

## ORIGINAL PAPER



## Current opportunities and new horizons into the genetic study of infertility

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### Abstract

**Introduction:** An estimated 12.5% of couples experiencing fertility problems and almost 12% of reproductive age women have turned to health services at least once due to infertility. First trimester miscarriage is the most common clinical manifestation of infertility associated with a genetic cause. **Patients, Materials and Methods:** The scientific research was conducted at A.S. Medical Center in Bucharest, Romania, between January 2016 and December 2018, on a representative group of 1264 Caucasian patients diagnosed with infertility, from which the study group was selected, consisting of 273 patients who were further genetically investigated. **Results:** Chromosomal instability, identified in 14% of patients, has been encountered most frequently in women (7%), and least often in fetuses (2%), unlike other chromosomal anomalies, identified in 55% of patients, which were more common in fetuses (27%) and least frequently in men (9%). Recurrent pregnancy loss due to genetic causes was identified in 53% of cases, being determined by chromosomal instability in 16% of cases and by other chromosomal anomalies in 37% of cases. Infertility due to a genetic cause was identified in 83% of cases, being determined by chromosomal instability in 17% of cases and by other chromosomal anomalies encountered in 66% of cases. In genetic risk pregnancies in evolution, fetal chromosomal anomalies were detected in 94% of cases, the most frequent being aneuploidy and polyploidy. Cytogenetic studies carried out on tissue fragments taken from aborted products of conception revealed the presence of a genetic cause in 57% of cases, an abnormal chromosome number being the most common (36%). The analysis of microdeletions of the long arm of the Y chromosome indicated that 5.5% of men with infertility are affected by this condition. **Conclusions:** Although genetic tests are considered complex and expensive laboratory investigations, they are crucial in identifying the etiology of over 40% of infertility cases associated with genetic factors, as well as in the correct and effective management of infertility.

**Keywords:** infertility, miscarriage, high risk pregnancy, chromosomal instability, chromosomal anomalies, microdeletion.

### Introduction

Infertility is defined as the inability to conceive after 12 months of regular and unprotected intercourse for women under the age of 35 or after six months in case of women over 35 years, the partner's age not being taken into consideration. Conversely, the existence of recurrent miscarriages and/or multiple stillbirths or nonviable fetuses are also regarded as infertility [1–3].

It is estimated that approximately 12.5% of reproductive age couples experience fertility issues and about 12% of women have addressed to health care specialists at least once to seek fertility evaluation and management.

One common manifestation of infertility is early pregnancy loss. Establishing the cause of miscarriage is possible in almost 2/3 of the cases [4]. These causes can be mainly genetic (chromosomal abnormalities of the embryo, numerical or structural) or maternal (chronic diseases or gynecological disorders) [5, 6].

The causes of infertility can be unraveled in about 90% of cases, notwithstanding that multiple investigations are performed, about 10% of couples will never find out why they cannot conceive naturally [7, 8]. It is roughly estimated that one third of all cases of infertility is due to the female partner, another third is due to the male partner, and the remaining third is attributed to both partners, cases of mixed causes, or unexplained infertility [9, 10].

The major cause of early pregnancy loss is in all likelihood genetic, given that over 50% of miscarriages during the first trimester of pregnancy are associated with the presence of chromosomal abnormalities of the fetus [11, 12].

Chromosomal abnormalities are common even among the gamete stage [13]. According to data from the literature, 10% of sperm cells and 25% of oocytes have a chromosomal abnormality [14].

Approximately 10–20% of clinical pregnancies are miscarried, with most losses occurring between the 7<sup>th</sup> and the 11<sup>th</sup> week of gestation [15]. After the 8<sup>th</sup> week of pregnancy, only 3.2% of pregnancies are lost, and after the 11<sup>th</sup> week, only 1% of fetuses are spontaneously aborted [16]. In case of late miscarriages, after 20 weeks of pregnancy, 5% of fetuses are cytogenetically abnormal.

The study of beta-human chorionic gonadotropin ( $\beta$ -hCG) hormone values in large groups of women who ovulate spontaneously, indicating the existence of pregnancy, revealed that the rate of early, menstrual abortion is very high. Thus, out of the total number of pregnancies diagnosed by  $\beta$ -hCG hormone testing, 13–14% were certainly miscarried during the first weeks of gestation, but, in all probability, it seems that down to 80% of these embryos would have been aborted at some time during gestation [17]. Most embryos aborted early in the first trimester appear morphologically unaffected, but have various chromosomal abnormalities, of number or structure, such as aneuploidies (trisomy, monosomy) or chromosomal translocations, and amidst embryos with morphological abnormalities, 90% have a chromosomal abnormality [18, 19].

### Aim

The scientific objective of this research was the study of the genetic heterogeneity of chromosomal abnormalities highlighted in patients diagnosed with infertility, recurrent miscarriages of unknown etiology, oligo/azoospermia or oligo-astheno-teratospermia, including the study of micro-deletions of the long arm of the Y chromosome in men diagnosed with infertility, as well as the identification and evaluation of genetic heterogeneity amongst fetal chromosomal abnormalities in high-risk pregnancies in evolution and tissue fragments taken from aborted products of conception.

### ☒ Patients, Materials and Methods

The scientific research was conducted at A.S. Medical Center in Bucharest, Romania, from January 2016 till December 2018, on a representative cluster of 1264 Caucasian patients diagnosed with infertility who presented at the Clinic for interdisciplinary consultation.

A signed informed consent document was obtained from the patients and the study was performed according to the Declaration of Helsinki and the ethical guidelines of A.S. Medical Center in Bucharest.

From this cohort, the study group was selected, consisting of 273 patients with infertility who were furthermore genetically investigated.

The patients from the study group were referred to the Clinic for the following reasons: a history of recurrent miscarriage of unknown etiology, a history of fetal malformations or stillbirths, genetic risk pregnancies in evolution, and the inability to conceive in at least one member of the couple.

The data regarding the particularities of the study group were recorded in the patient's medical files and in the Clinic Archive, from which we selected: age and gender, the grounds for the genetic investigation, the type of biological product analyzed, and the result obtained from its genetic tests. These data were statistically computerized using Microsoft Excel.

Peripheral blood karyotype was performed for the study of parental chromosomes. Approximately 2–5 mL of peripheral blood was drawn on anticoagulant (Sodium Heparin) from the elbow fold, in aseptic conditions.

According to the protocol for peripheral blood lymphocytes culture, we used 1 mL of blood sample, added in a mixture of 7 mL of Gibco RPMI-1640 (GIBCO-BRL/Life Technologies, Rockville, MD, USA) growth medium, 100 mg/mL solution of 0.01 mL Penicillin, and 0.02 mL Streptomycin, 0.002 M solution of 0.1 mL L-Glutamine and 0.1 mL Phytohemagglutinin M, incubated for 72 hours at 37°C.

