Doctoral School in Medical Sciences

Manuscript title

C.Z.U.: 616.697-02:616.69-008.6-07:575.224(043.2)

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GENETIC VARIATIONS IN MALE INFERTILITY WITH AZOOSPERMIA

315.02 – MOLECULAR BIOLOGY AND MEDICAL GENETICS

Summary of Ph.D. Thesis in Medical Sciences

CHISINAU, 2023

The thesis was developed at the Department of Molecular Biology and Human Genetics of the "Nicolae Testemițanu" State University of Medicine and Pharmacy

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CONTENT

CONCEPTUAL LANDMARKS OF THE RESEARCH

The actuality of the topic and the importance of the problem addressed

Reproductive health continues to be a vital priority for human evolution, which is the basis of the general health of the population, the prosperity and development of mankind. The World Health Organization (WHO) has declared infertility a global health problem, due to its high prevalence worldwide and especially due to the extent of its negative consequences on the quality of life [1].

Epidemiological research worldwide estimates that about 15% of couples of reproductive age face problems related to sterility and infertility, with the male factor being involved in about 50% of cases [2].

The background of male infertility is extremely heterogeneous, most commonly caused by spermatogenesis disorders, clinically manifested by severe azoospermia and oligospermia. Azoospermia is identified in 1% of the male population, while the frequency of azoospermia in the infertile male population ranges from 10 to 15% [3]. Genetic factors explain approximately 30% of cases of male infertility associated with azoospermia [4], the high frequency being justified by the involvement of numerous genes in the control of sexualization and reproduction. Among the multiple genetic causes involved in spermatogenic insufficiency, some of the most clinically relevant are chromosomal anomalies, microdeletions of the Y chromosome and mutations of the CFTR gene (the cystic fibrosis transmembrane conductance regulator gene). The importance of genetic diagnosis in men with azoospermia has increased significantly due to advances in assisted reproductive techniques such as intracytoplasmic sperm injection (ICSI) and microsurgical sperm extraction (microTESE) which help infertile couples have their own biological children [5].

The lack of national norms for the multilateral approach to male infertility justified the reason to initiate a multidisciplinary study with professionals in the field of genetics and reproductive medicine for the optimization of assisted reproduction methods in couples with azoospermia, for the indications for genetic testing, to properly manage an infertile couple with azoospermia. These findings argue the relevance and necessity of researching the theme "Genetic variations in male infertility with azoospermia" for contemporary medicine, as well as for couples facing infertility who want to conceive healthy children.

The aim of study: Study of clinical, paraclinical aspects and genetic variations in men with azoospermia in order to optimize assisted reproduction methods in infertile couples.

Objectives of the study:

1. Analysis of the change trend in the qualitative and quantitative parameters of the semen;
2. Clinical and paraclinical evaluation of men with azoospermia who addressed for medico-genetic consultation;
3. Cytogenetic, molecular genetic and biochemical testing of men with azoospermia and the interpretation of the results obtained in the context of medical-genetic consultation;
4. Correlation of cytogenetic and molecular-genetic variations with phenotypic manifestations in men with azoospermia;
5. Optimizing the genetic diagnosis algorithm of azoospermia and assisted reproduction methods in male infertility with azoospermia caused by chromosomal variations, Y microdeletions and mutations in the CFTR gene.

Scientific research methodology. To achieve the objectives, two scientific studies have been carried out. The descriptive study was carried out based on the assessment of semen analysis results n=5767 from an assisted human reproduction center, during the years 2012-2020. This study allowed the assessment of the trend by linear regression of the indicators of semen quality and quantity. The transversal study included the evaluation of n=96 patients with azoospermia, where clinical and paraclinical methods were used: medical-genetic consultation; semen analysis; hormonal evaluations; genetic methods; invasive methods such as testicular biopsy, ICSI, IVF. Cytogenetic study of numerical, balanced structural chromosomal abnormalities, chromosomal polymorphisms, also molecular genetic studies for the detection of different mutations that cause infertility in men with azoospermia included the correlation with their associated forms of infertility.

The novelty and scientific originality of the obtained results. Significant genetic contribution was demonstrated in patients with severely impaired semen analysis - azoospermia. The research allowed the identification of chromosomal abnormalities and polymorphisms, the highlighting of gene mutations, and the classification of cytogenetic and molecular-genetic forms in correlation with phenotypic manifestations in patients with infertility caused by azoospermia.

The applied value of the research. In order to make a complex etiopathogenetic diagnosis of infertility, men with azoospermia were investigated by a multidisciplinary team consisting of reproductive specialists, gynecologists, urologists, geneticists and cytogeneticists.

The men included in the investigation benefited from karyotype testing to highlight the profile of chromosomal abnormalities/chromosomal polymorphisms as well as molecular-genetic investigation to identify microdeletions of the Y chromosome, mutations in the CFTR gene, involved in the etiology of male infertility, establishing their correlations with phenotypic manifestations and their share in the population of the Republic of Moldova.

Appropriate strategies were initiated in the consultation of infertile men with azoospermia, which included all aspects regarding the individual type of anomaly, its clinical relevance, possible inheritance, genetic risk for offspring, treatment opportunities and, in case of pregnancy, prenatal diagnosis.

In the context of the intensification of the use of assisted reproduction techniques in our country, the genetic evaluation of the infertile male partner is important both for the correct selection of patients for this treatment to obtain maximum results and for the assessment of genetic risk. Optimizing the genetic diagnostic algorithm in the evaluation of infertile men with azoospermia caused by chromosomal abnormalities and gene mutations will be a methodological support in order to optimize genetic counseling and facilitate informed decision-making when the couple opts for medically assisted reproduction.

The theoretical value of research. The results obtained as a result of the investigations will contribute to the development of a qualitatively new scientific direction in the medical science of the Republic of Moldova — the diagnostic management of men with infertility caused by azoospermia, considering the investigation of the interconnection between the contribution of both chromosomal abnormalities and polymorphisms, as well as gene mutations, with the efficiency of assisted reproduction techniques for infertile couples due to azoospermia.

Approval of the scientific results. The positive opinion of the Research Ethics Committee Nicolae Testemițanu SUMPh was obtained for the study (minutes no. 48, to no. 60, dated 12.04.2018). The research was carried out within the Department of Molecular Biology and Human Genetics, Nicolae Testemițanu SUMPh. The approval of the obtained results was carried out in accordance with the fundamental stages of the study, as well as within national and international scientific conferences and congresses as follows: National Pediatrics Conference 2018 – Current Affairs in Pediatrics. Bucharest, Romania, 2018; Pediatric Medical School with international participation VI edition Iasi, 2018; International Conference, European Human Genetics, Milan Italy, 2018; The 5th Congress of Medical Genetics with international participation from Romania, 2018; The annual scientific conference of SUMPh faculty memebers and students "Nicolae Testemițanu", Chisinau, 2018; The 20th SNPCAR-Congress, the Society of Neurology and Psychiatry of the Child and Adolescent in Romania, 2019; Biennial International Pediatric Conference Sibiu-Chisinau, Romania, 2019; Nicolae Testemițanu SUMPh Days, Chisinau, 2019; The 8th International Medical Congress for Students and Young Doctors, Chisinau, 2020; "Pro Invent" international invention conference, Cluj-Napoca, Romania, 2020; A – IV National Conference of the Association of Laboratory Medicine from Romania, 2020; Congress Dedicated to the 75th Anniversary of the Foundation of Nicolae Testemițanu SUMPh, Chisinau, 2020; XXI Congress of SNPCAR, Romania, 2021; 13th National Conference of the Association of Laboratory Doctors from Romania, Brasov, 2022; International Conference on Pediatrics "Actualities in Pediatric Practice: Challenges and Successes", 2022. International category awards, awarded by the Romanian Society of Medical Genetics, 2019; Laureate of the "research performance" contest, Nicolae Testemițanu SUMPh, 2020; 2 Gold Medals,

2 Silver Medals, 1 Bronze Medal: International Specialized Invention Salons, 2021; Government Excellence Scholarship 2022.

Publications on the thesis topic. 45 scientific papers were published on the topic of the thesis, including: 16 articles, of which 5 in journals from international databases, (1 article in SCOPUS cited journal); 11 articles in magazines from the National Register of professional magazines, (category B+, B, C); 21 theses in the proceedings of international and 8 national scientific conferences and congresses. 1 Invention Patent, 12 innovator certificates, 6 implementation documents were obtained. The implementation of the results was recorded in the scientific-practical process in the following subdivisions: in the scientific-didactic process of the Department of Molecular Biology and Human Genetics of Nicolae Testemițanu SUMPh; in the Molecular Genetics Laboratory of the Repromed Medical Center; in the Human Molecular Genetics Laboratory IMSP Mother and Child Institute.

THESIS CONTENT

1. THE PARTICULARS OF MALE INFERTILITY WITH AZOOSPERMIA

In this section, the general aspects of male infertility with azoospermia are analyzed. Emphasis is placed on highlighting the conceptual evolution of the given topic. Also, the landmarks of the theoretical and practical course of azoospermia are revealed. The main genetic causes of azoospermia are substantiated: chromosomal variations, microdeletions of the Y chromosome and CFTR gene mutations of azoospermia, in the context of the latest scientific developments in the field.

2. CLINICAL DATA AND RESEARCH METHODS

To capitalize on each objective of the research, 2 types of study were carried out: the descriptive study and the transversal study.

1. **The descriptive study** included the research of semen analysis results n=5767, carried out during 2012–2020 period. The results of the semen analysis were extracted from the sperm analysis register, within the Repromed Medical Center Institution for the years 2012–2020. Linear regression was used to examine time trends in sperm parameters: sperm concentration; motility; morphology and vitality. An analysis of variance (ANOVA) test was performed to see if there were overall differences in the mean of sperm parameters, for each year from 2012 to 2020, and for the medians (ANOVA according to Kruskal Wallis). The study carried out allowed the evaluation of the tendency to change the quality of the semen.

2. The cross-sectional study was carried out between 2018 and 2020, on a sample of 96 infertile men with azoospermia, over 18 years old, from the IMSP Mother and Child Institute, the Center for Reproductive Health and Medical Genetics and the Health Center, "Repromed".

The calculation of the representative batch was carried out by applying Cochran's formula:

n=d[$\tilde{\pi}$(1−$\tilde{\pi}$)]*($z\alpha/w$)^2

d - design-efect = 1

$\tilde{\pi}$ = 0,06 (according to the laboratory data of the Center for Reproductive Health and Medical Genetics, the share of azoospermia in men with infertility during 2013-2017 is on average 6.0%)

$z\alpha$ =1.96 for the confidence interval of 95,0%

w – the work was done based on the evaluation of the frequencies and arranging them according to the relative values, we need the confidence interval of 95.0%, w=0,05

Entering the data into the formula we obtained:

n = 1*[0,06*0,94]*(1,96/0,05)^2 = 87 and 10.0% non-response rate - 96 infertile men with azoospermia meeting the inclusion and exclusion criteria. After the evaluation of the genetic examinations, the general group was divided into two subgroups: Subgroup 1 included infertile men with azoospermia with etiology with genetic variations and Subgroup 2 - men with azoospermia without genetic variations through cytogenetic and molecular genetic tests.

The scientific research was carried out consecutively in five stages:

Stage I - *Clinical and paraclinical evaluation of men with infertility,* who apply for medico-genetic consultation at the IMSP Mother and Child Institute, Center for Reproductive Health and Medical Genetics and "Repromed" Health Center.

1. Male anamnesis (general data, family anamnesis, fertility, pubertal, urogenital, sexual, iatrogenic diseases, etc.).

2. Semen evaluation (confirmation of azoospermia after at least 2 sperm analysis, centrifuged, according to WHO 2010 requirements).

3. Hormonal profile examination: (FSH - Follicle Stimulating Hormone, reference values (2.0-10.0), measurement units (mIU/ml); LH - Luteinizing Hormone, reference values (3.0-12.0), measurement units (mIU/ml); Prolactin, reference values (1.8-17.0), measurement units (ng/ml); Testosterone, reference values (2.0-6.9), measurement units (ng/ml); Free testosterone, reference values (4.5-42.0), measurement units (pg/ml).

4. Instrumental methods: (testicular, transrectal, abdominal ultrasound).

Stage II - *Selection of infertile men with azoospermia,* after obtaining the results of clinical and paraclinical evaluation, which included 96 patients.

The criteria for including patients in the research: signing the acceptance agreement for participation in the research; men with infertility caused by azoospermia; adult age (greater than or equal to 18 years).

Criteria for excluding patients from research: lack of consent to participate in research; men with infertility without azoospermia; minors under the age of 18.

Stage III - *Evaluation of the cytogenetic and molecular genetic examination of men with azoospermia.*

The aim of the cytogenetic examination was the analysis of chromosome variations: assessment of genetic sex; numerical chromosomal anomalies; identification of some aneuploidy of sex chromosomes, such as Klinefelter Syndrome; abnormalities of autosomal chromosomes, robertsonian translocations and reciprocal translocations; unbalanced structural chromosomal anomalies (deletions of the Y chromosome in the euchromatin region) and balanced abnormalities (deletions of the Y chromosome in the heterochromatin region); chromosomal polymorphisms.

The molecular-genetic examination for the identification of microdeletions of the Y chromosome was carried out by applying the multiplex PCR (Polymerization Chain Reaction) method. Through this test, using specific primers, deletions of the 3 regions of AZF (AZFa, AZFb, AZFc) can be detected. Thus, microdeletions in 12 loci were analyzed, of which SRY was used as an internal control and allowed the diagnosis of XX males. The target sequences used initially are the following sY84 and sY86 (AZFa), sY127 and sY134 (AZFb), sY254 and sY255 (AZFc) and SRY and ZFX/ZFY (for control). According to the target sequences, the following genes were tested: sY84 - DYS 388 gene and sY86 - DYS148 gene; sY127- DYS218 gene and sY13- DYS224 gene; sY254 - DAZ gene and sY255 - DAZ gene. The following target sequences are newly introduced sDBY1 and sY620 (AZFa), sY153 and sY158 (AZFc), sY117 and sY143 (AZFb). The genes tested thanks to the newly introduced target primers are the following: sDBY1-DBY gene and sY620-USP9Y; sY153-gene DYS237 and sY158-gene DYS 241; sY117-gene DYS209 and sY143-gene RBM1. The identification of CFTR gene mutations was carried out by applying the PCR method - delF508 and G542X mutations were analyzed. In case of confirmation of the mutation, the other partner was tested to assess the risk of recurrence in the child.

Based on the analysis of the results of cytogenetic and molecular-genetic investigations, the general research group of (n=96) men with azoospermia was divided into two subgroups, as follows: subgroup 1 included infertile men with azoospermia with chromosomal genetic variations, Y microdeletions and detected CFTR mutations (n=35); subgroup 2 included infertile men with azoospermia without chromosomal genetic variations, Y microdeletions and detected CFTR mutations (n=65).

Stage IV - *The statistical processing of the obtained data* was carried out by performing comparative analyses, between subgroup 1 - infertile men with genetically confirmed azoospermia and subgroup 2 - infertile men with unconfirmed azoospermia of genetic etiology.

Stage V - *Formulation of conclusions and recommendations.* Based on the results obtained, conclusions, practical recommendations, appropriate strategies in the consultation of infertile men with azoospermia were developed, which included all aspects regarding the individual type of anomaly, its clinical relevance, possible inheritance, genetic risk for offspring and prenatal diagnosis. The proposed recommendations can be used to optimize the genetic diagnosis algorithm of azoospermia and assisted reproduction methods in male infertility with azoospermia caused by chromosomal variations, Y microdeletions and CFTR mutations.

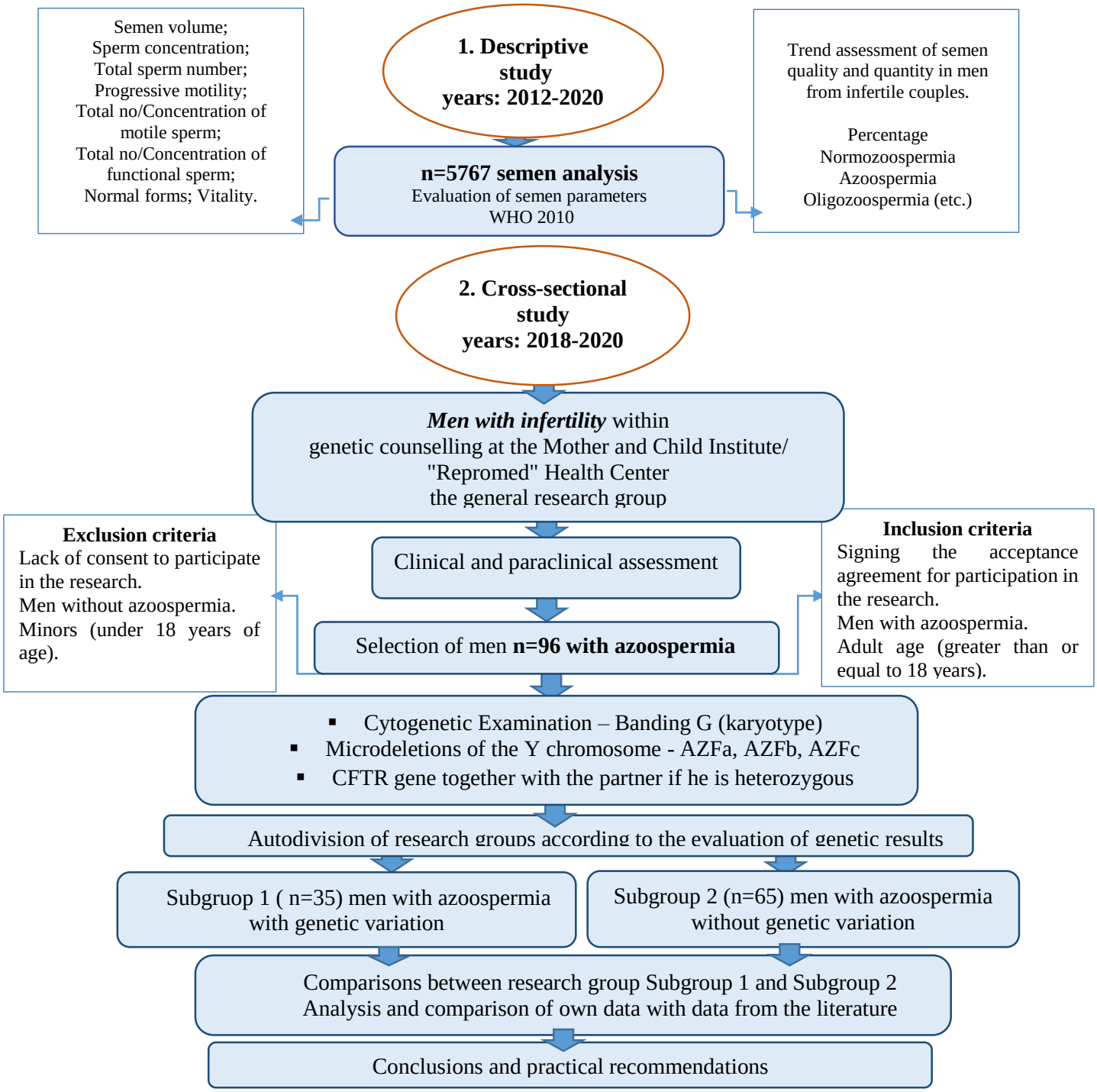


Figure 1. **The general design of the study**
Note: CFTR- the cystic fibrosis transmembrane conductance regulator gene, AZF- azoospermia factor

Characteristics of the research group (n=5767 semen analysis): The results of sperm parameters (n=5767) performed in a reproductive center during the years 2012-2020 were analyzed. The number of analyzed semen analysis distributed by year are as follows: 200 in 2012, 7003 in 2014, 852 in 2014, 794 in 2015, 703 in 2016, 685 in 2017, 685 in 2018, 630 in 2018, 640 in 2019 and 560 in 2020, which shows that the number of analyzed results is relatively constant.

