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Correlation Analysis Between Maternal Age and Pathogenic Copy Number Variations in Prenatal Diagnosis

Huang Shun-ting[1], Yuan Si-min[1], Zhong Hui-zhu[1], Yi Cui-xing[1], Li Ru[1], Zhang Yong-ling[1], Fu Fang[1], Zhou Hang[1], Yang Xin[2, *]

[1]Prenatal Diagnostic Center, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, China

[2]Prenatal Diagnostic Center, Shenzhen Longgang District Maternal and Child Health Hospital, Shenzhen, China

Email address:

yangsunrise@163.com (Yang Xin)

[*]Corresponding author

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Abstract: *Objective:* To investigate the correlation between maternal age and pathogenic copy number variations (pCNVs) in prenatal diagnosis. *Method:* This is a retrospective study, from 2009.6 to 2022.4, thirty-five thousand and seventeen invasive procedures have been performed due to high risk of down syndrome screening, advanced maternal age, ultrasound structural defects and so on. All the pregnant information, clinical indications and outcome of prenatal diagnosis were recorded in our prenatal database. The correlation between maternal age and chromosomal abnormalities/pathogenic copy number variations was analyzed by binary logistics regression. *Results:* In 35017 prenatal samples, karyotyping was performed in 34676 cases, which showed chromosomal abnormalities in 2704 cases (7.80%, 2704/34676). CMA was performed in 5041 cases, which showed chromosomal abnormalities in 646 cases (12.81%, 646/5041) including 233 pathogenic copy number variations (4.96%, 233/4700). The detection rate of CMA was significantly higher than that of karyotyping (P=0.000, X^2=143.437). In this study, karyotyping and CMA were both performed in 4700 cases, which showed a 4.36% (205/4700) increase in chromosomal abnormalities detected by CMA compared with karyotyping. By regression analysis, the incidence of chromosomal abnormalities increased significantly with the increase of the maternal age (P=0.000; Exp (B)=1.055; CI 95% 1.048-1.062). However, the proportion of pCNVs decreased greatly (P=0.000; Exp (B)=0.947; CI 95% 0.921-0.974). *Conclusion:* The detection rate of CMA was higher than traditional karyotype analysis in prenatal diagnosis. There was a significant correlation between maternal age and chromosomal abnormalities, but the incidence of pathogenic copy number variations did not increase with the increase of the maternal age.

Keywords: Prenatal Diagnosis, Maternal Age, Chromosomal Abnormalities, Pathogenic Copy Number Variations

1. Introduction

Chromosomal anomalies are a prevalent cause of abnormal fetal development. Advanced maternal age is a well-known risk factor for fetal chromosomal anomalies. In China, pregnant women over the age of 35 are advised to receive genetic counseling to detect possible fetal chromosomal anomalies. The primary methods for detecting fetal chromosomal anomalies include non-invasive prenatal testing in cell-free DNA, chromosome karyotype analysis, fluorescence quantitative PCR, and chromosome microarray analysis (CMA). CMA is often preferred for prenatal diagnosis due to its capacity to detect small copy number variations (CNVs) that may not be detected by karyotyping. It is well known that the occurrence of fetal chromosomal abnormalities, particularly chromosome aneuploidies, rises significantly as maternal age increases. However, it is still controversial whether there is an increase in the incidence of pathogenic copy number variations (pCNVs) in fetuses with advancing maternal age. Additionally, it remains unclear whether a combination of karyotyping and CMA testing will have a better yield compared to karyotyping alone for elderly pregnant women. It has been reported [1] that CMA can detect

