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Split protocol for next successful pregnancy after spontaneous abortions and pregnancy loss in Genetic Counselling Unit Split

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Abstract

Aim: Spontaneous abortion, early neonatal death and stillbirth are tragic events for the whole family. Sporadic abortion in the general population occurs at 12-15%, before the 12th weeks. The percentage increases with the age of the mother, up to 23%. Successful next pregnancy is the goal.

Methods: In a retrospective analysis from 1985 to 2010, 451 couples with one or more SAs before the 16th week of pregnancy were examined at the Genetic Counselling Unit, Pediatrics Clinics, UH Split.

Results: The highest number of SAs was recorded in the period 8-10th weeks of gestation. Furthermore, 69% of women and 66% of men had a positive family history (especially in a second-generation relative) for SA or sterility. Adverse habits (smoking, alcohol consumption) and exposure to pollutants and / or teratogens (chemicals, exposure to excessive heat or cold) did not affect the incidence of SA. Examination of serological signs (IgM + and EA +) in the presence of reactivation of infection with viruses most commonly revealed reactivation to viruses of the genus herpes viride such as HSV1, CMV and EBV, more often in women. Sideropenia or anemia with very low ferritin values were found in 40% of tested women.

Conclusion: The theory of "two hits" (multifactorial inheritance) is still in the basis of SA. Under these circumstances, it is possible to achieve a normal pregnancy as needed with prenatal or preimplantation diagnostics. If there is a need for assisted fertilisation, the same procedure should be followed.

Keywords: spontaneous abortions (SA), pregnancy loss (RPL), etiology, genetic counselling

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1. Introduction

Genetic information is a process during which patients and their relatives can find out about the risks of a trait or hereditary disease. It consists of a diagnostic procedure with later support and follow-up. In many ways it is special, very personal and important for the individual but also for the whole family. It is permanent and discriminatory and it can also have the ability to predict human destiny. The main postulates are: voluntariness, information, privacy and confidentiality of genetic information. Before the conversation, it is necessary to obtain information within the family about spontaneous abortions, stillbirths, hereditary and chronic diseases, sudden death, especially in childhood, and bring all relevant findings.

Spontaneous abortion (SA), early neonatal death and stilbirth are tragic events for the whole family and are a common reason for genetic testing of parents and their biological relatives. Such a pregnancy outcome can be influenced by various factors of a genetic, infectious, anatomical, endocrine and immune nature (Čulić V. 2010). The term abortion means termination of pregnancy at a certain gestational stage before the fetus becomes capable of independent living. Sporadic abortion in the general population occurs at a frequency of 12-15%, mostly before the 12th week of pregnancy. The percentage increases with the age of the mother, so it is up to 23% in women aged 40 (De La Rochebrochard E. & Thonneau P. 2002).

1.1 The etiology of miscarriages:

1.1.1 Chromosome number changes

Most commonly aneuploidies of chromosomes 16 and 21 are generated by chromosome non-disjunction. In trisomic spontaneously aborted fetus specimens (trisomy of chromosomes 2, 7, 15, 16 and 22), trophoblastic hyperplasia similar to a hydatiform mole is most commonly detected by pathohistological analysis (Čulić V. 2010).

1.1.2 Teratogens

Teratogens cause gene mutations, breakages and chromosome splitting, affect postzygotic mitoses, and cause de novo aneuploidies, translocations, or mosaicism (Lassi Z.S: et al 2014)). After exposure to teratogen cell death can occur, altered tissue growth (hyperplasia, hypoplasia, asynchronous growth) and changes in differentiation and morphogenesis. The teratogens found in the environment are drugs and chemical, physical and infectious agents. If different teratogens influence embryogenesis during the same period, they will have the same effect on the fetus (Čulić V. 2010). In the 3rd to 6th week of gestation 40% of fetuses die and by the 12th week of gestation 20% of them.

1.1.3 Infections

Perinatal infections occur in the general population at a frequency of 0.5 to 2.5%. They are most commonly caused by infections of the listeria bacterium and ascending bacterial infections of urogential tract of the pregnant woman. Other causes of perinatal infections are:toxoplasma, rubella virus, cytomegalovirus (CMV), Epstein-Barr virus (EBV), parvovirus B19, human herpesvirus 6 (HHV6), human papilloma virus (HPV6), coxsackievirus, influenza virus, respiratory syncytial virus (RSV), rotavirus, varicella zoster virus, herpes zoster virus, measles virus and human immunodeficiency virus (HIV). Depending on the timing and severity of the infection intrauterine fetal infection can cause miscarriage, fetal death or damage of organs and systems. Sometimes the infection of the mother can be transmitted to fetus and sometimes it can continue after birth as acute, chronic or latent (Check J.H. 2010, Čulić V. et al 2009.). Some pathogens pass the formed placenta (i.e. the causative agent of syphilis) and some do not (i.e. the influenza virus). The immune response is different in pregnancy (due to immunomodulation), and there is a significant difference in the specificity of the inflammatory response of each person, including the pregnant woman.

