Diagnostic Accuracy Study

Medicine

OPEN

Analysis of the impact of noninvasive prenatal testing for trisomies 21 and 18 in twin pregnancies undergoing artificial reproductive technology

Cuiyu Yang, MM[a,b], Linhua Hu, BM[a,b], Shudan Jiang, BM[a,b], Fengbing Liang, MM[a,b], Songying Zhang, MD, PHD[a,b,*]

Abstract

Purpose: The purpose of this study was to evaluate the performance and impact of noninvasive prenatal screening (NIPS) on twin pregnancies.

Patients and methods: Twin pregnancies after artificial reproductive technology(ART) were tested by NIPS for screening trisomy 21, 18, and 13 in a single medical center in Hangzhou. Positive NIPS results were confirmed by karyotyping, while negative results were interviewed after delivery.

Results: From January 2019 to December 2020, 474 twin pregnancies were tested by NIPS for screening trisomy 21, 18, and 13 in a single medical center in Hangzhou. The performance of NIPS had been evaluated compared to the invasive diagnostic results. The positive predictive value (PPV) of NIPS for chromosome 21 and 18 aneuploidies is 80% (95CI, 36.09–96.59) and 100%, respectively. The incidence of trisomy 21, and 18 chromosome aneuploidies among the twin pregnancies undergoing ART was 0.84% and 0.21%, respectively.

Conclusion: The performance of NIPS was substantially accurate among the twin pregnancies after ART in this study, and NIPS potentially avoided a considerable part of aneuploidies liveborn in twin pregnancies in Hangzhou.

Abbreviations: ART = artificial reproductive technology, DCDA = dichorionic diamniotic, IVF-ET = in vitro fertilization-embryo transfer, NIPS = noninvasive prenatal screening, NT = nuchal translucency, PPV = positive predictive value.

Keywords: artificial reproductive technology, NIPS, prenatal screening, twin pregnancies

1. Introduction

Trisomy 21 (Down's syndrome) is the most common chromosomal malformation in neonatal infants and is characterized by severe intellectual disability and other serious abnormalities.[1] The total prevalence of trisomy 21 is about 10 per 10,000 livebirths all over the world.[2] Trisomy 18 (Edward's syndrome), and trisomy 13 (Patau's syndrome) are also common chromosomal disorders among fetuses.[3] Using cell-free DNA genomic sequencing analysis, noninvasive prenatal screening (NIPS) for trisomy 21, 18, and 13 achieves much better performance, in terms of high sensitivity and specificity in pregnancies, than conventional standard screening tests,[4,5] which are based on serological markers, ultrasound, maternal age, and maternal history.[6–8] American College of Medical Genetics and Genomics has recommended replacing traditional biochemical screening tests with NIPS for trisomy 21, 18, and 13 across the maternal age spectrum.[9] In China, some municipal governments advocate using NIPS as the primary prenatal screening test for chromosomal abnormalities, and therefore to relieve the potential financial burden during and after pregnancy. The potential impact of NIPS on the landscape of prenatal diagnosis and the livebirth prevalence of chromosomal abnormalities is more and more dramatic since sequencing costs reduce gradually,[10,11] government funds increase,[12,13] and the implementation of NIPS as a primary screening test rather than a contingent screening test widely spread.[14]

This study was supported by funding from the Key Research and Development Program of Zhejiang Province (LGF18H040005).

The authors declare no conflict of interest.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

This study was approved by the Medical Ethics Review Board (20210628-31) of Sir Run Run Shaw Hospital (Hangzhou, China), and the study was carried out in compliance with the Helsinki Declaration. Written informed consent was obtained from all participants before recruitment.

[a] Assisted Reproduction Unit, Department of Obstetrics and Gynecology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China, [b] Key Laboratory of Reproductive Dysfunction Management of Zhejiang Province, China.

**Correspondence: Songying Zhang, Assisted Reproduction Unit, Department of Obstetrics and Gynecology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Key Laboratory of Reproductive Dysfunction Management of Zhejiang Province, No. 3 Qingchun East Road, Shangcheng District, Hangzhou 310016, China (e-mail: zhangsongying@zju.edu.cn).*

How to cite this article: Yang C, Hu L, Jiang S, Liang F, Zhang S. Analysis of the impact of noninvasive prenatal testing for trisomies 21 and 18 in twin pregnancies undergoing artificial reproductive technology. Medicine 2022;101:33(e29985).

Received: 8 February 2022 / Received in final form: 12 June 2022 / Accepted: 22 June 2022

http://dx.doi.org/10.1097/MD.0000000000029985

However, the test in twin pregnancies is more complex than that in singleton pregnancies due to the confounding fetal fraction, and there are relatively fewer reports about the NIPS performance in twin pregnancies.[15] Given that the rate of twin birth dramatically increased with the use of ART, the implementation of NIPS to screen for fetal aneuploidy in twin pregnancies is especially desirable and even still rapidly expanding.[16,17] Moreover, the risk of aneuploidies and unexpected miscarriage from invasive diagnosis are considerably higher in twin pregnancies than in singletons.[18] We aim to assess the feasibility and clinical application of NIPS in twin pregnancies, on the prenatal screening and livebirth prevention of aneuploidy, based on 474 twin pregnancies undergoing ART from 2019 to 2020 in a single medical center in Hangzhou, China.