an additional 5-6% of small fragment copy number variations in prenatal diagnosis compared with karyotyping. A meta-analysis of 19 studies showed that [2] there was no significant difference in the incidence of syndrome-related submicroscopic chromosomal aberrations between patients tested due to advanced maternal age (AMA) and those tested due to parental anxiety (ANX), which supported the hypothesis that the risk for submicroscopic imbalances was irrelevant to the age of the pregnant woman. Pregnant women under the age of 36 had a higher risk of developing submicroscopic pathogenic chromosomal aberrations than Down syndrome. However, drawing the conclusion that the risk for submicroscopic imbalances was irrelevant to the age of the pregnant woman from the comparison between AMA and ANX is not enough. According to a study conducted by Li Yu [3] et al. in China, the study analyzed the interventional prenatal diagnosis results of 467 advanced maternal age pregnant women and found that the incidence of pathogenic chromosome abnormalities increased with the increase of age. However, this study could not clearly distinguish the relationship between the incidence of chromosome aneuploidy and the advanced maternal age from the relationship between the incidence of pathogenic copy number variations and the advanced maternal age. Zhang Na [4] et al. retrospectively analyzed the interventional prenatal diagnosis results of 1335 advanced maternal age pregnant women, among which 207 were tested for both karyotyping and CMA. The results showed that the additional detection rate of CMA was 1.45%, and there was no statistically significant difference in the detection rate of CMA and karyotype analysis.

The aim of this study was to investigate the correlation between maternal age and pathogenic copy number variations (pCNVs) by reviewing interventional prenatal diagnosis results in our hospital in the last 10 years, with the main objective of clinical support.

2. Methods

2.1. Research Object

Thirty-five thousand and seventeen invasive procedures, including 34676 cases of Karyotyping, 5041 cases of CMA and 4700 cases of using both karyotyping and CMA have been carried out in Guangzhou Women and Children's Medical Center from 2009.6 to 2022.4, among which CMA has been implemented since 2013. Puncture site contains 24735 cases of amniotic fluid (70.6%), 5877 cases of villi (16.8%), and 4405 cases of cord blood (12.6%).

The indications [5-8] of invasive procedures included: (1) nuchal translucency (NT) thickening (≥ 3.0 mm) in the first trimester; (2) fetal structural abnormalities detected by ultrasound in the first or second trimester; (3) a history of adverse pregnancy with pathogenic chromosome copy number variations; (4) high risk for Down syndrome; (5) high risk of non-invasive prenatal testing (NIPT), and (6) advanced maternal age (≥ 35 years old). All pregnant women were informed of the risks, advantages and limitations as well as signed an consent form before interventional prenatal diagnosis. Peripheral blood of both husband and wife was extracted before surgery to exclude maternal cell contamination.

2.2. Chromosome Karyotype Analysis Technique

Culture the amniotic fluid cells routinely, make the G-banding slide, and count 30 chromosomes of the metaphase mitotic images for each patient. Determine the abnormal chromosome karyotype according to the 1955 International System for Human Cytogenetic Nomenclature (ISCN) criteria.

2.3. Genomic DNA Extraction

Genomic DNA was extracted from amniotic fluid and umbilical cord blood samples according to Qiagen kit instructions.

2.4. CMA Detection

Digestion, amplification, purification, fragmentation, tagging, hybridization, washing and scanning of genomic DNA in strict accordance with Affymetrix CytoScanTMHD chip testing standard operating procedures, and use ChAS 2.0 software for data analysis.

2.5. Analysis of CMA Results

Fragments with CNV≥ 700 kb were compared and analyzed, with reference to the following databases: Online Mendelian Inheritance in Man (OMIM), Database of Chromosomal Imbalance and Phenotype in Humans using Ensemble Resources (DECIPHER), Database of Genomic Variant (DGV), University of California Santa Cruz (UCSC) Gene browser, Gene Reviews, PubMed, etc. According to the American College of Medical Genetics and Genomics (ACMG) guidelines, CNVS are classified as pathogenic, variants of unknown significance, benign, likely pathogenic, and possibly benign [9].

2.6. Definition of Key Words and Research Methods

All prenatal diagnosis results were recorded in the workstation of the prenatal diagnosis center of our hospital, and the age of pregnant women, indications for interventional prenatal diagnosis, karyotyping results, and CMA results were included in this study. In this study, chromosomal abnormalities mainly referred to the abnormal chromosomal fetuses discovered by karyotyping. Pathogenic copy number variations (pCNVs) referred to pathogenic small segments of deletion/duplication (≤ 10Mb) that karyotyping fail to detect but were found out by CMA. All pCNVs referred to in this article do not include variants of unknown significance, benign, likely pathogenic, and possibly benign CNVS. In this study, we aimed to investigate the correlation between maternal age and pathogenic copy number variations (pCNVs) by retrospectively reviewing the detection rate of chromosomal abnormalities and pCNVs.