The Epstein-Barr virus persists asymptomatically throughout the life in almost all adults. It is the cause of infectious mononucleosis and oral leukoplakia, but is also associated with the development of several types of neoplasms. The virus genome (double-stranded DNA) can be either highly methylated (latent-lysogenic virus cycle) or unmethylated (lytic virus cycle). During latent infection, serological response to EBV antigens can be achieved. Infection of the mother with EBV during pregnancy can cause organ malformation and fetal death (Čulić V. 2004.). In any case, pregnant women are advised to avoid staying near a person with acute infectious mononucleosis and not to plan pregnancy during antibody seroconversion or the presence of antibodies to an early virus antigen. Characteristic pathohistological changes caused by active maternal infections during pregnancy are signs of inflammation in the placenta, lymphoplasmacytoid infiltrate of the stroma (rubella virus, CMV and treponema), vasculitis, hemosiderin deposition in the stroma (CMV) and calcium deposition. Infection can also be demonstrated by special pathohistological staining of inclusions in stroma or endothelial cells (CMV, rubella) and immunohistochemical, cytometric or molecular methods (Čulić V. et al 2009., Čulić V. 2004.).

The most common infections (symptomatic and asymptomatic) are the urinary tract (7-10%) caused by E. coli. As many as 20-30% of children become infected by passing through the birth canal; develop a clinical picture of newborn septicemia and meningitis. Sexual infections can spread by blood (listeria infections - 10% sepsis in newborns, group B and D streptococci - Enterococcus, S. faecalis, S. faecium and S. viridans groups A, C or G) and ascending (Chlamydia trachomatis infections and Mycoplasmom hominis). They can cause miscarriages, stillbirths and various fetal infections. Sexual infections are often asymptomatic (C. trachomatis, M. hominis,

Ureaplasma urealyticum) (Čulić V. et al 2009., Čulić V. 2004.).
Intra-amniotic infection syndrome arises ascending from the cervix. If pregnant women symptoms of threatening miscarriage is not recognised, acute septic form of the disease may occur (Čulić V. et al 2009., Čulić V. 2004.).

2. Results

Spontaneous abortion most commonly occurs in couples who have normal constitutional karyotypes. In a retrospective analysis from 1985 to 2010, 451 couples with one or more SAs before the 16th week of pregnancy were examined at the Genetic Counselling Unit, Pediatrics Clinics, UH Split. The highest number of SAs was recorded in the period 8-10th weeks of gestation, a slightly lower number from weeks 4 to 7, and from weeks 11 to 13, and the lowest percentage was observed between weeks 14 and 16. Maternal and paternal age did not influence the onset of SA. Furthermore, 69% of women and 66% of men had a positive family history (especially in a second-generation relative) for SA or sterility (Mišković S. et al 2012). Adverse habits (smoking, alcohol consumption, exposure to pollutants) and / or teratogens (chemicals, exposure to excessive heat or cold) did not affect the incidence of SA.
Examination of serological signs (IgM + and EA +) in the presence of reactivation of infection with viruses most commonly revealed reactivation to viruses of the genus herpes viride such as HSV1, CMV and EBV, and more often in women than in men. Sideropenia or anemia with very low ferritin values were found in 40% of tested women.
Cytogenetic examination of constitutional karyotype in women revealed changes in 58 subjects: 13 mosaicism, 7 balanced translocations and 38 population polymorphisms. The same type of study found changes in men in 43 subjects: two mosaicism, seven balanced translocations, one change on chromosome Y, and 33 chromosomal variations in the population. The number of Robertsonian translocations t(13;14) and t(13;21) were equal in women and men. Other chromosomes included in balanced translocations were 1, 4-6, 8-11, 15-18, and 21. There was evidence of cytogenetic cause of SA in these couples and preimplantation genetic testing (not performed in the Republic of Croatia) before the next pregnancy is recommended. Subjects in constitutional karyotypes have variations in the population, especially pericentric inversion of chromosome 9 and excess heterochromatin on chromosome 9. This is a specific feature of the studied population group in southern Croatia, unlike in Rijeka where there were more 16qh+ in the SA. Polymorphisms within the centromeric region are thought to be closely related to the mechanisms of epigenetic control and under the influence of some external factors (viruses, chronic inflammation, urinary tract, cytokine damage network, folate deficiency) and may have a direct effect on methylation and split spindle, which may ultimately result in chromosome aneuploidy (Dana M. & Stojan V. 2012, Hong Y. et al 2011, Caglayan A.O. et al 2010).
Cytogenetic analysis was also performed on 96 samples of aborted material. Changes were found in 40, most commonly trisomies of autosomes. Triploidy was reported in four cases. Only four women who had a genital tract infection (with normal constitutional karyotypes) were found to have aneuploidy in abortive material (47, XX, +22; 47, XX, + 5; 47, XY, +7; and 47, XY, + 20) which was 12.5% of the total number of aneuploidy in the SA or de novo changes. Comparing hereditary changes (balanced translocations of 1.7% in women or 1.8% in men) to de novo changes occurring in those with urinary tract infections, the ratio is seven times higher. All population polymorphisms involved a change on chromosome 9. A combination of two cytogenetic changes was observed in three samples. Similar data have been reported in other scientific papers (Alonso Lopez A.G. et al 2011, Zhang H.K. et al 2011.).