Characteristics of the research group (n=96 men with azoospermia): All patients were diagnosed with azoospermia after at least 2 semen analysis. The mean age of azoospermic men experiencing couple infertility in the entire sample (n=96) was 33.8 ±5.3 years, (95% CI: 32.7 – 34.9; median: 33.0). The average history of infertility in the entire sample was 6.5 ± 4.6 years, (95% CI: 5.6 – 7.5). The average age at which infertility was diagnosed for the entire sample is 27.3 ±3.8 years, (95% CI: 26.5 – 28.0). This fact can be explained by the fact that at this age couples more often plan to conceive children.

Research methods used: *General research methods:* comparison method; analytical method; observation method; the biostatistical method. *Paraclinical, clinical - genetic methods:* medical-genetic consultation; semen evaluation; hormonal evaluations; cytogenetic, molecular genetic methods; testicular biopsy/ICSI/IVF. *Data collection methods - direct:* interview (anamnesis); medical-genetic consultation; filling out the form (by the investigator) - *indirect*: bibliographic data (official statistics, reports, studies, summaries); extracting data from the medical documentation: the Registry (on paper) of sperm analysis records, Repromed Medical Center Institution for the years 2012 – 2020, (collection of sperm parameters); Form No. 209/e (cytogenetic, molecular genetic results); Form No. 235/e (endocrine marker results); Form No. 218/e (analysis of urogenital infections (from smear)); Form No. 210/e (summary urine examination) - Approved by the Ministry of Health of the Republic of Moldova no. 828 of 31.10.2011, Medical reports/urology AOP-F08 (primary examinations of urologists).

Statistical evaluation methods: The results of the semen parameters of n=5767 patients, from the years 2012-2020 were entered into the Microsoft Access 2016 database. The clinical and paraclinical results of n=96 patients with azoospermia were accumulated in the Excel 2016 program. Statistical analysis of the data for both databases was carried out using the SPSS 22.0 program (SPSS Inc). The following methods were used: arithmetic mean; standard deviation (SD), interquartile range; correlation coefficient (Pearson); the Student test; $\chi 2$ test (chi square; ANOVA procedure; Kruskal-Wallis ANOVA procedure; simple and multiple linear regression; correlation analysis.

3. ANALYSIS OF CHANGING TRENDS OF SEMEN PARAMETERS, YEARS 2012-2020

3.1. Evaluation of semen quality and quantity in the whole sample

At the global level, the level of male fertility is continuously decreasing, and the Republic of Moldova is part of the general European trends. Studying the indicators of the seminal material allow the description of the extent of the phenomenon of fertility and male reproductive health. According to the WHO, the diagnosis of infertility with the involvement of the male factor is established when the results of the repeated analysis of the semen are below the normal values established by the WHO [6]. The quality and quantity of semen material is used as an indirect factor for assessing male fertility potential. The results of the evaluation of semen parameters in the male population (n=5767) clearly demonstrate that the quantity and quality of semen decreases during the study period 2012-2020 [7].

The average age of the men at the time of the semen analysis for the entire sample (n=5767) was 37.4 ±6.3 years, (95% CI: 37.3– 37.6; median: 37). The average annual rate of decrease of the average age being 2.1%. It is difficult to say that the year-on-year addressability of younger patients is due to the increase in the infertility rate in the younger population or the population's awareness of the problem of infertility, but the trend of decreasing age is evident during the study period.

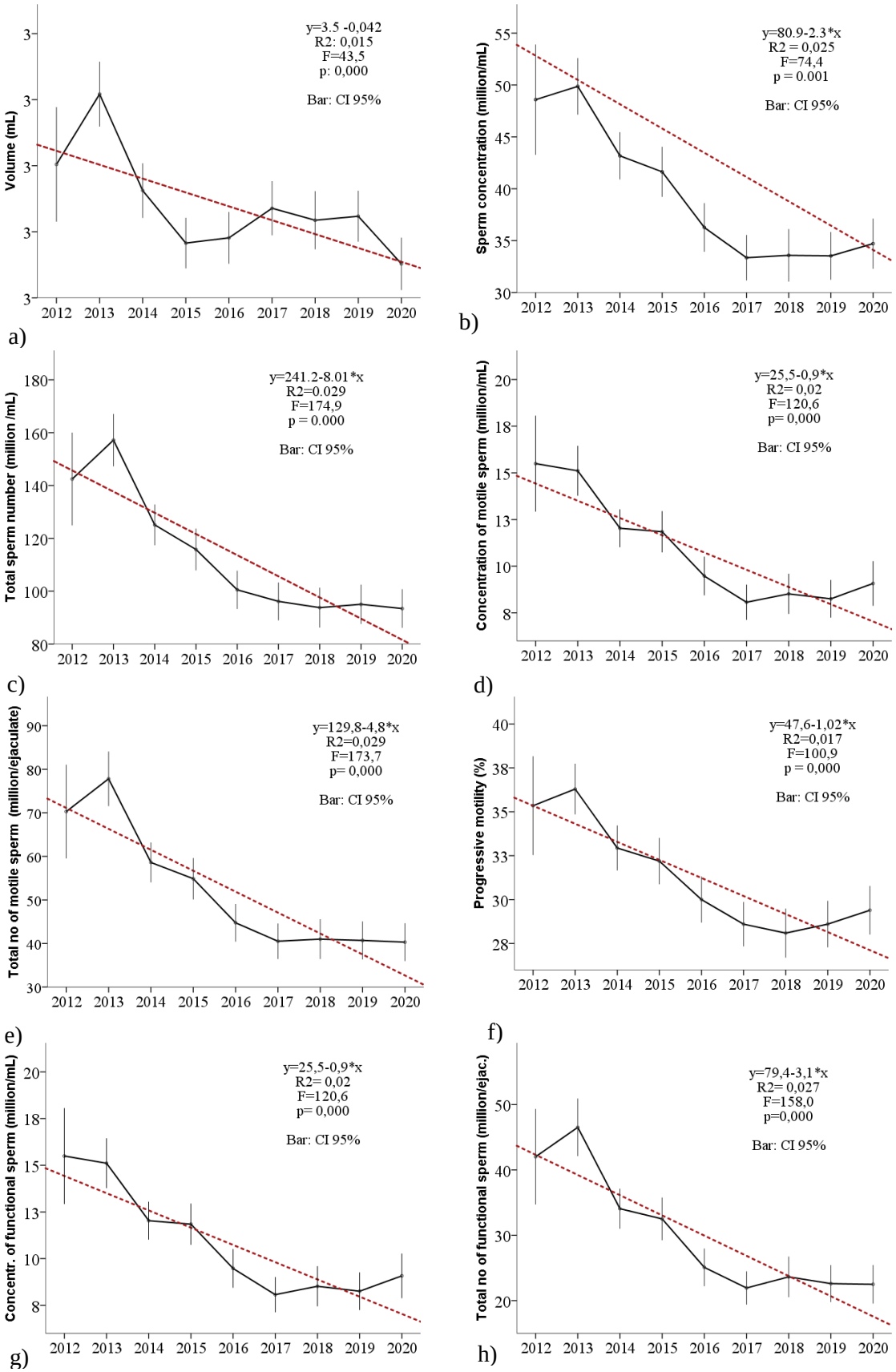


Figure 2. **Linear regression for: a) volume ejaculat; b) sperm concentration; c) total sperm number; d) concentration of motile sperm; e) total number of motile sperm; f) progressive motility; g) concentration of functional sperm; h) total number of functional sperm, between 2012-2020**

The results of the evaluation of semen parameters (n=5767) in the male population clearly demonstrate that the quantity and quality of semen decreases during the study period of 2012-2020. Decreasing trends were significant for all sperm parameters: semen volume (3,0 ± 1,2 mL to 2,7 ± 1,0 mL, 1,1% per year); sperm concentration (48,6 ± 38,1 million/mL to 34,7 ± 29,1 million/mL, 3,2% per year); total sperm number (142,5 ± 125,9 million/ejaculate to 93,4 ± 88,1 million/ejaculate, 3,8% per year); progressive motility (35,3 ± 20,2 % to 29,4 ±16,7 %, 1,9% per year); concentration of motile sperm (25,2 ± 27,5 million/mL to 16,6 ± 23,1 million/mL, 3,6% per year); total number of motile sperm (70,3 ±77,1 million/ejaculate to 40,3 ±52,4 million/ejaculate, 4,7% per year); concentration of functional sperm (15,5 ± 18,4 million/mL to 9,1 ± 14,4 million/mL, 3,9% per year); total number of functional sperm (42,2 ± 52,1 million/ejaculate to 22,5 ± 35,3 million/ejaculate, 5,2% per year); normal forms (24,6 ± 14% to 20,7 ± 12%, 1,8% per year) and vitality (58 ± 36 % to 30 ± 34 %, 3,8% per year) .

3.3. Evaluation of semen quality and quantity in the normozoospermic sample

The diagnosis of normozoospermia refers to the semen parameters that meet the criteria defined by the WHO in the semen analysis. In the current study, men with normozoospermia were selected according to the 2010 WHO semen analysis reference values, excluding the presence of leukocytes. From the entire sample (n=5767) of the analyzed semen analysis, the results with sperm parameters within normal limits were (n=1685).

The results of the evaluation of the semen parameters in the male population with normozoospermia (n=1685) show that the quantity and quality of the semen decreases during the study period. Decreasing trends were significant for all sperm parameters except one for viability: semen volume (0,05ml / 1,04% per year); sperm concentration (1,14 mln/ml / 1,4% per year); total sperm number (6,6 mln / 2,4% per year); progressive motility (0,4% / 0,7% per year); concentration of motile sperm (0,7 mln/m / 1,7 % per year); total number of motile sperm (4,6 mln / 3,2% per year); concentration of functional sperm (0,6 mln/ml / 2,8% per year); total number of functional sperm (3,4 mln / 4,0% per year); mobility index (3,1 / 1,4% per year) and normal forms (0,3% / 0,8% per year) (Table 1).

Table 1. **Simple linear regression of sperm parameters in men with normospermia in 2012-2020 period**

Semen parameters	**Constant**	**B**	**R2**	**F**	**P**
Volume (mL)	3,8	-0,05	0,011	18,2	0,000
pH	7,7	0,01	0,001	2,4	0,122
Sperm concentration (million/mL)	93,3	-1,14	0,013	22,5	0,000
Total sperm number (million/ejaculate)	330,9	-6,61	0,022	38,6	0,000
Progressive motility (%)	57,1	-0,41	0,015	25,4	0,000
Concentration of motile sperm (million/mL)	50,7	-0,67	0,006	10,3	0,001
Total number of motile sperm (million/ejaculate)	190,6	-4,55	0,023	40,3	0,000
Concentration of functional sperm (million/mL)	34,5	-0,65	0,009	14,8	0,000
Total no. of functional sperm (million/ejaculate)	124,7	-3,41	0,024	40,8	0,000
Mobility index	254,8	-3,13	0,013	22,8	0,000
Normal forms (%)	39,3	-0,31	0,014	23,7	0,000
Vitality (%)	89,5	0,52	0,012	20,4	0,000

3.4. Evaluation of semen analysis results, according to WHO 2010

In the years 2012-2020, from the total number (n=5767) of analyzed sperm analysis, 29,2% (n=1685) showed normal values of the semen - normozoospermia and 70,8% (n=4082) spermatogenesis disorders. During the entire study period, significant statistical differences were reported X2=352.7; gl=40; p=0.000. The most frequent abnormality of spermatogenesis recorded was asthenozoospermia in 35% (n=2016), followed by oligozoospermia in 27,8% (n=1606), azoospermia 3,9% (n=224), oligoasthenozoospermia 2,5% (n=143) and oligoasthenoteratozoospermia 1,6% (n=93), (Table 2).

Table 2. **Distribution of men according to semen analysis results by aggregated periods**

Semen analysis diagnosis	Years						X^2; gl; p
	2012-2015		2016-2020		Total		
	n	%	n	%	n	%	
Normozoospermia	986	38,7	699	21,7	1685	29,2	$X^2=2,2$; gl=1; p=0,136
Oligozoospermia	587	23,0	1019	31,7	1606	27,8	$X^2=12,1$; gl=1; p=0,001
Oligoasthenoteratozoospermia	39	1,5	54	1,7	93	1,6	$X^2=3,3$; gl=1; p=0,071
Asthenozoospermia	756	29,7	1260	39,2	2016	35,0	$X^2=0,4$; gl=1; p=0,530
Oligoasthenospermia	60	2,4	83	2,6	143	2,5	$X^2=0,1$; gl=1; p=0,795
Azoospermia	121	4,7	103	3,2	224	3,9	$X^2=3,2$; gl=1; p=0,075
Total	2549	100	3218	100	5767	100	$X^2=224,1$; gl=5; p=0,000

In 2012 normozoospermia was recorded in 46,5% (n=93) and in 2020 it reaches 14,5% (n=81). A decline in the normal values of spermatogenesis is observed, with an average annual rate of decrease of 7,6%, in the years 2012-2015 being 38,7% (n=986) and in the years 2016-2020 of 21,7% (n=699). Asthenozoospermia was detected in 21.5% in 2012 and 47.2% in 2020 (13.2% increase per year). Oligozoospermia - we observe an increase over the years of the study, from 21% to 30,9%, (increases by 5,2%), including oligoasthenozoospermia from 2,4% to 2,6% and oligoasthenoteratozoospermia from 1,5 to 1,6% (Figure 2).

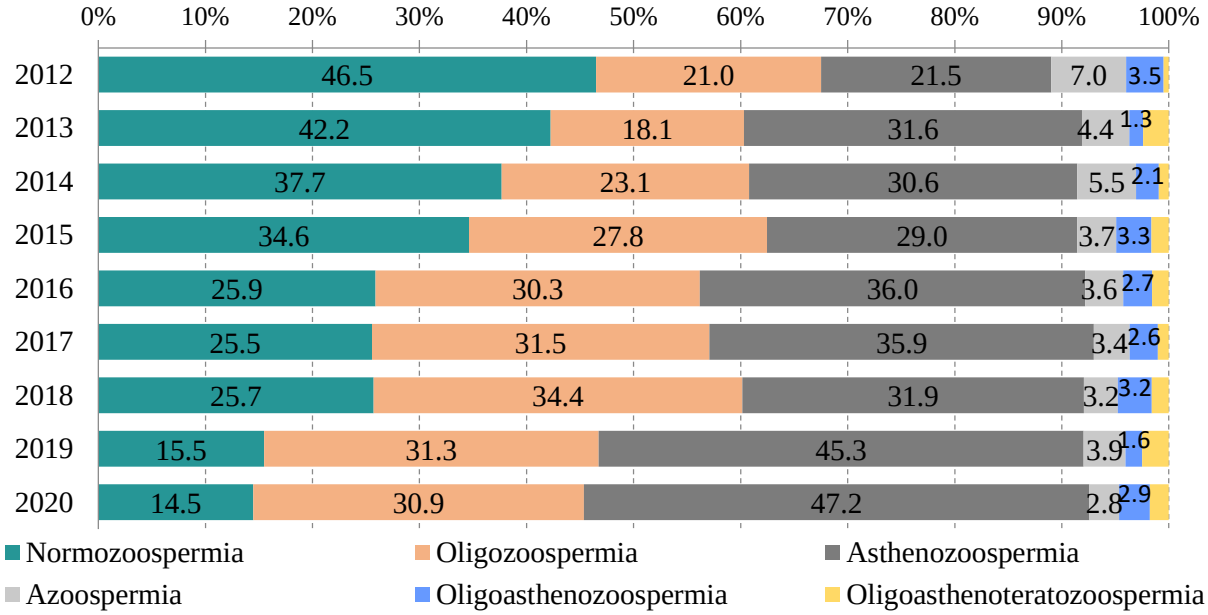


Figure 2. **Classification of semen according to diagnostic criteria between 2012 and 2020**

The representation of the qualitative and quantitative indicators of semen material through regression in a significant number (n=5767) of men described the situation at the present time and demonstrates the deterioration of male reproductive health in the Republic of Moldova. The data presented are substantial indicators that can serve as evidence for health authorities to make informed decisions to focus on the management and prevention program of male infertility.

4. PHENOTYPICAL AND GENETIC ASPECTS OF MEN WITH AZOOSPERMIA

4.1. Phenotypic characteristics of azoospermic men with karyotype variations, AZF deletions, detected CFTR mutations and no detected genetic variations

Following molecular genetic (Y chromosome microdeletion testing and CFTR gene testing) and cytogenetic (G-banding karyotyping) investigations, the investigated men with azoospermia (n=96) were divided into two subgroups: subgroup 1 – (n=35) confirmed patients by the proposed genetic tests and subgroup 2 – (n=61) patients who were not confirmed by the available genetic tests. Of the genetically confirmed patients, 25% have an abnormal karyotype, 10,4% have Y microdeletions and 3,1% have mutations in the CFTR gene (Figure 3).

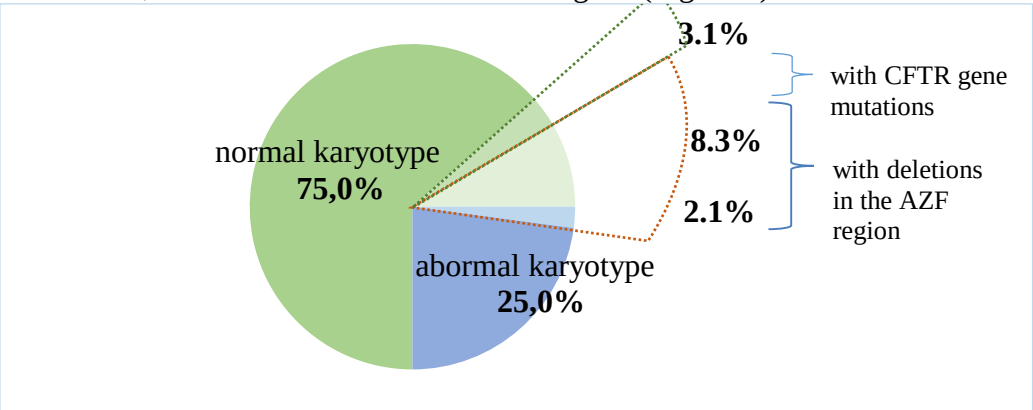


Figure 3. **Structure of the incidence of genetic causes diagnosed by molecular-genetic and cytogenetic tests in men with azoospermia (n=96)**

We analyzed the particularities of the subgroups depending on the age of the patients and the history of infertility. The mean age of azoospermic men experiencing couple infertility in study subgroup 1 (n=35) was 34,1 ± 4,3 years, (95% CI: 32,6 – 35,6; median: 34,0) and in subgroup 2 of 33,6 ±5,8 years, (95% CI: 32,1 – 35,1; median: 33,0). The mean history of infertility in study sample 1 was 6,5 ±3,6 years, (95% CI: 5,2 – 7,7; median 7,0) and study sample 2 was 6,4 ±5,1 years, (95% CI: 5,1 – 7,7; median 4,0). In study subgroup 1 the average age at which infertility was diagnosed is 27,3 ±3,6 years, (95% CI: 26,4 – 28,9; median 28,0) and in reference subgroup 2 was 27, 3 ±3,3 years, (95% CI: 26,3 – 28,0; median 27,0), (Table 3).

