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Tekrarlayan Düşüklerde Array Karşılaştırmalı Genomic Hibridizasyonun Yapısal Anomalileri Saptamadaki Önemi: Retrospektif Bir Çalışma

Importance of Structural Abnormalities Detected by Array-Comparative Genomic Hybridization in Recurrent Miscarriage: A retrospective Study

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ÖZ

Amaç: Kromozomal anomaliler, tekrarlayan düşüklerin en önemli risk faktörleri arasındadır. Vakaların %5.5'inde, ebeveynlerden birinin sitogenetik anormalliği mevcuttur ki bu oran genel nüfusa göre %0.55'tir. Son literatür verileri, düşüklere neden olan sebeplerin tespiti için a-CGH yöntemini kullanmış olsa da, kromozomal yeniden düzenlemenin ve tekrarlayan düşüklerin korelasyonunu değerlendirmek için özel olarak yapılan az sayıda çalışma bulunmaktadır; ancak bu konuda net bir kanıt bulunmamaktadır. Bu çalışmada, a-CGH yöntemi kullanılarak açıklanamayan tekrarlayan düşükleri olan çiftlerde kromozomal yeniden düzenlenmenin korelasyonunu amaçladık.

Yöntem: Beşten fazla düşüğü olan 74 hastanın karyotipleme ve a-CGH verileri retrospektif olarak analiz edildi. Hücre kültürleri, karyotipleme için standartlaşmış prosedürler takiben 400-550 bant düzeyinde hücre hasadı ve G-bantlama işlemleri gerçekleştirildi. Anne veya baba DNA'sının ekstrakte edilen miktarı ve kalitesi sırasıyla spektrofotometre ve jel elektroforezi ile ölçüldü.

Bulgular: Toplam 74 hasta arasında, çalışmaya 50 kadın ve 24 erkek dahil edildi. A-CGH sonuçları, erkeklerin 22'sinde (%91.7) ve kadınların 46'sında (%92) normal olarak bulundu ve normal hastaların, duplikasyon ve delesyon anormallikleri (Duplikasyon: 4q12, 2(p15-p14), 17q12; Delesyon: 1(q21.1-q21.2), 16p11.2, Xp22.31) olan hastalarla karşılaştırıldığında dağılımı anlamlı değildi ($P > 0.05$).

Sonuç: Anne ve baba adaylarının kromozmlarının aCGH ile araştırılması sonucunda düşük oranda delesyon ve dublikasyon anormallikleri izlenmiştir. Hastalara gerekli danışmanlığın verilebilmesi için bu anormalliklerin klinik önemi araştırılmalıdır.

Anahtar Kelimeler: Dizi karşılaştırmalı genomik hibridizasyon, Tekrarlayan düşük, Kromozomal anomaliler, Kromozomal duplikasyon, Kromozomal delesyon.

ABSTRACT

Objective: Chromosomal anomalies are among the most important risk factors of recurrent miscarriage. In 5.5% of the cases, one of the parents has cytogenetic anomaly in contrast to 0.55% of the general population. Recent literature data have used a-CGH for detection of cause of abortion, but there are few studies specifically conducted to evaluate the correlation of chromosomal rearrangement and recurrent miscarriages; yet there is no clear evidence on this issue. In this study, we aimed on the correlation of chromosomal rearrangement in couples with unexplained recurrent miscarriage by a-CGH.

Methods: The karyotyping and aCGH data of 74 patients with more than five abortions were analyzed retrospectively. Cell cultures, harvesting, and G-banding at the level of 400-550 bands for karyotyping were performed following standardized

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procedures. The extracted maternal or paternal DNA concentration and quality were measured with the spectrophotometer and gel electrophoresis, respectively.

Results: A total of 74 patients, 50 women with male partners of 24 were included in the study. The results of a-CGH were normal for 22 males (91.7%) and 46 females (92%) and the distribution of the normal patients were not significant between the genders when patients with duplication and deletion anomalies (Duplication: 4q12, 2(p15-p14), 17q12; Deletion: 1(q21.1-q21.2), 16p11.2, Xp22.31) were compared ($p > 0.05$).

