrent genetic factors with potential clinical applications.

Materials and Methods

The prospective study was conducted between 2013 and 2017. It was endorsed by the Ethics Committee of Arad County Hospital and the Scientific Commission for the Scientific Research of UVVG ("Vasile Goldis" Western University of Arad). Our study included

male patients who agreed to the study and signed an informed consent after having received detailed information about the purpose of the study and how it was performed. We included in our study all patients of all ages, who addressed an andrological examination but also patients who addressed other urological problems but were diagnosed with male infertility or with modified spermograms. Our study did not apply selection criteria for age, ethnicity, socio-economic status etc.

All patients were clinically examined and all anomalies (eg varicocele) or malformations (eg hypospadias, cryptorchidia) were noted in the evaluation sheet. Also, in all patients, a screening ultrasound of the urogenital apparatus was performed to identify other associated urological pathologies. The spermogram was performed from all patients in the study group to have a starting point in the analysis of a possible infertility. Biological samples were re-evaluated for hypothalamic-pituitary-gonadal axis assessment: total testosterone, free testosterone, FSH, LH, prolactin.

Results

There is no conclusive and recent data showing the prevalence of male infertility in Romania, which is why this study aims to create a starting point for a wider evaluation and to draw attention to the complex issues related of male fertility disorders in the demographic context of a downward trend. Thus, the present paper has proposed a multidisciplinary approach to male infertility disorders on a representative sample for the male population in Romania.

The study was conducted over a period of 5 years with a total number of investigated patients of 8207 men, representing 0.08% of the total male population in Romania (9 746 000 are men, according to INS 2014 (ins.ro). These patients come from Arad County and represents 1.7% of the county's total population according to INS 2016 [15], 3.56% of the total male population of Arad county and 4.64% of the male population aged 15-69. Patients diagnosed with male infertility ware 206, which represents an average of 2.51% of all investigated patients (Figure 1).

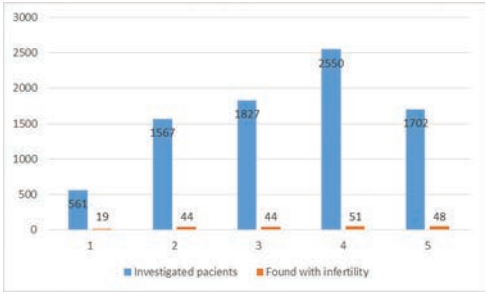


Fig. 1. Prevalence of infertility disorders in the male population in Arad County.

It has to be said that this prevalence during the five years of study is relative because some of the male infertility patients choose to be diagnosed in private medical centers where data are not available or in other large health centers such as Timisoara, Bucharest. Thus, based on our data, we can estimate that the prevalence of infertility disorders in Arad County is about 3% and at national level it could reach 5%. This estimate is based on the size of the male population in Bucharest [16].

The incidence of male infertility cases was relatively constant during the investigation period, which shows that this problem remains in the population and should be an alarm signal for short-term demographic programs at national level.

Of the total male urologic patients, 206 men with an average age of 32.5 years were selected. At age extremes, there is a 10-year-old patient with micropenis and testicular hypoplasia, subsequently diagnosed with chromosomal abnormality, and a 50-year-old patient who referred to urology for infertility problems. Most of the patients included in the study came from the urban area of Arad County (Figure 2).

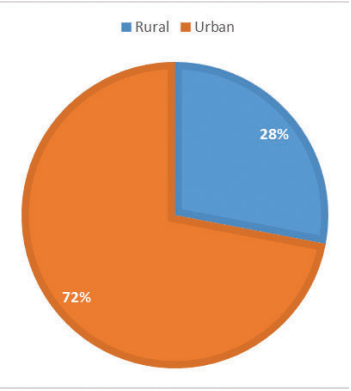


Fig. 2. The provenance environment of the study group.

An important factor affecting male fertility is smoking. From the study group 55% are smokers (Figure 3). Of this sub-group 90.2% are under 40 years of age. Smoking has harmful effects on the reproductive system. The quality of sperm is decreased in smokers by changes in semen sperm counts, low motility or abnormal sperm morphology compared to non-smokers or moderate smokers. Thus, it is possible that in our data, a cause of male infertility as a whole is due to smoking. However, literature data contradict the direct action of smoking on male infertility, measured by the number of pregnancies. But if we talk about patients who are at the limit of infertility, it is very likely that smoking will make a difference to male infertility.