Table 3. **Age and history of infertility in men with azoospermia in the study cohort**

		Subgroup 1	Subgroup 2	Total
		n=35	n=61	n=96
Age (years)	Mean ± SD	34,1 ± 4,3	33,6 ±5,8	33,8 ±5,3
	CI 95%	32,6 – 35,6	32,1 – 35,1	32,7 – 34,9
	Median	34,0	33,0	33,0
	Percentile 25 – 75	31,0 – 36,0	29,0 – 37,0	30,0 – 36,0
	Minimum – Maximum	28,0 – 47,0	19,0 – 52,0	19,0 – 52,0
History of infertility (years)	Mean ± SD	6,5 ±3,6	6,4 ±5,1	6,4 ±4,6
	CI 95%	5,2 – 7,7	5,1 – 7,7	5,5 – 7,4
	Median	7,0	4,0	5,0
F= 0,002; P=0,968	Percentile 25 – 75	3,0 – 8,0	2,0 – 10,0	3,0 – 9,0
	Minimum – Maximum	1,0 – 14,0	1,0 – 20,0	1,0 – 20,0
Age at diagnosis (years)	Mean ± SD	27,7 ±3,6	27,2 ±3,3	27,4 ±3,4
	CI 95%	26,4 – 28,9	26,3 – 28,0	26,7 – 28,1
	Median	28,0	27,0	27,0
F=0,415; p=0,521	Percentile 25 – 75	25,0 – 30,0	25,0 – 30,0	25,0 – 30,0
	Minimum – Maximum	21,0 – 35,0	18,0 – 34,0	18,0 – 35,0

We analyzed the characteristics of the patients' semen in both subgroups. According to the requirements of the WHO, the qualitative performance of the semen analysis requires the patient to observe a period of abstinence lasting 3-7 days before the sampling. Thus, the duration of abstinence in study subgroup 1 was 4,5 ±1,3 days, (CI 95%: 4,1 – 5,0; median 5,0), and in reference subgroup 2 – 4,9 ±1,4 days, (CI 95%: 4,6 – 5,3; median 5,0), without statistically significant difference between

groups (F=1,96; p=0,164). The semen analysis was partially sampled only in one case from study subgroup 1 and 2 cases in subgroup 2, in the other participants it was sampled completely (Table 4).

Table 4. **Semen parameters in men with azoospermia allocated to research groups**

Semen parameters		Subgroup 1 n=35	Subgroup 2 n=61	Total n=96
Abstinence (days)	Mean ± SD	4,5 ±1,3	4,9 ±1,4	4,8 ±1,4
	CI 95%	4,1 – 5,0	4,6 – 5,3	4,5 – 5,0
	Median	5,0	5,0	5,0
F=1,96;	Percentile 25 – 75	3,0 – 5,0	4,0 – 6,0	4,0 – 6,0
p = 0,164	Minimum – Maximum	2,0 – 7,0	3,0 – 7,0	2,0 – 7,0
Semen volume (ml)	Mean ± SD	2,3 ±0,8	2,9 ±1,3	2,7 ±1,2
	CI 95%	2,0 – 2,6	2,5 – 3,2	2,4 – 2,9
	Median	2,4	2,9	2,6
F=5,13;	Percentile 25 – 75	1,5 – 2,8	2,0 – 3,5	2,0 – 3,3
p = 0,026	Minimum – Maximum	0,3 – 3,8	0,3 – 8,0	0,3 – 8,0
Liquefaction time (min)	Mean ± SD	37,4 ±8,0	38,3 ± 10,4	38,0 ±9,5
	CI 95%	34,7 – 40,2	35,6 – 40,9	36,0 – 39,9
F=0,75;	Median	40,0	40,0	40,0
p = 0,676	Percentile 25 – 75	30,0 – 45,0	35,0 – 45,0	32,5 – 45,0
	Minimum – Maximum	20,0 – 60,0	10,0 – 60,0	10,0 – 60,0
pH	Mean ± SD	7,5 ±0,4	7,7 ±0,5	7,6 ±0,5
	CI 95%	7,3 – 7,6	7,6 – 7,8	7,5 – 7,7
F=6,29;	Median	7,5	7,8	7,7
p = 0,014	Percentile 25 – 75	7,1 – 7,8	7,6 – 8,0	7,4 – 7,9
	Minimum – Maximum	6,7 – 8,2	5,5 – 9,0	5,5 – 9,0
Leukocytes (mln/ml)	Mean ± SD	0,7 ±0,9	0,8 ±0,9	0,8 ±0,9
	CI 95%	0,4 – 1,0	0,6 – 1,1	0,6 – 1,0
	Median	0,6	0,7	0,6
F=0,26;	Percentile 25 – 75	0,1 – 1,0	0,1 – 1,0	0,1 – 1,0
p = 0,606	Minimum – Maximum	0 – 5,0	0 – 4,9	0 – 5,0

The liquefaction time of the semen did not differentiate essentially between the subgroups, being in subgroup 1 of the study – 37,4 ±8,0 min, (CI 95%: 34,7 – 40,2; median 40) and in subgroup 2 of reference – 38,3 ±10,4 min, (CI 95%: 35,6 – 40,9; median 40), respectively no statistically significant differences are observed between subgroups (F=0,75; p=0,676), (Table 4).

The volume of the semen analysis in patients from subgroup 1 proved to be statistically significantly lower than in those from reference subgroup 2 (F=5,13; p=0,026). Thus, in study subgroup 1 the volume was 2,3 ±0,8 ml, (CI 95%: 2,0 – 2,6; median 2.4), while in study subgroup 2 – 2,9 ± 1,3 ml, (95% CI: 2,5 – 3,2; median 2,9), (Table 4, Figure 4).

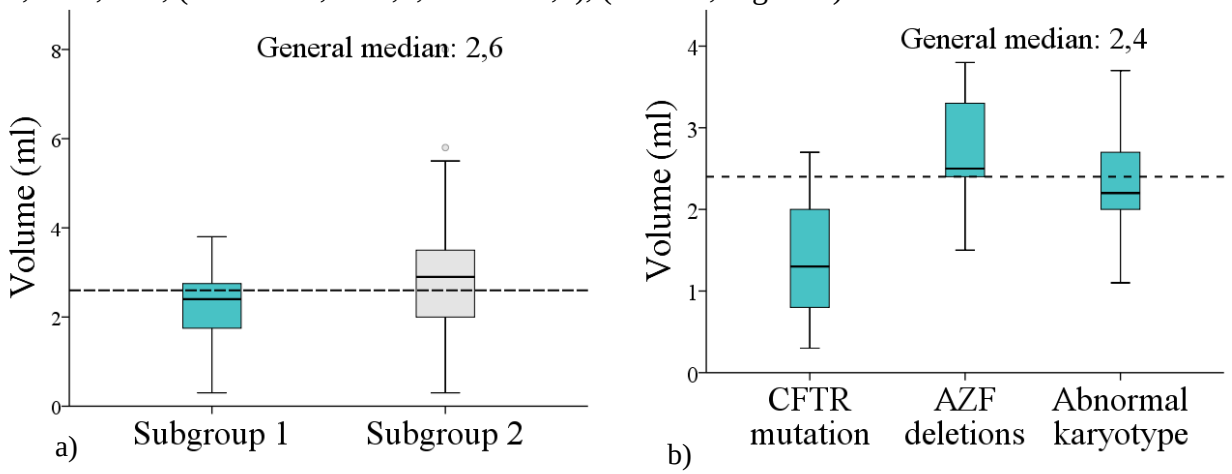


Figure 4. **Box plot analysis of volume (ml) data distributed across study subgroup 1 (a, b) and study subgroup 2 (a)**

The pH value of the sperm in both subgroups is basic (alkaline) with a value of 7,5 ± 0,4, (95% CI: 7,3 – 7,6; median 7,5) in study subgroup 1 and 7,7 ± 0,5, (95% CI: 7,6 – 7,8; median 7,8) in subgroup 2 of the study, showing a statistically significant difference between subgroups (F=6,29; p=0,014), (Table 4, Figure 5).

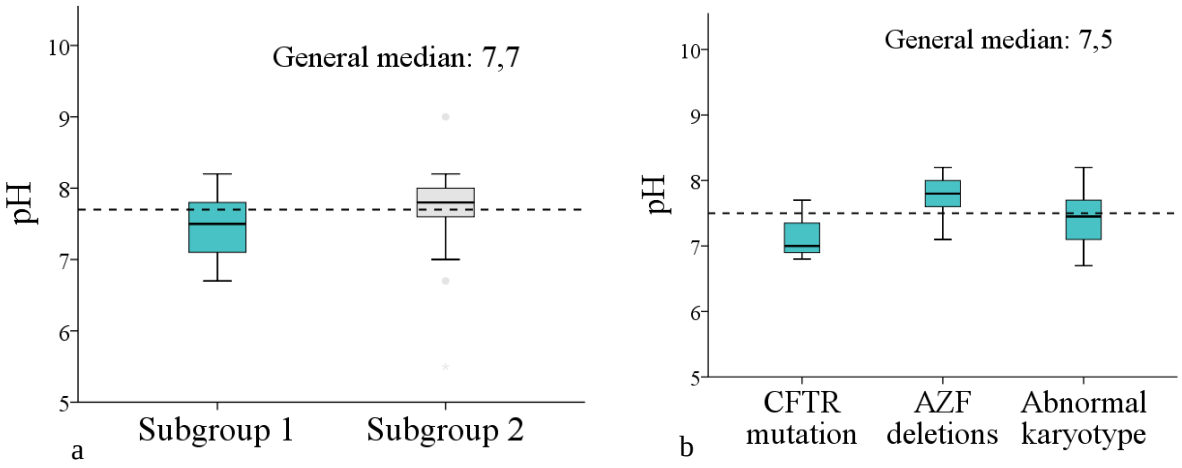


Figure 5. **Box plot analysis of pH values distributed on subplot 1 (a, b) and subplot 2 of the study (a)**

The data from the patients' anamnesis were analyzed in order to identify possible factors that could have affected the fertility status. Approximately 2/3 of the research participants did not suffer diseases in childhood, which presuppose the impairment of fertility: in study subgroup 1 – 22 (62,9%) patients and in study subgroup 2 – 39 (63,9%) patients, no statistically significant difference between groups ($\chi2$=0,11; gl=1; p=0,916). Mumps in study subgroup 1 presented in 61,5% (n=8) and subgroup 2 of 50,0 (n=11). Viral orchitis was present in 22,7% (n=5) in subgroup 1 and 15,4% (n=2) in subgroup 2.

Some investigated men mentioned the presence of genital trauma in the anamnesis, which, likewise, could influence fertility: in study subgroup 1 - 11,4% (n=4) and in reference subgroup 2 - 31,7% (n=19), without statistically significant difference between groups ($\chi2$=4,96; gl=1; p=0,026) (Table 5). Surgical interventions in the pelvic region are mentioned in 14,3% (n=5) of the participants from subgroup 1 of the study and 21,3% (n=13) from subgroup 2, which does not determine a statistically significant difference between them ($\chi2$=0,721; gl =1; p=0,396). Genital infections such as epididymitis or urethritis were present in 17,1% (n=6) of participants in study subgroup 1 and 24,6% (n=15) in study subgroup 2 with no statistically significant difference between them ($\chi2$= 0,722; gl=1; p=0,396). Gonadotoxic exposures, such as radiation therapy, chemotherapy, recent fever, or current medications had 25,7% (n=9) of patients in study subgroup 1 and 32,8% (n=20) of reference subgroup 2, statistically significant difference between the subgroups is not attested ($\chi2$=0,528; gl=1; p=0,468). Approximately the same proportion of participants from both subgroups suffer from smoking: in study subgroup 1 – 42,9% (n=15) of patients and in study subgroup 2 – 37,7% (n=23) with no statistically significant difference between them ($\chi2$=0,247; gl=1; p=0,619). Taking into account the influence of a possible family history of congenital anomalies, mental retardation, reproductive disorders or cystic fibrosis on fertility, participants were also questioned on this matter. The following affirmative answers were received: in study subgroup 1 in 25,7% (n=9) cases and in study subgroup 2 – 14,8% (n=9) cases (Table 5).

Table 5. **The association of some risk factors that can affect fertility in the researched groups**

Risk factors from personal history		Subgroup 1		Subgroup 2		Total	
		n=35	%	n=61	%	n=96	%
Genital trauma	not	31	88,6	41	68,3	72	75,8
	yes	4	11,4	19	31,7	23	24,2
Surgical interventions in the pelvic region	not	30	85,7	48	78,7	78	81,3
	yes	5	14,3	13	21,3	18	18,8
Genital infections	not	29	82,9	46	75,4	75	78,1
	yes	6	17,1	15	24,6	21	21,9
Gonadotoxic exposures	not	26	74,3	41	67,2	67	69,8
	yes	9	25,7	20	32,8	29	30,2
Smoking	not	20	57,1	38	62,3	58	60,4
	yes	15	42,9	23	37,7	38	39,6
Family history of congenital anomalies	not	26	74,3	52	85,2	78	81,3
	yes	9	25,7	9	14,8	18	18,8

The analysis of clinical manifestations in the urogenital sphere of the patients allowed the identification of clinical signs suggestive of certain nosological entities. Although in both subgroups half of the participants had normal testicle size and consistency, however, the presence of changes determined a statistically significant difference between the subgroups (χ2=12,1; gl=3; p=0,007). Shrunken testes were recorded in 8,6% (n=3) of patients in study subgroup 1 and 29,5% (n=18) in study subgroup 2 (Table 6).

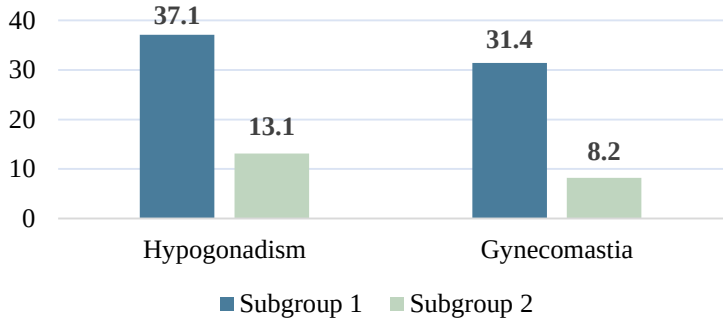


Figure 6. **The prevalence of hypogonadism and gynecomastia in both research group**

The presence of hypogonadism being more frequent in 37.1% (n=13) in study subgroup 1 compared to reference subgroup 2 - 13,1% (n=8). Among patients with genetic pathologies, the most commonly hypogonadism was recorded in patients with an abnormal karyotype - in 50,0% (n=11), followed by patients with deletions in the AZF region - 20,0% (n=2), (Table 6).

The presence of gynecomastia is statistically significantly more frequent in study subgroup 1 of 31,4% (n=11) compared to subgroup 2 of 8,2% (n=5), (χ2=8,642; gl=1; p=0,003), (Figure 6). In subgroup 1 of the study, gynecomastia is more frequent in patients with an abnormal karyotype - in 45,5% (n=10), followed by 10,0% (n=1) in patients with microdeletions of the Y chromosome, in patients with mutations in the gene CFTR it was not recorded (χ2=5,5; gl=2; p=0,063), (Table 6).

Table 6. **Distribution of clinical manifestations in the research group**

Clinical manifestations		Subgroup 1		Subgroup 2		Total	
		n = 35	%	n =61	%	n=96	%
The size and consistency of the testicles	normal	19	54,3	32	52,5	51	53,1
	reduced in volume	3	8,6	18	29,5	21	21,9
	hypogonadism	13	37,1	8	13,1	21	21,9
χ2=12,1; gl=3; p=0,007	lack of/testicular cyst			3	4,9	3	3,1
Body habitus	normosthenic	15	42,9	37	60,7	52	54,2
	hypersthenic	15	42,9	18	29,5	33	34,4
χ2=2,838; gl=2; p=0,242	asthenic	5	14,3	6	9,8	11	11,5
Gynecomastia	lack	24	68,6	56	91,8	80	83,3
χ2=8,642; gl=1; p=0,003	present	11	31,4	5	8,2	16	16,7
The deferent channel	It is palpable	32	91,4	59	96,7	91	94,8
χ2=1,26; gl=1; p=0,261	it is not palpable	3	8,6	2	3,3	5	5,2
Varicocele	lack	33	94,3	54	88,5	87	90,6
χ2=0,86; gl=1; p=0,351	present	2	5,7	7	11,5	9	9,4
Clinical of the prostate	fără modificari	24	68,6	42	68,9	66	68,8
χ2=0,001; gl=1; p=0,977	prezintă modificari	11	31,4	19	31,1	30	31,3

Although in subgroup 2 in 60,7% compared to subgroup 1 of 42,9% the normosthenic constitution predominates, the body habitus does not differ statistically significantly between the subgroups (χ2=2.838; gl=2; p=0.242). Hypersthenic constitution is more common in study subgroup 1- 42.9% (n=15); in the case of patients with mutations in the CFTR gene 66,7% (n=2), with abnormal karyotype 54,7% (n=12) and deletions in the AZF region 10% (n=1). The bilateral congenital presence of the vas deferens is palpable in the majority of patients in both study subgroups: in study subgroup 1 in 91,4% (n=32) patients and in subgroup 2 in 96,7% (n=59) patients, (χ2=1,262; gl=1; p=0,261). On the other hand, if we analyze patients with genetic pathologies, the deferent duct is palpable in all patients with abnormal karyotype and deletions in the AZF region and is not palpable in all patients with mutations in the CFTR gene, (χ2=35,0; gl=2; p= 0,000). From the total number of participants in each study subgroup, the clinical examination of the prostate shows no changes: in

study subgroup 1 – 68,6% (n=24) cases and in study subgroup 2 – 68,9% (n=42) cases, (χ2=0,001; gl=1; p=0,977), (Table 6).

The values of endocrine markers (FSH, LH, prolactin and testosterone) were analyzed in patients with azoospermia. The mean value of the FSH hormone level in study subgroup 1 was 9,9 ±9,1 mIU/ml, (95% CI: 6,8-13,0; median 7,3) with a minimum value of 1,1 mIU /ml and the maximum value of 38,4 mIU/ml, and in subgroup 2 the level of the average value was 8,1 ±7,8 mIU/ml, (95% CI: 6,1-10,0; median 4,7) with a minimum value of 0,9 mIU/ml and a maximum value of 40,7 mIU/ml, (F=1,16; p=0,283). The LH hormone level registered a mean value of 10,1±8,1 mIU/ml, (95% CI: 7,3-12,8; median 7,5) in study subgroup 1 with a minimum value of 1,1 mIU /ml and the maximum value of 28,6 mIU/ml, and in subgroup 2 of – 7,6±5,6 mIU/ml, (95% CI: 6,1-9,2; median 6) with a minimum value of 1,0 mIU/ml and the maximum value of 27,7 mIU/ml, (F=2,74; p=0,101), (Table 7).