Table 1: Number of unsuccessful pregnancies (UP) per couple.

Number of abortions per couple	Number of couples	Number of UP
1	76	76
2	265	530
3	65	195
4 and more	36	179
Total	442	980

The total number of unsuccessful pregnancies (UP) found in 442 couples was 980 (Table 1).
The number of unsuccessful pregnancies (UP) in relation to the gestational period when it occurred is of the total 980 UP 747 (76.2%) and happened before 16th week of pregnancy. From 4-7 w. 210 (28,11%); 350 (46,85%) from 8-10 w., 139(18,61%) 11-13 w. and 48 (6,42%) from 14-16 week of pregnancy.The largest number of UP occurred between 8-10 week of pregnancy. For 111 (14,86%) we had no data and 43 (5,75%) occured after 16 week of pregnanacy. In 79 (10,58%) cases it was ectopic pregnancy.

Table 2: Serum iron values in 65 tested women

Serum iron	No. of women
10.0-30.0	47
3.8-10	17
>30	1
Total	65
Normal range for women older than 20 years: 10.0-30 µmol/L	

Despite physiologic variations, the serum ferritin concentration is currently the most reliable non-invasive marker of iron reserve in pregnancy and postpartum. The serum ferritin and iron values were determined in 65 tested women (Tables 2 and 3). The majority of tested women had normal iron val-ues. Seventeen women had iron values below the normal value, while one woman had increased serum iron value.

Table 3: Serum ferritin values in women

Serum ferritin	No. of women
10.0-120.0	48
2.4 – 10.0	17
10.1– 40.41	45
>50.0	2
>70.0	1
Total	65
Normal range for women from 20 to100 years: 10.0 -120.0 µg/L	

Tables 2 and 3 show that the women with normal serum iron values had normal serum ferritin values as well. Seventeen women had decreased values of both serum iron and ferritin. They were immediately started with treatment with full dose of iron medication with the supplement of vitamin C and folic ac-id. The treatment lasted for 1 - 3 months, namely until serum iron values reached normal values. Alt-hough most of the tested women had normal ferritin values, in almost 45 tested women ferritin reserves were less than 50 □g/ml, which is not sufficient for the next pregnancy. For this reason, those women were started with a treatment with half of the previously mentioned medication (iron with the supple-ment of vitamin C and folic acid). The treatment lasted for 3 to 12 months, i. e until the serum ferritin values reached values of 50 □g/ml.