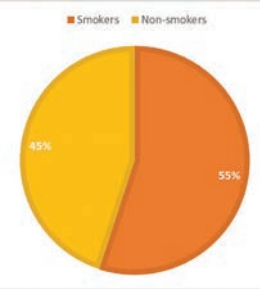


Fig. 3. Share of smokers in the study group.

Of the total infertile patients included in the study group 88.3% are young and very young <40 years. This distribution is interesting given that these age groups are the most sexually active and optimal chances of procreation. Although the number of patients in the study group is moderate, the data are quite eloquent regarding the tendency for male infertility to manifest in the young population, a trend that is steadily increasing.

In approximately 40% of infertile couples there is no apparent cause. In the study group 68% of the couple infertility is attributed to the male partner for whom the cause was identified (Figure 4). For 32% of investigated infertile men, the etiology of infertility could not be identified.

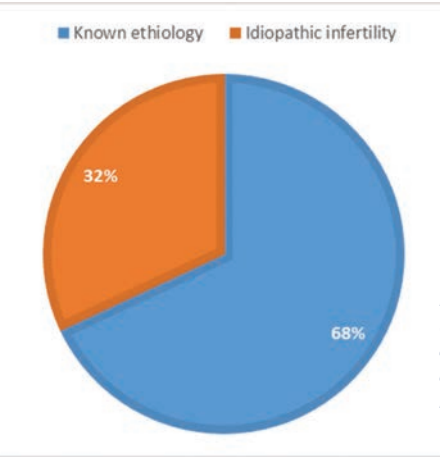


Fig. 4. The ratio between known and unknown etiology of the male infertility in the study group.

Current knowledge of varicocele's impact on testicular function is largely based on studies on subfertile and infertile men, making it difficult to extrapolate the impact of varicocele on the general population. The link between varicocele and male infertility has been under fierce debate for more than half a century. Varicocele is considered the most common corrective cause of male infertility, but some men with varicocele are able to have children, even without intervention. In addition, improving the quality of semen after varicocelectomy does not always result in spontaneous pregnancy. Studies on possible pathophysiological mechanisms behind varicocele induced infertility attempted to address these controversies. Oxidative stress seems to be a central mechanism; however, no theory is able to explain the differential effect of varicocele on infertility. As a consequence, careful selection of patients for treatment based on couples' fertility status, varicocele grade and semen quality is essential to gain a chance of further pregnancy.

30% of patients in the study group presented the varicocele as a clear cause of infertility (Figure 5). Of these, the most common was the second grade varicocele followed by grade III varicocele (Fig.6)



Fig. 5. Proportion of patients with varicocele

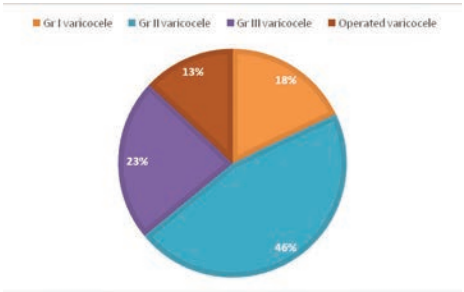


Fig. 6. Types of varicocele and frequency in the study group.

The lack of large population studies makes it difficult to extrapolate the impact of varicocele on reproductive health in the human population. Therefore, it is necessary to know the associations between varicocele and hormonal or semen changes especially in the young male population.

Among the factors responsible for male infertility, hypogonadotropic hypogonadism (HH) is a treatable cause and is found in a small number of individuals. HH may be congenital (eg, Kallmann's syndrome) or acquired as a result of pituitary lesion, hyperprolactinemia, or excessive use of steroidal anabolic agents. Patients have a reduced testosterone level with modified spermograms (oligospermia or azoospermia) secondary to low pituitary gonadotropins: luteinizing hormone (LH) and follicle stimulating hormone (FSH). According to the literature the percentage of HH patients is small (4%).

Other causes identified in the infertile study group

are shown in Figure 7. Among them, varicocele and urogenital infections are the most common causes identified as associated with male infertility.

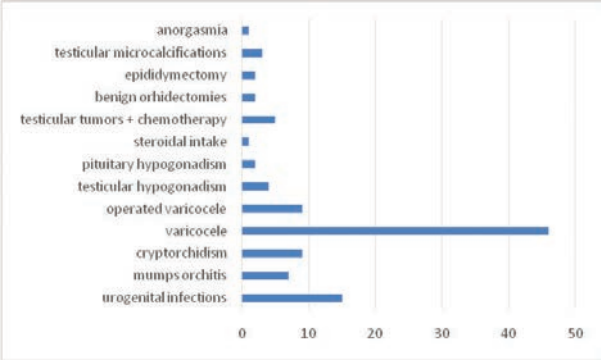


Fig. 7. Known cases of male infertility identified in the study group.