Table 7. **Characteristics of endocrine markers in research groups**

Endocrine markers		Subgroup 1	Subgroup 2	Total
		n=35	n=61	n=96
FSH	Mean ± SD	9,9 ±9,1	8,0 ±7,8	8,7 ±8,3
2,0 –10,0 mIU/ml	CI 95%	6,8 – 13,0	6,0 – 10,0	7,0 – 10,4
	Median	7,3	4,7	5,2
F=1,16;	Percentile 25 – 75	2,5 – 15,1	3,0 – 10,5	3,0 – 11,6
p = 0,283	Minimum – Maximum	1,1 – 38,4	0,9 – 40,7	0,9 – 40,7
LH	Mean ± SD	10,1 ±8,1	7,6 ±5,6	8,6 ±6,7
3,0 – 12,0 mIU/ml	CI 95%	7,3 – 12,8	6,1 – 9,2	7,2 – 10,0
	Median	7,5	6,0	6,4
F=2,74;	Percentile 25 – 75	3,5 – 13,8	4,0 – 9,8	4,0 –11,2
p = 0,101	Minimum – Maximum	1,1 – 28,6	1,0 – 27,7	1,0 – 28,6
Prolactin	Mean ± SD	14,6 ±6,3	19,5 ±12,4	17,5 ±10,6
1,8 – 17,0 ng/ml	CI 95%	12,4 – 16,8	16,0 – 23,0	15,3 – 19,8
	Median	12,9	16,0	15,9
F=4,423;	Percentile 25 – 75	11,0 – 19,5	11,4 – 20,5	11,3 – 19,7
p = 0,038	Minimum – Maximum	4,3 – 31,0	5,6 – 65,0	4,3 – 65,0
Testosterone	Mean ± SD	3,5 ±1,2	2,9 ±1,3	3,1 ±1,3
2,0 – 6,9 ng/ml	CI 95%	3,1 – 4,0	2,5 – 3,2	2,9 – 3,4
	Median	3,2	3,0	3,0
F=6,386;	Percentile 25 – 75	2,7 – 4,6	2,0 – 3,5	2,4 –3,7
p = 0,013	Minimum – Maximum	1,2 – 5,6	0,7 – 6,2	0,7 – 6,2
Free testosterone	Mean ± SD	11,7 ±9,2	13,3 ±8,4	12,7 ±8,6
	CI 95%	5,1 – 18,3	8,9 – 17,8	9,2 – 16,1
F=0,21;	Median	8,4	13,8	13,5
p = 0,644	Percentile 25 – 75	2,8 – 18,5	7,0 – 16,4	4,1 – 17,5
	Minimum – Maximum	2,8 – 28,8	1,7 – 35,5	1,7 – 35,5

The prolactin level in study subgroup 1 had an average value of 14,6 ±6,3 ng/ml, (95% CI: 13,1-17,4; median 12,9) with the lowest level of 4,3 ng/ml and the highest of 310 ng/ml, and in reference subgroup 2 – 19,5 ±12,4 ng/ml, (95% CI: 16,0-23,0; median 16,0) with the lowest level of 5,6 ng/ml and the highest of 65,0 ng/ml. The difference between groups is statistically significant (F=4,423; p=0,038). There is also a statistically significant difference between the groups in the mean values of the testosterone level (F=6,386; p=0,013). Thus, in subgroup 1 of the study, the average value of the testosterone level is equal to 3,5±1.2 ng/ml, (CI 95%: 3,1-3,9; median 3,2), oscillating between 1,2 ng/ml and 5,6 ng/ml, and in study subgroup 2 – 2,9 ±1,3 ng/ml; (95% CI: 2,4-3,3), ranging from 0,7 ng/ml to 6,2 ng/ml (Table 7).

Among patients with genetic pathologies, the highest mean value of 12,2 ± 10,5 mIU/ml, (95% CI: 7,6 – 16,9; median 10,0) of FSH level was recorded in patients with abnormal karyotype, followed by patients with deletions in the AZF region - 6,4 ±3,5 mIU/ml, (95% CI: 3,9 – 8,9; median 6,3) and mean value of 4,7 ±4,6 mIU/ml, (95% CI: 0 – 16,1; median 2,0), the lowest was registered in patients with mutations in the CFTR gene. The highest mean LH 12,8 ±8,9 mIU/ml, (95% CI: 8,9 – 16,8; median 10,8) was also recorded in patients with abnormal karyotype, followed by those with

AZF deletions 6,2 ±3,2 mIU/ml, (95% CI: 3,9 – 8,5; median 7,1) and with CFTR mutations 2,6 ±1,4 mIU/ml, (95% CI: 0 – 6,1; median 3,0), (Figure 7).

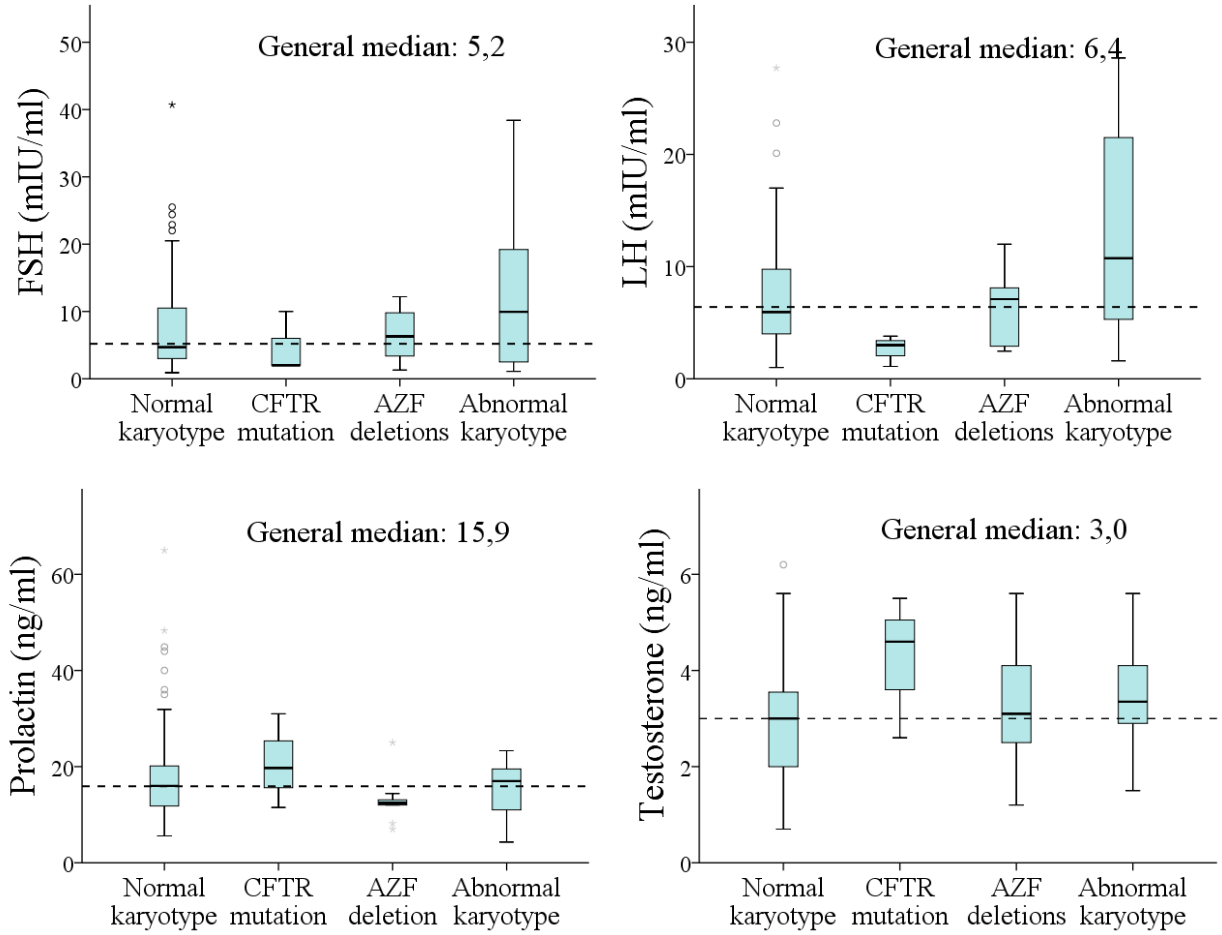


Figure 7. Mean and median values for endocrine markers in azoospermic patients with normal karyotype and genetic and chromosomal variations

4.2. Chromosomal variations in men with azoospermia diagnosed by cytogenetic examination

In all men in the study (n=96), the karyotype was analyzed through the examination of peripheral blood cells, using the technique of G banding of chromosomes. According to the cytogenetic examination of the total number of (n=96) infertile men with azoospermia, 75% (n=72) had a normal karyotype 46,XY and 25% (95% CI: 24,1 – 25,9), (n=24) showed variations in the number or structure of chromosomes. It should be noted that the results obtained are comparable to other specialized studies in Bulgaria 20,7% (19,3 – 22,1), Romania 30,0% (CI 95%: 29,1 – 30,9), Turkey 19,2% (18,2 – 20,2), Ukraine 35,0% (95% CI: 32,7 – 37,3) etc. (Figure **8**).

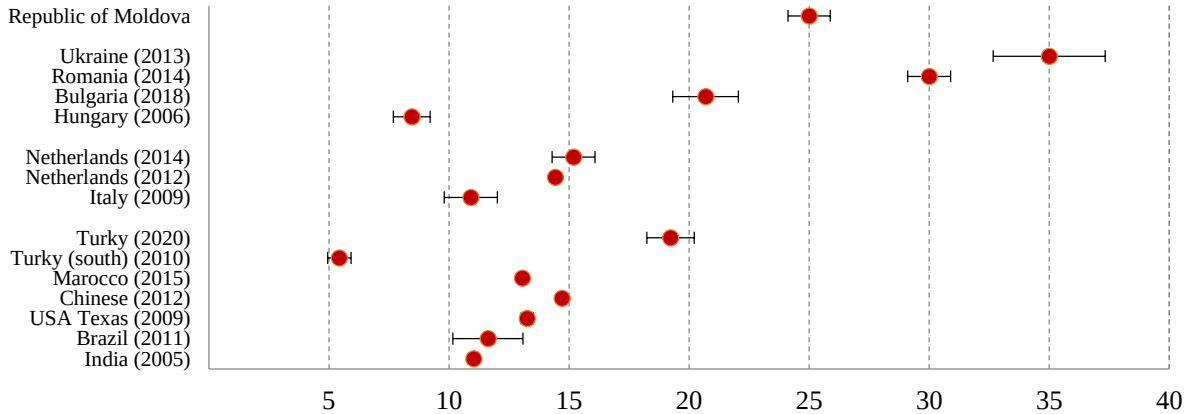


Figure 8. **Forest plot for the prevalence of chromosomal abnormalities in researched azoospermic patients compared to other similar studies**

The classification of chromosomal abnormalities and their distribution by age groups did not determine significant statistical differences $\chi 2=11.7$; gl=8; p=0.161. Patients with numerical chromosomal abnormalities were diagnosed in 66,7% at age ≥40 years and 57,9% (n=11) at age 30 - 39 years. Structural anomalies were identified in 100% (n=2) at the age of 19 -29 years, in 33,3% (n=1) at the age ≥40 years and 21,1% (n=4) at the age of 30 - 39 years old (Table 8, Table 9).

Table 8. **Frequency of chromosomal variations in men with azoospermia by age group**

Cytogenetic results test	Age group (years)							
	19 - 29		30 - 39		≥40		Total	
	n=22	%	n=64	%	n=10	%	n=96	%
Klinefelter syndrome/XXY			10	15,6	1	10,0	11	11,5
Jacobs syndrome/XYY			1	1,6			1	1,04
45,X/46,XY					1	10,0	1	1,04
Robertsonian translocations			1	1,7			1	1,04
Reciprococal translocations	1	4,5	1	1,6			2	2,1
Inverstion	1	4,5	1	1,6			2	2,1
46,XX+SRY					1	10,0	1	1,04
46,Xdel(Y)(q11.21)			1	1,6			1	1,04
Chrs. polymorphisms			3	4,7			3	3,13
Fragile site			1	1,6			1	1,04
46,XY	20	90,9	45	70,3	7	70,0	72	75,0

Table 9. **Classification of age-related cytogenetic variants in men with azoospermia**

Cytogenetic results test		Age group (years)							
		19 – 29		30 – 39		≥40		Total	
		n =22	%	n=64	%	n=10	%	n=96	%
The type of chr. involved	Gonosomal, inclusive:			13	68,4	3	100	16	66,7
	SK/ XXY			10	76,9	1	33,3	11	68,7
	47,XYY			1	7,7			1	4,2
	46,XX +SRY					1	33,3	1	4,2
	45,X/46,XY					1	33,3	1	4,2
	46,Xdel(Y)(q11.21)			1	7,7			1	4,2
	46,XYqh+			1	7,7			1	4,2
	Autozomal, inclusive:	2	100	6	31,6			8	33,3
	45,XY,rob(13/14)			1	16,7			1	4,2
	46,XY, t(1;19)(23.2q;q12.4)			1	16,7			1	4,2
	46,XY,15ps+			1	16,7			1	4,2
	46,XY,22sts			1	16,7			1	4,2
	46,XY,der(5),t(9;5)	1	50,0					1	4,2
	46,XY,fra(17)(p12)			1	16,7			1	4,2
	46,XY,inv(9)(p11q12)	1	50,0					1	4,2
	46,XY,inv(9)(p13.21)			1	16,7			1	4,2

It is known that abnormalities of sex chromosomes are the most common causes of infertility associated with chromosomes [8]. In our research, among the total number (n=96) of patients with azoospermia, the prevalence of sex chromosome abnormalities was identified in 16,7% (n=16) cases and 8,3% (n=8) cases presented autosomal abnormalities (Figure 9).

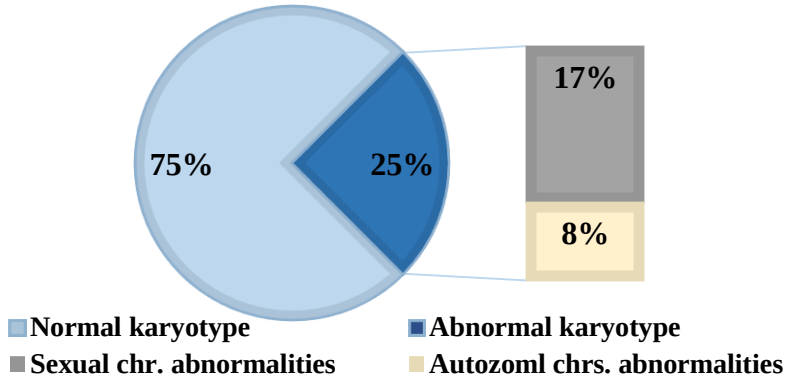


Figure 9. **The proportion of chromosomal abnormalities in men with azoospermia (n=96)**

Table 10. **The characteristics of gonosomal compared to autosomal abnormalities in patients with azoospermia**

Parameters	Gonosomal chrs. ab.	Autosomal crs. ab.	Total	p
Nr. pacienților	n=16	n=8	n=24	
Age	36,2 ±4,9	32,0 ±2,9	34,8 ±4,8	**p=0,037**
(years)	35,5 (32,5-39,0)	32,0 (29,5-34,5)	34,5 (31,0-36,0)	
Volume	2,2 ±0,7	2,4 ±0,8	2,2 ±0,7	p=0,447
(ml)	2,2 (1,5-2,5)	2,4 (2,0-2,9)	2,2 (1,8-2,6)	
FSH	15,4 ±10,3	4,9 ±4,8	11,9 ±10,1	**p=0,012**
(mIU/ml)	15,5 (7,5-20,6)	2,9 (1,8-6,7)	9,2 (2,9-19,1)	
LH	15,3 ±8,9	6,7 ±4,0	12,4 ±8,6	**p=0,018**
(mIU/ml)	13,0 (8,1-23,3)	5,8 (3,9-9,2)	9,9 (5,9-19,5)	
Prolactin	12,9 ±6,5	15,9 ±5,6	13,9 ±6,2	p=0,294
(ng/ml)	11,3 (7,0-19,1)	18,0 (11,3-19,6)	11,8 (7,9-19,5)	
Testosterone	3,4 ±0,9	3,9±1,2	3,6 ±1,0	p=0,281
(ng/ml)	3,4 (2,9-3,8)	3,7 (2,8-4,9)	3,4 (2,9-4,1)	
Free testosterone	7,1 ±6,8	15,9±10,5	9,6 ±8,3	p=0,231
(ng/ml)	2,8 (2,8-8,4)	15,9 (8,4-23,3)	8,4 (2,8-18,5)	

Notă: Mean ±SD; Median (IIQ)

The mean age of patients with gonosomal chromosomal abnormalities (n=16) was 36.2 ±4.9 years, (IQ: 32,5 – 39,0; median: 35,5) and in patients with autosomal chromosomal abnormalities (n=8) of 32,0 ±2,9 years, (IQ: 29,5 – 34,5; median: 32,0, (F=4,917; p=0,037). For the volume of semen, no significant statistical differences (F=0,601; p=0,447), on the other hand, for the FSH hormone, significant statistical differences (F=7,406; p=0,012) are observed between the described groups. The average FSH being 15,4 ±10,3, (IQ: 7,5 – 20,6; median: 15,5) in subjects who presented sex chromosome abnormalities and 4,9 ±4,8, (IQ: 1,8 – 6,7; median: 2,9) to autosomal abnormalities. Similarly, for the LH hormone, statistical differences are also found (F=6,576; p=0,018), in patients with gonosomal chromosomal abnormalities with an average of 15,3 ±8,9, (IQ: 8,1 – 23,3; median: 13,0) and autosomal of 6,7 ±4,0, (IQ: 3,9 – 9,2; median: 5,8). The mean for the hormone prolactin in subjects with gonosomal abnormalities is of 12,9 ±6,5, (IQ: 7,0 – 19,1; median: 11,3) and in those with autosomal 15,9 ±5,6, (IQ: 11,3 – 19,6; median: 18,0), (F=1,158; p=0,294). Testosterone was with mean of 3,4 ±0,9, (IQ: 2,9 – 3,8; median: 3,4) in subjects with gonosomal chromosomal abnormalities and a mean of 3,9 ±1,2, (IQ: 2,8 – 4,9; median: 3,7) with autosomal, (F=1.224; p=0,281), (Table 10).