Conclusion: The maternal and paternal aCGH analysis yielded low rate of duplication and deletion anomalies of the chromosomes. The clinical significance of the yielded abnormalities need to be evaluated for patient consultation.

Key words: Array comparative genomic hybridization, Recurrent miscarriage, Chromosomal anomalies, Chromosomal duplication, Chromosomal deletion.

1. INTRODUCTION

During the first trimester miscarriage complicates about 20% of all clinically detected pregnancies (1). Recurrent miscarriage or recurrent abortion occurs in one percent of pregnant women and defined as more than one consecutive pregnancy loss (2,3). More than half of the women attending clinical specialists are depressed due to consequent failures of getting pregnant and this can be frustrating for the physician as well (4).

Miscarriage has multifactorial factors such as increased maternal and paternal age, uterine anatomic abnormalities, immunologic factors, maternal systemic metabolic or endocrine disorders, tobacco usage, chemical toxicity, and microbial infections, which have been discussed in detail before (5, 6). Today, the standard evaluation of recurrent miscarriage includes evaluation of each of the parents for chromosomal translocations, autoimmune diseases, anatomic abnormalities and for inherited thrombophilia (7,8) Chromosomal anomalies are among the most important risk factors of recurrent miscarriage. In 5.5% of the cases, one of the parents has cytogenetic anomaly in contrast to 0.55% of the general population (9). It is generally assumed that the chromosomal abnormalities are inherited by the offspring from one of the parents; which leads to the miscarriage (10). It is officially recommended by the American Colleges of Obstetricians and Gynecologists to practice a clinical karyotyping for both parents who experience recurrent miscarriages (8). Numerical fetal anomalies such as trisomy and monosomy comprise approximately 90% of all chromosomal abnormalities (11).

Comparative genomic hybridization (CGH) is becoming a popular alternative method for cytogenetic analysis of recurrent miscarriages (12). In CGH, the DNA extracted from the miscarriage material is compared to control DNA across the metaphase for imbalances which in turn corresponds to chromosomal copy number variants such as trisomies and monosomies (13).

On the other hand array CGH (aCGH) is used to rapidly detect abnormalities of specific regions of specific chromosomes without the need of live cells (14). Metaphase analyses is needed in standart karyotyping whereas aCGH can be applied on nondiniding cells without any need for cell culture (12). These advantages makes aCGH the test of choice especially in cases of abortion where embryo is not living, and cell cultures may fail. Yet there is no consensus on the routine use of this technology (15-22).

In this retrospective study, we focused on the correlation of chromosomal rearrangement in couples with unexplained recurrent miscarriage by a-CGH.

2. MATERIALS AND METHODS

Among 2300 patients who applied to the Gynecology and Obstetrics department for an obstetric history of unexplained recurrent miscarriages over a 3 -year period, karyotyping and aCGH data of 74 patients with more than five miscarriages collected from Medical Genetics department were analyzed retrospectively. During gynecological examination, clinical history was recorded and blood tests regarding autoimmune, endocrine, and infectious diseases were performed on the maternal or paternal blood samples. Anatomical causes were excluded by gynecological examination, transvaginal ultrasound and hysterosalpingography. After exclusion of these factors, those parents who accepted to give blood samples for the study were recriuited and informed consent was obtained.. This study was conducted in compliance with Helsinki Declaration and approved by Alaaddin Keykubat University Non-invasive Clinical Research Ethics Committee.

Cytogenetic Analysis

Cell cultures, harvesting, and G-banding at the level of 400-550 bands for karyotyping were performed following standardized procedures (23). Chromosome observations were performed using Olympus microscope and CytoVision analysis software.