2.7. Statistical Analysis

Use SPSS 22.0 software for statistics analysis. The counting data was expressed in frequency and rate, the measurement data in accordance with normal distribution was expressed in x ± s, and the measurement data in nonnormal distribution was expressed in M (min~max). The incidence of chromosomal abnormalities and pCNVs was counted according to maternal age. Regression analysis was used to study the correlation between the incidence of chromosomal abnormalities and maternal age, as well as the correlation between the incidence of pCNVs and maternal age. P<0.05 indicated a statistically significant difference. Use curve estimation in SPSS to estimate the model with the highest goodness of fit, and GraphPad Prism 9 to perform nonlinear regression analysis according to the optimal model.

3. Results

3.1. Prenatal Diagnosis Results

From June 2009 to April 2022, a total of 35017 cases of interventional prenatal diagnosis were performed at Guangzhou Women and Children's Medical Center, including 24735 cases of amniotic fluid (70.64%), 5877 cases of villi (16.78%), and 4405 cases of cord blood (12.57%). Karyotyping revealed 2704 (7.80%) chromosomal abnormalities in 34676 cases, including 2122 (78.48%, 2122/2704) common chromosome number abnormalities (trisomy 21, 18, 13 and sex chromosome abnormalities). CMA revealed 646 (12.81%, 646/5041) chromosomal abnormalities in 5041 cases, including 233 (4.96%, 233/4700) pCNVs. Comparative analysis showed that the positive detection rate of CMA was significantly higher than that of karyotyping (P=0.000, X^2=143.437). A total of 4700 cases underwent both karyotyping and CMA, and 205 pCNVs were detected (4.36%, 205/4700).

3.2. Analysis of Correlation Between Maternal Age and Fetal Chromosomal Abnormalities and pCNVs

All prenatal diagnosis results were grouped according to maternal age to get the incidence of fetal chromosomal abnormalities and pCNVs. The model with the highest goodness of fit value was obtained by SPSS curve estimation for the data in Table 1 and GraphPad Prism 9 conducted nonlinear regression analysis according to the optimal model and obtained Figure 1. Binary logistic regression analysisin dicated that there was a significant positive correlation between chromosomal abnormalities and maternal age (P=0.000; Exp (B)=1.055; CI 95% 1.048-1.062) and the incidence of pCNVs was negatively correlated with maternal age (P=0.000; Exp (B)=0.947; CI 95% 0.921-0.974).