4.2.1 Klinefelter syndrome (KS) 47,XXY

Out of the total group (n=96) of cytogenetically investigated azoospermic men, 11 presented disomy X, representing 11,5% (Table 9). The results of the research are comparable to the literature data reporting the same high frequency of Klinefelter syndrome among azoospermic men of 10-15%. The mean age of patients with KS was 35,5 ± 4,9 years (95% CI: 32,3– 38,8; median: 35,0) with a minimum value of 30 years and a maximum of 47 years. The mean of their infertility was 6,3 ±3,1 years (95% CI: 4,2– 8,3; median: 6,0) with a minimum value of 3 years and a maximum of 14 years. The phenotype of patients with KS is extremely heterogeneous, being influenced by the type of karyotype as well as the patient's age. During childhood, KS frequently remains undiagnosed due to the nonsuggestive clinical picture. In the current research childhood diseases, such as cryptorchidism, viral orchitis and mumps, were presented in 63,6% (n=7), however no patient was diagnosed during this period. The clinical manifestations take shape in adulthood when gynecomastia, hypogonadism and infertility respectively appear. All patients in the current research were diagnosed in adulthood due to infertility. In the current research, gynecomastia is present in 90,9% (n=10), hypogonadism in 81,8% (n=9), hypersthenic constitution in 72,7% (n=8), (Figure 10).

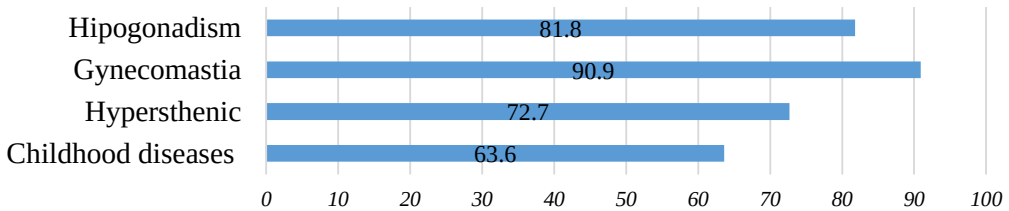
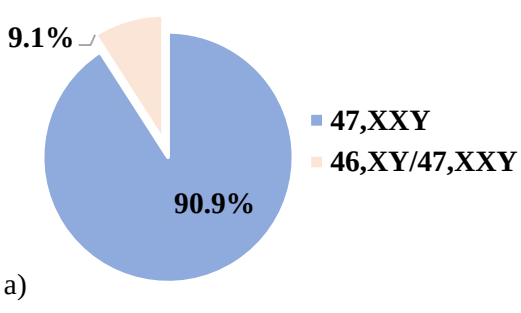
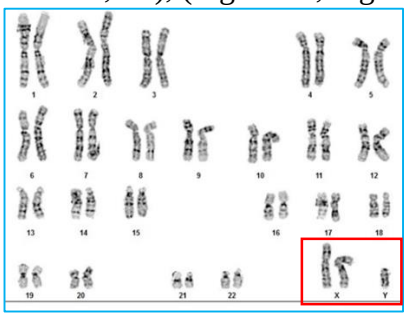


Figure 10. **Prevalence of clinical manifestations in patients with Klinefelter Syndrome**

According to the cytogenetic results, the most frequent chromosomal variant diagnosed in 11 patients with KS was the homogeneous form of trisomy 47,XXY (10 cases – 90,9%), followed by the mosaic form, the same classical forms 47,XXY/46,XY (1 case – 9,1%), (Figure 11, Figure 12).



a)



b)

Figure 11. **a) The structure of cytogenetic variants diagnosed in patients with Klinefelter Syndrome; b) Karyotype 47,XXY in patient no. 1, age 31**

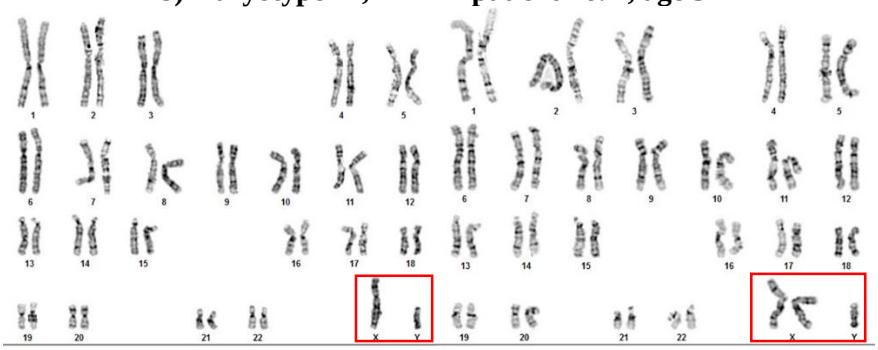


Figure 12. **Karyotype 46,XY/47,XXY in patient no. 25, age 31 years**

The cytogenetic variants diagnosed in the current research come in confirmation of data from the literature reporting the same high incidence of 80-90% of the classical 47,XXY form of KS and about 20% of the mosaic forms. Aneuploidy 47,XXY is the most common chromosomal abnormality, with an incidence that varies from 1/500 to 1/1,000, cytogenetic variants that include X polysomies are rare: 48,XXXY (1/50,000); 49,XXXXY (1/85,000) [9].

4.2.2 Jacobs syndrome 47,XYY

Out of the total group (n=96) of cytogenetically investigated azoospermic men, one case was diagnosed with disomy Y with a frequency of 1.04% (Table 8). According to the cytogenetic examination, the presence of the additional Y chromosome was identified in all analyzed cells, with the karyotype formula 47,XYY (Figure 13). 47,XYY syndrome is relatively common, seen in 1 in 1000 male newborns, being the second most common sex chromosome abnormality after Klinefelter syndrome. However, up to 85% of XYY males go undiagnosed. The diagnosis of these patients is usually late, with the average age of diagnosis being 17,1 years, according to a Danish cohort study. This is due to the apparently normal phenotype in most cases [10]. According to the bibliographic data, the majority of those with karyotype 47,XYY have normal spermatogenesis, while the minority may have varying degrees of spermatogenesis impairment from normal to azoospermia [11]. The extra Y chromosome is eliminated in the early stages of spermatogenesis, therefore in these patients the need for pre-implantation genetic diagnosis (PGD) before treatment for assisted reproduction could be argued [12].

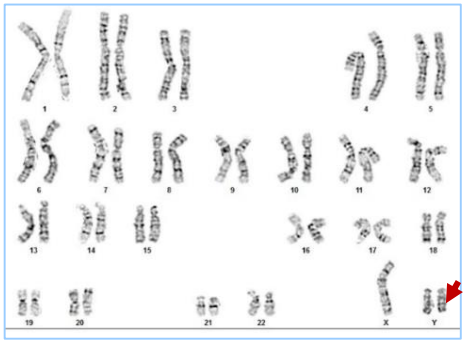


Figure 13**. Karyotype 47,XYY, age 35years**

4.2.3 Chapelle syndrome, sex inversion in a 46,XX male

In one azoospermic man from the total number (n=96) the karyotype 46,XX (1,04%) was identified (Table 8). The frequency of XX men in the general population being very rare (1 in 20.000), it is identified in case of azoospermic men in 0,9% and in 1-3% of normozoospermic men [13].

Chapelle syndrome is a sex-reversal syndrome characterized by a female karyotype discordant with a male phenotype. More frequently, this syndrome is described in the specialized literature as sporadic cases [14]. The mechanism of disease production in most cases 80% is caused by an unequal crossing-over between the X and Y chromosomes during the recombination of the genetic material in prophase 1 of meiosis, resulting in the translocation of the SRY gene from the Y chromosome to the X chromosome or chromosomes autosomes [15]. Due to this fact two categories are found, SRY positive in 80% of patients and in the remaining 20% - SRY negative [13]. The presented case refers to the positive SRY category, the presence of the SRY gene being detected through the multiplex PCR molecular genetic examination, through the FISH test it was possible to identify the SRY gene on the X chromosome. Due to the relatively normal phenotype and sexual organs, the early diagnosis of this syndrome is difficult. Most patients are diagnosed after puberty when hypogonadism, azoospermia and the inability to conceive children are evident. The described case was diagnosed at the late age of 40 years due to the same previously mentioned cause of azoospermia and infertility. Azoospermia is due to the lack of genes in the AZF region of the Y-chromosome Yq11.23 [13].

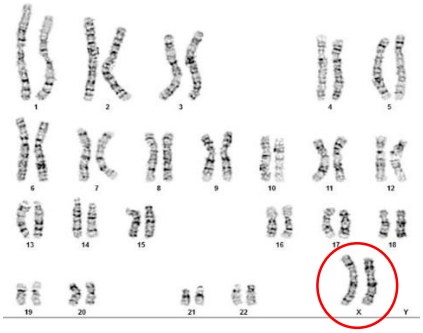


Figure 14. **Karyotype *46,XX* in all cells analyzed in a 40 year old men**
(Metaphases counted: 33; Karyotyped metaphases: 10; Band resolution level: 575 – 570)

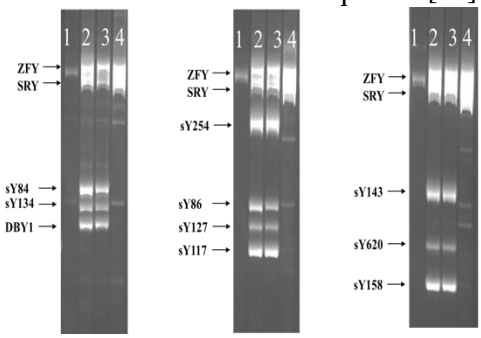


Figure 15. The **rezult of electrophoresis for detection of Y markers in the man with 46,XX**
1 – Female control; 2, 3 – Normal male;
4 – Male with deletions of the AZF (a, b, c) region

During the genetic counseling regarding the possibility of conceiving a child, the possibility of using sperm from the donor was recommended. In patients with testicular DSD, extraction of testicular sperm is not recommended, residual spermatogenesis may not be present in the testes. Histological evaluation of the testicular tissue of XX males shows the presence of Sertoli cell syndrome and Leydig cell hyperplasia. In the treatment of 46,XX patients with testicular failure, a multidisciplinary approach should be considered, psychological support is an important part of the holistic approach [13].

4.2.4 Mixed gonadal dysgenesis in the 45,X/46,XY male

The 45,X/46,XY mosaic was identified in a patient with azoospermia (Table 8) which occurs rarely with a frequency of 1/15,000 newborns. The significance of the 45,X/46,XY mosaic in the bibliographic sources is controversial and presents a great clinical challenge, as it presents clinical manifestations of variable severity from fertility problems, ambiguous genitalia to phenotypically normal men [14].

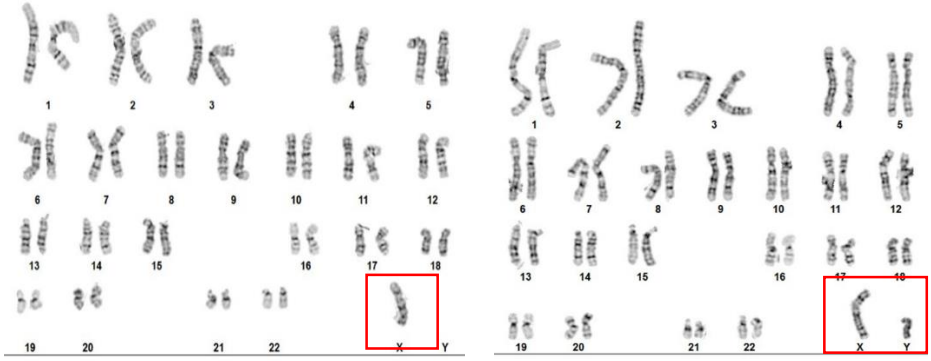


Figure 16. **45,X[3]/46,XY[12] mosaic karyotype of 46-year-old patient**

The phenotype of patients with such condition is extremely variable from sexual ambiguity, predominantly female genitalia, to normal phenotype. Phenotypic variation in individuals with the

45,X/46,XY karyotype may correlate with the degree of blood and testis mosaicism, the ratio of 45,X vs 46,XY cell counts. In some cases, men with the X /XY mosaic can benefit from ART (Assisted Reproduction Techniques), with the risk of passing on some genetic defects to their offspring, a situation that requires genetic counseling and prenatal diagnosis [8, 16].

4.2.5 Microscopic structural variations of the Y chromosome

Through cytogenetic techniques, two cases with structural variations of the Y chromosome were identified: 46,X,del(Y)(q11.21) (Y≤21) and 46,XYqh+(Y ≥ 18) (Figure 17). Polymorphic variants of the Y chromosome (Yqh+) are mentioned in a few studies on male infertility mainly in men with azoospermia and severe oligozoospermia [17] Yqh+ represents a variation of the constitutive heterochromatin region in the Y chromosome, which cannot directly explain spermatogenesis disorders. Conversely, in Y(≤21) cases, similar to the normal Y chromosome in morphology and banding, as well as in size, genes associated with spermatogenesis may be missing [18]. In the case of the patient with karyotype 46,X,del(Y)(q11.21) - Y(≤21), the genes in the AZFb+c region were deleted (confirmed by multiplexPCR).

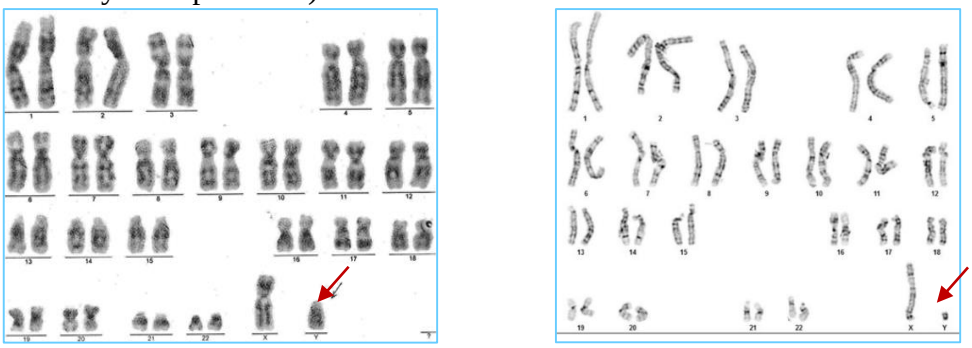
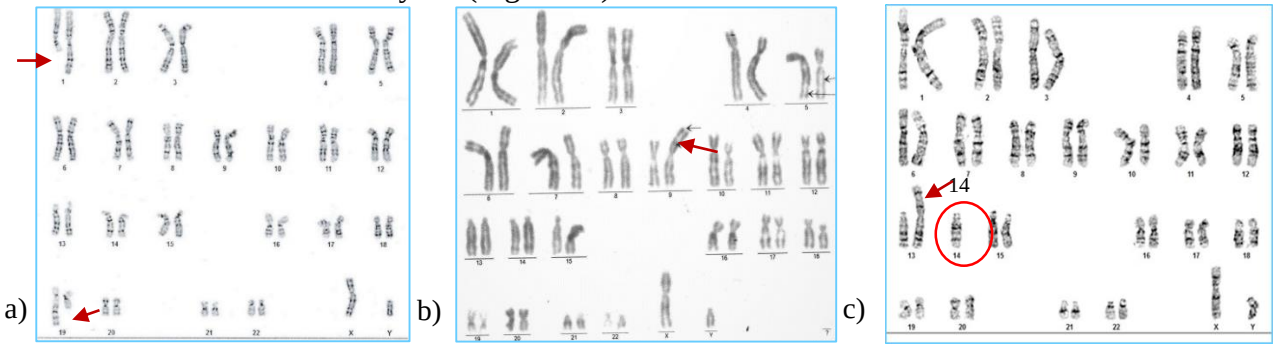


Figure 17. a) **Karyotype: 46,XYqh+(Y ≥ 18) age 30 years b) 46,Xdel(Y)(q11.21) (Y≤21) age 36 years**

4.2.6 Structural variations of autosomal chromosomes

Cytogenetic testing of patients with azoospermia revealed variations in the structure of some autosomal chromosomes: 46,XY,t(1;19)(23.2q:q12.4); 46,XY,der(5),t(9,5); 45,XY,rob(13/14); 46,XY,inv(9)(p11q12); 46,XY,inv(9)(p13.21); 46,XY,15ps+; 46,XY,22 sts; 46,XY,fra(17)(p12). In specialized studies, variations of autosomal chromosomes are described in patients with infertility, which frequently do not express themselves through phenotypic changes. In the current study of men with azoospermia, 8,3% (n=8) cases are found (Table 9). The most common autosomal chromosomal abnormalities detected were balanced chromosomal rearrangements in 5 cases. Translocations are the most common balanced chromosomal abnormalities. In the current research of 96 men with azoospermia simple balanced translocations were detected in 2,1% (n=2) cases- t(1;19) and t(9;5). In one case, a Robertsonian translocation, with 45 chromosomes, involving the long arms of one chromosome from pair 13 and one from pair 14, was identified in all rob(13/14) cells analyzed. Chromosomal inversions are the most common balanced chromosomal rearrangements after translocations. In the current study, 2,1% (n=2) of the total number of men with azoospermia were identified. In both patients, pericentric inversion involving both arms of a chromosome from pair 9 was identified in all cells analyzed (Figure 18).



a) b) c)

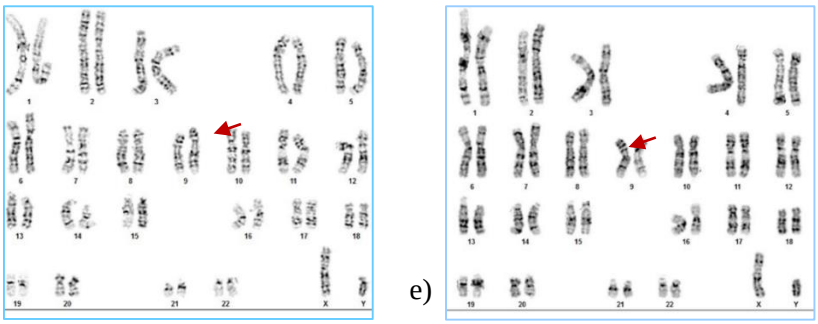


Figure 18. Karyotype: a) ***46,XY,t(1;19)(q23.2;q13.4)*** **age 35 years; b)** ***46,XY,der(5),t(9;5)*** **age 29 years; c)*45,XY,rob(13;14)(q10;q10)*** **age 31 years; d)** ***46,XY,inv(9)(p11q12)*** **age 35 years; e)** ***46,XY,inv(9)(p13q21)*** **age 31 years**

Chromosomal polymorphisms still remain one of the most interesting topics in genetics [5]. In the current study, 2,1% (n=2) cases of 96 men with azoospermia were identified. One case with an enlarged satellite region on the short arm of one of chromosome pair 15 (15ps+) and another of chromosome pair 21 (21ps+), in all cells (Figure 19).

Figure 19. Karyotype: **a)** ***46,XY,15ps+*** **age 33 years; b)** ***46,XY,22ps+*** **age 32 years; c)** ***46,XY,fra(17)(p12)*** **age 36 years**

In one case in a 36-year-old azoospermic man, a male karyotype with 46 chromosomes was detected, showing a fragile site at the level of the short arm of one of the chromosomes in pair 17 (region 17p12), in all cells analyzed (Figure 19).

4.3. Variations in AZF and CFTR genes in azoospermic patients tested molecularly-genetically

All patients (n=96) with azoospermia were molecular-genetically tested for the analysis of variations in the genes of the AZF region of the Y chromosome (mutiplex PCR) and for delF508 and G542X mutations of the CFTR gene (PCR).

4.3.1 Microdeletions of the Y chromosome in men with azoospermia

In the current research, chromosomal variations were diagnosed in 25% (24/96), of which 11,5% (11/96) were associated with Klinefelter Syndrome. In the molecular-genetic analysis, deletions of the AZF region of the Y chromosome were detected in 10,4% (10/96), being similar to that reported in patients from the USA (10,4%), Japan (11,1%) and China (11,7%), (Figure 20). Y microdeletions in the AZF region represent the second genetic cause of male infertility, after chromosomal anomalies and especially SK, data confirmed in this study [19].