According to the European guidelines on male infertility management, semen analysis is an important element in determining the diagnosis of infertility. The study group presented various types of spermogram changes. The quantitative and qualitative analysis of the spermogram in patients diagnosed with infertility led to their classification in three major groups: 65% of the infertile patients had oligosospermia, the most common condition reported in the male infertile population; 20% of patients were enrolled in the azoospermia group; 15% of patients had other changes in the spermogram, yet they were unable to conceive in the couple relationship.

At a closer analysis of the study group, an appropriate distribution was performed according to the assessment of sperm counts (Table 1).

Oligozoospermia is one of the most common abnormalities in infertile men. Between 40% and 50% of infertile couples, the male partner is responsible for infertility. However, there appears to be a geographical variation in fertility couples such as 59% in France [17], 26% -32% in the UK and Kashmir Valley in India and around 36% in South Africa, Indonesia and Finland. Other studies have shown that there is also a regional variation in mean sperm concentration in men in different regions of the USA and France [18-20].

Geographic, ethnic, climatic and occupational factors were suggested to be responsible for regional differences in sperm counts in males [19,21]. Unfortunately, in Romania there is no data to prove the male infertility rate, the less we have not found data to show the distribution of infertile couples on geographic areas.

More than 20 factors associated with infertility in the study group could be identified, but they were not a direct cause of infertility (Figure 8). Among these, the most frequently encountered were: obesity, microlitis or renal lithiasis, bilateral hernias, etc.

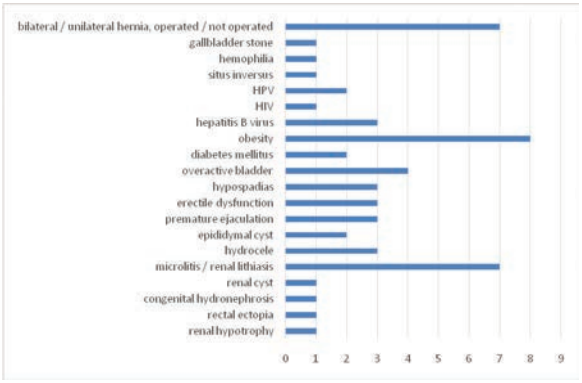


Fig. 8. Pathologies associated with infertile patients in the study group.

Clinical diagnosis	Characteristics	No. of patients
Oligosospermia	<10 mil / ml spermatozoa	43
Azoospermia	complete absence of sperm	50
Aspermia	without ejaculated seminal fluid	1
Asthenozoospermia	> 40% of low-motility sperm	31
Teratozoospermia	< 4-5% of normal morphology sperms	6
Oligoastenozoospermia	sperm motility < 8 mil / mL	58
Hematospermia	RBC in semen	17
Piospermia	leukocytes in semen	0
Polizoospermia	high sperm count	0

Table 1: Clinical diagnosis of infertility based on spermogram analysis in patients in the study group.

More and more literature suggests that obesity contributes to male infertility. The prevalence of obesity has steadily increased over the past 35 years and is currently a major health problem. Only in the US more than one third of the population is obese. Obesity is associated with low sperm counts. Obesity alters the hypothalamic-pituitary-gonadal axis both centrally and peripherally, leading to hypogonadotropic, hyperestrogenic hypogonadism. Factors derived from adipose tissues, such as leptin and adipokine, regulate testosterone production and inflammation, respectively. The increase in systemic inflammation results in an increase in the number of reactive oxygen species and the fragmentation of sperm DNA. Increasing testicular temperature due to body habitus and inactivity affects spermatogenesis. The degree to which obesity affects hormonal levels, semen parameters, sperm DNA integrity, and pregnancy rates vary, which may be the result of other comorbid conditions. Weight loss had incoherent results. Multiple interdependent mechanisms contribute to the detrimental effect of obesity on male fertility. Studies on large, randomized cohorts are needed to better characterize the therapeutic benefits of weight loss to restore male reproductive potential.

Discussions

Approximately 15% of reproductive couples fail to conceive over a 12-month period. In 50% of couples, abnormal semen parameters indicate an incidence associated with male infertility [22].

There is a clear need to understand the heterogeneous causes behind male infertility and the need to improve individualized treatment of the condition. Currently, there are only a few large-scale epidemiological studies addressing this subject [23, 24].