DNA extraction

DNA was extracted from product of the maternal or paternal blood using DNeasyBlood&Tissue kit (Qiagen, Hilden, Germany) method. Concentration and quality of the extracted DNA were measured with the (NanoDrop ND-1000; NanoDrop Technologies, Wilmington, DE) spectrophotometer and gel electrophoresis, respectively.

Array CGH analysis

Array CGH analysis was performed using the oligo based CytoSure Syndrome Plus ISCA Design (v2) Microarray 4x44K (Oxford Gene Technology, Oxford, UK) according to the manufacturer's recommendations. Data analysis was performed using CytoSure visualization software (Oxford Gene Technology, Oxford, UK). CNVs were interpreted according to public databases and literature mining. Benign CNVs were excluded by screening against Database of Genomic Variants (DGV).

Statistical analysis

GraphPad Version 3.06 2003 program was used for statistical analysis. Two-sided Chi-square Test and the Yates' corrected Chi-square were used to compare qualitative data. $p<0.05$ level was considered significant.

3. RESULTS

A total of 74 patients, 50 women with male partners of 24 were included in the study. The mean of maternal age was 32.9 ± 4.9 (range: 24-48) and paternal age was $34.8 \pm 6,7$ (range 24-56). The median of prior live births was 1 (range: 0-4) and the median of prior miscarriages was 5 (range 4-16). Most of the parents (44%) had one live birth and 42% of patients had fourth

birth miscarriages (Table 1). One woman (2%) had diabetes mellitus, three (6%) had hyperthyroidism and one (2%) had hypertension as a comorbidity. Most of the patients (86%) including men were smokers. Patients with uterine anomaly were excluded from the study.

Table 1. Demographic data of the patients

Feature		
Age	Mean ± SD [Range]	
Maternal		32.9 ± 4.9 (24-48)
Paternal		34.8 ± 6,7 (24-56)
Prior miscarriages	N (%)	
4		21 (42%)
5		12 (24%)
6		7 (14%)
7 or more		10 (20%)
Prior live births	N (%)	
0		18 (36%)
1		22 (44%)
2		7 (14%)
3		1 (2%)
4 or more		2 (4%)
Systemic disease	N (%)	
Diabetes mellitus		1 (2%)
Hyperthyroidism		3 (6%)
Hypertension		1 (2%)
Smoking	N (%)	43 (86%)

Results of a-CGH

The results of a-CGH were normal for 22 males (91.7%) and 46 females (92%) (Table 2) and the distribution of the normal patients were not significant when compared with the patients with duplication and deletion anomalies ($p > 0.05$). In a couple (2.7%) with recurrent miscarriage with no known risk factor, the female was found to have 4q12 duplication. This duplication was reported to be a benign micro mutation in a newborn (24).

Duplication of 2(p15-p14) was detected in a female with recurrent miscarriage with no known risk. Similar duplications (2p14–p16.1 and 2p16.1–p22.1) were reported to be associated with mental retardation in a 9-year-old boy and in a 17-year-old girl, respectively (25). In a couple with recurrent IVF failure with no known cause, the male and female were both found to have 16p11.2 deletion; and the male additionally had 1(q21.1-q21.2) deletion. As shown in Table 3, the distribution of duplications was not significant among the gender and not differed from the other anomalies ($p > 0.05$). Although neither of these mutations is known to cause miscarriage, a combination of them was reported in a case to cause developmental delay and congenital anomalies (26).

In a couple with recurrent intrauterine death without any known cause, the male was found to have Xp22.31 deletion; which was associated with X-linked ichthyosis, mental retardation, and neurological problems, as well as recurrent miscarriage in one case (27-30). A woman with recurrent miscarriage with no known risk factor had 17q12 duplication, which was associated with a wide spectrum of presentations, including congenital anomalies, disabilities of learning, motor skills, and psychiatric and neurological features (31, 32). As shown in Table 3, the distribution of deletions was also not significant among the gender and not differed from the other anomalies (p> 0.05).