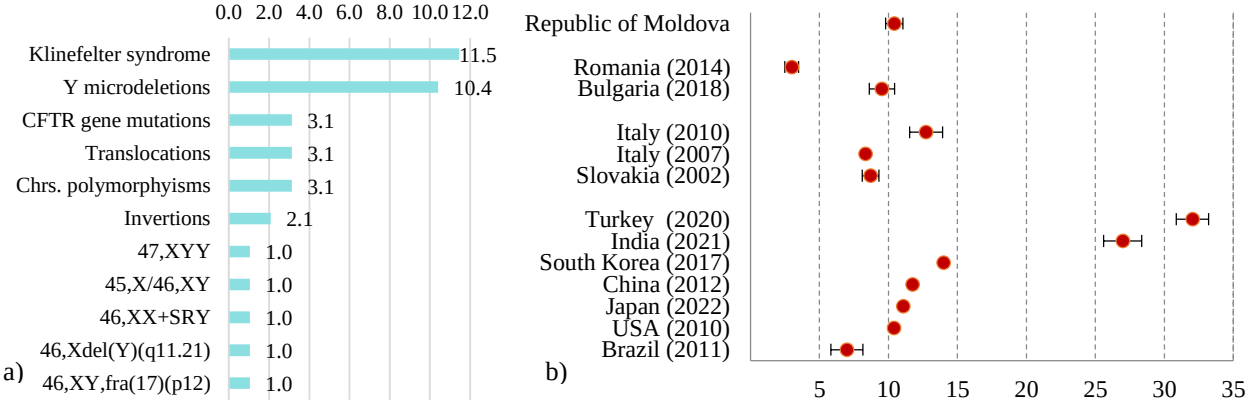


Figure 20. **a) Prevalence of Y microdeletions relative to other genetic causes in men with azoospermia, b) Forest plot for the prevalence of Y microdeletions in this study compared to other similar studies**

Y microdeletions are more common in infertile men than in the general population, found in 3–5% of patients with oligozoospermia and 6–16% of patients with azoospermia. The high incidence of Y microdeletions among men with severely affected spermograms is directly due to deletions in the AZF region affecting genes that control spermatogenesis [20].

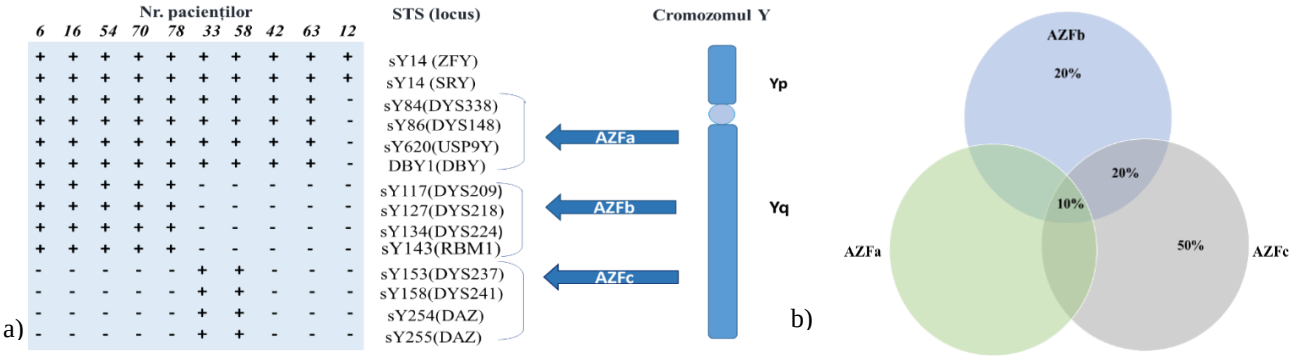


Figure 21. **a) Schematic diagram illustrating different deletion types of STS markers in patients with AZF deletions. +: PCR product present; –: missing PCR product, b) The Venn diagram represents the prevalence of different types of AZF deletions**

Of the total number (n=10) of patients with Y microdeletions, the most common being microdeletions of the AZFc locus, the missing markers were sY153, sY158, sY254 and sY255. Isolated deletions of this locus were diagnosed in 50% (n=5) of (n=10) azoospermic patients. In 20% (n=2) they were detected with the deletion of the AZFb region, the missing markers being sY117, sY127, sY134, sY143. Deletions affecting both AZFb and AZFc loci were identified in 20% (n=2). In a single case, 10% (n=1) microdeletions were detected in each region of AZFa-sY84, sY86, sY620, DBY1; AZFb-s Y117, sY127, sY134, sY143; AZFc-sY153, sY158 sY254, sY255; and the presence of the SRY gene (the sex-determining region on the Y). Isolated complete deletions of the AZFa region were not detected in any of the patients (Figure 21). Males with AZFc deletions show the most variable phenotype ranging from complete azoospermia to mild oligozoospermia. However, in most carriers with AZFc deletions, spermatogenesis is completed, but on a reduced scale, usually less than 2 million/ml, resulting in severe oligozoospermia. According to reports from multiple studies in patients with AZFc deletions, mature spermatozoa are obtained in approximately 50% by the TESE technique, despite reduced fertilization rates and poorer embryo scores after ICSI [21]. In the current study, testicular biopsy was performed in 3 patients with AZFc deletion for sperm retrieval, however, we did not find spermatozoa mature enough for ICSI in any patient. Deletion of AZFb may be associated with arrest of germ cell development at the pachytene stage resulting in azoospermia or SCOS [19].

4.3.2 Mutations in the CFTR gene in men with azoospermia

Following the molecular genetic evaluation of delF508 and G542X mutations of the CFTR gene in patients (n=96) with azoospermia, in 3.1% (n=3) cases they were detected as carriers of CFTR-N/ΔF508 mutations (Figure 22). Cystic fibrosis (CF) is the most common autosomal recessive disorder affecting approximately 70,000 people worldwide characteristic for the Caucasian population. The clinical manifestations of the disease are caused by defects in the Cystic Fibrosis Transmembrane Conductance Regulator protein, determined by mutations in the CFTR gene. The ΔF508 mutation is the most common - about 50-80% [22].

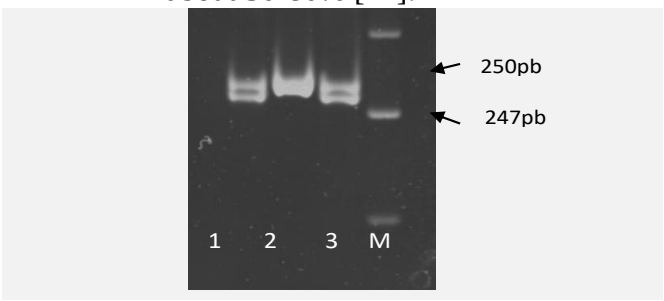


Figure 22. **Electrophorogram for the del F508 mutation in the CFTR gene**
1, 3: heterozygous male (N/delF508); 2: homozygous male (N/N)

Mutations in the CFTR gene disrupt the function of chloride channels, preventing them from regulating the flow of ions and water across cell membranes. As a result, cells in the male genital tract produce mucus of a viscous consistency. All heterozygous patients with N/ΔF508 alleles underwent testicular biopsy, where spermatozoa were identified for ICSI and subsequently opted for IVF. For couples in which the male partner has mutations in the CFTR gene, testing of the female partner and genetic counseling are very important before ART to estimate risks and possible genotype-phenotype correlations. Thus, for the calculation of the risk of recurrence in the offspring, their wives, who were homozygous NN, were also investigated. All (n=3) patients carrying ΔF508 mutations had bilateral absence of vas deferens (CBAVD). CBAVD is a congenital developmental disease (1:1000 males) characterized by the absence of both vas deferens. The prevalence of CBAVD in azoospermic men is estimated at 4-17% and increases to 25% in obstructive azoospermia. Absence of vas deferens is clinically asymptomatic and when CBAVD is the only manifestation in a patient then in most cases it harbors at least one CFTR gene mutation. This condition is known as the genital form of fibrosis and may be referred to as FC-CBAVD [23].

4.5. Genetic consultation in infertile couples due to azoospermia in the context of assisted reproduction

Azoospermia is the most severe cause of male infertility in couples, azoospermic men suffer from the absence of sperm in the ejaculate. Confirmation of a clinical diagnosis by genetic testing can lead to personalized medical management. Similar clinical symptoms may be the result of different genetic variations. Specifically, in rarer clinical situations, genetic evaluations can help to specifically identify the disease or confirm a suspected diagnosis [24].

Table 11. **Reproductive treatment options for patients with microdeletions of the Y chromosome within Assisted Reproduction Techniques** [21] [24]

Phenotype	**Genetic cause**	**Frequency**	**Test**	**ART**	**Inheritance**
Normal male FSH ↔ ↥ LH Normozoospermia-Azoospermia	Y chromosome microdeletions	1/2.500 10-15% in azoospermia; 3-7% în oligozoospermia	Diagnosis Moleculrar genetic PCR STS region Y; ZFY; SRY	According type of deletion	
Azoospermia/ Oligozoospermia severe	Y-AZF c deletion	AZFc del- 60%	STSY- region AZFc sY254- gene DAZ sY255- DAZ sY153- DYS237 sY158- DYS241	micro-TESE + ICSI	De novo/ Y linked Genetic counseling/ AZFc del-transmission in boys; PGD
Azoospermia/ Blockage of spermatogenesis	Y-AZF b deletion	AZFb del 1-5%	STSY- region AZFb sY117-DYS209 sY127-DYS218 sY134-DYS224 sY143-RBMY		
Azoospermia/ Sertoli cell-only syndrome-SCO / Blockage of spermatogenesis	Y-AZFb+c deletion	AZFbc del- 22%	STSY- region AZFc combine with AZFb	Sperm donor IVF	De novo It is not transmitted to offspring
Azoospermia/ SCO syndrome	Y-AZFa deletion	AZFa del- 3%	STSY- region AZFa sY84- DYS388 sY86- DYS148 DBY1- DBY sY620- USP9Y		
Azoospermia/ SCO syndrome	Y-AZFabc Deletion	AZFabc del- 1%	STSY- region AZFc combine with AZFb și AZFc	Sperm donor IVF	

TRA- SCO - Sertoli cell-only syndrome; PCR – Chain polymerization reaction; PGD- Preimplantation Genetic Diagnosis; FSH - Follicle Stimulating Hormone; LH - Luteinizing Hormone; ↥ - increased; ↔ the norm; IVF- Fertilization in vitro

Genetic counseling in reproductive techniques should be based on the following directions:

i. A couple where the male has infertility due to Y chromosome microdeletions may be offered the option of in vitro fertilization using ICSI (intracytoplasmic sperm injection). In this procedure, sperm collected from ejaculate (in men with oligozoospermia) or extracted from testicular biopsies (in men with azoospermia) are injected by ICSI into an egg harvested through IVF (in vitro fertilization) [24]. In clinical practice, the indication for surgical TESE or micro-TESE for

azoospermic patients should be considered based on the results of molecular genetic testing of the deletion type AZFa, AZFb, AZF b,c and AZFc (Table 11).

ii. If the man is a carrier of a balanced autosomal abnormality, the couple should be informed that the success rate is variable and that there is an increased risk of spontaneous abortion. The couple should be advised that their fetus may be: affected by a chromosomal abnormality which may cause multiple birth defects, mental retardation or; the fetus may be normal or; the fetus may have the same chromosomal abnormality as the father, leading to reduced fertility or infertility. If the man is a carrier of a structural abnormality involving only the sex chromosomes, there is an increased risk of passing on infertility and possibly other disorders or malformations (Table 12, Table 13).

Table 12. **Reproductive treatment options for patients with balanced chromosomal abnormalities within Assisted Reproduction Techniques** [5][18]

Phenotype	Genetic cause / frequency	Test	ART
Normospermia up to azoospermia; FSH ↔; Testosterone ↔; Majority normal phenotype	*Balanced structural chrs. anomalies 5% of infertile men*	Cytogenetic examination/karyotyping FISH	IVF oligozoospermia microTESE/TESE azoospermia PGD
	Reciprocal translocations: 0,9/1000 n.b. 0,5- 0,9% azoospermie, 0,6% oligozoospermie,	46,XY,t(1;19) 46,XY,t(9;5) etc.	
	Robertsonian translocations: 0,5% azoospermie, 1,6% oligozoospermie.	45,XY,rob(13;14) 45,XY,rob(14;21) etc.	
	Inversion:	46,XY,inv(9)(p11q12) 46,XY,inv(9)(p13.21) etc.	

PGD - Preimplantation Genetic Diagnosis; FSH - Follicle Stimulating Hormone; LH - Luteinizing Hormone; ↥ - increased; ↔ the norm; IVF- Fertilization in vitro; microTESE - Microscopic testicular retrieval of sperm

iii. Azoospermic men with mutations in the CFTR gene can conceive through ART. An affected male (aa) will pass on one disease-causing mutated allele of the CFTR gene to each of his offspring. CFTR molecular genetic testing should be offered to the reproductive partner to determine her CFTR carrier status. If the reproductive partner is a carrier of the mutation in the CFTR gene, their offspring will be at risk for CF or CBAVD in 25% [25].

Table 13. **Reproductive treatment options for patients with chromosomal abnormalities within Assisted Reproduction Techniques** [5] [16]

Phenotype	Genetic cause / frequency	Test	ART	Inheritance
Azoospermia, oligozoospermia; FSH ↥, LH ↥ Testosterone ↧; tall stature, small testicles, infertility, gynecomastia; neurocognitive disorders, metabolic syndrome, etc.	XXY/ Klinefelter syndrome 1/700 male newborns; 10-15% in azoospermia, 2-5% oligozoospermia.	Cytogenetic examination/karyotyping; FISH The classic form 47,XXY (80-90%) Mosaic form 46,XY/47,XXY (6-7%) Polysomies X 48,XXXY 49,XXXXY etc. (3-8%)	- in patients with oligozoospermia/IVF + ICSI - in patients with azoospermia up to the age of 35/micro TESE+ ICSI - in patients diagnosed with prepuberty during puberty, genetic counseling regarding cryopreservation of spermatozoa	De novo
Azoospermia, Oligozoospermia, FSH ↔ ↥, LH ↥, Testosterone ↔ ↧; short stature, male genitalia, cryptoorchidism, hypospadias, gynecomastia, infertility.	XX in male/ Chapelle syndrome Rare cases 1:20.000 newborns	Cytogenetic examination/karyotyping 46,XX^{+}SRY (80%); FISH –presence gene SRY; PCR- lack of region AZFa, AZFb, AZFc, presence gene SRY 46,XX^{-}SRY (20%); FISH – lack gene SRY; PCR- lack of region AZFa, AZFb, AZFc	Use of donor sperm (subsequently IVF)	AD Is not clear
Normospermia up to Azoospermia, FSH ↔ ↥, Testosterone ↔ ↧; Majority normal phenotype, minority tall stature, ASD.	XYY/Jacobs syndrome 1/1.000 newborns 0,4% oligozoospermia	Cytogenetic examination/karyotyping; FISH Homogeneous form 47,XYY Mosaic form 46,XY/47,XXY	IVF or ICSI in patients with oligozoospermia micro TESE+ ICSI in patients with azoospermia	Is not clear

ASD - Autism Spectrum Disorders; PGD- Preimplantation Genetic Diagnosis; FSH - Follicle Stimulating Hormone; LH - Luteinizing Hormone; ↥ - increased; ↔ the norm; ↓ decreased; IVF- In Vitro Fertilizatio; microTESE - Microscopic testicular retrieval of sperm

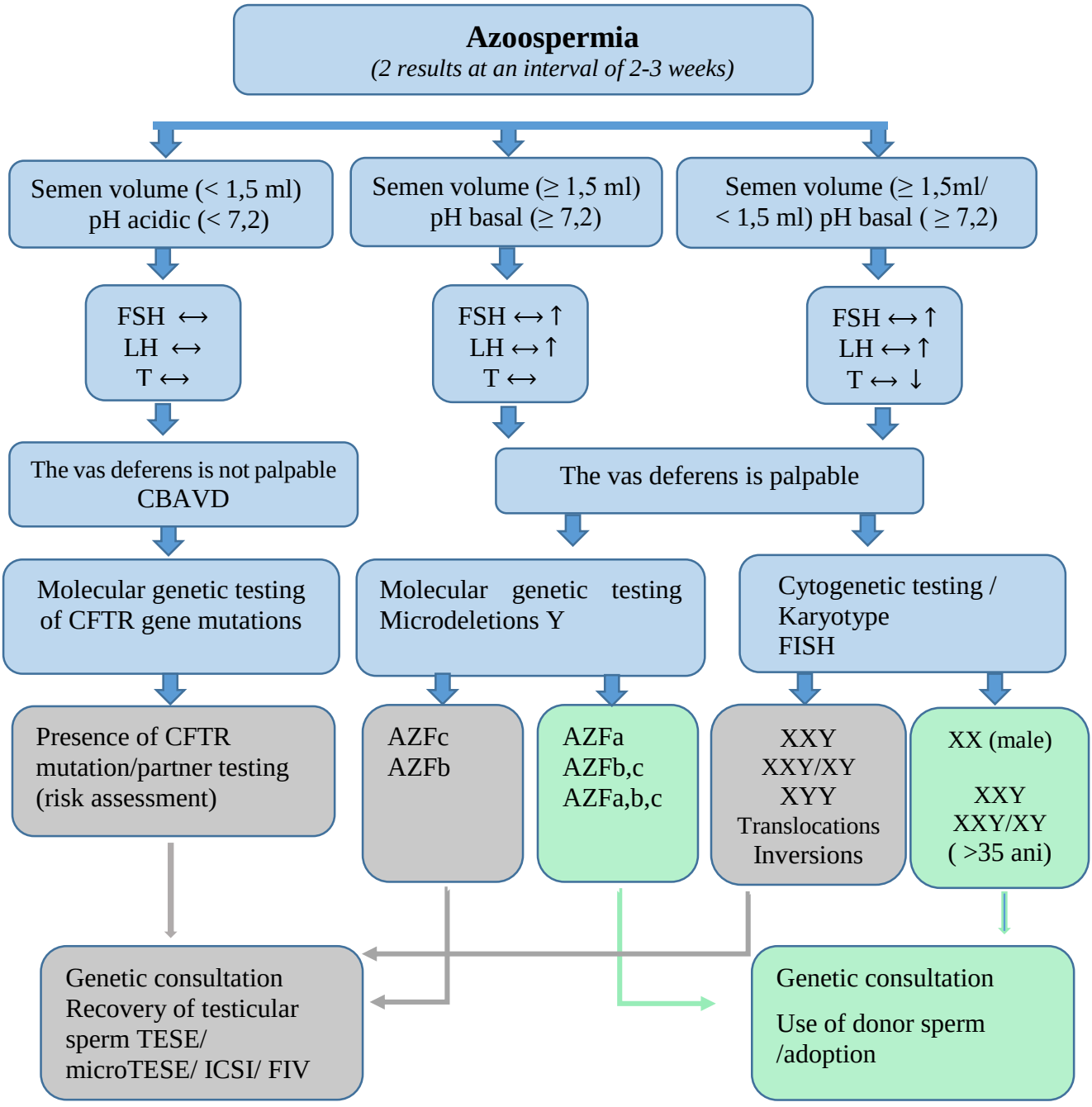


Figure 23. **Genetic diagnostic algorithm for CFTR gene mutations, Y microdeletions and chromosomal abnormalities in men with azoospermia**

FSH - Follicle Stimulating Hormone; LH - Luteinizing Hormone; ↥ - increased; ↔ the norm; ↓ decreased; IVF- In Vitro Fertilizatio; TESE - Testicular Sperm Extraction; microTESE - Microscopic testicular retrieval of sperm; CBAVD - Congenital Bilateral Absence of the Vas Deferens; CFTR - Cystic Fibrosis Transmembrane Conductance Regulator

GENERAL CONCLUSIONS

1. The study of semen parameters by evaluating 5676 spermograms, during the years 2012-2020, demonstrated a statistically significant decline ($p < 0.05$) for all the qualitative and quantitative indicators of the spermogram; a decrease in normozoospermia by 7,6% per year; an increase in oligozoospermia by 5,2%, asthenozoospermia by 13,7% and oligoasthenozoospermia by 1,0% per year; an increased addressability of young patients (in 2012 – the average age was 41,4 ± 5,5 years, and in 2020 it was 33,5 ± 5,5 years); which would indirectly represent a regression of male reproductive health.