There is a growing tendency to thoroughly underestimate male infertile partners before opting for assisted reproductive technology (ART). As a consequence, men with medical pathologies that can be the main cause of suboptimal quality of semen are not properly diagnosed and treated. Inappropriate assessment of the causes of male infertility leads to a common situation where the female partner is subjected to invasive, stressful and expensive ART procedures, without considering alternative solutions to manage the infertility of the couple.

Known causes of male infertility are: congenital or acquired urogenital abnormalities, malignancies, urogenital tract infections, elevated scrotal temperature, endocrine disorders, immunological factors and some characteristic genetic anomalies. However, in 30 to 40% of cases, the etiology of male infertility remains unknown and is called male idiopathic infertility [24]. Despite the fact that these men do not have a history of diseases that affect fertility and show normal physical examinations and endocrine, genetic and biochemical laboratory tests, semen analysis often reveals abnormal semen parameters. The most common anomalies of routine semen analysis are: the absence of sperm (azoospermia); very low number of sperm in ejaculate (oligosospermia); abnormal morphology (teratosospermia) and / or abnormal sperm motility (astenosoospermia). These anomalies of idiopathic sperm are supposed to be caused by several factors, including reactive oxygen species (ROS), unknown genetic and epigenetic abnormalities, and endocrine disturbances through ecological pollution [26]. The state of absence of sperm in ejaculation is called azoospermia and is identified in 11% of infertile men. In 40% of cases, azoospermia is caused by physical blocking of the male eccentric duct system (obstructive azoospermia, OA), and here the etiology is mostly known. The remaining cases are called non-structural azoospermia (ANO) and are frequently associated with testicular failure, characterized by a lower volume of testicles and elevated LH and FSH concentrations [27]. However, many times the cause of infertility remains unknown and may be due to genetic anomalies. These genetic defects may result in a total absence of spermatogenesis, also called Sertoli Cell Only (SCO), or in a stop to the maturation of spermatogenesis.

Conclusions

1. The incidence of male infertility cases was relatively constant (3%) over the investigation period, which shows that this problem remains in the population and should be an alarm signal for short-term demographic programs at national level.

2. Male infertility is characteristic of the 35-40 year old group. Of the total infertile patients included in the study group, 88.3% are young and very young <40 years. This distribution is interesting given that these age groups are the most sexually active and optimal chances of procreation. Although the number of patients in the study group is moderate, the data are quite eloquent regarding the tendency for male infertility to manifest in the young population, a trend that is steadily increasing.

3. An important factor affecting male fertility is smoking. From the study group 55% are smokers. Of

this sub-group 90.2% are under 40 years of age. Smoking has harmful effects on the reproductive system. The quality of sperm is decreased in smokers by changes in semen sperm counts, low motility or abnormal sperm morphology compared to non-smokers or moderate smokers.

4. According to the data reported in the literature and also our data in 68% of the male infertility patients the cause has been identified.

5. For 32% of investigated infertile men the etiology of infertility could not be identified. Our inability to find the causes of infertility does not mean that there is no cause for this disorder. Management of infertile couples with idiopathic causes requires individualized treatment. Several key variables, including age, history of infertility, treatment history, costs and risks, should be considered when selecting the appropriate treatment plan. However, the main problem of treatment for these couples is disagreement among physicians about the management of inexplicable subfertility.

6. Varicocele remains the main cause of male infertility, being present in over 30% of infertile men.

7. Oligospermia or oligozoospermia is the most common form under which male infertility is manifested. In the study group, 64.56% of patients are in this category. Of these, the most frequent change in sperm counts was low sperm motility in semen - the oligoasthenozoospermia.

8. The quality of sperm can be influenced by a number of factors. More than 20 factors associated with infertility in the study group, but without being a direct cause of infertility, were identified in the research.