Table 2. Frequencies of the results of array Comparative Genomic Hybridization (a-CGH)

Finding N (%)	**Male (n=24)**	**Female(n=50)**	**Total (n=74)**
Normal	22 (29.7)	46 (62.2)	68 (91.9)
Duplication, Total	0	3 (4.1)	3 (4.1)
4q12	0	1 (1.4)	1 (1.4)
2(p15-p14)	0	1 (1.4)	1 (1.4)
17q12	0	1 (1.4)	1 (1.4)
Deletion, Total	2 (2.7)	1 (1.4)	3 (4.1)
1(q21.1-q21.2) **16p11.2**	1 (1.4)	0	1 (1.4)
Xp22.31	0	1 (1.4)	1 (1.4)
	1 (1.4)	0	1 (1.4)
p value	0.218		

Table 3. Comparisons of the data of array Comparative Genomic Hybridization (a-CGH) by gender

Finding N (%)	**OR**	**95%CI**	**P value**
Normal vs Duplication	3.39	0.17-68.47	0.322
Normal vs Deletion	0.24	0.02-2.78	0.262
Duplication vs Deletion	0.09	0.003-3.104	0.200

4. DİSCUSSION

In spite of the state of art medical techniques, recurrent miscarriage remains to be a significant issue. It affects approximately 1% of couples and can cause depression and family problems (6). Chromosomal anomalies have been found to be the most important risk factor for recurrent miscarriage (10). Inheritance of chromosomal anomalies to the fetus can cause the fetus to develop abnormally, causing miscarriage. Karyotyping both parents with recurrent miscarriage is recommended by the American Colleges of Obstetricians and Gynecologists (8). However, effects of specific micro mutations have not been discussed in details before. We used array comparative genomic hybridization (a-CGH) to analyze the correlation of chromosomal rearrangement in patients with unexplained recurrent miscarriage.

Micro mutations in 50 females and their partners (24 males) were evaluated. We were not able to test every female's partner, which was a handicap for our results on the correlation of structural anomalies and unexplained recurrent miscarriage. We performed gynecological examination and blood tests regarding anatomical, endocrine, and infectious problems on our patients. After exclusion of these factors, we performed karyotyping; yet the cause of recurrent miscarriage remained obscure. After this, we looked for structural abnormalities in their chromosomes. Structural abnormalities were detected in 4 females and 2 males.

It was interesting that, in one couple with recurrent IVF failure, the female and the male both had 16p11.2 deletion and the male had 1(q21.1-q21.2) deletion. It is possible that these mutations were passed to the embryos; which caused abnormalities, resulting in IVF failure. This is supported by the report of developmental delay and congenital anomalies in a patient with combination of these two mutations (15).

A female with have 4q12 duplication, a female with 2(p15-p14) duplication, a female with 17q12 duplication, and a male with Xp22.31 deletion were also detected. There is not enough evidence about the effects of these mutations; yet they were all similarly associated with mental retardation and an enormous spectrum of clinical outcomes (15, 16, 18-22, 24). Our results are not sufficient to decide whether any specific micro mutation mentioned above is correlated with recurrent miscarriage, as we had a small sample size and did not perform further tests to explain functions of the affected genes on fetal development. We also could not test all individuals in the couples. Cytogenetic evaluation of more patients with recurrent miscarriage should be performed and molecular tests should be used to explain the cause-effect relationship of specific structural anomalies.

We believe the importance of cytogenetic analysis in recurrent miscarriage should be understood by clinicians and researchers; so that people suffering from it can be adequately informed and treated. Future studies on this topic may lead to a better understanding and several treatment options for couples with different chromosomal rearrangements, opening way for personalized medicine.

Previous studies showed that CGH can be used in case the conventional cytogenetic analysis were not conclusive. The possible causes of failure of conventional cytogenetic analysis are maternal cell DNA contamination, miscarriage tissue put in formalin or paraffin and failure of cell cultures(13, 33-37) . In such situations CGH was proved to have improved accuracy higher success and fewer errors due to maternal contamination (36). Balanced structural chromosome rearrangements and polyploidy cannot be identified with this CGH.