2. The mean age of men with azoospermia in the entire sample (n=96) was 33,8 ±5,3 years, (95% CI: 32,7 – 34,9; median: 33,0), mean history infertility of 6,5 ±4,6 years, (CI 95%: 5,6 – 7,5).

3. Cytogenetic investigations carried out in patients with azoospermia (n=96) identified karyotype variations in 25,0% of cases, of which 16,7% had sex chromosome abnormalities: 47,XXY – Klinefelter syndrome (11,5%) ; microscopic structural variations of the Y chromosome (2,1%); one case each: 47,XYY – Jacobs syndrome; 46,XX – male sex inversion, 45,X/46,XY - mixed gonadal dysgenesis; and with autosomal chromosome abnormalities in 8,3%: translocations – (3,1%); inversions – (2,1%), chromosomal polymorphisms – (2,1%) and one case with 46,XY,fra(17)(p12).

4. Evaluation of the AZF region of the Y chromosome by the multiplex PCR technique in patients with azoospermia (n=96) identified deletions in the AZF region in 10,4% of cases: deletion of the AZFc region (5,2%); AZFb (2,08%), AZFbc (2,08%), AZFabc (1,0%);

5. Evaluation of CFTR gene mutations (delF508 and G542X) by PCR test in patients with azoospermia (n=96) identified heterozygous N/ΔF508 mutation in 3,1% of cases, and testing partners with the same CFTR mutations identified homozygous status, without risk of Cystic fibrosis in offspring.

6. Correlation of the phenotypic characteristics of patients with azoospermia (n=96) with the results of genetic tests, highlights: values <1,5 ml of the volume and values < 7,2 of the pH of the seminal material in patients with mutations in the CFTR gene, compared with abnormal karyotype or AZF deletions; high values of FSH, LH in subjects with abnormal karyotype versus patients with AZF deletions or mutations in the CFTR gene; hypogonadism and gynecomastia were recorded more frequently in patients with abnormal karyotype; CBAVD was present in all patients with mutations in the CFTR gene.

7. The genetic approach of the infertile couple offers the possibility of confirming the clinical diagnosis, elucidating the cause of infertility, assessing the risk of transmission of genetic abnormalities to offspring, choosing a personalized reproductive diagnostic-therapeutic strategy.

PRACTICAL RECOMMENDATIONS

1. Simultaneous assessment of the female and male partner in couples experiencing infertility.
2. Complex clinical, paraclinical and genetic approach to infertile couples due to azoospermia within multidisciplinary teams that include specialists in the fields of andrology, urology, reproductive medicine, psychology and genetics.
3. Personalized approach to the patient with azoospermia in order to elucidate the cause of infertility, assess the genetic risk in the case of the association of genetic variations and correctly select the options in the treatment of assisted reproduction.
4. Patients with azoospermia presenting:
   - with volume < 1,5 ml and pH < 7,2 (acidic) and normal FSH values must be investigated for CFTR gene mutations; in patients
   - with volume ≥ 1,5 ml and pH ≥ 7,2 (basal) and normal FSH values should be investigated for deletions in the AZF region;
   - with volume ≥ or < 1,5 ml and pH ≥ 7,2 (basal) and normal or elevated FSH values should be investigated cytogenetically.
5. In clinical practice, the indication for surgical TESE or micro-TESE for azoospermic patients should be considered based on the results of cytogenetic and molecular genetic tests.
   - in patients with numerical or structural chromosomal abnormalities and patients with microdeletions of the Y chromosome - AZFb and AZFc deletion, microTESE is recommended;
   - patients with mutations in the CFTR gene can recover sperm through TESE;
   - for young patients with Klinefelter's syndrome, microTESE and sperm cryopreservation are recommended

SELECTIVE BIBLIOGRAPHY

1. DATTA, J. et al. Prevalence of infertility and help seeking among 15 000 women and men. In: *Human reproduction Oxford, England*. 2016. Vol. 31, nr. 9, pp. 2108-2118. DOI 10.1093/HUMREP/DEW123. ISSN:1460-2350
2. AZIMI, C., KHALEGHIAN, M., FARZANFAR, F. A retrospective chromosome studies among Iranian infertile women: Report of 21 years. In: *Iranian Journal of Reproductive Medicine*. 2013. Vol. 11, nr. 4, pp. 315. ISSN:2008-2177
3. AMERICAN SOCIETY FOR REPRODUCTIVE M. Report on evaluation of the azoospermic male. In: *Fertility and sterility*. 2006. Vol. 86, nr. 5 Suppl 1, pp. S210-S215. DOI 10.1016/J.FERTNSTERT.2006.08.030. ISSN:1556-5653
4. HAMADA, A.J., ESTEVES, S.C., AGARWAL, A. A comprehensive review of genetics and genetic testing in azoospermia. In: *Clinics*. 2013. Vol. 68, nr. Suppl 1, pp. 39. DOI 10.6061/CLINICS/2013(SUP01)06. ISSN:1807-5932
5. KATE, U. V. et al. Chromosomal Aberrations and Polymorphic Evaluation in Males with Primary Infertility from Indian Population. In: *Journal of Clinical and Diagnostic Research*. 2014. Vol. 8, nr. 10, pp. SC01-SC06. DOI 10.7860/JCDR/2014/8644.4933. ISSN:0973-709X
6. RACOVITA, S. et al. Development of semen quality in male partners of infertile couples in the Republic of Moldova. In: *One Health & Risk Management*. 2020. Vol. 1, nr. 1, pp. 36-41. DOI 10.38045/OHRM.2020.1.06. ISSN:2587-3466
7. RACOVITA, S., SPRINCEAN, M., MOSIN, V. Decline of semen quality among males consulted for couple infertility in the Republic of Moldova. In: *Archives of the Balkan Medical Union*. 2022. Vol. 57, nr. 2, pp. 152-162. DOI 10.31688/ABMU.2022.57.2.04. ISSN:1584-9244
8. RACOVITA, S. et al. Chromosomal abnormalities in men with azoospermia. In: *Moldovan Medical Journal*. 2021. Vol. 64, nr. 1, pp. 50-55. DOI 10.5281/zenodo.4527139.
9. RACOVIȚĂ, S. et al. Variații cromozomiale la bărbații infertili. In: *Buletinul Academiei de Ştiinţe a Moldovei. Ştiinţe Medicale*. 2020. Vol. 67, nr. 3, pp. 78-82. ISSN:1857-0011
10. FLANNIGAN, R., SCHLEGEL, P.N. Genetic diagnostics of male infertility in clinical practice. In: *Best Practice & Research Clinical Obstetrics & Gynaecology*. 2017. Vol. 44, pp. 26-37. DOI 10.1016/J.BPOBGYN.2017.05.002. ISSN:1521-6934
11. KIM, I.W. et al. 47,XYY Syndrome and Male Infertility. In: *Reviews in Urology*. 2013. Vol. 15, nr. 4, pp. 188. DOI 10.3909/riu0580. ISSN:1523-6161
12. RACOVIȚĂ, S. et al. Sindromul 47,XYY asociat cu infertilitatea masculină: raport de caz clinic. In: *Buletin de Perinatologie*. 2020. Vol. 86, nr. 1, pp. 112-115. ISSN:1810-5289
13. RACOVIȚĂ, S. et al. Bărbat 46,XX, raport de caz clinic. In: *Buletin de Perinatologie*. 2019. Vol. 83, nr. 2, pp. 104-107. ISSN:1810-5289
14. AKINSAL, E.C. et al. Ten cases with 46,XX testicular disorder of sex development: single center experience. In: *International Brazilian Journal of Urology*. 2017. Vol. 43, nr. 4, pp. 770. DOI 10.1590/S1677-5538.IBJU.2016.0505. ISSN:1677-6119
15. DOMINGUEZ-LOPEZ, M. et al. Clinical case report: male patient with SRY-positive 46,XX testicular disorder of sex development. In: *Endocrine Abstracts*. 2013. Vol. 32, pp. 288. DOI 10.1530/ENDOABS.32.P288. ISSN:1470-3947
16. KILIC, S. et al. Assisted reproductive treatment applications in men with normal phenotype but 45,X/46,XY mosaic karyotype: clinical and genetic perspectives. In: *Taiwanese journal of obstetrics & gynecology*. 2010. Vol. 49, nr. 2, pp. 199-202. DOI 10.1016/S1028-4559(10)60042-3. ISSN:1875-6263
17. RACOVIȚĂ, S. et al. Clinical and genetic study in male infertility with azoospermia. In: *Abstract book*. Universitatea de Stat de Medicină și Farmacie „Nicolae Testemițanu" din Republica Moldova, 2021. p. 393. ISBN:978-9975-82-223-7.
18. LI, D. et al. Chromosomal abnormalities in men with pregestational and gestational infertility in northeast China. In: *Journal of Assisted Reproduction and Genetics*. 2012. Vol. 29, nr. 8, pp. 829-836. DOI 10.1007/S10815-012-9783-1. ISSN:1058-0468
19. RACOVITA, S. et al. Y chromosome microdeletions in men with severe spermatogenetic defects. In: *International Journal of Current Research*. 2021. Vol. 13, nr. 03, pp. 16428-16433. DOI 10.24941/ijcr.40899.03.2021.
20. ELSAID, H.O.A. et al. Detection of AZF microdeletions and reproductive hormonal profile analysis of infertile sudanese men pursuing assisted reproductive approaches. In: *BMC Urology*. 2021. Vol. 21, nr. 1, pp. 1-9. DOI 10.1186/s12894-021-00834-3. ISSN:1471-2490
21. CHOI, D.K. et al. Detection of Y chromosome microdeletion is valuable in the treatment of patients with nonobstructive azoospermia and oligoasthenoteratozoospermia: Sperm retrieval rate and birth rate. In: *Korean Journal of Urology*. 2013. Vol. 54, nr. 2, pp. 111-116. DOI 10.4111/KJU.2013.54.2.111. ISSN:2005-6737
22. CHEN, H. et al. Regulation of male fertility by CFTR and implications in male infertility. In: *Human reproduction update*. noiembrie 2012. Vol. 18, nr. 6, pp. 703-713. DOI 10.1093/HUMUPD/DMS027. ISSN:1460-2369
23. CIOPPI, F., ROSTA, V., KRAUSZ, C. Genetics of azoospermia. In: *International Journal of Molecular Sciences*. 2021. Vol. 22, nr. 6. DOI 10.3390/ijms22063264. ISSN:14220067
24. KURODA, S. et al. Genetic disorders and male infertility. In: *Reproductive Medicine and Biology*. 2020. Vol. 19, nr. 4, pp. 314-322. DOI 10.1002/rmb2.12336. ISSN:14470578
25. MOSKOWITZ, S.M. et al. Clinical practice and genetic counseling for cystic fibrosis and CFTR-related disorders. In: *Genetics in medicine: official journal of the American College of Medical Genetics*. 2008. Vol. 10, nr. 12, pp. 851-868. DOI 10.1097/GIM.0B013E31818E55A2. ISSN:1530-0366

LIST OF SCIENTIFIC PUBLICATIONS AND EVENTS
where have been presented the research results of the doctoral thesis in medical sciences with the topic "Genetic variation in male infertility with azoospermia"

carried out within the Department of Molecular biology and human genetics by Racoviță Stela,
„Nicolae Testemițanu" State University of Medicine and Pharmacy, Republic of Moldova

SCIENTIFIC WORKS

- **Articles in scientific journals abroad:**

✓ **articles in ISI, SCOPUS and other international databases***

1. Racoviță S., Sprincean M., Mosin V. Decline of semen quality among males consulted for couple infertility in the Republic of Moldova. *Archives of the Balkan Medical Union*. 2022;57(2):152-162. ISSN: 1584-9244. DOI: 10.31688/ABMU.2022.57.2.04. (IF:0,23)

✓ **articles in accredited foreign scientific journals:**

2. Racoviță S., Capcelea S., Boiciuc K., Moșin V., Revenco N., Hadjiu S., Sprincean M. Clinical and genetic particularities in male infertility. *Romanian Journal of Child and Adolescent Neurology and Psichiatry*. 2019;25 (3): 61-70. ISSN: 2068-8040.

3. Racoviță S., Moșin V., Hadjiu S., Revenco N., Sprincean M. Behavioral and psychiatric aspects of infertile 47,XYY patient. *Romanian Journal of Child and Adolescent Neurology and Psychiatry*. 2020;26(2):75-85. ISSN: 2068-8040.

4. Racoviță S. Clinical and cytogenetic polymorphism in Klinefelter Syndrome. *Romanian Journal of Child and Adolescent Neurology and Psychiatry*. 2022;28(2):34-42. ISSN: 2068-8040.

5. Sprincean M., Hadjiu S., **Racoviță S.**, Halabudenco E., Samoilenco T., Mișina A., Egorov V., Lupușor N., Grîu C., Feghiu L., Cuznеț L., Călcîi C., Revenco N. Differential diagnosis of chromosomal pathologies in children. *Romanian Journal of Child and Adolescent Neurology and Psychiatry*. 2020;26(2):47-63. ISSN: 2068-8040.

- **Articles in accredited national scientific journals:**

✓ **Articles in category B+ journals**

6. Racoviță S., Moșin V., Capcelea S., Mișina A., Sprincean M. Chromosomal abnormalities in men with azoospermia. *Moldovan Medical Journal*. 2021;64(1):50-55. ISSN: 2537-6373. DOI: 10.5281/zenodo.4527139.

✓ **Articles in category B journals**

7. Racoviță S., Moșin V., Capcelea S., Ponetenco D., Boiciuc K., Sprincean M. 46,XX male, clinical case report. *Bulletin of perinatology*. 2019; 2(83):104-107. ISSN 1810-5289.

8. Racoviță S., Moșin V., Hadjiu S., Poneatenco D., Revenco N, Sprincean M. Syndrome 47,XYY associated with men's infertility: clinical case report. *Bulletin of perinatology.* 2020, 1(86):112-115. ISSN 1810-5289.

9. Sprincean M., Hadjiu S., Halabudenco E., Fuior L., **Racoviță S**., Egorov V., Barbova N., Calcii C., Revenco N. Role of medico-genetic counseling and prenatal screening in diagnosis of renal urinary pathology in fetus. *Bulletin of perinatoogy*. 2020;2(87):32-38. ISSN: 1810-5289.

10. Racoviță S., Sprincean M., Poneatenco D., Gorduza EV., Mosin V. Development of semen quality in male partners of infertile couples in the Republic of Moldova. *One Health and Risk Management.* 2020;1(1):36–41. ISSN:2587-3458. Doi:10.38045/OHRM.2020.1.06. (DOAJ)

✓ **Articles in category C journals**

11. Racoviță S., Sprincean M., Moșin V., Hadjiu S., Barbova N., Halabudenco E., Mișina A., Aamoilenco T., Chesov E., Guțuleac R., Revenco N. Clinical Polymorphism and Cytogenetic Variation in Male Infertility Caused by Klinefelter Syndrome. *Bulletin of the Academy of Sciences, Medical Sciences*. 2018;1(58):44-48. ISSN 1857-0011.

12. Racoviță S., Capcelea S., Moșin V., Halabundenco E., Mișina A., Samoilenco T., Hadjiu S., Sprincean M. Chromosomal variations in infertile men. *Bulletin of the Academy of Sciences, Medical Sciences*. 2020;3(67):78-82. ISSN 1857-0011.

- **Articles in the proceedings of scientific conferences:**

✓ **national with international participation**

13. Racoviță A., Sprincean M., Moșin V., Capcelea S., Hadjiu S., Revenco N. 45,X/46,XY in a male with infertility: a clinical case report. *Materials of the national conference with international participation Biennial Chisinau-Sibiu, 5th edition: "Interdisciplinary management of the child"*. Chisinau: 2022, p. 145-149. ISBN 978-9975-58-274-2.

14. Sprincean, M., Hadjiu, S., Călcîi, C., Lupușor, N., Grîu, C., Feghiu, L., Cuznеț, L., Racoviță, S., Tihai, O., Revenco, N., Groppa, S. Resources to improve the development of children with some genetic pathologies. *Materials of the national conference with international participation Biennial Chisinau-Sibiu, 5th edition: "Interdisciplinary management of the child"*. Chisinau: 2022, p. 51-61.

15. Hadjiu, S., Călcîi, C., Lupușor, N., Feghiu, L., Grîu, C., Cuznеț, L., Racoviță, S., Tihai, O., Sprincean, M., Revenco, N. Clinical-diagnostic peculiarities of mitochondrial encephalopathies. *Materials of the national conference with international participation Biennial Chisinau-Sibiu, 5th edition: "Interdisciplinary management of the child"*. Chisinau: 2022, 66-75.

16. Mariana S., Hadjiu S., Racovița, Tihai O., Popa C., Dumitraș A., Revenco N., Bioethical aspects in medico-genetic consultation. *Proceedings of the International Scientific Conference*, Health, Medicine and Bioethics in contemporary society: inter- and pluridisciplinary studies 5th edition. Chișinău: 2022, p. 185-191.

- **Summaries/abstracts/theses in the works of national and international scientific conferences**

✓ **In international conferences**

1. Racoviță S., Moșin V.; Gorduza EV., Hadjiu S., Chesov E., Revenco N., Sprincean M. Clinical-genetic aspects in male infertility. *Volume of Abstracts, Medical School of Pediatrics*. Romania Iasi: 2018, p. 57.

2. Racoviță S., Revenco N., Hadjiu S., Moșin V., Barbova N., Halabudenco E., Mișina A., Samoilenco T., Sprincean M. Clinical-genetic aspects in Klinefelter Syndrome. *Volume LXVII, Supplement Romanian Journal of Pediatrics.* Bucharest, Romania: 2018, p. 130. ISSN 1454-0398.