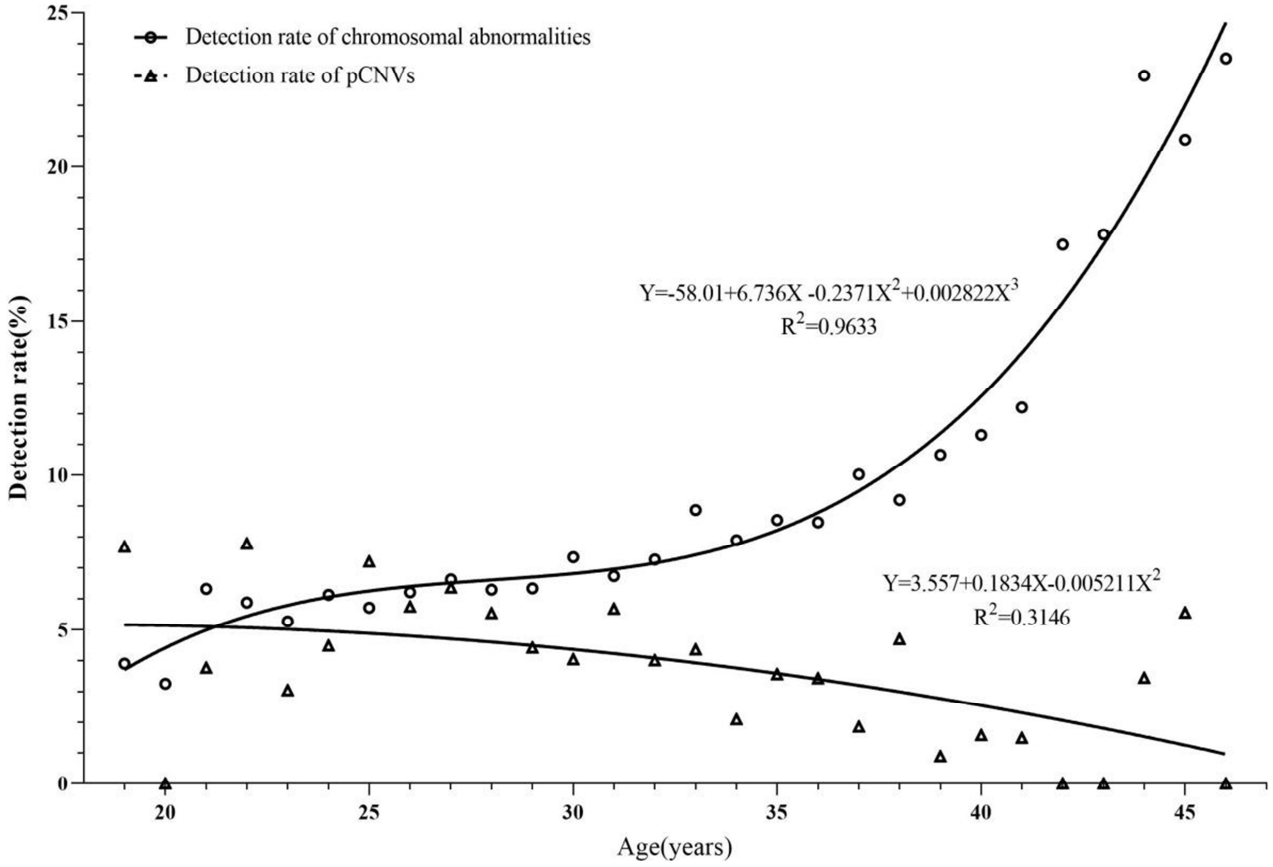

Figure 1. *Analysis of correlation Between Maternal Age and Fetal Chromosomal Abnormalities and pCNVs.*

Table 1. *Maternal age and the incidence of fetal chromosomal abnormalities and pCNVs.*

age	chromosomal abnormalities			pCNVs		
	total[a]	N[b]	Rate (%)	total[c]	N[d]	Rate (%)
19	128	5	3.91	13	1	7.69
20	276	9	3.26	33	0	0.00
21	506	32	6.32	53	2	3.77
22	748	44	5.88	77	6	7.79
23	1061	56	5.28	131	4	3.05
24	1418	87	6.14	178	8	4.49
25	1785	102	5.71	208	15	7.21
26	2026	126	6.22	261	15	5.75
27	2427	161	6.63	330	21	6.36
28	2493	157	6.30	397	22	5.54
29	2254	143	6.34	384	17	4.43
30	2177	160	7.35	321	13	4.05
31	2076	140	6.74	352	20	5.68
32	1911	139	7.27	274	11	4.01
33	1735	154	8.88	252	11	4.37
34	1696	134	7.90	238	5	2.10
35	1731	148	8.55	224	8	3.57
36	1581	134	8.48	204	7	3.43
37	1457	146	10.02	161	3	1.86
38	1283	118	9.20	149	7	4.70
39	1153	123	10.67	112	1	0.89
40	981	111	11.31	126	2	1.59
41	655	80	12.21	67	1	1.49
42	429	75	17.48	47	0	0.00
43	292	52	17.81	41	0	0.00
44	148	34	22.97	29	1	3.45
45	91	19	20.88	18	1	5.56
46	34	8	23.53	5	0	0.00

a: The total number of cases performing karyotyping in this age group. b: The number of chromosomal abnormalities cases in this age group. c: The total number of cases performing both karyotyping and CMA in this age group. d: The number of pCNVs cases with normal karyotype in this age group.