In a review to identify studies that have recorded monogenic genetic contributions to pregnancy loss in euploid pregnancies, evidence for genetic causes of pregnancy loss was established which adds to Mendelian causes of pregnancy loss (38). After analyzing 50 studies, causative variants were found in a range of genes, including DYNC2H1 (dynein, cytoplasmic 2, heavy chain 1), CHRNA1 (cholinergic receptor, nicotinic, alpha polypeptide 1), and RYR1 (ryanodine receptor 1), which were identified in multiple studies. A casual link with copy number variants and recurrent miscarriage was also identified. For appropriate counselling of the couples, for understanding the biology of these pathways, for designing a diagnostic sequencing panel for patients with recurrent pregnancy loss and planning possible treatment strategies, identification of these candidate genes are utmost important (38).

The limitation of aCGH is that failure todetect polyploidy, low grade mosaicism and balanced rearrangements (39). Although low grade mosaicism and balanced rearrangements are unlikely causes of pregnancy loss, polyploidy accounts for 8%–15% of miscarriages (19, 40). G-banded karyotyping with quantitative fluorescence- polymerase chain reaction (QF-PCR) and a-CGH can be used to overcome this limitation for the conception material. On the other hand in this study we have found an 8% rate of deletions and dublications in couples experiencing recurrent miscarriage.

5. CONCLUSION

Clinical importance of found dublications and deletions in couples with recurrent miscarriage is yet to be explained for any possible causative relationship.

Conflict of Interest

The authors have no conflicts of interest relevant to this article.

KAYNAKLAR

1. National Collaborating Centre for Women's and Children's Health (2012). Ectopic Pregnancy and Miscarriage: Diagnosis and Initial Management in Early Pregnancy of Ectopic Pregnancy and Miscarriage. NICE Clinical Guidelines, 154, 23.
2. Practice Committee of the American Society for Reproductive M. (2008). Definitions of infertility and recurrent pregnancy loss. *Fertil Steril, 89*(6), 1603.
3. Stirrat G.M. (1990). Recurrent miscarriage. *Lancet, 336*(8716), 673-675.
4. Rai R., & Regan L. (2006). Recurrent miscarriage. *Lancet, 368*(9535),601-611.
5. Garcia-Enguidanos A., Calle M.E., Valero J., Luna S., & Dominguez-Rojas V. (2002). Risk factors in miscarriage: A review. *Eur J Obstet Gynecol Reprod Biol, 102*(2), 111-119.
6. Alijotas-Reig J., & Garrido-Gimenez C. (2013). Current concepts and new trends in the diagnosis and management of recurrent miscarriage. *Obstet Gynecol Surv, 68*(6), 445-466.
7. Sierra S, & Stephenson M.(2006). Genetics of recurrent pregnancy loss. *Semin Reprod Med, 24*(1), 17-24.
8. Practice Committee of the American Society for Reproductive M. (2012). Evaluation and treatment of recurrent pregnancy loss: a committee opinion. *Fertil Steril, 98*(5), 1103-1111.
9. Menasha J., Levy B., Hirschhorn K., & Kardon N.B.(2005). Incidence and spectrum of chromosome abnormalities in spontaneous abortions: new insights from a 12-year study. *Genet Med, 7*(4), 251-263.
10. Carp H., Feldman B., Oelsner G., & Schiff E.(2004). Parental karyotype and subsequent live births in recurrent miscarriage. *Fertil Steril, 81*(5), 1296-1301.
11. Nagaishi M., Yamamoto T., Iinuma K., Shimomura K., Berend S.A., & Knops J. (2004). Chromosome abnormalities identified in 347 spontaneous abortions collected in Japan. *J Obstet Gynaecol Res, 30*(3) ,237-241.
12. Hyde K.J., & Schust D.J. (2015). Genetic considerations in recurrent pregnancy loss. *Cold Spring Harb Perspect Med, 5*(3), a023119.