Into the following steps, in the peripheral blood lymphocytes culture, we added Gibco KaryoMAX Colcemid 10  $\mu$ g/mL solution, 0.075 M Potassium Chloride (KCl) solution for hypotonic shock and a mixture of one part of glacial Acetic Acid and three parts of Methanol, as a fixing solution, after which the chromosomes were highlighted by Giemsa staining with 2% Giemsa solution and identified by microscopic examination.

Fetal cells were withdrawn either by amniocentesis or by chorionic villus sampling to obtain the fetal karyotype.

The amniotic cell cultures and their subsequent cytogenetic analysis require a period of time between 10 and 14 days, while fetal cells are cultured on Gibco Amnio MAX medium (GIBCO-BRL/Life Technologies, Rockville, MD, USA) are carefully examined at the microscope and then cytogenetically studied.

When chorionic villus sampling is carried out, the karyotype can be determined in two–three days, because chorionic villi are a rapidly multiplying tissue, with enough cells in division. The main advantage of this test over amniocentesis is that the prenatal cytogenetic diagnosis is made in the first trimester of pregnancy, which gives the couple the opportunity and time required to analyze their options earlier, in the event of an abnormal cytogenetic result.

To determine the etiology of a miscarriage, the cytogenetic study of tissue fragments taken from aborted products of conception was performed. The tissue samples were obtained using a sterile technique, the sample being immediately delivered, under appropriate conditions, to the cytogenetics laboratory, otherwise, the success of the cell culture would have been compromised. Frozen or Formalin-preserved tissue fragments cannot be used for cell cultures.

For the analysis of microdeletions of the long arm of the Y chromosome in infertile men, the peripheral blood sample collected by venipuncture on anticoagulant [Ethylenediaminetetraacetic acid (EDTA)] was processed for genomic deoxyribonucleic acid (DNA) extraction using the Promega Wizard Genomic DNA Purification Kits (Promega Corporation, WI, USA).

The DNA sample obtained was used as a template in the multiplex polymerase chain reaction (PCR) amplification reaction, which performs the specific amplification of some target regions of the Y chromosome structure, called sequence-tagged site (or STS, a short region sequence of high specificity at cellular DNA level), using the Promega Y Chromosome Deletion Detection System kit (Promega Corporation, WI, USA).

The Y Chromosome Deletion Detection System is a quick method for the detection of specific regions from the human Y chromosome. This system allows the identification

of deletions located on the long arm of the Y chromosome in the azoospermia factor (AZF) region, the system comprising of 20 pairs of primers, homologous to the STSs, identified and mapped.

During the PCR, primers amplify the short segments of non-polymorphic DNA from the Y chromosome, being combined into five sets to be used in multiple PCRs, making it possible to determine (the presence or absence) all 20 sequence-tagged sites using five PCR amplifications at the same time.

Deletions located on the long arm of the Y chromosome amplified by these sets of primers have been associated with male infertility. The Y Chromosome Deletion Detection System covers all loci recommended by the *European Academy of Andrology* (EAA), is certified and scientifically endorsed by the *European Quality Monitoring Network Group*.

To rule out the possibility of a false negative result, the patient's amplified DNA sample was processed in parallel with a negative control of a healthy man (with Y chromosome morphologically normal, without deletions) using the same analysis conditions.

After amplification by PCR, the samples were separated on 4% Agarose gel and examined after Ethidium Bromide staining and finally, the result was confirmed by comparative analysis of the sample and the control probe.

## Results

The study group consisting of 273 patients diagnosed with infertility and genetically investigated, included 201 (74%) women and 72 (26%) men, of whom 34 were couples.

Regarding the distribution by age, most infertile patients belonged to the age ranges 26–30 years (93 patients, 34% of cases), and 31–35 years (93 patients, 34% of cases), respectively. The percentage of patients assigned between 36–40 years (68 patients, 25% of cases) was not negligible at all, which indicates that the age at which pregnancy is desired, or children are conceived is utterly advanced, this alone being a risk factor for the fetus.

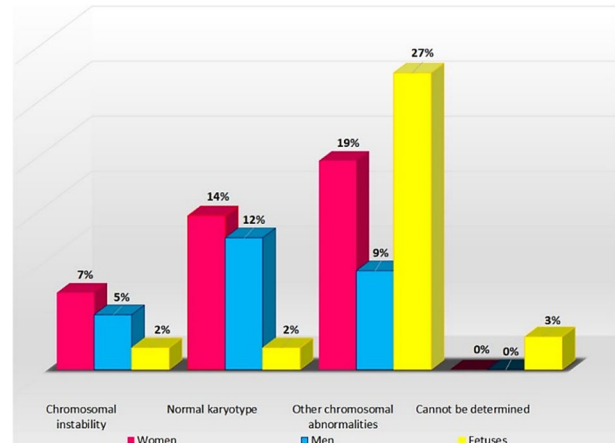
Furthermore, we ascertained that most women were cytogenetically investigated between 26 and 30 years (74 patients, 27% of cases), while most men between 31 and 35 years (35 patients, 13% of cases). The largest discrepancy between the number of women (74 patients, 27% of cases) and the number of men (19 patients, 7% of cases) genetically investigated was found in the age group 26–30 years, a discrepancy that could be explained by a greater willingness of women to have children at this age.

The most frequent biological product taken for genetic testing was peripheral blood (182 cases, 67% of patients), which emphasizes that in case of infertility most cytogenetic investigations begin with the couple itself, and the fewest biological product was tissue samples taken from aborted products of conception (10 cases, 4% of patients). To establish the fetal karyotype, the most frequently used technique for fetal cell sampling was amniotic fluid extraction by amniocentesis (67 cases, 24% of patients), followed by chorionic villus sampling (14 cases, 5% of patients).