3.3. Data Description of Fetal Chromosomal Abnormalities and pCNVs in Isolated Advanced Maternal Age Pregnant Women

In order to exclude the influence of other factors in this study and further confirm the correlation between maternal age and pathogenic chromosome copy number variation, we removed the influence of ultrasonic structural abnormalities, adverse reproductive history, high risk of Down's screening, high risk of NIPT and other factors on the positive detection rate, and selected cases with isolated advanced maternal age from the database. A total of 2001 pregnant women accepted karyotyping because of isolated advanced maternal age in the database, where 73 (3.65%, 73/2001) chromosomal abnormalities were found. A total of 193 pregnant women accepted CMA because of isolated advanced maternal age in the database, where 7 (3.63%, 7/193) chromosomal abnormalities and 2 pCNVs (1.04%, 2/193) were found. 192 isolated advanced maternal age pregnant women accepted both karyotyping and CMA, in which 2 pCNVs (1.04%, 2/192) were detected. There was no great difference in the detection rate of fetal chromosomal abnormalities between karyotyping and CMA for isolated advanced maternal age pregnant women. The detection rate of pCNVs in the fetuses of isolated advanced maternal women was comparable to that of the low-risk population.

4. Discussion

As we all know, advanced maternal age is one of the most important factors associated with fetal chromosomal abnormalities. Studies have shown that the risk of chromosomal abnormalities increases by 5-7 times in pregnant women over the age of 35. Therefore, all pregnant women over the age of 35 are recommended to exclude the risk of fetal chromosomal abnormalities in domestic clinical work. With the rapid development of medical technology, the use of chromosome microarray analysis (CMA) has become increasingly prevalent in clinical practice. CMA utilizes DNA probe hybridization to diagnose chromosome diseases and is particularly advantageous in detecting small segments of chromosomal deletion/duplication that can cause genetic syndromes such as 22q11 microdeletion syndrome, Prader-Willi syndrome, Angelman syndrome, etc. Numerous papers have shown that CMA can detect an additional 5-10% of chromosomal diseases compared with karyotyping. Even in low-risk populations, CMA has been found to have an additional detection rate of 1-2%. Although advanced maternal age is strongly associated with chromosomal diseases, it is still controversial whether advanced maternal age increases the risk of pCNVs. A meta-analysis of CMA in low-risk populations [2] showed that the pooled estimated indication rate of pathogenic clinically significant submicroscopic aberration was 0.84% (95%CI 0.55-1.3%) in advanced maternal age (AMA) /anxious (ANX) pregnant women. By comparing AMA and ANX pregnant women, there was no significant difference in the incidence of submicroscopic chromosomal aberrations associated with syndromal disorders (0.38% vs 0.43%, X^2=0.17, P=0.681), suggesting that the risk of submicroscopic imbalances was not significantly related to the age of the pregnant women. Marta Larroya et al. [10] retrospectively analyzed 189 pregnancies: 63 pregnancies in the group with abnormal CMA result and 126 pregnancies in the control group, and concluded that there was no significant difference in the proportion of the two groups between non-elderly and elderly fathers or mothers, while the incidence of pCNVs in fetuses with ultrasound anomalies was associated with advanced paternal or maternal age. In China, Yang Shuting [11] et al. reviewed the CMA results of 562 advanced maternal age pregnant women, which suggested that there was no statistical significance in the detection rate of the chromosome CNVs between advanced maternal age and non-advanced maternal age pregnant women, and between isolated advanced maternal age and non-isolated advanced maternal age pregnant women (P > 0.05).

In this study, the detection rate of fetal chromosomal abnormalities was 7.80%, while the positive detection rate of

CMA was 12.93%. Statistical analysis showed that CMA significantly increased the detection rate of fetal chromosomal abnormalities. This study again confirmed that the incidence of fetal chromosomal abnormalities increased with the increase of maternal age. However, the incidence of pathogenic copy number variations did not increase with the increase of the maternal age, but decreased. Statistical analysis showed that this negative correlation was statistically significant. In other words, the results of this study suggested that the incidence of pCNVs gradually decreased with the increase of maternal age. Theoretically, more than 80% of fetal chromosome number abnormalities are caused by chromosome division errors in the follicles of pregnant women during meiosis [12-15], and most of the small segments of chromosomal deletion/duplication are new mutations occurring in the division process of fertilized oocytes [16-17]. These distinct pathological mechanisms provide some explanations for the correlation between advanced maternal age and fetal chromosomal abnormalities.