13. Hu Y., Chen X., Chen L.L., Xu Z.F., Wang X., & Cui H.(2006). Comparative genomic hybridization analysis of spontaneous abortion. *Int J Gynaecol Obstet*, *92*(1), 52-57.
14. Oostlander A.E., Meijer G.A., & Ylstra B. (2004). Microarray-based comparative genomic hybridization and its applications in human genetics. *Clin Genet*, *66*(6), 488-495.
15. Bagheri H., Mercier E., Qiao Y., Stephenson M.D., & Rajcan-Separovic E. (2015). Genomic characteristics of miscarriage copy number variants. *Mol Hum Reprod*, *21*(8), 655-661.
16. Dhillon R.K., Hillman S.C., Morris R.K., McMullan D., Williams D., Coomarasamy A. et al. (2014). Additional information from chromosomal microarray analysis (CMA) over conventional karyotyping when diagnosing chromosomal abnormalities in miscarriage: a systematic review and meta-analysis. *BJOG*, *121*(1), 11-21.
17. Donaghue C., Davies N., Ahn J.W., Thomas H., Ogilvie C.M., & Mann K. (2017). Efficient and cost-effective genetic analysis of products of conception and fetal tissues using a QF-PCR/array CGH strategy; five years of data. *Mol Cytogenet*, *10*, 12.
18. Ozawa N., Sago H., Matsuoka K., Maruyama T., Migita O., Aizu Y. et al. (2016). Cytogenetic analysis of spontaneously discharged products of conception by array-based comparative genomic hybridization. *Springerplus*, *5*(1), 874.
19. Wou K., Hyun Y., Chitayat D., Vlasschaert M., Chong K., Wasim S. et al. (2016). Analysis of tissue from products of conception and perinatal losses using QF-PCR and microarray: A three-year retrospective study resulting in an efficient protocol. *Eur J Med Genet*, *59*(8), 417-424.
20. Sudhir N., Kaur T., Beri A., & Kaur A. (2016). Cytogenetic analysis in couples with recurrent miscarriages: a retrospective study from Punjab, north India. *J Genet*, *95*(4), 887-894.
21. De Krom G., Arens Y.H., Coonen E., Van Ravenswaaij-Arts C.M., Meijer-Hoogeveen M., Evers J.L. et al. (2015). Recurrent miscarriage in translocation carriers: no differences in clinical characteristics between couples who accept and couples who decline PGD. *Hum Reprod*, *30*(2), 484-489.
22. Najafi K., Gholami S., Moshtagh A., Bazrgar M., Sadatian N., Abbasi G. et al. (2019). Chromosomal aberrations in pregnancy and fetal loss: Insight on the effect of consanguinity, review of 1625 cases. *Mol Genet Genomic Med*, *7*(8), e820.
23. Moorhead P.S., Nowell P.C., Mellman W.J., Battips D.M., & Hungerford D.A. (1960). Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp Cell Res*, *20*, 613-616.
24. Chen C.P., Su Y.N., Chen Y.T., Chen W.L., Hsu L.J., & Wang W. (2011). Prenatal diagnosis of directly transmitted benign 4q12-q13.1 quadruplication associated with tandem segmental amplifications of the LPHN3 gene. *Taiwan J Obstet Gynecol*, *50*(3), 401-404.
25. Kasnauskiene J., Cimbalistiene L., Utkus A., Ciuladaite Z., Preiksaitiene E., Peciulyte A. et al. (2013). Two new de novo interstitial duplications covering 2p14-p22.1: clinical and molecular analysis. *Cytogenet Genome Res*, *139*(1), 52-58.
26. Brisset S., Capri Y., Briand-Suleau A., Tosca L., Gras D., Fauret-Amsellem A.L. et al. (2015). Inherited 1q21.1q21.2 duplication and 16p11.2 deletion: a two-hit case with more severe clinical manifestations. *Eur J Med Genet*, *58*(9), 497-501.
27. Hung C., Ayabe R.I., Wang C., Frausto R.F., Aldave A.J. (2013). Pre-Descemet corneal dystrophy and X-linked ichthyosis associated with deletion of Xp22.31 containing the STS gene. *Cornea*, *32*(9), 1283-1287.