Table 4. Number of individuals (both gender) with high titers of EBV (IgM, EA, IgG and EBNA) seropositivity

Serology	Women	Men
IgM+, EA+, IgG>170, EBNA+ or >200	102 (51%)	49 (33%)
	199 100%)	146 (100%)
IgG>170 only	60 (30.3%)	41 (28%)
	199 (100%)	146 (100%)
EBNA >200 only	37 (18.7%)	56 (39%)
	199 (100%)	146 (100%)
Total number of individuals with very high positive titers	199 (55.6%)	146 (50.5%)
Total number of individuals with positive titers	359(100%)	289 (100%)

359 women and 289 men had positive viral serology findings for EBV (IgM, IgG, EA (early antigen), EBNA)

2.1 Split protocol for next pregnancy

1. Chromosome analysis of both partners, materials of miscarriage and CMA;
2. Pathohistological analysis of the placenta (the search for hydropic and degenerative changes and inflammatory cells);
3. Create a family tree and collect information on abortions, stillbirths and malformations from relatives I, II and III generations;
4. Before the next pregnancy, stop with harmful habits and exposure to teratogens at home and / or in workplace;
5. Treat asymptomatic urinary tract infections in both partners;
6. Monitor the serological titer of antibodies specific for virus infections (for both partners);
7. Examine the status of genes responsible for the propensity of thrombophilia in both partners;

8. Check serum iron and ferritin values in women. If necessary, treat them with iron, when the values of feritin are 50 mg/ml before and 70 mg/ml during pregnancy.

3. Discussion

There is a lot of literature data on changes caused by viruses at the chromosomal level such as chromosome breaks, chromosomal instability due to the action on the dividing spindle mostly found in cancer cells. When talking about idiopathic spontaneous abortion, we believe that there is not a single mechanism for every gene or chromosomal change in embryo.

Rather it depends on the time that passed between conception to abortion or the birth of a sick child. Similar data were obtained by other researchers (Niroumanesh S. et al 2011, El-Dahtory F.A.2011). The result is a multifactorial disease - recurrent pregnancy loss (Al-Buthori M. et al 2011).

ToRCH (toxoplasmosis, rubella, CMV, HSV) are the most common causes of congenital infections. Primary infections during pregnancy have wide ranges of clinical symptoms dependent on the stage of pregnancy.

During the early stages of pregnancy infections cause congenital malformations, intrauterine growth restriction (IUGR) or fetal death. Infections during the later stages of pregnancy may result in asymptomatic babies at birth, which also may progress to signs of infection at a later stage. The incidence rate of congenital toxoplasmosis is 1.5 cases per 1000 livebirths (Al-Buthori M. et al 2011). Rubella virus is a major cause of birth defects and fetal death following infection in pregnant women (congenital rubella syndrome - CRS). About 0,5% of infants are born with congenital CMV infection.

Some of them will usually have health problems later in life. Neonatal HSV infection incidence varies from 0.3 to 0.05 permille.

Cytogenetic analysis of Burkitt's lymphoma EBV positive and negative cells revealed a significant increase in dicentric chromosomes, chromosome fragments, chromatid gaps and increase of telomere size and telomere fusion in EBV-positive cells (Kamranvar S. et al 2007.).

Re-stimulation of T lymphocytes results in telomere damage that eventually leads to growth arrest or replication arrest, which then leads to activation of factors that initiate rest in memory T cells between episodes of viral reactivation responsible for long-term maintenance of T cell memory (EBV and CMV). Antibody production can take a lifetime. Human B lymphocytes proliferate into plasma cells in response to polyclonality which then allows serological memory to be maintained for life.

EBV reactivation is closely related to cell cycle arrest in the Go / G1 phase (Bernasconi N.L. et al 2002, Lin Z. et al 2004, Szymula A. et al 2018). CMV infection is a risk factor for centromere aberration in peripheral blood lymphocytes (Voon-Kwan Siew et al (2009), Gao L. et al 2009.).

A causal link between viral infections and autoimmunity has been studied for a long time and the role of some viruses in the induction or exacerbation of systemic lupus erythematosus (SLE) has been proved in genetically predisposed patients.The strength of the association between different viral agents and SLE is variable. Epstein-Barr virus (EBV), parvovirus B19 (B19V), and human endogenous retroviruses (HERVs) are involved in SLE pathogenesis, whereas other viruses such as Cytomegalovirus (CMV) probably play a less prominent role (Quaglia M. et al, 2021.).

Epstein-Barr virus infection is predominantly latent; however, lytic infection is detected in healthy seropositive individuals and becomes more prominent in certain pathological conditions (McKenzie J.& El-Guindy A. 2015., Sinclair A.J. 2006.).

Studies from Turkey found values with atypical serological findings for EBV in individuals with autoimmune diseases. The establishment and maintenance of Epstein-Barr Virus latent infection requires distinct viral gene expression programs, chromatin structure, epigenetic modifications and chromatin remodeling (Varıcı Balcı F.K. et al 2017., Abrahamyan S. et al 2020., Sompallae R. et al 2010.).

