Acta Scientifica Balcanica, **2022, 3(1)**
DOI: 10.7251/ASB220301023I **UDC: 575.224:364.444.058.73(497.6PC)**
Original scientific paper

CHROMOSOMAL ABERRATIONS IN COUPLES WITH STERILITY AND HABITUAL ABORTIONS AT THE UNIVERSITY CLINICAL CENTER OF THE REPUBLIC OF SRPSKA

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Summary

The cytogenetic laboratory of the University Clinical Center of the Republic of Srpska (UCCRS) in Banja Luka is the primary institution where the karyotypes of patients' peripheral blood are analyzed. In the period from 2009 to 2019, a total of 3842 karyotype analyzes of patients were performed in it, of which 1956 had a referral diagnosis of sterility and habitual abortion, and therefore the inability to achieve pregnancy. The importance of cytogenetic analysis of patient's karyotypes is great because the presence of chromosomal aberrations in them can be the cause of sterility and spontaneous abortions in patients. Therefore, the aim of the work was to determine the presence of chromosomal aberrations in the karyotypes of patients at the University Hospital of RS who had a referral diagnosis of sterility or habitual abortion. The total number of processed karyotype samples of patients in the Cytogenetic Laboratory at the UCCRS in the period from 2009 to 2019 grew by year. The study found a significant difference in the frequency distribution of patients with a diagnosis of sterility, which was twice as many as compared to patients with a diagnosis of habitual abortion. The percentage of patients who are treated for sterility and habitual abortion with the presence of chromosomal aberrations in their karyotypes compared to those without aberrations is on average low. An equal distribution of male and female patients with aberrant karyotypes who were treated for sterility and habitual abortions was determined. The most prevalent chromosomal aberrations in the karyotypes of both male and female patients treated for sterility and habitual abortion were inversions, followed by mosaic aberrations, then translocations; then followed by trisomies, Robertsonian translocations and finally additions.

Key words: chromosomal aberrations, inversions, Klinefelter's syndrome, Down's syndrome, mosaicism

INTRODUCTION

According to the charter of the World Health Organization and international law, basic human right of individuals and couples is to decide freely and responsibly the number of their children as well as intervals between births (Mau-Holzmann, 2005). Failure to achieve pregnancy among couples is a growing problem today. The frequency of failure to achieve pregnancy after one year of regular sexual intercourse in couples is 15-17%, while true

sterility accounts for 10-12% of stated percentage (Šimunić *et al*., 2012). In most cases, diagnosis remains unclear due to a large number of factors that lead to sterility (Shah *et al*., 2003). Primary sterility is the impossibility of achieving pregnancy during the reproductive period with regular and unprotected intercourse for a year. If after one previously achieved pregnancy, spontaneous abortion, ectopic pregnancy is an impossibility of achieving pregnancy occurs; in that case we speak of secondary sterility (Stipoljev, 2007). Large help of specialist doctors, gynecologists or clinical geneticists is usually sought after two years of not achieving pregnancy or after spontaneous abortions, and targeted treatment usually begins after four years (Indore Infertility Clinic, 2018). The World Health Organization defined miscarriage as loss of a fetus or embryo weighing less than 500g, which normally corresponds to a fetus aged 20-22 weeks of gestation (WHO, 1977). Chromosomal aberrations are a common cause of early spontaneous abortions in the first twelve weeks of pregnancy, and disorders at the level of gene functioning in the second or third trimester of pregnancy (Jauniaux *et al*., 2006). Based on this, it is very important to make a classification of spontaneous abortions determined according to weeks of pregnancy. Aim of this is obtaining adequate genetic advice, as well as optimal diagnostics and treatment (Van den Boogaard, 2014; Kolte *et al*., 2015). Speaking about factors that lead to sterility, we can divide them into: male factors, female factors, combination of both and there is also sterility of unknown cause, idiopathic (Heuser *et al*., 2010). When we talk about factors that cause female sterility, it is usually a combination of two or more causes, and most common are: hormonal disorder due to thyroid gland dysfunction, ovulation disorder due to hormonal imbalance, endometriosis, poor quality of oocytes, polycystic ovary syndrome, fallopian tube problems, presence of polyps and/or fibroids, immunological causes, autoimmune diseases (Coccia *et al*., 2015). Causes that can lead to habitual abortions are: disorders of luteal phase, endocrine disorders, such as insulin resistance, immunological disorders, such as lupus anticoagulant or anticardiolipin antibodies, maternal inheritance of thrombophilia, as well as exposure to toxins. In addition to all mentioned causes, age of women can also be a reason for sterility, since in women after 35 years of age, ability to conceive significantly decreases due to reduced number and quality of oocytes (McPherson, 2016). Male infertility can be of different origin: hormonal disorders, absence of spermatogenesis, poor quality of spermatozoa, disorder in transport of spermatozoa in vas deferens, psychological problems, etc. (Sharma *et al*., 2013). Today, analyzing sperm fluid, it is possible to observe anomalies in morphology, number and mobility of spermatozoa, which can also lead to male infertility (Practice Committee of the American Society for Reproductive Medicine, 2015). Most common cause of infertility related to genetic factors is chromosomal aberrations and chromosomal diseases. Chromosome pathologies in the human population occur in about 1% of cases, while in couples with problems of sterility it is almost fifteen times higher. In cases of sterility of unknown origin or in couples with repeated spontaneous abortions, karyotype is done for both spouses (Grimes and Lopez, 2007). For this reason, genetic counseling centers in the world represent an important step in the direction of discovering causes of infertility. Determining genetic factors of infertility is very helpful in making a correct assessment of risk for a certain chromosomal or genetic disorder of both the couple and the future fetus. A very important role of genetic counseling centers is to choose the optimal approach in diagnosis and treatment of infertility, because genetic problems are very different from couple to couple. Chromosomes are structures that distinguish one species

from another and are responsible for transmitting genetic information from one generation of individuals of a certain species to the next. During formation of gametes, each mature oocyte and sperm receives a unique set of parental genes. Thanks to technical achievements and standardization of tissue cultivation and preparation of chromosome preparations, an intensive development of cytogenetics began (Miller and Therman, 2000). Advances in technology, the development of banding techniques, have made it possible to identify individual chromosomes, and in addition to changes in number of chromosomes, it is also possible to establish changes in structure itself, such as loss or excess of certain parts of chromosome. The most famous tape technique used is G tape technique (GTG) which is also used in UKCRS, Banja Luka. While the entire categorization of description of chromosomal aberrations is harmonized with the world standards prescribed by the International System for Human Cytogenetic Nomenclature (ISCN), first published in 1995 (Alberts *et al*., 2007). Chromosome aberrations represent changes in the number or structure of chromosomes, and depending on whether changes will be passed onto offspring or not, chromosome aberrations are divided into hereditary and non-hereditary. If the abberations are newly discovered, i.e. were not found in family members, then we speak of de novo aberrations. Chromosome aberrations of size 4Mb can be detected under a light microscope, while molecular-cytogenetic and molecular methods are used for changes smaller than 4Mb. Chromosomal aberrations are divided into numerical and structural; they can be linked to autosomes or sex chromosomes. Numerical aberrations change the overall number of chromosomes in a cell. Structural aberrations lead to the loss or creation of excess chromosomal material, or to its balanced redistribution (Alberts *et al*., 2002). Structural aberrations of chromosomes mainly occur in the interphase of cell cycle when chromatin material is in replication phase. As a consequence of lack or excess of genetic material, a clinical picture occurs, disorders of which are most often pathological. Structural aberrations can be interchromosomal and intrachromosomal (Guć-Šekić and Radivojević, 2009). Interchromosomal ones arise as a result of transfer of chromosomal material from one chromosome to another and can be: reciprocal, Robertsonian and non-reciprocal. Reciprocal translocations occur when there is a break in at least two chromosomes, and then exchange of their fragments and formation of two new chromosomal derivatives. Carriers of these translocations do not have phenotypic changes, they are mostly balanced. Robertsonian translocations or centric fusions are the result of breaks on two acrocentric chromosomes D (chromosomes 13, 14, 15) or group G (chromosomes 21, 22) in centromeres or in their immediate vicinity and fusion of their long arms. They occur with a frequency of 1:1000 in the human population, of which the largest percentage is inherited, while only 10% are de novo abberations. Non-reciprocal, insertional translocations occur when a certain chromosomal segment is removed from one chromosome and another is inserted. Carriers of this change have a balanced karyotype, but also a high risk of even 50% for having offspring with partial trisomy or monosomy for the given segments (Turnpenny and Ellard, 2012). Intrachromosomal changes occur within one chromosome and include deletions, duplications, inversions (paracentric and pericentric) and isochromosomes. Deletions represent loss of part of a chromosome, which leads to monosomy for that segment. If more than 2% of the total haploid genome is lost through deletion, such deletion will end fatally. Duplications are structural aberrations where an additional copy of a certain chromosomal segment is present. Inversions occur as a result of a double break on the same arm of the chromosome, after which