Cytogenetic investigations were performed more often in female patients (110 cases, 40% of patients), and the fewest were performed on tissue samples taken from

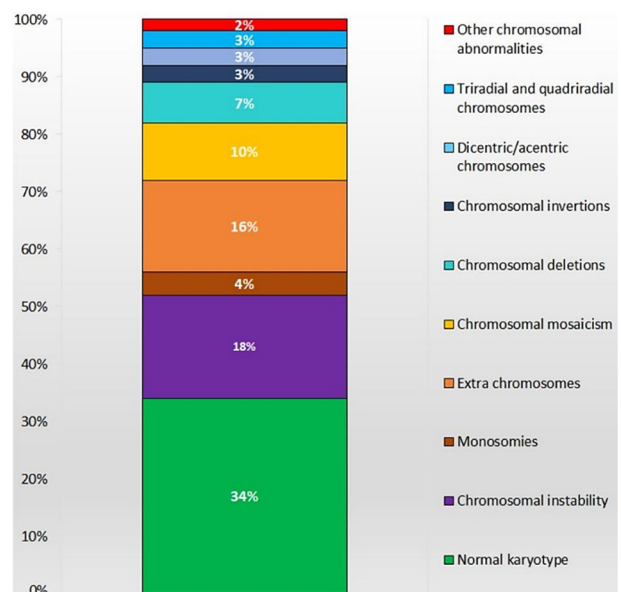
aborted products of conception (21 cases, 8% of patients), although the most common reason for genetic testing was a history of recurrent miscarriages. Also, 72 (26%) men and 70 (26%) fetuses were studied cytogenetically.

The results of cytogenetic investigations of the study group revealed that chromosomal instability was identified in 38 (14%) patients, most frequently in women (7%) and least frequently in fetuses (2%), while other chromosomal anomalies, such as multiple chromosomal aberrations, identified in 150 (55%) patients, were most frequently found in fetuses (27%) and least frequently in men (9%). Normal karyotype was identified in only 76 (28%) patients, most frequently in women (14%) and least frequently in fetuses (2%) (Figure 1).



**Figure 1 – Results of cytogenetic investigations in the study group.**

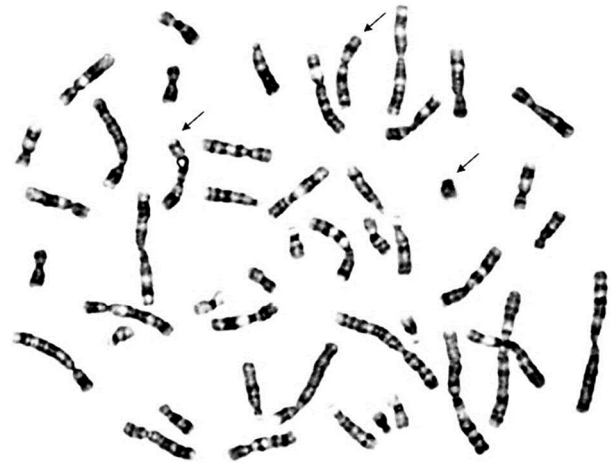
Cytogenetic investigations performed on women indicated that chromosomal instability (19 patients, 18% of cases) and supernumerary chromosomes (18 patients, 16% of cases) are the most commonly encountered cytogenetic abnormalities, whilst structural chromosomal abnormalities (deletions, inversions, dicentric/acentric chromosomes, and triradial/quadriradial chromosomes) were most rarely highlighted (Figures 2 and 3). Normal karyotype was identified in 38 (34%) patients.



**Figure 2 – Results of cytogenetic investigations of women in the study group.**



**Figure 3 – Karyotype of female with 47,XXX chromosomal constitution. Tissue: peripheral blood; GTG banding (banding score >6, 550 bands); Slide: F1-2, metaphase 2. GTG: Giemsa–Trypsin–Giemsa.**



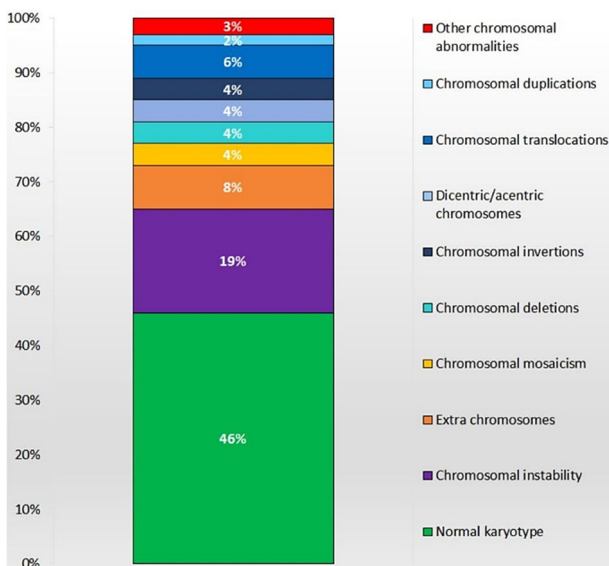
**Figure 5 – Metaphase plate obtained from culture of human blood cells. Cytogenetic analysis identifies: XXY trisomy. Tissue: peripheral blood; GTG banding (banding score >6, 550 bands); Slide: F2-3, metaphase 2. GTG: Giemsa–Trypsin–Giemsa.**

Cytogenetic investigations carried out on male patients showed that chromosomal instability (14 patients, 19% of cases) was the most common cytogenetic abnormality, and normal karyotype was also more frequent in men (33 patients, 46% of cases) than in women (38 patients, 34% of cases) (Figure 4). Extra chromosomes were identified in six (8%) patients, whilst structural chromosome abnormalities (translocations, deletions, inversions, and duplications) were most rarely highlighted (Figures 5 and 6).

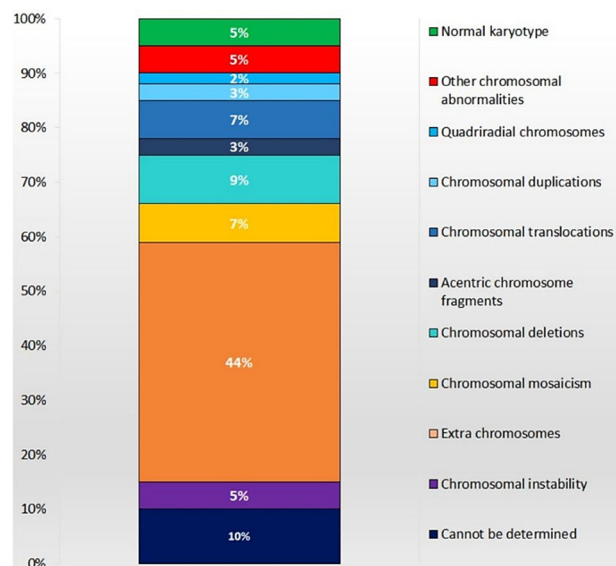
Regarding fetuses, cytogenetic investigations have shown that the most common fetal chromosomal abnormalities were extra chromosomes (27 patients, 44% of cases), aneuploidy, and polyploidy (Figures 7–10). Structural chromosome abnormalities (deletions, translocations, inversions, acentric chromosomes, duplications, and quadriradial chromosomes) were identified in 15 (24%) patients, whilst chromosomal instability and other chromosomal abnormalities (ring chromosomes and dicentric chromosomes) were identified in only three (5%) patients (Figure 11). Also, normal karyotype was identified in three (5%) cases.