Placental oxidative stress, with necrosis and apoptosis of trophoblastic epithelium and placenta takes place in a low oxygen (O2) environment. In abortion the development of the placental-decidual membrane is severely impaired due to maternal blood flow and large oxidative degeneration (Jauniaux E. et al 2006.). The existence of the placental microbiota explains the colonization of the fetus / placenta by both pathogenic and commensal microbes (de Lima Kaminski V. et al 2019.).

Presence of an entire additional chromosome or chromosome loss can affect the global genome methylation level. These results point out to possible link between aberrant epigenetic processes and etiology of mitotic non-disjunction (Tolmacheva E.N. et al 2020., Kashevarova A.A.et al.2011.).

Women with RPL had lower s-ferritin than the comparison group, 39.9 μg/ml versus 62.2 μg/ml, and had a higher prevalence of low iron stores (s-ferritin <30 μg/L), 35.7% versus 13.7%. They found an inverse relationship between s-ferritin level and number of pregnancy losses, but also did not find s-ferritin level to be associated with ability to conceive in either group. Nor did s-ferritin level predict the risk of losing the first pregnancy after referral for RPL. Whether low s-ferritin is causally related to RPL and if such women could benefit from iron supplementation to achieve a live birth needs further investigation (Georgsen M. et al. 2021.)

4. Conclusion

Genetic information is a process during which patients and their relatives can find out about the risks

of a trait or hereditary disease. The theory of "two hits" is still in the basis of SA. Chron-ic infection (urogenital) can lead to iron deficiency. Fe starvation promotes degradation battery of Fe dependent metabolism and Fe storage. It is important to prevent iron deficiency in the fetus by prevent-ing iron deficiency in the pregnant women. Iron supplement should be taken before next pregnancy to get serum levels of ferritin 50µgr/ml and to have 70µgr/ml during first trimester to avoid repeated spontaneous abortions. EBV is a causative agent of autoimmune diseases such as MS characterized by serological polyclonality. It probably acts in the same way in development of SA with or without chromosomes abnormalities, depending on the time of viral reactivation, other coinfections and the time of conception. Under these circumstances, it is possible to achieve a normal pregnancy as needed with prenatal or preimplantation diagnostics (if there is hereditary disease or chromosome aberration). If there is a need for assisted fertilisation, the same procedure should be followed.

Conflicts of Interest

The authors have no conflicts of interest to declare.

5. References

Abrahamyan S. et al (2020). Complete Epstein-Barr virus seropositivity in a large cohort of patients with early multiple sclerosis. J Neurol Neurosurg Psychiatry, 0:1–6. doi:10.1136/jnnp-2020-322941

Al-Buhtori M. et al (2011). Viral Detection in Hydrops Fetalis, Spontaneous Abortion, and Unexplained Fetal Death In Utero. Journal of Medical Virology, 83:679-684.

Alonso López A.G. et al (2011). Cytogenetic diagnosis of first trimester spontaneous abortion. Ginecol Obstet Mex; 79: 779-84.

Bernasconi N.L. et al (2002). Maintenance of Serological Memory by Polyclonal Activation of Human Memory B Cells. Science, 298(5601):2199-2202. doi: 10.1126/science.1076071.

Caglayan A.O. et al (2010). Are heterochromatin polymorphisms associated with recurrent miscarriage? J Obstet Gynaecol Res; 36: 774-6.

Check J.H. (2010). A practical approach to the prevention of miscarriage. Part 4-role of infection. Clin Exp Obstet Gynecol; 37: 252-5.

Culic V. et al (2009). Genitourinary diseases prior to spontaneous abortion as a risk factor for recurrent pregnancy loss. Coll Anthropol; 1: 187-92.

Čulić V. (2004). Diagnosis and monitoring of intrauterine infections. Paediatr Croat; 48: 180-91.

Čulić V. (2010). The importance of genetic testing and information in pregnancy planning (when and how to start thinking about procreation). Paediatr Croat; 54: 122-8.

De La Rochebrochard E. & Thonneau P. (2002). Paternal age and maternal age are risk factors for miscarriage; results of a multicenter European study. Hum Reprod; 17: 1649-56.

de Lima Kaminski V., et al. (2019). Extracellular vesicles in host-pathogen interactions and immune regulation-exosomes as emerging actors in the immunological theater of pregnancy. Heliyon, doi: 10.1016/j.heliyon.2019.e02355

El-Dahtory F.A. (2011). Chromosomal abnormalities as a cause of recurrent abortions in Egypt. Indian J Hum Genet; 17: 82-4.