the broken part rotates. The most common inversion in the human population is pericentric inversion of chromosome 9 (inv9p12q13). It is an inversion that has no phenotypic expression, because it covers the heterochromatin region of secondary constriction. Chromosomal mosaicism represents the presence of two or more cell lines with different karyotypes, originating from one zygote. Two or more cell lines means that karyotypes in cells of same person are different: eg. One person has a karyotype of 46, XX in one of his cells and 45, XO in others. Mosaicism occurs due to non-separation of chromosomes in mitosis. Chimerism is the existence of one or more cell lines derived from two or more zygotes forming a single fetus in early stages of embryogenesis. Thus, the fetus has a different genetic origin and therefore different cell lines. There are two types of chimeras in the human population: dispermic chimeras and blood chimeras. They arise as a result of double fertilization, i.e. joining of two zygotes into one embryo. If a zygote is of a different sex, a chimeric embryo can develop into a person with true hermaphroditism 46, XX/46, XY. Their formation occurs due to the exchange of cells through placenta between dizygotic twins in the uterus (Salazar *et al*., 2011).

MATERIALS AND METHODS

In this study, a retrospective analysis of karyotypes of couples with sterility and habitual abortions who were referred to the Cytogenetic Laboratory of the UCCRS during a ten-year period from 2009-2019 was performed. The main goal of the analysis was to determine the presence of chromosomal aberrations as potential causes of sterility and habitual abortions. Conduct of this study was approved by the competent Ethics Committee of UCCRS Board in Banja Luka, protocol number: 01-19-246-2/20. In order to examine karyotypes and potential existence of chromosomal aberrations in couples with sterility and habitual abortions, 1.5-2 ml of peripheral blood was sampled from subjects. Immediately before blood collection, syringes with 0.2 ml of heparin previously diluted with medium are prepared for each patient.

Blood sampling was performed according to procedure PR-06-019-36 by the UCCRS laboratory technician for sampling and sample handling at the Institute for Clinical Laboratory Diagnostics. After taking biological samples, peripheral blood is seeded in a nutrient medium enriched with 20% fetal calf serum, to which phytohaemagglutinin (Phytohaemagglutinin - PHA) is added, which triggers the dedifferentiation of lymphocytes, as well as antibiotics penicillin and streptomycin. The seeded sample is cultivated in a thermostat at a temperature of 37˚C for 69-72 hours. After cultivation, 0.1 ml of colcemid was added to seeded cultures in each test tube in order to stop cells in metaphase of mitosis. Action of colcemid lasts for 1-1.5 hours, after which sample processing begins. Half an hour before start of processing, hypotonia is prepared at a temperature of 37℃. Each sample is processed in the laboratory, which means treatment with hypotonic solution, triton and fixative. In a hypotonic solution, the volume of the cell increases, as a result of which chromosomes are better arranged. Triton lyses erythrocytes, while cold fixative improves visibility of chromosome contours. Microscopic preparations are made from obtained suspension, by dropping onto a glass slide. After preparations of metaphase chromosomes from peripheral blood lymphocytes have been made, they are classically stained with gimzo (Merck, USA) and the GTG band technique is applied. The GTG band implies treatment of prepared preparations with proteolytic enzyme

trypsin, after which Giemsa dye is added. After treatment and drying of preparation, a detailed analysis of the G bands on each individual chromosome is performed. Cytogenetic analysis is performed on a high-quality light microscope under an immersion objective of magnification 100 (Figure 1).

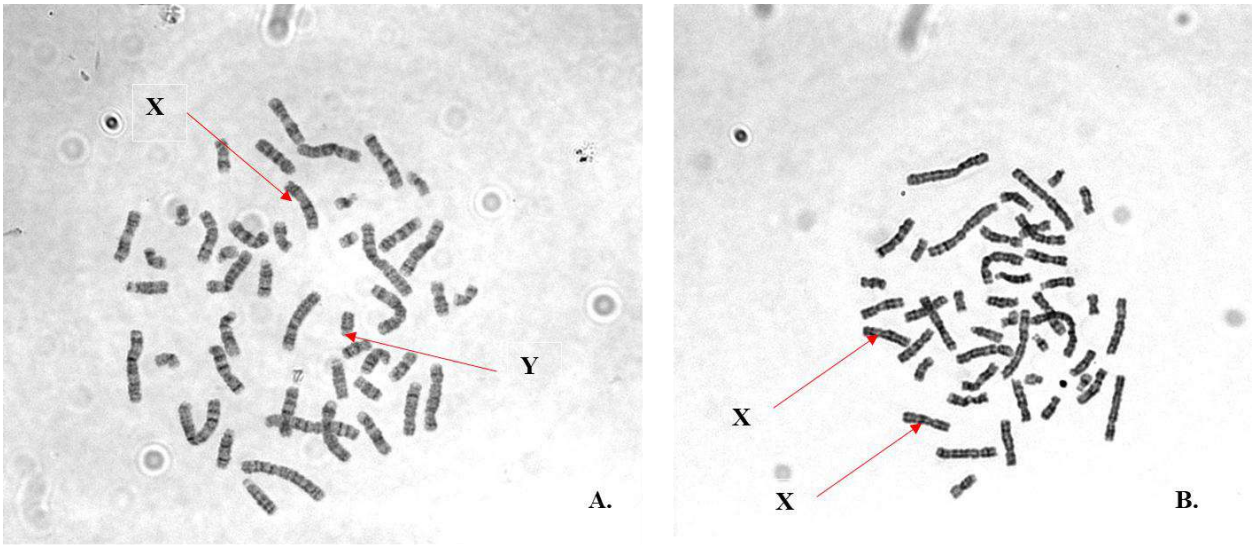

Figure 1. Normal male (A) and female (B) karyotypes

Eyepiece magnification is 10x, so chromosomes are analyzed under a total magnification of 1000x. 2-3 metaphases are photographed for each patient. Chromosomal aberrations were defined using the rules of the International System for Human Cytogenetic Nomenclature (ISCN, 2013). The SPSS program package was used for statistical data processing; graphics were created in Excel, while the Chi-square test was applied from the OriginPro 2019b package and the QuickCalcs online program.

RESULTS

In the period from January 5, 2009 to December 31, 2019, a total of 3842 karyotype analyzes from peripheral blood were performed in the UCCRS Cytogenetic Laboratory. Of the total number of analyses, 1956 were samples of patients with a referral diagnosis of habitual abortion and impossibility of achieving pregnancy, i.e. sterility. Remaining 1886 analyzes were samples that had instructions for karyotype analysis at UCCRS Cytogenetic Laboratory in Banja Luka due to family anamnesis, suspicion of de novo chromosomal aberrations (Figure 2).