**Figure 6 – Karyotype of male with 47,XXY chromosomal constitution. Tissue: peripheral blood; GTG banding (banding score >6, 550 bands); Slide: F2-3, metaphase 2. GTG: Giemsa–Trypsin–Giemsa.**



**Figure 4 – Results of cytogenetic investigations of men in the study group.**



**Figure 7 – Results of cytogenetic investigations of fetuses in the study group.**



**Figure 8 – Metaphase plate obtained from amniotic cell culture. Cytogenetic analysis identifies polyploidy (triploidy); Tissue: amniotic fluid; RHG banding (score >4); Slide: F1-1, metaphase 1. RHG: R-bands after heat denaturation and Giemsa.**



**Figure 11 – Metaphase plate obtained from amniotic cell culture. Cytogenetic analysis identifies ring chromosome and acentric chromosome fragments. Tissue: amniotic fluid; G banding; Slide: 3; Cell: 2.**



**Figure 9 – Karyotype of fetus with 69,XXX chromosomal constitution. Tissue: amniotic fluid; RHG banding (score >4); Slide: F1-1, metaphase 2. RHG: R-bands after heat denaturation and Giemsa.**

The results of cytogenetic investigations of patients with a prior history of recurrent miscarriages have shown that repeated miscarriages genetically linked constitute the majority, being brought upon by chromosomal instability in 22 (16%) patients and by other chromosomal anomalies in 50 (37%) patients (Figures 12 and 13). Among them, in 56 (41%) cases the karyotype was normal, and in eight (6%) cases it could not be performed.

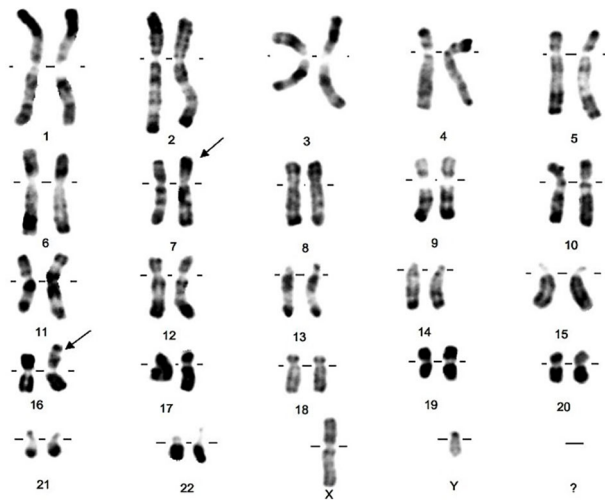
The results of cytogenetic investigations showed that infertility attributed to genetics was recorded in 30 (83%) patients, being determined by chromosomal instability highlighted in six (17%) patients, and by other chromosomal abnormalities present in 24 (66%) patients (Figures 14 and 15). Normal karyotype was identified in only six (17%) patients. Note that among the cases of infertility patients with no history of prior pregnancy were included, but men investigated for oligo/azoospermia and oligo-asthenoteratospermia were not included.



**Figure 10 – Metaphase plate obtained from amniotic cell culture. Cytogenetic analysis identifies polyploidy (tetraploidy), dicentric chromosomes, deletions, acentric chromosome fragments, and chromosomal translocation; Tissue: amniotic fluid; G banding; Slide: 2; Cell: 5.**



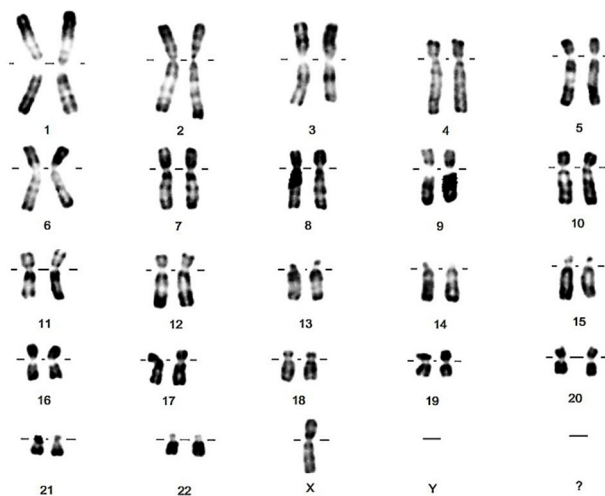
**Figure 12 – Metaphase plate obtained from culture of human blood cells. Cytogenetic analysis identifies reciprocal translocations between two non-homologous chromosomes t(7;16); Tissue: peripheral blood; GTG banding (banding score >6, 550 bands); Slide: F1-5, metaphase 1. GTG: Giemsa–Trypsin–Giemsa.**



**Figure 13** – Karyotype of male with reciprocal translocation  $t(7;16)$ :  $46,XY,t(7;16)(p14;p11.1)$ . Tissue: peripheral blood; GTG banding (banding score  $>6$ , 550 bands); Slide: F1-5, metaphase 1. GTG: Giemsa–Trypsin–Giemsa.



**Figure 14** – Metaphase plates obtained from culture of human blood cells. Cytogenetic analysis identifies monosomy X. Tissue: peripheral blood; GTG banding (banding score  $>6$ , 550 bands); Slide: F1-3, metaphase 3. GTG: Giemsa–Trypsin–Giemsa.

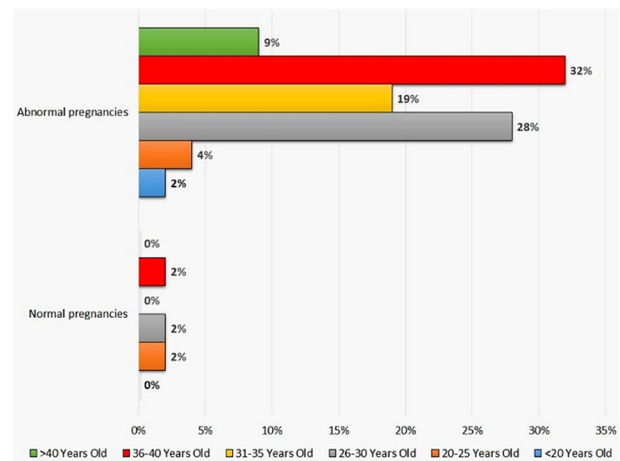


**Figure 15** – Karyotype of female with  $45,X$  chromosomal constitution. Tissue: peripheral blood; GTG banding (banding score  $>6$ , 550 bands); Slide: F1-3, metaphase 3. GTG: Giemsa–Trypsin–Giemsa.