In order to exclude the influence of other factors in this study, 2001 cases with clinical indications of isolated advanced maternal age were selected from the database. Data description showed that the detection rate of chromosomal abnormalities was 3.65% (73/2001) by karyotyping, and 3.63% (7/193) by CMA, which indicating that there was no great difference in the detection rate of fetal chromosomal abnormalities between karyotyping and CMA for isolated advanced maternal age pregnant women. Further analysis was performed in 192 cases accepting both karyotyping and CMA simultaneously, among which two pCNVs (1.04%) were found in 183 fetuses with normal karyotype, which was generally consistent with a previous report showing that the additional detection rate of CMA in low-risk populations was 1-2% [2]. This result showed that the added value of CMA was not obvious compared to using karyotyping alone in the positive detection rate of fetal chromosomal abnormalities for advanced maternal age pregnant women, which also further verified the conclusion above that the incidence of pCNVs did not increase with the increase of the maternal age.

This study is the largest retrospective study based on prenatal diagnostic data in China. The results once again confirmed the correlation between chromosomal abnormalities and the age of pregnant women, and also found that the incidence of pCNVs did not increase with the increase of the age of pregnant women, but decreased. However, there were some shortcomings in this study, mainly reflected in: 1. All prenatal diagnosis statistics were from the high-risk population. Actually, we can neither get nor study the prenatal diagnosis statistics of the low-risk population, which may result in a high positive detection rate of all statistical outcomes. 2. With only 193 cases of isolated advanced maternal age pregnant women tested for CMA, this sample size is actually very small, and its statistical results are not sufficient to explain the association between maternal age and fetal pathogenic copy number variation, so in this part, we only carried out data description. 3. For the five CMA results, we only concentrated on the results of pathogenic CNVs and did not include variants of unknown significance and likely pathogenic CNVs, possibly leading to a lower detection rate. 4. The resolution ratio of the chromosome microarray analysis technique in this study is approximately 700kb, which means that smaller fragments of pathogenic chromosome copy number variations may be missed, leading to a lower detection rate. 5. The clinical penetrance of different pCNVs is different, and some small fragments of pCNVs may have no clinical symptoms even though their CMA results are pCNVs. 6. Due to the sharp reduction number of cases over the age of 41 in our database, it may result in overall data bias and a negative correlation between age and pCNVs.

5. Conclusion

In conclusion, with the increase of maternal age, the incidence of fetal chromosomal abnormalities, especially aneuploidy, increases significantly. In prenatal diagnosis cases, CMA can detect more fetuses with chromosomal abnormalities, especially pathogenic chromosome copy number variations, but the incidence of pathogenic chromosome copy number variations did not increase with the increase of maternal age.

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References

[1] Levy B, Wapner R. Prenatal diagnosis by chromosomal microarray analysis. Fertil Steril. 2018; 109 (2): 201-212. doi: 10.1016/j.fertnstert.2018.01.005.

[2] Srebniak MI, Joosten M, Knapen MFCM, et al. Frequency of submicroscopic chromosomal aberrations in pregnancies without increased risk for structural chromosomal aberrations: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2018; 51 (4): 445-452. doi: 10.1002/uog.17533.

[3] Li Yu, Song Ting-ting, Xu Ying, Dang Ying-hui, Wan Shan-ning, Zhang Jian-fang. Application of chromosomal microarray analysis in prenatal diagnosis of advanced maternal age pregnant women. Journal of Lanzhou University: Medical Edition. 2021, 47 (3): 5. doi: 10.13885/j.issn.1000-2812.2021.03.016.