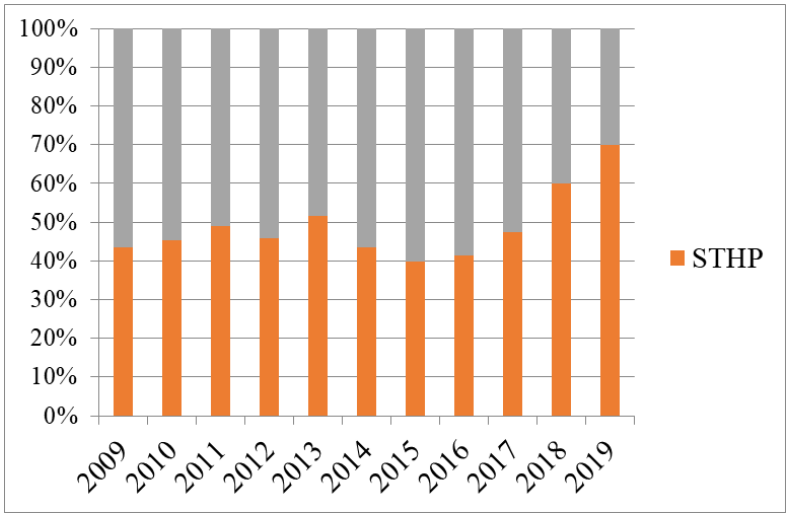

Figure 2. Presentation of the percentage share of STHP (sterility and habitual abortions) samples in the UCCRS Cytogenetic Laboratory in Banja Luka

In the first years, percentage representation of other samples (samples that are not STHP) is higher compared to STHP samples and it decreases with age, so that at the end of period, percentage representation of STHP samples is higher than others. Percentage frequency of appearance of chromosomal aberrations in karyotypes of patients with sterility and habitual abortion by age is shown in Figure 3. There were extremely significantly more samples of karyotypes with a diagnosis of sterility (1295) than those with habitual abortion (661), which was also confirmed by application of Chi-square test ($\chi 2=205.499$, $p=0.0001318$, $p<0.001$).

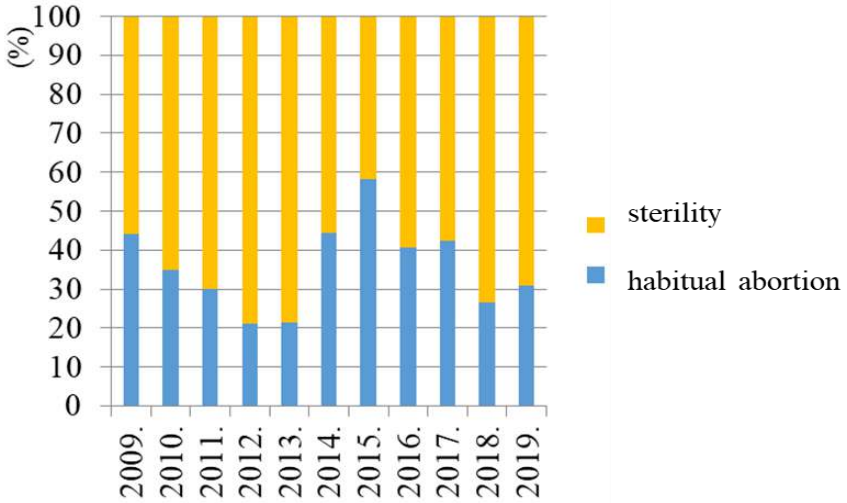

Figure 3. Presentation of the percentage of samples of persons with a diagnosis of sterility and habitual abortion referred to the UKCRS cytogenetic laboratory in Banja Luka

Percentage of patients with a diagnosis of sterility (Figure 4) in whom aberrations (STA+) were confirmed in their karyotypes compared to those whose karyotypes were without aberrations (STA-). Percentage of karyotypes of patients diagnosed with sterility and with aberrations (mean value±standard deviation) for ten years is 6.33±2.8, is much lower

compared to the percentage of karyotypes of patients with same diagnosis who do not have aberrations (mean value±standard deviation) was 93.67±11.63.

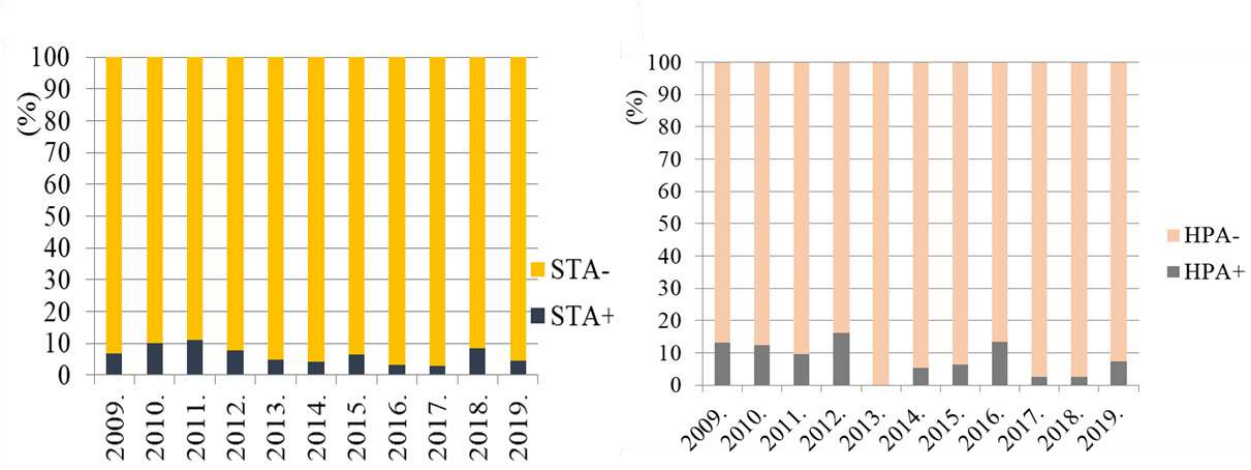

Figure 4. Representation of the percentage of patients with a diagnosis of sterility who had confirmed aberrations (STA+) in their karyotypes compared to those whose karyotypes were without aberrations (STA-) and the percentage of patients with a diagnosis of habitual abortion who had confirmed aberrations in their karyotypes karyotypes (HPA+) compared to those whose karyotypes were without aberrations (HPA-)

A similar result was obtained in determination of the percentage of karyotypes of patients with a diagnosis of habitual abortion in which aberrations (HPA+) were confirmed in their karyotypes compared to those whose karyotypes were without aberrations (HPA-). Percentage of karyotypes of patients with a diagnosis of habitual miscarriage and with aberrations (7.41±2.35) is much lower compared to percentage of karyotypes of patients with the same diagnosis who do not have aberrations (92.59±12.16). There are statistically significantly more patients with a diagnosis of sterility and confirmed chromosomal aberrations in karyotype (n=82) than those patients with a diagnosis of habitual abortion with also confirmed chromosomal aberrations (n=49). This statistically significant difference in the number of patients with STA+ and HPA+ was confirmed using the Chi-square test ($\chi 2=8.313$, $p=0.0039$, $p<0.005$). Presentation of sex structure of patients with a diagnosis of sterility and habitual abortion (Figure 5) and presence of chromosomal aberrations is very important because some chromosomal aberrations are related to autosomes and others to sex chromosomes. Thus, 131 karyotypes from total number of all analyzed karyotypes (1886) contained a confirmed chromosomal aberration. In percentage terms, it is 6.95% of the karyotypes of patients of both sexes.