The results of cytogenetic investigations of high-risk pregnancies in evolution indicated the presence of fetal chromosomal abnormalities in most patients (68 cases, 94% of patients), especially those belonging to the age group 36–40 years (23 cases, 32% of patients), which proves once again that advanced maternal age is an independent risk factor in the appearance of chromosomal abnormalities in offspring (Figures 16–20).

The results of cytogenetic investigations carried out on tissue samples taken from aborted products of conception have identified the presence of a genetic cause in 12 (57%) cases, the chromosomal anomalies most frequently encountered were abnormal chromosome number (six cases, 36% of patients) and less frequent, structural chromosomal abnormalities (two cases, 7% of patients) (Figure 21).

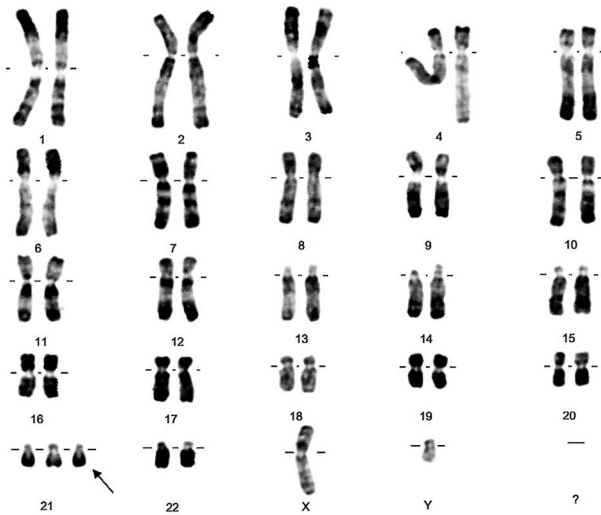
The test results for the determination of microdeletions of the long arm of the Y chromosome performed on patients with oligo/azoospermia and oligo-astheno-teratospermia indicated that 5.5% of men (five cases) with infertility have this microdeletion. At the same time, their cytogenetic analysis indicated the presence of other chromosomal abnormalities associated with microdeletions of the long arm of the Y chromosome.



**Figure 16** – Results of cytogenetic investigations in high-risk pregnancies in evolution according to age group.



**Figure 17** – Metaphase plate obtained from amniotic cell culture. Cytogenetic analysis identifies the presence of a third copy of chromosome 21: trisomy 21. Tissue: amniotic fluid; RHG banding (score  $>4$ ); Slide: F1-1, metaphase 1. RHG: R-bands after heat denaturation and Giemsa.



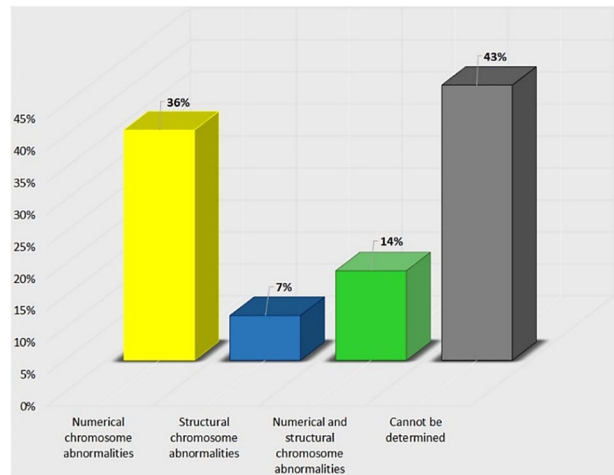
**Figure 18** – Karyotype of male with 47,XY,+21 chromosomal constitution. Tissue: amniotic fluid; RHG banding (score >4); Slide: F1-1, metaphase 1. RHG: R-bands after heat denaturation and Giemsa.



**Figure 19** – Karyotype of male with 46,XY,1q+ chromosomal constitution. Tissue: amniotic fluid; G banding; Slide: 3; Cell: 7. Metaphase plate obtained from the culture of human blood cells. Cytogenetic analysis identifies the presence of additional genetic material in the submetacentric region of one of the two chromosomes in pair 1.



**Figure 20** – Metaphase plate obtained from amniotic cell culture. Cytogenetic analysis identifies chromosomal deletion and acentric chromosome fragments. Tissue: amniotic fluid; G banding; Slide: 1; Cell: 3.



**Figure 21** – Results of cytogenetic investigations of tissue samples taken from aborted products of conception.

**Discussions**

Infertility is a cause for concern for almost 186 million people worldwide [20].

According to the *World Health Organization* (WHO), one in six couples of reproductive age struggles with infertility and the chances to conceive decrease significantly starting with the maternal age of 35, are greatly reduced after 40 years, and nearly 10% of women are infertile or have difficulty in carrying the pregnancy till term [21, 22].

According to the *World Bank*, if in 1990, the fertility rate in Romania was equal to the European average rate, registering a value of 1.96, after that year the fertility rate in Romania decreased, remaining today below the European average. The lowest fertility rate in Romania, 1.27 was registered in 2001–2002, while the average rate in Europe and Central Asia was 1.54–1.56. The latest statistical data for 2019 indicate a fertility rate of 1.63 in Romania, less than the European average rate [23].

The etiology of infertility is based on a variety of causes. There are multifactorial, intricate causes ranging from minor local diseases to complex systemic disorders [24, 25]. Thereby, management and treatment options are very diverse and highly individualized [26, 27].

One of the most common and nowadays frequently encountered cause of infertility is advanced maternal age. It is the condition that is naturally impossible to treat, and which can only be prevented by the decision not to postpone pregnancy, given that any fertile, clinically healthy woman after 35 years has a gradual decline in her ability to conceive due to the decreasing number and quality of oocytes [28, 29].

Cytogenetic analysis allows the identification of chromosomal abnormalities of the couple and assess the extent to which they are involved in infertility, allow detection of the possibility of passing on the chromosomal abnormality to future offspring, especially if members of the couple benefit from an assisted reproductive technique, and assess whether the highlighted chromosomal abnormality is compatible or not with normal growth and development of the future fetus [30–34].

To find out if there are unstable parental chromosomal abnormalities, which can lead to the impossibility of

conceiving or to miscarriage due to a genetic cause, the karyotype of the couple and of the fetus were performed in cases of high-risk pregnancy [35–37].

Analyzing the results from this study, we ascertained that women are primarily genetically investigated for infertility, unlike male patients, which does not necessarily mean that they are more commonly affected by genetic infertility, but that they are more frequently investigated to elucidate as early as possible and to treat as quickly and efficiently as possible the causes of their infertility [38].

Recurrent miscarriages of unknown etiology were more frequently encountered in the age group 31–35 years, but we cannot say that with advancing age the frequency of miscarriages increases, because we do not have enough data regarding the total number of women who become pregnant during this age period [39].