Gao L. et al (2009). Changes of peripheral blood chromosomal centromere aberration in patients with cytomegalovirus infection after anti-viral treatment. (Article in Chinese) Nan Fang Yi Ke Da Xue Xue Bao, 29(9):1846-7.

Georgsen M. et al. (2021). Serum ferritin level is inversely related to number of previous pregnancy losses in women with recurrent pregnancy loss. Fertil Steril, 115(2):389-396. doi:10.1016/j.fertnstert.08.1410.

Hong Y. et al (2011). Do polymorphic variants of chromosomes affect the outcome of in vitro fertilization and embryo transfer treatment? Hum Reprod; 26: 933-40.

Jauniaux E. et al (2006). Placental-related diseases of pregnancy: involvement of oxidative stress and implications in human evolution. Hum Reprod Update, (12),6:747- 755. doi: 10.1093/humupd/dml016.

Kashevarova A.A.et al. (2011). [Methylation profiling of the cell cycle regulating genes in placenta of human embryos with chromosomal mosaicism]. Mol Biol (Mosk),45(2):316-324. Russian. PMID: 21634119.

Kamranvar S.A. et al (2007). Epstein-Barr virus promotes genomic instability in Burkitt's lymphoma. Oncogene, 26:5115–5123.

Lassi Z.S. et al (2014). Preconception care: caffeine, smoking, alcohol, drugs and other environmental chemical/radiation exposure. Reprod Health; 11 (Suppl 3): S6.

Lin Z., Yin Q. & Flemington E. (2004). Identification of a Negative Regulatory Element in the Epstein-Barr Virus Zta Transactivation Domain That Is Regulated by the Cell Cycle Control Factors c-Myc and E2F1. J Virol, 78(21):11962-11971. doi: 10.1128/JVI.78.21.

McKenzie J. & El-Guindy A. (2015). Epstein-Barr Virus Lytic Cycle Reactivation. Curr Top Microbiol Immunol, 391:237-61. doi: 10.1007/978-3-319-22834-1-8.

Miskovic S., et al. (2012). Positive reproductive family history for spontaneous abortion: a predictor for recurrent miscarriage in young couples. Eur J Obstet Gynecol Reprod Biol; 161: 182-6.

Niroumanesh S. et al (2011). A cytogenetic study of couples with repeated spontaneous abortions. Ann Saudi Med; 31: 77-9.

Quaglia M. et al (2021). Viral Infections and Systemic Lupus Erythematosus: New Players in an Old

Story. Viruses,11;13(2):277. doi: 10.3390/v13020277.

Sinclair A.J. (2006). Unexpected structure of Epstein–Barr virus lytic cycle activator Zta. Trends in Microbiology, (14),7:289-291. doi: 10.1016/j.tim.2006.05.003.

Sompallae R. et al (2010). Transcription Profiling of Epstein-Barr Virus Nuclear Antigen (EBNA)-1 Expressing Cells Suggests Targeting of Chromatin Remodeling Complexes. PLoS One,10;5(8) doi: 10.1371/journal.pone.0012052

Tolmacheva E.N. et al (2020). Aneuploidy and DNA Methylation as Mirrored Features of Early Human Embryo Development. Genes,11:1-21.1084; 10.3390/genes11091084.

Dana M. & Stoian V. (2012). Association of pericentric inversion of chromosome 9 and infertility in the romanian population. Maedica (Buchar); 7: 25-9.

Szymula A. et al (2018). Epstein-Barr virus nuclear antigen EBNA-LP is essential for transforming naïve B cells, and facilitates recruitment of transcription factors to the viral genome. PLOS Pathogens, 14(2) doi.org/10.1371/journal.ppat.1006890.

Voon-Kwan Siew et al (2009). Human cytomegalovirus UL76 induces chromosome aberrations. Journal of Biomedical Science, 16:107.

Varıcı Balcı F. K. et al (2017). Atypical profile problem in serological diagnosis of EBV. [Article in Turkish]. Mikrobiyoloji bülteni, 51(4):378-386. DOI: 10.5578/mb.58662.

Zhang H.K. et al., (2011). Analysis of fetal chromosomal karyotype and etiology in 252 cases of early spontaneous abortion. Chi J Med Genet, 28: 575-8.