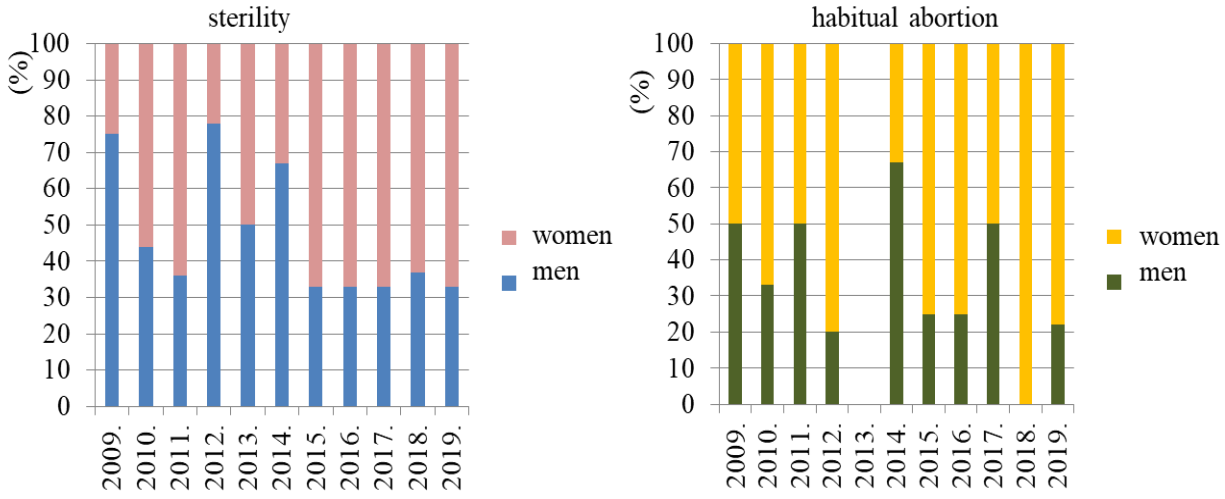

Figure 5. Representation percentage of patients with their gender in a diagnosis of sterility in and with a diagnosis of habitual abortion in whom aberrations were confirmed

In the last five years, there were twice as many women who were treated for infertility and in whom an aberration in karyotype was confirmed compared to men, in contrast to the situation in the previous five years. In total, there were karyotypes of patients treated for sterility with aberrations: in men n=37 and in women n=45. Chi-square test showed that there was no statistically significant difference in representation of the sexes ($\chi 2$= 0.780, p=0.3770, p>0.05) in patients with aberrations treated by sterility. The different percentage representation of male and female patients who were treated for habitual abortion and had chromosomal aberrations was analyzed. In the same way, the total frequency of obtained aberrant karyotypes in men (n=16) and women (n=33) diagnosed with habitual abortion was analyzed. Chi-square test showed that there are statistically significant differences in gender representation ($\chi 2$= 5.898, p=0.0152, p<0.05) in patients with aberrations treated for habitual abortion. Types of chromosomal aberrations diagnosed in the karyotypes of patients with sterility and habitual miscarriage were: inversions, additions, mosaics, translocations, Robertsonian translocations and numerical - trisomies. In our study all types and number of chromosomal aberrations detected in patients with sterility and habitual abortion in relation to gender of patient are shown in Figure 6. It is shown that the largest number of chromosomal aberrations in patients with sterility are inversions, n=32 in total (16 for women and 16 for men). Chromosomal aberration of addition type is least represented, only one confirmed in a male patient. In study, there was a significantly higher number of mosaic aberrations (n=16) in women compared to men (n=2). There were n=11 translocation cases and they were equally represented: n=5 in women and n=6 in men. It is important to note that in our study, the existence of other types of chromosomal aberrations in patients treated for sterility and habitual abortion was not recorded.

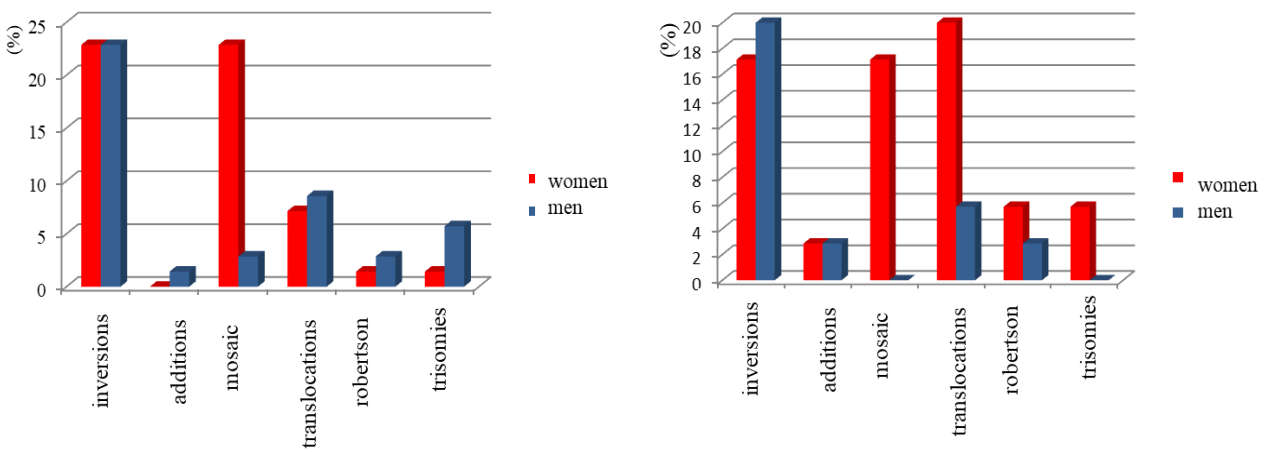

Figure 6. Representation of types of aberrations in relation to total number of detected aberrations in karyotypes of persons with sterility and habitual abortion in relation to gender

All types and number of chromosomal aberrations detected in patients with habitual abortion cumulatively for a period of ten years in relation to sex of patient prove that the largest number of chromosomal aberrations is inversions (total 13.6% in women and 7% in men). Chromosomal aberration of addition type is least represented (one in men and one in women) in patients with habitual abortion. In summary, based on representation of all types of chromosomal aberrations in patients treated for sterility and habitual abortions from our study, it can be concluded that inversions are the most common. After them, mosaic aberrations are represented in the highest percentage, significantly more in women than in men. Inversions in both sexes and mosaic aberrations in women are the most common causes of sterility and habitual abortions in couples treated at UCCRS in Banja Luka.

Table 1. Presentation of altered karyotypes within each type of aberration in persons with sterility in the period from 2009 to 2019 analyzed at UCCRS in Banja Luka

aberrations :				
inversions	women	46, XX, inv(9)(p12q13)	46, XX, inv(x)(q11.2q26)	46, XX, inv(12)(p13q13)
	men	46, XY, inv(9)(p12q13)	46, XY, inv(1)(q25. 1,q32.1)	46, XY, inv(5)(q13.1q15)
additions	women			
	men	46, XY, add(10)(q11.2)		
mosaic	women	mos45, x(3)/46, XX(97)	mos45, X/47 XXX,	46, XX(28)/ mos47, XXX(2)
		46, XX(27), mos45, XXX	46, XX(16), mos45, XXX	46, XX(47), mos45, XXX
		46, XX(97), mos45, XXX del (x)(11.2)	48, XX(2), mos48, XXXX	
	men	mos47, XXY(3)/46, XY(98)	mos47,XXY(3)/46, XY(97)	
translocations	women	46, XX, t(3,19)(q27,p13)	46, XX, t(5,14)(q31,p32)	46, XX, t(9,21)(q21q22)
		46, XX, t(1,8)(q25,q21.2)	46, XX, t(1,5)(p22,q31)	
	men	46, XY, t(15,16)(q26p11.2)	46, XY, t(15,18)(q24q23)	46, XY, t(10,20)(p11.2q13)
		46, XY, t(7,11)(q32q23) inv(9)(p12q13)	46, XY, t(17,18)(q23q12.2)	46, XY, t(2,15)(p13q26)
robertson	women	45, XX, rob(21,22)(q10,q10)		
	men	45, XY, rob(13, 14)(q10,q10)	45, XY, rob(21,22)(q10,q10)	
numerical	women	47, XXX Down's syndrome		
	men	47, XXY, (26)/46, XY(4); Klinefelter syndrome	47, XXY (92)/46, XX(8); Klinefelter syndrome	47, XXY (98)/46, XY(2); Klinefelter syndrome
		47, XXY Down's syndrome		

From Table 1, it can be seen that in diagnosis of sterility, most chromosomally altered karyotypes belong to translocations (Figure 7) with n=11 types of different types, n=5 in women and n=6 in men. Immediately in second place are mosaic aberrations with ten different types, of which n=8 were in women and only two in men. The fewest chromosomal additions were only one case of 46, XY, add(10)(q11.22) addition, in a male member of a couple treated for infertility.