In cases of clinically recognized miscarriages, fetal chromosomal number abnormalities were identified in 43% of cases, structural chromosomal abnormalities in 2% of cases, combined fetal abnormal karyotypes in 54% of cases, and in only 1% of cases fetal chromosomal abnormalities were not detected [16, 40].

Consistent with current literature data, fetal chromosomal number abnormalities were the main genetic anomaly identified on cytogenetic examinations of tissue fragments taken from aborted products of conception, as opposed to structural chromosomal abnormalities that were less frequent [12, 41, 42]. The remaining cytogenetic results could not be compared with those in the literature, because among the patients of the group we studied, no miscarriages with a normal karyotype were registered and the studies presented in the literature did not include the cases in which both types of chromosomal abnormalities were considered.

“Chromosomal instability”, because of cytogenetic examination, involves a change in the amount of genetic material, either by loss or by excess of genetic material, represented by whole chromosomes or fragments of chromosome, which subsequently rearrange into an abnormal shape, thus forming numerous chromosomal abnormalities in number and structure, identified in cell cultures [43].

The result “other chromosomal abnormalities” includes chromosomal deletions, translocations, duplications, inversions, monosomies, trisomies or chromosomal mosaicism, detected on cell cultures as such and not associated with other chromosomal abnormalities [44]. These “other chromosomal abnormalities” might also cause chromosomal instability [45].

Chromosomal instability is associated with numerous degenerative diseases, malignancies, and age-related disorders, being a determining factor in the initiation and progression of the disease [46].

Male infertility, a multifactorial disorder, with an etiology still incompletely elucidated, affects globally about 7% of the total male population. The multiple and various causes of male infertility, both genetic and non-genetic, range from gene mutations, and approximately 2000 genes are involved in spermatogenesis, to systemic diseases and current lifestyle [47, 48].

Genetic studies have shown that some of the infertile men with severe forms of azoospermia or oligospermia

have microdeletions of the long arm of the Y chromosome, without there being a direct and proved correlation between the position and size of the microdeletion and the severity of the disorder [49].

Notwithstanding the number of men genetically investigated to highlight the microdeletions of the long arm of the Y chromosome was relatively low, however, the results obtained which indicate that 5.2% of them have this microdeletion, can be considered to have statistical value approaching the value presented in the literature, where it is estimated that microdeletions of the long arm of the Y chromosome are encountered in approximately 7% of the infertile men [50].

Although worldwide, male infertility is responsible in nearly 50% of cases, infertility still remains a psychosocial burden of the woman [51–54].

Following the dynamics of the investigations carried out to identify the etiology of infertility, we found that the total number of patients investigated between January 2016 and December 2018 gradually increased, and in terms of the number of people genetically investigated, it increased significantly in the last six months. This fact demonstrates that, although genetic tests are expensive laboratory procedures, their benefit in accurately diagnosing genetic infertility is undeniable, as genetic testing plays a crucial role not only in the diagnosis but also in the effective and targeted management of genetic infertility.

## ☒ Conclusions

Infertility, a multifactorial disorder, with great etiopathogenic heterogeneity, registers in over 80% of cases a genetic determinism conditioned both by the presence of chromosomal instability and by other forms of chromosomal abnormalities. In the case of recurrent miscarriages of unknown etiology, the genetic factor is present in over 50% of cases and high-risk pregnancies are associated in over 90% of cases with fetal chromosomal abnormalities. Male infertility, much less genetically investigated compared to female infertility, assumes a genetic etiology objectified by the presence of microdeletions of the long arm of the Y chromosome in 5.5% of cases. Although genetic investigations are complex and expensive laboratory tests, they are crucial in identifying the etiology of over 40% of infertility cases, which proved to have a genetic nature, as also demonstrated by the growing number of patients who request and benefit from the results of genetic tests. The correct and efficient approach to infertility management can only be done through a complex spectrum of investigations, among which genetic investigations are absolutely necessary and mandatory.

## ☒ Conflict of interests

The authors declare that they have no conflict of interests.

## References

- [1] Rochon M. Sterilité et infertilité: deux concepts [Sterility and infertility: two concepts]. *Cah Que Demogr*, 1986, 15(1):27–56. PMID: 12268268
- [2] Tabong PTN, Adongo PB. Understanding the social meaning of infertility and childbearing: a qualitative study of the perception of childbearing and childlessness in Northern Ghana. *PLoS One*, 2013, 8(1):e54429. <https://doi.org/10.1371/journal.pone.0054429> PMID: 23342158 PMCID: PMC3546987