28. Carrascosa-Romero M.C., Suela J., Alfaro-Ponce B., Cepillo-Boluda A.J. (2012). [X-chromosome-linked ichthyosis associated to epilepsy, hyperactivity, autism and mental retardation, due to the Xp22.31 microdeletion]. *Rev Neurol, 54*(4), 241-248.
29. Ben Khelifa H., Soyah N., Ben-Abdallah-Bouhjar I., Gritly R., Sanlaville D., Elghezal H. et al. (2013). Xp22.3 interstitial deletion: a recognizable chromosomal abnormality encompassing VCX3A and STS genes in a patient with X-linked ichthyosis and mental retardation. *Gene, 527*(2), 578-583.
30. Malla T.M., Pandith A.A., Dar F.A., Zargar M.H., Shah Z.A. (2014). De novo Xp terminal deletion in a triple X female with recurrent spontaneous abortions: a case report. *J Genet, 93*(3), 819-822.
31. Li R., Fu F., Zhang Y.L., Li D.Z., Liao C. (2014). Prenatal diagnosis of 17q12 duplication and deletion syndrome in two fetuses with congenital anomalies. *Taiwan J Obstet Gynecol, 53*(4), 579-582.
32. Rasmussen M., Vestergaard E.M., Graakjaer J., Petkov Y., Bache I., Fagerberg C. et al. (2016). 17q12 deletion and duplication syndrome in Denmark-A clinical cohort of 38 patients and review of the literature. *Am J Med Genet A, 170*(11),2934-2942.
33. Daniely M., Aviram-Goldring A., Barkai G., & Goldman B. (1998). Detection of chromosomal aberration in fetuses arising from recurrent spontaneous abortion by comparative genomic hybridization. *Hum Reprod, 13*(4), 805-809.
34. Barrett I.J., Lomax B.L., Loukianova T., Tang S.S., Lestou V.S., & Kalousek D.K. (2001). Comparative genomic hybridization: a new tool for reproductive pathology. *Arch Pathol Lab Med, 125*(1), 81-84.
35. Bell K.A., Van Deerlin P.G., Haddad B.R., & Feinberg R.F. (1999). Cytogenetic diagnosis of "normal 46,XX" karyotypes in spontaneous abortions frequently may be misleading. *Fertil Steril, 71*(2), 334-341.
36. Lomax B., Tang S., Separovic E., Phillips D., Hillard E., Thomson T. et al. (2000). Comparative genomic hybridization in combination with flow cytometry improves results of cytogenetic analysis of spontaneous abortions. *Am J Hum Genet, 66*(5), 1516-1521.
37. Bell K.A., Van Deerlin P.G., Feinberg R.F., du Manoir S., & Haddad B.R. (2001). Diagnosis of aneuploidy in archival, paraffin-embedded pregnancy-loss tissues by comparative genomic hybridization. *Fertil Steril, 75*(2), 374-379.
38. Colley E., Hamilton S., Smith P., Morgan N.V., Coomarasamy A., & Allen S. (2019). Potential genetic causes of miscarriage in euploid pregnancies: a systematic review. *Hum Reprod Update, 25*(4), 452-472.
39. Schaeffer A.J., Chung J., Heretis K., Wong A., Ledbetter D.H., Lese Martin C. (2004). Comparative genomic hybridization-array analysis enhances the detection of aneuploidies and submicroscopic imbalances in spontaneous miscarriages. *Am J Hum Genet, 74*(6), 1168-1174.
40. Jia Y., Zhao H., Shi D., Peng W., Xie L., Wang W. et al. (2014). Genetic effects of a 13q31.1 microdeletion detected by noninvasive prenatal testing (NIPT). *Int J Clin Exp Pathol, 7*(10), 7003-7011.