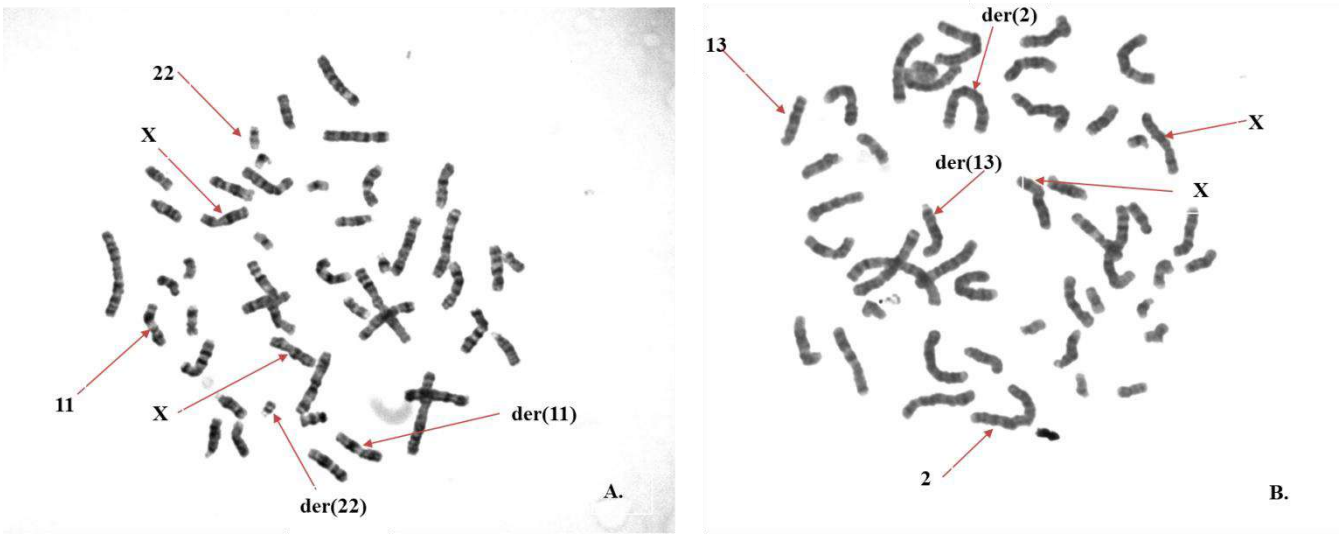

Figure 7. Micrographs: A: 46,XX,t(11;22)(q23;q11.2) and B: 46,XX,t(2;13)(p21;q32), G - Bend

Table 2. Presentation of altered karyotypes within each type of aberration in persons with habitual abortion in the period from 2009 to 2019 analyzed at UCCRS in Banja Luka

aberrations:				
inversions	women	46, XX, inv(9)(p12q13)		
	men	46, XY, inv(1)(p36.1q21)	46, XY, inv(9)(p12q13)	
additions	women	46, XX, add(16)(p11.2)		
	men	46, XY, add(16)(p11.2)		
mosaic	women	mos45, X(4)/46, XX(94)	mos45, X(5)/46, XX(95)	mos45, X(3)/46, XX(27)
	men	mos45, X(3)/46, XX(47)	mos45, X(3)/46, XX(97)	
translocations	women	46, XX, t(11,22)(q23q11.2)	46, XX, t(17,18)(q23q23)	46, XX, t(1,2)(p32,q23)
		46, XX, t(2,3)(p21,q32)		
	men	46, XY, t(11,22)(q23,q11.2)	46, XY, t(4,12)(q33,q13)	
robertson	women	45, XX, rob(14,21)(q10q10)	45, XX, rob(13,14)(q10q10)	
	men	45, XY, rob(13,14)(q10q10)		
numerical	women	47, XXX(2)/ Down's syndrome	47, XXX, Down's syndrome	

From Table 2, it can be seen that most patients who were treated for habitual abortion had an inversion of type inv(9)(p12q13) n=35, (Figure 8), of total number of inversions n=45. Immediately in second place are translocations with six different types, four of which were in women and only two in men. At least, even with habitual abortion, there were additions in

chromosomes in only two cases 46, XY, add(16)(q11.2) and 46, XX, add(16)(p11,2) in one woman and one man. The reason for abortion in couples was chromosomal aberrations of Robertson type (Figure 9) in both sexes, as well as numerical aberrations of Down's syndrome type (Figure 9B) in women.

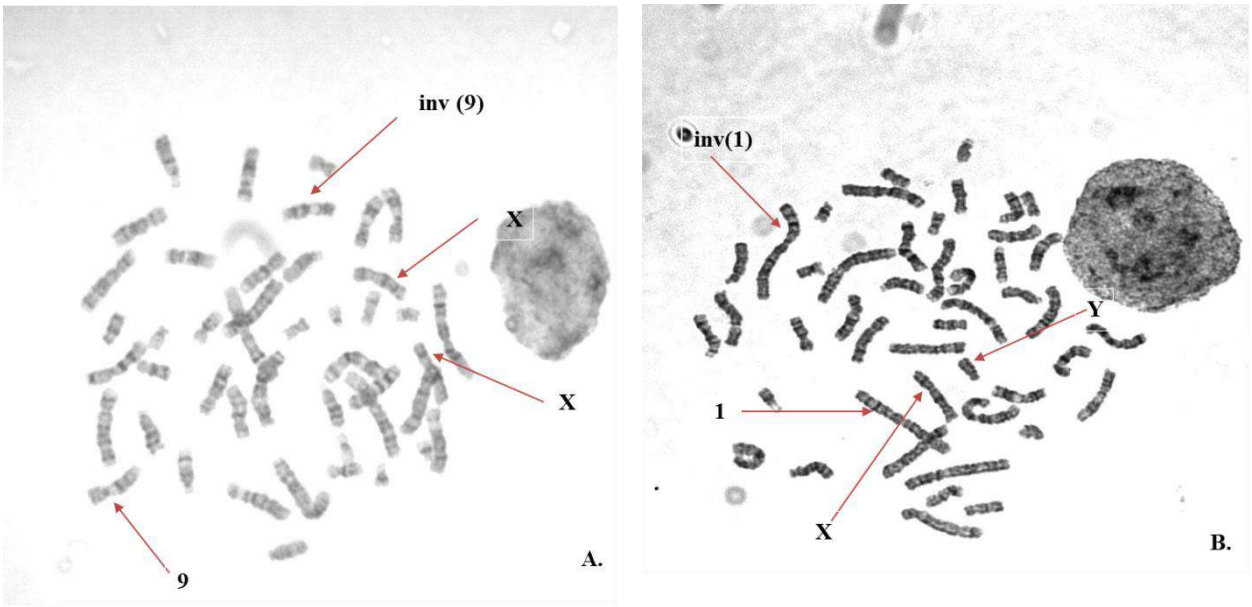

Figure 8. Micrographs, A: 46, XX, inv(9)(p12q13) and B: 46, XY, inv(1)(p13q21), G -Band