References

1. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, et al. *International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009.* Fertil Steril. 2009; 92:1520–4.
2. EUA, Guidelines 2018
3. Mascarenhas MN, Cheung H, Mathers CD, Stevens GA. *Measuring infertility in populations: Constructing a standard definition for use with demographic and reproductive health surveys. 2012;* Popul Health Metr. 2012 Aug 31;10(1):17. doi: 10.1186/1478-7954-10-17.
4. Calverton, Maryland, USA: *ORC Macro and the World Health Organization; 2004. World Health Organization. Infecundity, Infertility, and Childlessness in Developing Countries.* DHS Comparative Rep. No 9.
5. Stephen EH, Chandra A. *Declining estimates of infertility in the United States: 1982-2002.* Fertil Steril. 2006; 86:516–23.
6. Chandra A, Copen CE, Stephen EH. Hyattsville, MD: *National Center for Health Statistics; 2013. Infertility and impaired fecundity in the United States, 1982-2010:* Data from the National Survey of Family Growth. National Health Statistics Reports; No 67. Available from: http://www.cdc.gov/nchs/data/nhsr/nhsr067.pdf
7. Martin JA, Hamilton BE, Sutton PD, Ventura SJ, Menacker F, Kirmeyer S. Births: *Final data for 2004. Natl Vital Stat Rep.* 2006; 55:1–101.
8. Agarwal et al. *Reproductive Biology and Endocrinology. 2015*; 13:37
9. Martinez G, Daniels K, Chandra A. *Fertility of men and women aged 15-44 years in the United States: National Survey of Family Growth, 2006-2010.* Natl Health Stat Report. 2012;51:1–28.
10. Collins HP, Kalisch D. *The health of Australia's males. Health.* Canberra: Australian Institute of Health and Welfare; 2011.
11. Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Dohle G, et al. *European association of urology guidelines on male infertility: the 2012 - update.* Eur Urol. 2012; 62:324–32.
12. Olsen J, Kuppers-Chinnow M, Spinelli A. *Seeking medical help for subfecundity: a study based upon surveys in five European countries.* Fertil Steril. 1996; 66:95–100.
13. Rutstein SO, Shah IH. *Infecundity, infertility, and childlessness in developing countries. DHS Comparative Reports No. 9.* Geneva: World Health Organization; 2004.
14. Cooper TG, Noonan E, Eckardstein S, Auger J, Baker HWG, Behre HM, et al. *World Health Organization reference values for human semen characteristics.* Hum Reprod Update. 2010; 16:231–45.
15. www.insse.ro/cms/sites/.../populatia_romaniei_pe_localitati_la_1ianuarie2016_0.pdf
16. Esteves SC, Zini A, Aziz N, Alvarez JG, Sabanegh ES, Agarwal A. *Critical Appraisal of World Health Organization's New Reference Values for Human Semen Characteristics and Effect on Diagnosis and Treatment of Subfertile Men.* Urology. 2012; 79:16–22.
17. Thonneau P, Marchand S, Tallec A, Ferial ML, Ducot B, Lansac J, et al. *Incidence and main causes of infertility in a resident population (1,850,000) of three French regions (1988-1989).* Hum Reprod 1991; 6: 811–16.
18. Leke RJ, Oduma JA, Bassol-Mayagoitia S, Bacha AM, Grigor KM. *Regional and geographical variations in infertility: ef- fects of environmental, cultural, and socioeconomic factors.* Environ Health Perspect 1993; 101 Suppl 2: 73–80.
19. Fisch H, Goluboff ET. *Geographic variations in sperm counts: a potential cause of bias in studies of semen quality.* Fertil Steril 1996; 65: 1009–14.
20. Auger J, Jouannet P. *Evidence for regional differences of semen quality among fertile French men.* Federation Francaise des Centres d'Etude et de Conservation des Oeufs et du Sperme humains. Hum Reprod 1997; 12: 740–5.
21. Jorgensen N, Andersen AG, Eustache F, Irvine DS, Suominen J, Petersen JH, et al. *Regional differences in semen quality in Europe.* Hum Reprod 2001; 16: 1012–9.
22. Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Dohle G, Krausz C. *European Association of Urology Working Group on Male Infertility. European association of urology guidelines on male infertility: the 2012 update.* Eur Urol 2012;62:324–332.

23. Pierik FH, Van Ginneken AM, Dohle GR, Vreeburg JT, Weber RF. *The advantages of standardized evaluation of male infertility.* Int J Androl 2000;23:340–346.
24. Tüttelmann F, Nieschlag E. *Classification of andrological disorders.* In: Nieschlag E, Behre HM, Nieschlag S (eds). Andrology: Male Reproductive Health and Dysfunction, 3rd edn. Berlin: Springer-Verlag, 2010:87–92.
25. Nieschlag, E., Behre, H.M., Nieschlag, S., *2010. Male Reproductive Health and Dysfunction.* doi:10.1007/978-3-540-78355-8.
26. European Association of Urology, 2015. *Guidelines on Male Infertility.* doi:10.1007/978-1-60761-193-6.
27. Wosnitzer, M., Goldstein, M., Hardy, M.P., *2014. Review of azoospermia.* Spermatogenesis 4, e28218. doi:10.4161/spmg.28218.