3. Racoviţă S., Moşin V., Gorduza EV., Strătilă M., Halabudenco E., Samoilenco T., Mişina A., Sprincean M. Clinical importance of cytogenetic testing in infertile male. *Supplement Romanian Journal of Rare Diseases, 5th Medical Genetics Congress with international participation*. Gura Humorului, Romania: 2018, p 38.
4. Racoviță S., Sprincean M., Halabudenco E., Mișina A., Samoilenco T., Guțuleac R., Chesov E., Revenco N., Moșin V. The cytogenetic study in male infertility. *Proceedings of the International Conference, European Human Genetics Conferece*. Milano, Italia: 2018, p. 107.
5. Tihai O., Sprincean M., Hadjiu S., Eţco L., Halabudenco E., Racoviță S., Barbova N., Bejan N., Secrieru V., Nour V., Revenco N. Prenatal diagnosis in pregnancy with high risk of cerebral congenital malformations. *Volume LXVII, Supplement Romanian Journal of Pediatrics.* Bucharest, Romania: 2018, p. 136. ISSN 1454-0398.
6. Racoviţă S., Moşin V., Capcelea S., Cemortan I., Badan L., Boiciuc K., Gorduza EV., Sprincean M. Cytogenetic and Y chromosome microdeletion testing in infertile males. *Supplement Romanian Journal of Rare Diseases.* Timisoara, Romania: 2019, p. 49.
7. Racoviţă S., Capcelea S., Boiciuc K., Moşin V., Revenco N., Hadjiu S., Sprincean M. Clinical and genetic particularities in male infertility. *Supplement to the journal of Child and Adolescent Neurology and Psychiatry*. Romania: 2019, p. 81-82. ISSN 2344-3405.
8. Hadjiu S., Sprincean M., Lupuşor N., Grîu C., Racoviță S., Cuzneț L., Feghiu L., Egorov V., Calcîi C., Revenco N. Clinical characteristics of Guillain-barre syndrome in children. *Supplement to the journal of Child and Adolescent Neurology and Psychiatry*. Romania: 2019, p. 41-42. ISSN 2344-3405.
9. Sprincean M., Hadjiu S., Racoviță S., Burac N., Lupuşor N., Grîu C., Cuzneț L., Feghiu L., Egorov V., Calcîi C., Revenco N. Clinico genetic aspects in progressive muscular dystrophies. *Supplement to the journal of Child and Adolescent Neurology and Psychiatry*. Romania: 2019, p. 71-72. ISSN 2344-3405.
10. Racoviță S., Moşin V., Sprincean M. Molecular biomarkers of Y chromosome and CFTR gene in infertile males. *Colection of abstracts Biological Markers in Fundamental and Clinical Medicine*. Cehia, Praga: 2019, p. 89. ISN 2570-5911.
11. Racoviță S., Sprincean M. Defects in spermatogenesis of men with Y chromosome microdeletions. *Abstract book of the 8th International Medical Congress for Students and Young Doctors, MedEspera.* Chisinau: 2020, p. 290. ISBN 978-9975-151-11-5.
12. Mironiuc N., Racoviță S. Clinical and cytogenetic variations in male infertility caused by Klinefelter syndrome. *Abstract book of the 8th International Medical Congress for Students and Young Doctors, MedEspera.* Chișinau: 2020, p. 288. ISBN 978-9975-151-11-5.
13. Racovita S., Patrascu A., Capcelea S., Misina A., Samoilenco T., Sprincean M. Cytogenetic study in male infertility associated with azoospermia and severe oligosoospermia. *Abstract book of the XIth International Congress of Geneticists and Breeders.* Republic of Moldova: 2021, p. 32. ISBN 978-9975-933-56-8. DOI: 10.53040/cga11.2021.015.
14. Sprincean M., Hadjiu S., Racovita S., Burac N., Sacara V., Lupusor N., Griu C., Revenco N. Clinical-genetic particularities of progressive muscular dystrophies in children. *Abstract book of the XIth International Congress of Geneticists and Breeders.* Republic of Moldova: 2021. p. 62, ISBN 978-9975-933-56-8. DOI: 10.53040/cga11.2021.001.
15. Racoviță S., Moşin V., Gorduza EV., Sprincean M. Cytogenetic and Y chromosome microdeletion analysis in infertile males with azoospermia. *Supplement Romanian Journal of Laboratory Medicine*. Tîrgu Mures, Romania: 2020, p. 56. ISSN 1841-6624. (**IF: 0,945)**
16. Sprincean M., Hadjiu S., Racoviță S., Halabundeco E., Revenco N. Prenatal diagnosis in pregnacies with high genetic risk. *Supplement Romanian Journal of Laboratory Medicine*. Tîrgu Mures, Romania: 2020, p. 57. ISSN 1841-6624. (**IF: 0,945)**
17. Racoviță S., Moşin V., Capcelea S., Boiciuc K., Sprincian M. Molecular genetic method for detecting Y chromosome microdeletions in male infertility. *Puplicatia in Catalogue of Posters the 13t h Edition of Euroinvent European Exhibition of creativity and inovation*. Romania: 2021, p. 141.
18. Sprincean M., Hadjiu S., Calcii C., Lupusor N., Racovita S., Griu C., Feghiu L., Cuznet L., Revenco N., Groppa S. Immunoenzymatic expressivity of interleukin-6 in pediatric ischemic stroke. *Supplement of the European Stroke Journal.* 2021, p. 396. DOI: 10.1177/23969873211034932.
19. Racoviță S., Moşin V., Capcela S., Gorduza EV., Sprincean M. Semen quality of male partners of infertile couples in Republic of Moldova. Supplement Romanian Journal of Laboratory Medicine. Brasov Romania: 2022, p. 91-92. ISSN 1841-6624. (**IF: 0,945**)
20. Racovita S., Sprincian M., Hadjiu S. Neurological impairment and cytogenetic variations in Klinefelter Syndrome. *Abstracts Book of the 8th Congress of the European Academy of Neurology*. Vienna, Austria: 2022, p. 906. ISSN 1468-1331.
21. Racoviță S., Sprincean M., Moșin V., Hadjiu S., Revenco N. Genetic variations in male infertility. *Supliment la revista de Neurologie şi Psihiatrie a Copilului şi adolescentului.* Romania: 2022, p. 123. ISSN 2344-3405.
22. Racoviță S., Moșin V., Hadjiu S., Revenco N., Sprincean M. Clinical and cytogenetic polymorphism in Klinefelter Syndrome. Russian Pediatric Journal: Rusia, 2022, p. 368. eISSN: 2687-0843

✓ **in the proceedings of national scientific conferences with international participation**

23. Racoviță S., Moșin V., Capcelea S., Mișina A., Cemortan I., Sprincean M. Cytogenetic analysis in men with azoospermia. *Abstract book, The congress dedicated to the 75th anniversary of the founding of the Nicolae Testemițanu SUMPh. Chisinau:* 2020, p. 56.
24. Racoviță S., Moșin V., Hadjiu S., Mișina A., Sprincian M. Aspecte neurogenetice la bărbații infertili cu Sindromul Klinefelter. *Abstract book, The congress dedicated to the 75th anniversary of the founding of the Nicolae Testemițanu SUMPh*. Chisinu: 2020, p. 550.
25. Racoviță S., Moșin v., Hadjiu S., Revenco N., Calcii C., Cuznet L., Griu C., Feghiu L., Lupusor N., Sprincean M. Neurogenetic aspects in men with Klinefelter's syndrome. *Neuro Congres Issue, Moldovan Medical Journal*. Chisinău: 2021. p. 55. ISSN 2537-6373.
26. Feghiu L., Hadjiu S., Calcii C., Sprincean M., Lupusor N., Griu C., Cuznet L., Racoviță S., Groppa S. Drug-resistant epilepsies in children: clinical case. *Neuro Congres Issue, Moldovan Medical Journal.* Chisinău: 2021, p. 40. ISSN 2537-6373.

27. Mariana S., Hadjiu S., Lupusor N., Griu C., Cuznet L., Racovita S., Feghiu L., Calcii C., Revenco N., Groppa S. Immunoenzymatic changes in ischemic stroke in children. *Neuro Congres Issue, Moldovan Medical Journal.* Chisinău: 2021, p. 25. ISSN 2537-6373

28. Racoviță S., Moșin V., Capcelea S., Mișina A., Sprincian M., Clinical and genetic study in male infertility with azoospermia. *Abstract book, Biomedical and Health Research: Quality, Excellence and Performance Conference*. Chisinau: 2021, p. 393. ISBN 978-9975-82-223-7.

29. Racoviță S., Moșin V., Capcelea S., Sprincean M. Clinical and genetic evaluation in male infertility. *Collection of Abstracts, Biomedical and Health Research: Quality, Excellence and Performance Conference.* Chisinau: 2022, p. 12.

30. Racoviţă S., Moşin V., Sprincean M. Analysis of Y chromosome microdeletions and CFTR gene mutations as genetic markers in male infertility. *Abstract book, the National Conference with International Participation Life sciences in the dialogue of generations.* Chisinau: 2022, p 139.

- **Invention patents, materials at the invention salons**

✓ **Patent of inventions**

1. Racoviţă S., Moşin V., Capcelea S., Boiciuc K., Sprincean M. Molecular-genetic method for detecting microdeletions of the Y chromosome in male infertility, Invention Patent MD 1489 BI. In: *Official intellectual property bulletin no.1*/2021. 31.01.2021, pp. 58-59.

✓ **Innovative certificates:**

2. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome DBY gene mutation in male infertility.* No. 473 of 22.06.2020 granted in accordance with art. 16 of Law no. 138-XV of 10.05.2001.
3. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome DYS 209 gene mutation in male infertility.* No. 474 of 22.06.2020 granted in accordance with art. 16 of Law no. 138-XV of 10.05.2001.
4. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome DYS 237 gene mutation in male infertility.* No. 475 din 22.06.2020 granted in accordance with art. 16 of Law no. 138-XV of 10.05.2001.
5. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome DYS 241 gene mutation in male infertility.* No. 476 din 22.06.2020 granted in accordance with art. 16 of Law no. 138-XV of 10.05.2001.
6. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome RBMY1 gene mutation in male infertility.* No. 477 din 22.06.2020 granted in accordance with art. 16 of Law no. 138-XV of 10.05.2001.
7. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome USP9Y gene mutation in male infertility.* No. 478 din 22.06.2020 granted in accordance with art. 16 of Law no. 138-XV of 10.05.2001.
8. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome DBY gene mutation in male infertility.* No. 5770 from 23.06.2020, registred at Nicolae Testemițanu SUMPh.
9. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome DYS 209 gene mutation in male infertility.* No. 5771 from 23.06.2020, registred at Nicolae Testemițanu SUMPh.
10. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome DYS 237 gene mutation in male infertility.* No. 5772 from 23.06.2020, registred at Nicolae Testemițanu SUMPh.
11. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome DYS 241 gene mutation in male infertility.* No. 5773 from 23.06.2020, registred at Nicolae Testemițanu SUMPh.
12. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome RBMY1 gene mutation in male infertility.* No. 5774 from 23.06.2020, registred at Nicolae Testemițanu SUMPh.
13. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome USP9Y gene mutation in male infertility. No.* 5775 from 23.06.2020, registred at Nicolae Testemițanu SUMPh.

✓ **Implementing acts:**

1. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome DBY gene mutation in male infertility.* No. 44 from 22.06.2020.
2. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome DYS 209 gene mutation in male infertility.* No. 45 from 22.06.2020.
3. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome DYS 237 gene mutation in male infertility.* No. 46 from 22.06.2020.
4. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome DYS 241 gene mutation in male infertility.* No. 47 from 22.06.2020.
5. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome RBMY1 gene mutation in male infertility.* No. 48 from 22.06.2020.
6. Racoviță S., Sprincean M., Moșin V., Capcelea S., Sacara V., Boiciuc C. *Molecular-genetic method for detection of Y chromosome USP9Y gene mutation in male infertility.* No. 49 from 22.06.2020.

- **Participation with communications at scientific forums:**

✓ **international:**

1. Racoviță S., Moșin V., Gorduza EV., Hadjiu S., Chesov E., Revenco N., Sprincean M. Clinical-genetic aspects in male infertility. *Pediatric Medical School program with international participation, edition VI*. Iasi, Romania: May 15-17, 2018, p. 15.
2. Racoviță S., Moșin V., Gorduza EV., Strătilă M., Halabudenco E., Samoilenco T., Mișina A., Sprincean M. Clinical importance of cytogenetic testing in infertile male. *Program of the 5th Congress of Medical Genetics with international participation.* Gura Humorului, Romania: september 26-28, 2018, p. 7.

3. Racoviţă S., Capcelea S., Boiciuc K., Moşin V., Revenco N., Hadjiu S., Sprincean M., Clinical-genetic peculiarities in male infertility. *Program of the 20th SNPCAR Congress Society of Child and Adolescent Neurology and Psychiatry*, Romania: 18-21 september, 2019, p. 23.
4. Racoviță S., Sprincean M. Defects in spermatogenesis of men with y chromosome microdeletions. *MedEspera The 8th International Medical Congress for Students and Young Doctors*. Chisinau, Republic of Moldova: 24-26 septembrie, 2020.
5. Racoviță S. Clinical peculiarities and cytogenetic variations in Klinefelter Syndrome. *The 21st SNPCAR congress and the 43rd conference of neurology - child and adolescent psychiatry and associated professions with international participation*. Romania: 22-25 september, 2021.
6. Racoviță S., Veaceslav M., Capcela S., Gorduza EV., Sprincean M. Semen quality of male partners of infertile couples in Republic of Moldova. *The 13th National Conference of the Romanian Association of Laboratory Medicine, with international participation.* Brasov, Romania: 25-27 may, 2022, p. 13.
7. Racoviţă S., Sprincean M., Hadjiu S., Revenco N. Genetic variations in male infertility. *XXII SNPCAR congress and 44th conference of neurology - child and adolescent psychiatry and associated professions with international participation*. Romania: 21-24 september 2022, p.12.

Invited speaker

8. Racoviţă S., Moşin V., Gorduza EV, Sprincean M. Cytogenetic and Y chromosome microdeletion analysis in infertile males with azoospermia. *A-IV National Conference of the Association of Laboratory Medicine from Romania with International Participation.* Tîrgu-Mures, Romania: 9-11 september, 2020, p. 15.

✓ **at national conferences with international participation**

9. Racoviţă S., Moşin V., Capcelea S., Ponetenco D., Boiciuc K., Sprincean M. Male 46,XX, clinical case report. Biennial national conference with international participation Chisinau Sibiu, 3rd edition, interdisciplinary in pediatric infectious diseases. Chisinau: 16-18 may 2019, p. 3.
10. Racoviță S., Moșin V., Hadjiu S., Mișina A., Sprincean M. Neurogenetic aspects in Klinefelter Syndrome. Congress Dedicated to the 75th anniversary of the founding of Nicolae Testemițanu SUMPh. Chisinau: 21-23 octomber 2020, p. 35.
11. Racoviță S. Neurogenetic aspects in Klinefelter's syndrome in men. In program: *The 7th congress of the society of neurologists of the Republic of Moldova with international participation*. Chisinau: 16-18 september 2021, p.9.
12. Racoviță S. Peculiarities of clinical polymorphism and cytogenetic variations in Klinefelter syndrome. *International Conference on Pediatrics "Current Affairs in Pediatric Practice: Challenges and Successes"*. Kishinev: 16 september 2022, p.3.

✓ **national:**

13. Racoviță S. Identification of microdeletions in the Y chromosome by multiplex PCR. *Annual Scientific Conference Section No.1, Fundamental Problems of Medicine, Nicolae Testemițanu SUMPh*. Chisinau: 18 october 2018, p. 7.
14. Racoviță S. Clinical-genetic peculiarities in men with azoospermia. *Annual Scientific Conference Section No.1, Fundamental Problems of Medicine, Nicolae Testemițanu SUMPh*. Chisinau: 17 october 2019, p.7.
15. Racoviță S. Clinical and genetic evaluation in male infertility. Annual Scientific Conference Section No.1, Normal and Pathological Morphology, *Nicolae Testemițanu SUMPh*. Chisinau: 20 october 2022, p.2.

- **Posters at scientific conferences**

✓ **with international participation**

1. Racoviță S., Revenco N., Hadjiu S., Moșin V., Barbova N., Halabudenco E., Mișina A., Samoilenco T., Sprincean M. Clinical-genetic aspects in Klinefelter Syndrome. *National Pediatrics Conference 2018 – Current Affairs in Pediatrics.* Bucharest Romania: 22-24 march, 2018. P085
2. Racoviță S., Sprincean M., Halabudenco E., Mișina A., Samoilenco T., Guțuleac R., Chesov E., Revenco N., Moșin V. The cytogenetic study in male infertility. *International conference, European Human Genetics Conferece.* Milano Italia: 16-19 june, 2018. E-P01.07
3. Racoviţă S., Moşin V., Capcelea S., Cemortan I., Badan L., Boiciuc K., Gorduza EV., Sprincean M. Cytogenetic and Y chromosome microdeletion testing in infertile males. *Conference of the Romanian Society of Medical Genetics*. Timisoara, Romania: 18-20 september 2019.
4. Racoviță S., Sprincean M. Defects in spermatogenesis of men with Y chromosome microdeletions. *MedEspera The 8th International Medical Congress for Students and Young Doctors.* Chisinau, Republic of Moldova: 24-26 september 2020.
5. Nicoleta Mironiuc, **Racoviță S.** Clinical and cytogenetic variations in male infertility caused by Klinefelter syndrome. *MedEspera The 8th International Medical Congress for Students and Young Doctors.* Chisinau, Republic of Moldova: 24-26 september 2020.
6. Racoviță S., Moșin V., Capcelea S., Boiciuc K., Sprincean M. Molecular genetic method for detecting Y chromosome microdeletions in male infertility. *The 13t h Edition of Euroinvent European Exhibition of creativity and inovation*. Iasi, Romania, 22 may 2021.
7. Racoviță S., Sprincian M., Hadjiu S. Neurological impairment and cytogenetic variations in Klinefelter Syndrome. Congress of the European Academy of Neurology. Vienna, Austria: 25-26 june 2022. EPV-613

✓ **national with international participation**

8. Racoviță S., Moșin V., Capcelea S., Ponetenco D., Boiciuc K., Sprincean M. Male 46,XX, clinical case report. *Biennial national conference with international participation Chisinau Sibiu, 3rd edition, interdisciplinary in pediatric infectious diseases*. Chisinau: 16-18 may 2019.
9. Racoviță S., Moșin V., Hadjiu S., Mișina A., Sprincean M. Neurogenetic aspects in Klinefelter Syndrome. *The Congress dedicated to the 75th anniversary of the foundation Nicolae Testemițanu SUMPh*. Chisinău: 21-23 octomber 2020, p. 48.
10. Racoviță S., Moșin V., Capcelea S., Mișina A., Cemortan I., Sprincean M. Cytogenetic investigations in men with azoospermia. *The Congress dedicated to the 75th anniversary of the foundation Nicolae Testemițanu SUMPh*. Chisinău: 21-23 octomber 2020, p. 22.
11. Racoviță S., Moșin V., Capcelea S., Mișina A., Sprincean M. Clinical-genetic study in male infertility with azoospermia. The scientific conference dedicated to the 76th anniversary of its founding *Nicolae Testemițanu SUMPh*. Chisinau: 20-22 octomber 2021, p. 40.