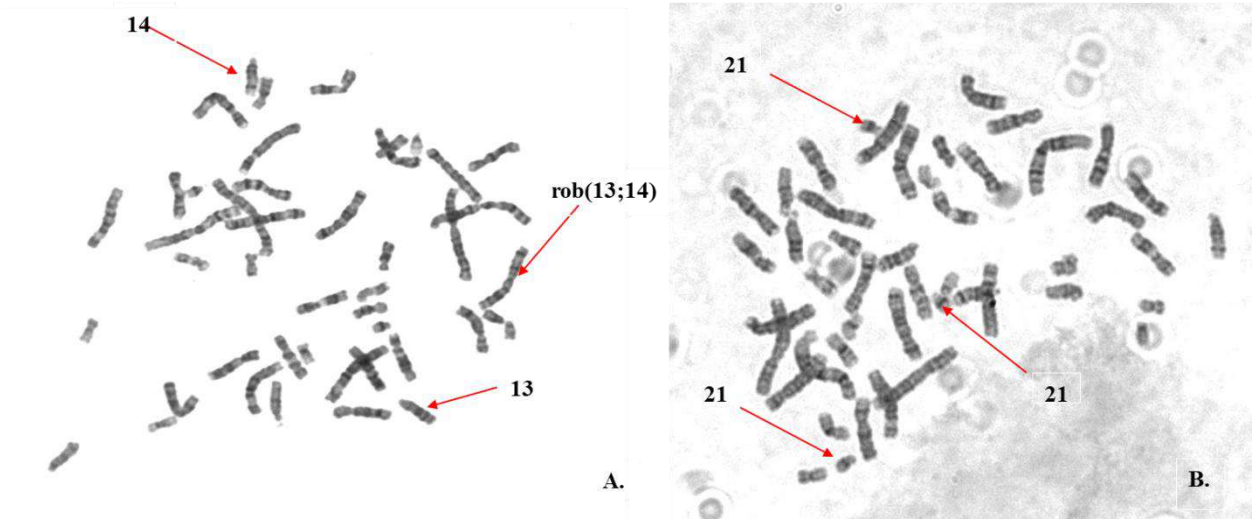

Figure 9. Micrograph, A: 45, XY, rob(13;14)(q10;q10) and B: 47, XXX, numerical trisomy, Down's syndrome, G-Band

DISCUSSION

In the Cytogenetic laboratory at UCCRS Banja Luka, analyses of a total of 3842 karyotypes from peripheral blood were performed in the period from 2009 to 2019. In addition, 1956 patients had referral diagnosis of sterility and habitual abortion. In that period, the number of samples with a referral diagnosis of sterility and habitual abortion of processed

karyotypes of patients at UCCRS Banja Luka grew by age. In recent years, the number of processed samples of patients with above-mentioned diagnoses has increased in relation to the number of all other diagnoses. Analysis of karyotypes is done together in men and women who plan to have offspring, which only increases the number of analyzes (Ford and Schust, 2009). Literature data prove that about 20% of clinically diagnosed pregnancies end in spontaneous abortion (Poppe *et al.,* 2008) for various reasons such as endocrine disorders, immunological disorders, maternal inheritance of thrombophilia, presence of chromosomal aberrations in parents' karyotypes (Jafari-Ghahfarokhi *et al*., 2015) or exposure to any endogenous toxins (Tinting *et al*., 2020). Of the total number of samples of couples from UCCRS Cytogenetic Laboratory who could not become parents, 33.79% had a diagnosis of habitual abortion, while the remaining 66.21% had a diagnosis of sterility. Literature data indicate that the percentage of habitual abortions is 46%, while sterility is represented by 54% in patients who want to become parents (Kovalvsky *et al*., 2004). Percentage of chromosomal aberrations in patients who are trying to have offspring and are being treated for sterility and habitual abortion has a very wide range, even from 2.3% to 15.4% (Gary *et al*., 2012). In our study, the mean value percentage of karyotypes with aberrations in patients diagnosed with sterility was 6.33% in relation to the total number of patients treated for sterility. While the mean value of percentage of patients diagnosed with habitual abortion was 7.41% in relation to the total number of patients with habitual abortion. The analysis of sex structure of patients with a diagnosis of sterility or habitual abortion that had chromosomal aberrations in their genotypes was determined for a reason because some chromosomal aberrations are related to autosomes, and some to sex chromosomes. Patients treated for infertility, and in which inversions, additions and translocations in their karyotypes were proven in cytogenetic laboratories, were both men and women (Zachaki *et al*., 2020). Women have a higher representation when it comes to mosaic and Robertsonian translocations (Caglayan *et al*., 2010). In men treated for sterility, presence of numerical aberration, trisomy is higher (Raziel *et al*., 2002). In our study, there are more women with aberrant karyotypes who are treated for infertility and habitual abortion than men. In literature, aberrant karyotypes of women and men who are treated for sterility are in a 1:1 ratio (Halder and Fauzdar, 2006). In our study, in patients who were treated for sterility, most common chromosomal aberrations were inversions, a total of n=32 out of n=70 aberrant genotypes. Additions were least common, only one was confirmed in men treated for sterility. In our study, a large number of mosaic aberrations were detected, eight times more in women than in men. There were n=11 translocations, approximately equally represented in both sexes; There were n=3 Robertsonian translocations. All numerical aberrations in men in our study were trisomies of the Klinefelter syndrome type, which are primarily sterile individuals due to the inability to synthesize high-quality spermatozoa (Liehr and Al-Rikabi, 2019). Inversions, additions and translocations in our study showed an approximately similar frequency in men and women who were treated for sterility, which means that these aberrations are equally involved in preventing fertility in both sexes, which is in accordance with data from literature (Misbah *et al*., 2019). Trisomies of sex chromosomes can lead to incurable sterility in men, which is unfortunately discovered late, only when he expresses the desire to have offspring (Hasanzadeh-Nazar *et al*., 2014). According to literature data, Robertsonian translocations and mosaic aberrations are more common in women than in men treated for sterility (Zarifian *et al*., 2012), however, in our

study there were more men than women with Robertsonian translocations and mosaic additions who were treated for sterility. In our study, the largest numbers of chromosomal aberrations in patients with habitual abortion were inversions, approximately equally distributed around 46% in women and around 54% in men. Additions were least represented. In couples treated for habitual abortion, the highest number of chromosomal aberrations was of inversion type - 13 out of 35, while smallest number was of the addition type, two out of 35. In our study, in patients treated for habitual abortion, mosaic aberrations were present only in women; there were a total of nine translocations and three Robertsonian translocations. Inversions and additions in our study showed an approximately similar frequency in men and women who were treated for habitual abortion, as stated in literature (Keymolen *et al*., 2014). Translocations showed uneven distribution between the sexes in our study when it comes to diagnosis of habitual abortion, in contrast to diagnosis of sterility, where distribution is almost half of the sexes. Reason for this distribution of aberrant karyotypes in patients treated for habitual abortion is that translocations cause a very low conception rate, abnormal fetal development and cessation of fetal heart contractions in very early stages of embryogenesis (Sciurano *et al*., 2019). Low frequency of trisomies in habitual abortion can be explained with their consequences on the fetus. They greatly burden embryonic development of the fetus or completely stop it, and that fetus perishes very early in embryogenesis (Puig *et al*., 2015). Literature reports prove that the highest frequency of inversion: inv(9)(p12q13) is as much as 64.29% compared to all other pericentric inversions (Babu *et al*., 2006). Other authors confirm that this type of inversion leads to congenital anomalies in children, embryo anomalies and spontaneous abortions of fetuses that have inherited inversion from their parents. In general, according to literature data, additions are the least common chromosomal aberrations in the human population (Yatsenko and Rajkovic, 2016). Mosaic aberrations are very common in couples undergoing treatment for infertility and habitual abortion. Mosaicism was more common in women in our study than in men, which is in accordance with literature (Signore *et al*., 2019). Chromosomal translocations are cited in literature as the main factors of male and female sterility because they lead to a lack of expression on certain parts of DNA (Wang *et al*., 2019; Khalafalla *et al*., 2020). Translocations with the same author can affect different chromosomes in different regions, leading to a large number of their forms. Robertsonian translocations can cause infertility in men and women because they can occur on sex chromosomes and then lead to sterility of the person (Zhang *et al*., 2019). High proportion of Klinefelter's syndrome among men with sterility is a fact that has been proven in literature (Krausz and Rosta, 2020; Santos *et al*., 2019). In our study, the most common form of numerical aberrations in women, as it was the case in the large body of work, is the numerical aberration of sex chromosomes (Jaseem, 2019).