- [3] Taylor A. ABC of subfertility: extent of the problem. *BMJ*, 2003, 327(7412):434–436. <https://doi.org/10.1136/bmj.327.7412.434> PMID: 12933733 PMCID: PMC188498
- [4] Agenor A, Bhattacharya S. Infertility and miscarriage: common pathways in manifestation and management. *Womens Health (Lond)*, 2015, 11(4):527–541. <https://doi.org/10.2217/whe.15.19> PMID: 26238301
- [5] Grossman TB, Chasen ST. Abortion for fetal genetic abnormalities: type of abnormality and gestational age at diagnosis. *AJP Rep*, 2020, 10(1):e87–e92. <https://doi.org/10.1055/s-0040-1705173> PMID: 32190411 PMCID: PMC7075712
- [6] Suzumori N, Sugiura-Ogasawara M. Genetic factors as a cause of miscarriage. *Curr Med Chem*, 2010, 17(29):3431–3437. <https://doi.org/10.2174/092986710793176302> PMID: 20712563
- [7] Quaaas A, Dokras A. Diagnosis and treatment of unexplained infertility. *Rev Obstet Gynecol*, 2008, 1(2):69–76. PMID: 18769664 PMCID: PMC2505167
- [8] Ray A, Shah A, Gudi A, Homburg R. Unexplained infertility: an update and review of practice. *Reprod Biomed Online*, 2012, 24(6):591–602. <https://doi.org/10.1016/j.rbmo.2012.02.021> PMID: 22503948
- [9] Deshpande PS, Gupta ASP. Causes and prevalence of factors causing infertility in a public health facility. *J Hum Reprod Sci*, 2019, 12(4):287–293. [https://doi.org/10.4103/jhrs.JHRS\\_140\\_18](https://doi.org/10.4103/jhrs.JHRS_140_18) PMID: 32038077 PMCID: PMC6937760
- [10] Zargar AH, Wani AI, Masoodi SR, Laway BA, Salahuddin M. Epidemiologic and etiologic aspects of primary infertility in the Kashmir region of India. *Fertil Steril*, 1997, 68(4):637–643. [https://doi.org/10.1016/s0015-0282\(97\)00269-0](https://doi.org/10.1016/s0015-0282(97)00269-0) PMID: 9341602
- [11] Hyde KJ, Schust DJ. Genetic considerations in recurrent pregnancy loss. *Cold Spring Harb Perspect Med*, 2015, 5(3):a023119. <https://doi.org/10.1101/cshperspect.a023119> PMID: 25659378 PMCID: PMC4355257
- [12] Soler A, Morales C, Mademont-Soler I, Margarit E, Borrell A, Borobio V, Muñoz M, Sánchez A. Overview of chromosome abnormalities in first trimester miscarriages: a series of 1,011 consecutive chorionic villi sample karyotypes. *Cytogenet Genome Res*, 2017, 152(2):81–89. <https://doi.org/10.1159/000477707> PMID: 28662500
- [13] Szczygiel M, Kurpisz M. Chromosomal anomalies in human gametes and pre-implantation embryos, and their potential effect on reproduction. *Andrologia*, 2001, 33(5):249–265. <https://doi.org/10.1046/j.1439-0272.2001.00438.x> PMID: 11683699
- [14] Pellestor F, Anahory T, Hamamah S. The chromosomal analysis of human oocytes. An overview of established procedures. *Hum Reprod Update*, 2005, 11(1):15–32. <https://doi.org/10.1093/humupd/dmh051> PMID: 15569701
- [15] Ford HB, Schust DJ. Recurrent pregnancy loss: etiology, diagnosis, and therapy. *Rev Obstet Gynecol*, 2009, 2(2):76–83. PMID: 19609401 PMCID: PMC2709325
- [16] Simpson JL, Carson SA. Genetic and nongenetic causes of pregnancy loss. *Glob Lib Women Med [Internet]*, 2009 (last updated: January 2013). <https://doi.org/10.3843/GLOWM.10319>
- [17] Marrs RP, Kletzky OA, Howard WF, Mishell DR Jr. Disappearance of human chorionic gonadotropin and resumption of ovulation following abortion. *Am J Obstet Gynecol*, 1979, 135(6):731–736. [https://doi.org/10.1016/0002-9378\(79\)90383-1](https://doi.org/10.1016/0002-9378(79)90383-1) PMID: 495673
- [18] Viotti M. Preimplantation genetic testing for chromosomal abnormalities: aneuploidy, mosaicism, and structural rearrangements. *Genes (Basel)*, 2020, 11(6):602. <https://doi.org/10.3390/genes11060602> PMID: 32485954 PMCID: PMC7349251
- [19] Munné S. Chromosome abnormalities and their relationship to morphology and development of human embryos. *Reprod Biomed Online*, 2006, 12(2):234–253. [https://doi.org/10.1016/s1472-6483\(10\)60866-8](https://doi.org/10.1016/s1472-6483(10)60866-8) PMID: 16478592
- [20] Inhorn MC, Patrizzo P. Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. *Hum Reprod Update*, 2015, 21(4):411–426. <https://doi.org/10.1093/humupd/dmv016> PMID: 25801630
- [21] Deatsman S, Vasilopoulos T, Rhoton-Vlasak A. Age and fertility: a study on patient awareness. *JBRA Assist Reprod*, 2016, 20(3):99–106. <https://doi.org/10.5935/1518-0557.20160024> PMID: 27584600 PMCID: PMC5264372
- [22] Tulandi T, Arronet GH, McInnes RA. Infertility in women over the age of 36. *Fertil Steril*, 1981, 35(6):611–614. [https://doi.org/10.1016/s0015-0282\(16\)45550-0](https://doi.org/10.1016/s0015-0282(16)45550-0) PMID: 7195826
- [23] Margan R, Popa ZL, Margan MM, Bratosin F, Moza A, Tudor A, Onofriescu M, Toma BF, Vlaicu B. Socio-economical, legal and ethical issues raised by infertility and assisted reproductive techniques in Romania. *Rev Cercet Interv Soc*, 2020, 70:214–227. <https://doi.org/10.33788/rcis.70.13>
- [24] Albu DF, Albu CC, Văduva CC, Niculescu M, Edu A. Diagnosis problems in a case of ovarian tumor – case presentation. *Rom J Morphol Embryol*, 2016, 57(4):1437–1442. PMID: 28174816
- [25] Masoumi SZ, Parsa P, Darvish N, Mokhtari S, Yavangi M, Roshanaei G. An epidemiologic survey on the causes of infertility in patients referred to infertility center in Fatemeh Hospital in Hamadan. *Iran J Reprod Med*, 2015, 13(8):513–516. PMID: 26568755 PMCID: PMC4637117
- [26] Văduva CC, Constantinescu C, Ţenovici M, Văduva AR, Niculescu M, Diţescu D, Albu CC, Albu DF. Delayed interval delivery in twin pregnancy – case reports. *Rom J Morphol Embryol*, 2016, 57(3):1089–1098. PMID: 28002529
- [27] Albu DF, Albu CC, Gogănu AM, Albu ŞD, Mogoantă L, Edu A, Diţescu D, Văduva CC. Borderline Brenner tumors associated with ovarian cyst – case presentation. *Rom J Morphol Embryol*, 2016, 57(2 Suppl):893–898. PMID: 27833989
- [28] Maheshwari A, Hamilton M, Bhattacharya S. Effect of female age on the diagnostic categories of infertility. *Hum Reprod*, 2008, 23(3):538–542. <https://doi.org/10.1093/humrep/dem431> PMID: 18308834
- [29] American College of Obstetricians and Gynecologists Committee on Gynecologic Practice and Practice Committee. Female age-related fertility decline. Committee Opinion No. 589. *Fertil Steril*, 2014, 101(3):633–634. <https://doi.org/10.1016/j.fertnstert.2013.12.032> PMID: 24559617
- [30] Jesus AR, Silva-Soares S, Silva J, Severo M, Barros A, Dória S. Reproductive success of assisted reproductive technology in couples with chromosomal abnormalities. *J Assist Reprod Genet*, 2019, 36(7):1471–1479. <https://doi.org/10.1007/s10815-019-01486-x> PMID: 31104291 PMCID: PMC6642249
- [31] Behbahany LK, Keshavarzi F, Homayounpour G. The frequency of chromosomal abnormalities in individuals with susceptibility to abortion and/or infertility by cytogenetics method. *Ann Clin Lab Res*, 2018, 6(3):258. <https://doi.org/10.21767/2386-5180.100258>
- [32] Albu CC, Albu DF, Muşat AR, Stancu IG, Albu ŞD, Pătraşcu A, Gogănu AM. The crucial role of SRY gene in the determination of human genetic sex: 46,XX disorder of sex development. *Rom J Morphol Embryol*, 2019, 60(4):1311–1316. PMID: 32239110
- [33] Foresta C, Ferlin A, Gianaroli L, Dallapiccola B. Guidelines for the appropriate use of genetic tests in infertile couples. *Eur J Hum Genet*, 2002, 10(5):303–312. <https://doi.org/10.1038/sj.ejhg.5200805> PMID: 12082505
- [34] Albu DF, Albu C, Severin E, Toma A, Dumitrescu M. P02.69: Genital ambiguity problems discovered through prenatal ultrasound diagnosis. Special Issue: 16<sup>th</sup> World Congress on Ultrasound in Obstetrics and Gynecology. *Ultrasound Obstet Gynecol*, 2006, 28(4):539. <https://doi.org/10.1002/uog.3501>
- [35] Albu CC, Cilievici SE, Albu DF, Albu SD, Patrascu A, Goganau AM. Impact of material serum screening in early prenatal diagnosis and management of congenital anomalies. *Rev Chim (Bucharest)*, 2019, 70(5):1534–1538. <https://doi.org/10.37358/RC.19.5.7164>
- [36] Albu D, Oncescu A, Albu C, Enache T, Enache A. P25.16: Correlation between abnormal karyotype 46,XY,del(12)(q24) and ultrasound findings in a fetus with a rare association of a multiple malformations: case report. Supplement: Abstracts of the 22<sup>nd</sup> World Congress on Ultrasound in Obstetrics and Gynecology, 9–12 September, Copenhagen, Denmark. *Ultrasound Obstet Gynecol*, 2012, 40(Suppl 1):267. <https://doi.org/10.1002/uog.12109>
- [37] Albu CC, Albu DF, Albu SD, Patrascu A, Musat AR, Goganau AM. Early prenatal diagnosis of an extremely rare association of Down syndrome and transposition of the great vessels. *Rev Chim (Bucharest)*, 2019, 70(7):2574–2578. <https://doi.org/10.37358/RC.19.7.7383>