[4] ZHANG Na, YAN Meizhen, WANG Yuanbai, JIANG Yuying, ZHUANG Jianlong. Karyotype and copy number variation analyses of amniotic fluid cells in pregnant women with advanced maternal age. Journal of Southwest Medical University. 2021, 044 (002): 144-149. doi: 10.3969/j.issn.2096-3351.2021.02.010.

[5] Wilson KL, Czerwinski JL, Hoskovec JM, et al. NSGC practice guideline: prenatal screening and diagnostic testing options for chromosome aneuploidy. J Genet Couns. 2013; 22 (1): 4-15. doi: 10.1007/s10897-012-9545-3.

[6] Ghi T, Sotiriadis A, Calda P, et al. ISUOG Practice Guidelines: invasive procedures for prenatal diagnosis. Ultrasound Obstet Gynecol. 2016; 48 (2): 256-268. doi: 10.1002/uog.15945.

[7] American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Obstetrics; Committee on Genetics; Society for Maternal-Fetal Medicine. Screening for Fetal Chromosomal Abnormalities: ACOG Practice Bulletin, Number 226. Obstet Gynecol. 2020; 136 (4): e48-e69. doi: 10.1097/AOG.0000000000004084.

[8] Society for Maternal-Fetal Medicine (SMFM). Electronic address: pubs@smfm.org, Dugoff L, Norton ME, Kuller JA. The use of chromosomal microarray for prenatal diagnosis [published correction appears in Am J Obstet Gynecol. 2017 Feb; 216 (2): 180]. Am J Obstet Gynecol. 2016; 215 (4): B2-B9. doi: 10.1016/j.ajog.2016.07.016.

[9] Kearney HM, Thorland EC, Brown KK, Quintero-Rivera F, South ST; Working Group of the American College of Medical Genetics Laboratory Quality Assurance Committee. American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. Genet Med. 2011; 13 (7): 680-685. doi: 10.1097/GIM.0b013e3182217a3a.

[10] Larroya M, Tortajada M, Mensión E, Pauta M, Rodriguez-Revenga L, Borrell A. Have maternal or paternal ages any impact on the prenatal incidence of genomic copy number variants associated with fetal structural anomalies?. PLoS One. 2021; 16 (7): e0253866. Published 2021 Jul 9. doi: 10.1371/journal.pone.0253866.

[11] Yang Shuting, Zhao Yali, Tang Xinxin, Wang Zhiwei, Liu Dengping, Zhang Jinglu, Gu Ying, Wang Leilei. Application of Chromosome microarray analysis in prenatal diagnosis of pregnant women with advanced age. Chin J Med Genet, 2021 February, 38 (2): 7. doi: 10.3760/cma.j.cn511374-20200323-00195.

[12] Blyth U, Craciunas L, Hudson G, Choudhary M. Maternal germline factors associated with aneuploid pregnancy loss: a systematic review. Hum Reprod Update. 2021; 27 (5): 866-884. doi: 10.1093/humupd/dmab010.

[13] Cheng JM, Liu YX. Age-Related Loss of Cohesion: Causes and Effects. Int J Mol Sci. 2017; 18 (7): 1578. Published 2017 Jul 22. doi: 10.3390/ijms18071578.

[14] Wang S, Liu Y, Shang Y, et al. Crossover Interference, Crossover Maturation, and Human Aneuploidy. Bioessays. 2019; 41 (10): e1800221. doi: 10.1002/bies.201800221.

[15] Mikwar M, MacFarlane AJ, Marchetti F. Mechanisms of oocyte aneuploidy associated with advanced maternal age. Mutat Res Rev Mutat Res. 2020; 785: 108320. doi: 10.1016/j.mrrev.2020.108320.

[16] Pös O, Radvanszky J, Buglyó G, et al. DNA copy number variation: Main characteristics, evolutionary significance, and pathological aspects. Biomed J. 2021; 44 (5): 548-559. doi: 10.1016/j.bj.2021.02.003.

[17] Hastings PJ, Lupski JR, Rosenberg SM, Ira G. Mechanisms of change in gene copy number. Nat Rev Genet. 2009; 10 (8): 551-564. doi: 10.1038/nrg2593.