CONCLUSION

In accordance with set goals of research, and based on obtained results, it is possible to conclude that new improved protocols in treatment of infertility in couples and long-term experience in application of methods in Cytogenetic Laboratory of patients at UCCRS in Banja Luka led to a significant increase in number of karyotype analyses. Our study showed that the percentage of patients treated for sterility and habitual abortion with presence of

chromosomal aberrations in their karyotypes was low on average. There were more patients with a diagnosis of sterility than patients with a diagnosis of habitual abortion. The distribution of male and female patients with aberrant karyotypes who were treated for sterility and habitual abortions is divided. Most prevalent chromosomal aberrations in karyotypes of both male and female patients treated for sterility and habitual abortion were inversions, then mosaic aberrations and translocations. Less common are trisomies, Robertsonian translocations and additions. Types of aberrant karyotypes of patients treated for sterility and habitual abortions are very different, and most common are inversions of inv(9)(p12q13) type. Knowing the presence of chromosomal aberrations in couples who are being treated for infertility and habitual abortion is very important in the procedure of their treatment.

REFERENCES

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K. & Walter, P. (2002). *Molecular Biology of the Cell,* 4th edition. New York: Garland Science.

Alberts, B., Karen, H., Jonson, A., Morgan, D. & Raff, M. (2007). *Essential cell biology,* 4th edition (pp. 656-712). New York, London: Norton and Company.

Babu, R. V., Kerketta, L., Korgaonkar, S. & Kanjaksha, G. (2006). Pericentric inversion of chromosome 9[inv(9)(p12q13)]: Its association with genetic diseases. *Indian Journal of Human genetics, 12*(3), 129-132.

Caglayan, A. O., Ozyazgan, I., Demiryilmaz, F. & Dundar, M. (2010). Cytogenetic Results of Patients with Infertility in Middle Anatolia, Turkey: Do Heterochromatin Polymorphisms Affect Fertility? *Journal of Reproduction and Infertility, 11*(3), 179-181.

Coccia, M. E., Rizzello, F., Capezzuoli, T., Spitaleri, M. & Riviello, C. (2015). Recurrent pregnancy losses and gestational age are closely related: an observational cohort study on 759 pregnancy losses. *Reproductive Sciences, 22*, 556-562. doi: 10.1177/1933719114553063

Ford, H. B. & Schust, D. J. (2009). Recurrent Pregnancy Loss: Etiology, Diagnosis and Therapy. *Reviews in Obstetrics and Gynecology, 2*(2), 76-83.

Gary, L., Harton, H. & Tempest, G. H. (2012). Chromosomal disorders and male infertility. *Asian Journal of Andrology, 14*(1), 32-39. doi: 10.1038/aja.2011.66

Grimes, D. A. & Lopez, L. M. (2007). Oligozoospermia, azoospermia and other semen analysis terminology: the need for better science. *Fertility and Sterility*, *88*(6), 1491–1494. doi: 10.1016/j.fertnstert.2007.04.013

Guć-Šćekić, M., Radivojević, D. (2009). *Priručnik iz medicinske genetike*. Beograd: Alta nova.

Halder, A. & Fauzdar, A. (2006). Skewed sex ratio & low aneuploidy in recurrent early missed abortion. *Indian Journal of Medical Research, 124*(1), 41-50.

Hasanzadeh-Nazar, M. A., Baghbani, F., Namazi, I. & Mirzaee, I. (2014). Robertsonian translocation between chromosomes (no.21/14) in relation to the history of spontaneous abortion in a family. *Iranian Journal of Reproductive Med*icine, *12*(8), 581-585.

Heuser, C., Dalton, J., Macpherson, C., Branch, D. W., Porter, T. F. & Silver, R. M. (2010). Idiopathic recurrent pregnancy loss recurs at similar gestational ages. *American Journal of Obstetrics and Gynecology, 203*(4), 343.e1-343.e5. doi: 10.1016/j.ajog.2010.05.010

Indore Infertility Clinic (2018). Top Causes of Male and Female Infertility. Retrieved from: https://www.indoreinfertilityclinic.com/causes-of-infertility/

Jafari-Ghahfarokhi, H., Moradi-Chaleshtori, M., Liehr, T., Hashemzadeh-Chaleshtori, M., Teimori, H. & Ghasemi-Dehkordi, P. (2015). Small supernumerary marker chromosomes and their correlation with specific syndromes. *Advanced Biomedical Research, 4*, 140. doi: 10.4103/2277-9175.161542

Jaseem, K. M. (2019). *Mapping genes involved in human hereditary infertility* (pp. 514-621). Peshawar: Khyber Medical University.

Jauniaux, E., Farquharson, R. G., Christansen, O. B. & Exalto, N. (2006). Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage. *Human Reproduction, 21*(9), 2216-2222. doi: 10.1093/humrep/del150

Keymolen, K., Staessen, C., Verpoest, W., Liebaers, I. & Bondulle, M. (2014). Preimplantation genetic diagnosis in female and male carriers of reciprocal translocations: clinical outcome until delivery of 312 cycles. *European Journal of Human Genetics, 20*(4), 376-380. doi: 10.1038/ejhg.2011.208

Khalafalla, K., Sengupta, P., Arafa, M., Majzoub, A. & Elbardisi, H. (2020). Chromosomal Translocations and Inversion in Male Infertility. In: Arafa, M., Elbardisi, H., Majzoub, A., Agarwal, A. (Eds.) *Genetics of Male Infertility* (pp. 207-219). Springer, Cham. https://doi.org/10.1007/978-3-030-37972-8_12

Kolte, A. M., Bernardi, L. A., Christansen, O. B., Quenby, S., Farquharson, R. G. & Goddijn, M. (2015). Terminology for pregnancy loss prior to viability: a consensus statement from the ESHRE early pregnancy special interest group. *Human Reproduction, 30*(3), 495-498. doi: 10.1093/humrep/deu299

Kovalevsky, G., Gracia, C. R. & Berlin, J. A. (2004). Evaluation of the association between hereditary thrombophilias and recurrent pregnancy loss. *Archives of Internal Med*icine, *164*(5), 558–563. doi: 10.1001/archinte.164.5.558

Krausz, C. & Rosta, V. (2020). Chromosome Abnormalities and the Infertile Male. Chapter 3 In: Aitken, R. J., Mortimer, D. & and Kovacs, G. (Eds.) *Male and Sperm Factors that Maximize IVF Success*. (pp. 28-40). Cambridge: Cambridge University Press. doi:10.1017/9781108762571.003

Liehr, T. & Al-Rikabi, A. (2019). Mosaicism: Reason for Normal Phenotypes in Carriers of Small Supernumerary Marker Chromosomes With Known Adverse Outcome. A Systematic Review. *Frontiers in Genet*ics, *10*, 1131. doi: 10.3389/fgene.2019.01131

Mau-Holzmann, U. A. (2005). Somatic chromosomal abnormalities in infertile men and women. *Cytogenetic and Genome Res*earch, *111*(3–4), 317-336. doi: 10.1159/000086906

McPherson, E. (2016). Recurrence of stillbirth and second trimester pregnancy loss. *American Journal of Medical Genetics, 170A*(5), 1174-1180. doi: 10.1002/ajmg.a.37606.

Miller, O. J. & Therman, E. (2000). *Human Chromosomes*. 4th Edition (pp. 210-263). New York: Springer.