- [38] \*\*\*. Physiopathological determinants of human infertility. *Hum Reprod Update*, 2002, 8(5):435–447. <https://doi.org/10.1093/humupd/8.5.435> PMID: 12398224
- [39] Kling C, Magez J, Hedderich J, von Otte S, Kabelitz D. Two-year outcome after recurrent first trimester miscarriages: prognostic value of the past obstetric history. *Arch Gynecol Obstet*, 2016, 293(5):1113–1123. <https://doi.org/10.1007/s00404-015-4001-x> PMID: 26796678 PMCID: PMC4829626
- [40] Zhang T, Sun Y, Chen Z, Li T. Traditional and molecular chromosomal abnormality analysis of products of conception in spontaneous and recurrent miscarriage. *BJOG*, 2018, 125(4):414–420. <https://doi.org/10.1111/1471-0528.15052> PMID: 29220118
- [41] Kim JW, Lee WS, Yoon TK, Seok HH, Cho JH, Kim YS, Lyu SW, Shim SH. Chromosomal abnormalities in spontaneous abortion after assisted reproductive treatment. *BMC Med Genet*, 2010, 11:153. <https://doi.org/10.1186/1471-2350-11-153> PMID: 21044350 PMCID: PMC2991301
- [42] Russo R, Sessa AM, Fumo R, Gaeta S. Chromosomal anomalies in early spontaneous abortions: interphase FISH analysis on 855 FFPE first trimester abortions. *Prenat Diagn*, 2016, 36(2): 186–191. <https://doi.org/10.1002/pd.4768> PMID: 26716606
- [43] Geigl JB, Obenauf AC, Schwarzbraun T, Speicher MR. Defining 'chromosomal instability'. *Trends Genet*, 2008, 24(2): 64–69. <https://doi.org/10.1016/j.tig.2007.11.006> PMID: 18192061
- [44] Thompson SL, Bakhoun SF, Compton DA. Mechanisms of chromosomal instability. *Curr Biol*, 2010, 20(6):R285–R295. <https://doi.org/10.1016/j.cub.2010.01.034> PMID: 20334839 PMCID: PMC3781365
- [45] Vargas-Rondón N, Villegas VE, Rondón-Lagos M. The role of chromosomal instability in cancer and therapeutic responses. *Cancers (Basel)*, 2017, 10(1):4. <https://doi.org/10.3390/cancers10010004> PMID: 29283387 PMCID: PMC5789354
- [46] Wilhelm T, Said M, Naim V. DNA replication stress and chromosomal instability: dangerous liaisons. *Genes (Basel)*, 2020, 11(6):642. <https://doi.org/10.3390/genes11060642> PMID: 32532049 PMCID: PMC7348713
- [47] Krausz C, Riera-Escamilla A. Genetics of male infertility. *Nat Rev Urol*, 2018, 15(6):369–384. <https://doi.org/10.1038/s41585-018-0003-3> PMID: 29622783
- [48] Fainberg J, Kashanian JA. Recent advances in understanding and managing male infertility. *F1000Res*, 2019, 8:F1000 Faculty Rev-670. <https://doi.org/10.12688/f1000research.17076.1> PMID: 31143441 PMCID: PMC6524745
- [49] Pryor JL, Kent-First M, Muallem A, Van Bergen AH, Nolten WE, Meisner L, Roberts KP. Microdeletions in the Y chromosome of infertile men. *N Engl J Med*, 1997, 336(8):534–540. <https://doi.org/10.1056/NEJM199702203360802> PMID: 9023089
- [50] Foresta C, Moro E, Ferlin A. Y chromosome microdeletions and alterations of spermatogenesis. *Endocr Rev*, 2001, 22(2): 226–239. <https://doi.org/10.1210/edrv.22.2.0425> PMID: 11294825
- [51] Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: a review of literature. *J Hum Reprod Sci*, 2015, 8(4):191–196. <https://doi.org/10.4103/0974-1208.170370> PMID: 26752853 PMCID: PMC4691969
- [52] Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reprod Biol Endocrinol*, 2015, 13:37. <https://doi.org/10.1186/s12958-015-0032-1> PMID: 25928197 PMCID: PMC4424520
- [53] Cui W. Mother or nothing: the agony of infertility. *Bull World Health Organ*, 2010, 88(12):881–882. <https://doi.org/10.2471/BLT.10.011210> PMID: 21124709 PMCID: PMC2995184
- [54] Fatima P, Rahman D, Hossain HB, Hossain HN, Mughli CR. Psychosocial consequences of infertility on infertile women. *Mymensingh Med J*, 2015, 24(4):704–709. PMID: 26620007

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