Misbah, I. H., Ayesha, K., Afsheen, A. & Erum, S. (2019). Cytogenetic investigation of couples with recurrent spontaneous miscarriages. *Pakistan Journal of Medical Sciences, 35*(5):1422-1427. doi: 10.12669/pjms.35.5.678

Poppe, K., Velkeniers, B. & Glinoer, D. (2008). The role of thyroid autoimmunity in fertility and pregnancy. *Nature Clinical Practice Endocrinology and Metabolism, 4*, 394–405. doi:10.1038/ncpendmet0846

Practice Committee of the American Society for Reproductive Medicine (2015). Diagnostic evaluation of the infertile male: a committee opinion. *Fertility and Sterility*, *103*(3), e18-25. doi: 10.1016/j.fertnstert.2014.12.103.

Puig, M., Casillas, S., Villatoro, S. & Caceres, M. (2015). Human inversions and their functional consequences. *Briefing in Functional Genomics, 14*(5), 369-379. doi: 10.1093/bfgp/elv020

Raziel, A., Shevach, F., Morey, S., Kasterstein, E., Strassburger, D. & Ron-El, R. (2002). Increased frequency of female partner chromosomal abnormalities in patients with high-order implantation failure after in vitro fertilization. *Fertility and Sterility*, *78*(3), 515-519. doi: 10.1016/s0015-0282(02)03298-3

Salazar, A. U., Almos, C. B., Arrigada, A. & Selman, E. C. (2011). Cytogenetic study of 677 spontaneous abortion. *Revista Anacem, 5*(2),74-77.

Santos, M. E., Martinez, M. H., Hernandez, A. E., Gonzalez, G. N., Rojas, A. M., Garcia, A. P. & Conde D. R. (2019). Chromosomal Studies In Individuals With Infertility. Revista Cubana de *Investigaciones Biomedicas*, *38*(1).

Sciurano, R. B., Rahn, I. M., Gonzalez, B. A., Valzacchi, G. R., Benavente, P. & Solari, A. J. (2019). Selective advantage of euploid spermatocytes I in an azoospermic 47,XXY man gonadal mosaicism. *Human Reproduction, 34*(3), 568-573. doi: 10.1093/humrep/dey387

Shah, K., Sivapalan, G., Gibbons, N., Tempest, H. & Griffin, D. K. (2003). The genetic basis of infertility. *Reproduction, 126*(1), 13-25. doi: 10.1530/rep.0.1260013

Sharma, R., Biedenharn, K. R., Fedor, J. M. & Agarwal, A. (2013). Lifestyle factors and reproductive health: taking control of your fertility. *Reproductive Biology and Endocrinol*ogy, *11*, 66. doi: 10.1186/1477-7827-11-66

Signore, F., Gulia, C., Votino, R., De Leo, V. & Zaami, S. (2019). The Role of Number of Copies, Structure, Behavior and Copy Number Variations (CNV) of the Y Chromosome in Male Infertility. *Genes, 11*(1), 40. doi: 10.3390/genes11010040.

Stipoljev, F. (2007). Genetski uzroci neplodnosti. *Medicina Fluminensis*, *43*(4), 279–284.

Šimunić,V. *et al.* (2012). *Reprodukcijska endokrinologija i neplodnost – Medicinski pomognuta oplodnja*. IVF (pp. 119–159). Zagreb: Školska knjiga.

Tinting, L., Haiquan, S., Guoming, C., Yuanyuan, Z., Manlong, Q, Xiaoliang, L., Wanting, C. & Yanyan, Z. (2020). Genotype-phenotype correlation in 75 patients with small supernumerary marker chromosomes. *Molecular Cytogenet*ics, *13*, 30. doi: 10.1186/s13039-020-00494-2

Turnpenny, P. D. & Ellard, S. (2012). *Emery's Elements of Medical Genetics*. (p. 445). Philadelphia: Elsevier/ Churchill Livingstone.

Van den Boogaard, E. (2014). Optimizing quality of care for couples with recurrent miscarriage. [Doctoral dissertation]. Faculty of Medicine, University of Amsterdam, Amsterdam.

Wang, R., Yu, Y., Wang, Q., Jiang, Y., Li, L. & Zhu, H. (2019). Clinical features of infertile men carrying a chromosome 9 translocation. *Open Medicine, 14*(1), 854-862. doi: 10.1515/med-2019-0100

WHO (1977). WHO: recommended definitions, terminology and format for statistical tables related to the perinatal period and use of a new certificate for cause of perinatal deaths. Modifications recommended by FIGO as amended October 14, 1976. *Acta Obstetricia et Gynecologica Scandinavica, 56*(3), 247-253. Retrieved from: https://pubmed.ncbi.nlm.nih.gov/560099/

Yatsenko, S. A. & Rajkovic, A. (2019). Genetics of human female infertility. *Biology of reproduction, 101*(3), 549-566. doi: 10.1093/biolre/ioz084

Zachaki, S., Kouvidi, E., Pantou, A., Tsarouha, H., Mitrakos, A., Tounta, G., Charalampous, I. & Kalliopi, N. (2020). Low-level X Chromosome Mosaicism: A Common Finding in Women Undergoing IVF. *In Vivo*, *34*(3), 1433-1437. doi: 10.21873/invivo.11925.

Zarifian, A., Farhoodi, Z., Roya, A., Selmeh, M. & Hassanzadeh-Nazarabadi, M. (2012). Balanced chromosomal rearrangement in recurrent spontaneous abortions: a case report. *International Journal of Molecular and Cellular Medicine, 1*(4), 225-228.

Zhang, H., Wang, R., Yu, Y., Zhu, H. & Li, L. (2019). Non-Robertsonian translocations involving chromosomes 13, 14, or 15 in male infertility. *Medicine*, 98(9):e14730. doi. doi: 10.1097/MD.0000000000014730.

HROMOZOMSKE ABERACIJE KOD PAROVA SA STERILITETOM I HABITUALNIM POBAČAJIMA U UNIVERZITETSKOM KLINIČKOM CENTRU REPUBLIKE SRPSKE

Sažetak

Citogenetička laboratorija Univerzitetskog kliničkog centra Republike Srpske u Banjoj Luci predstavlja primarnu instituciju u kojoj se analiziraju kariotipovi periferne krvi pacijenata. U periodu od 2009. do 2019. godine u njoj su urađene ukupno 3842 analize kariotipa pacijenata od kojih je 1956 imalo uputnu dijagnozu sterilitet i habitualni pobačaj pa samim tim i nemogućnost ostvarivanja trudnoće. Značaj citogenetičkih analiza kariotipova pacijenata je velik jer prisustvo hromozomskih aberacija u njima može da bude uzrok steriliteta i spontanih pobačaja kod pacijenata. Zato je cilj rada bio je da se utvrdi prisustvo hromozomskih aberacija u kariotipovima pacijenata na UKC RS koji su imali uputnu dijagnozu sterilitet ili habitualni pobačaj. Ukupan broj uzoraka obrađenih kariotipova pacijenata u Citogenetičkoj laboratoriji na UKCRS u Banjoj Luci u periodu od 2009. do 2019. godine rastao je po godinama. U studiji je utvrđena značajna razlika u raspodjeli učestalosti pacijenata sa dijagnozom steriliteta, kojih je bilo dva puta više u odnosu na pacijente sa dijagnozom habitualni pobačaj. Procenat pacijenata koji se liječe od steriliteta i habitualnog pobačaja sa prisustvom hromozomskih aberacija u njihovim kariotipovima u odnosu na one koji nemaju aberacije prosječno je nizak. Utvrđena je podjednaka raspodjela muških i ženskih pacijenata sa aberantnim kariotipovima, a koji su liječeni od steriliteta i habitulanih pobačaja. Najzastupljenije hromozomske aberacije u kariotipovima kako muških tako i ženskih pacijenata liječenih od steriliteta i habitualnog pobačaja bile su inverzije, nakon njih dolaze mozaične aberacije, translokacije, zatim slijede trizomije, Robertsonove translokacije i na kraju adicije.

Ključne riječi: hromozomske aberacije, inverzije, Klinefelterov sindrom, Daunov sindrom, mozaicizmi

Received October 17, 2022
Accepted November 28, 2022