2. Methods

2.1. Participants

From January 2019 to December 2020, in total 484 twin pregnancies after ART were recruited in the Department of Obstetrics and Gynecology of Sir Run Run Shaw Hospital, after clinical examination 10 cases were excluded in this study, and eventually 474 pregnancies were screened by NIPS in Hangzhou, China. All participants accepted the pre-test counseling and signed informed written consents before blood sampling. One month after the date of expected confinement, the pregnancy outcome was surveyed by telephone interview or other methods. This study was approved by the Medical Ethics Review Board (20210628-31) of Sir Run Run Shaw Hospital (Hangzhou, China).

According to the standard screening,[19] a patient undergoing in vitro fertilization-embryo transfer (IVF-ET)[20] was highly recommended to perform the NIPS test or G-banded karyotyping. While a patient with any of the following factors was not recommended to perform the NIPS test.

1. Gestational age lower than 12 weeks at the sample collection date.
2. Transplantation or stem cell therapy performed before.
3. Xenogenous blood transfusion within one year; xenogenous DNA-based cell immunotherapy within 4 weeks.
4. Pregnancy combined with any malignant tumor.
5. Other conditions under which a doctor may concern about the accuracy of NIPS results.

2.2 Test methods

2.2.1. Study population and sample collection. This is an observational study of NIPS performance in twin pregnancies after ART treatment in the department of Obstetrics and Gynecology of Sir Run Shaw Hospital. The sample inclusion criteria of this study were as follows: (1) pregnant women with twin pregnancies after ART from January 2019 to December 2020; (2) over 18 years old; (3) Gestational age ≥12 weeks; (4) voluntarily received NIPS screening for fetal trisomy 21 (T21),

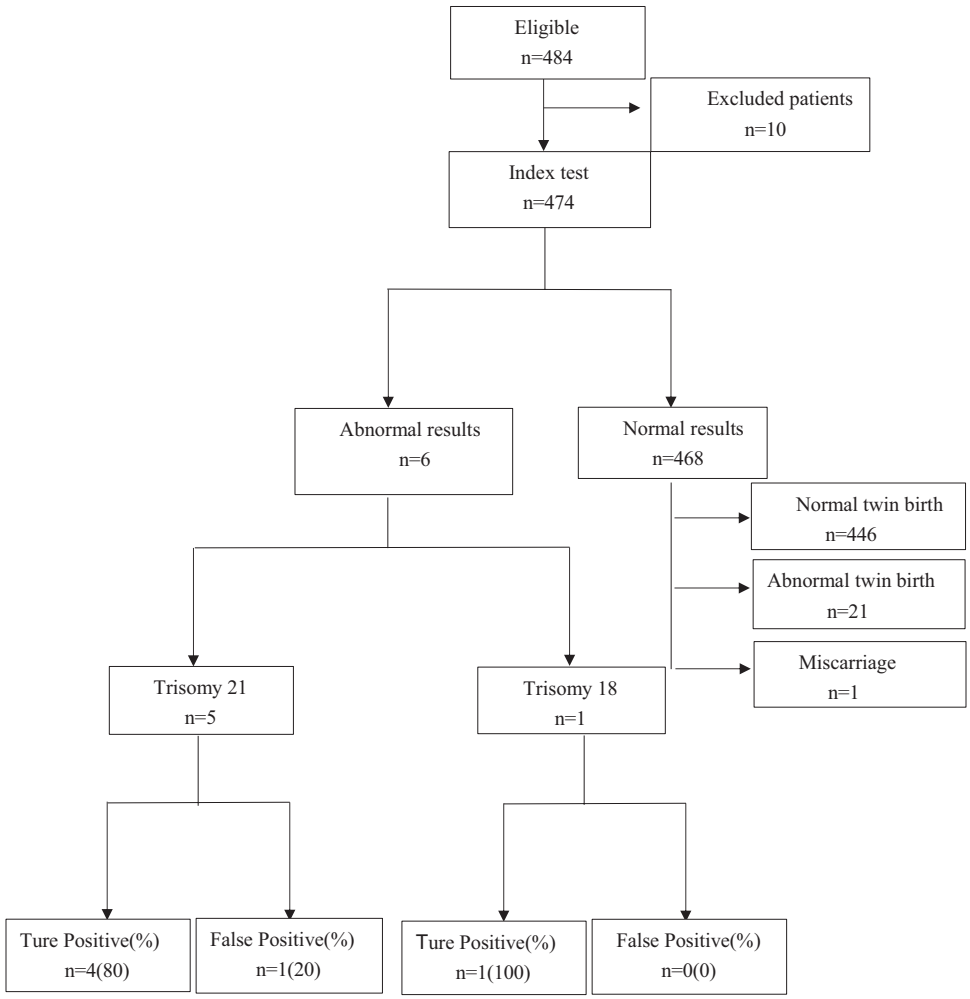

Figure 1. The flow diagram of the study.

trisomy 18 (T18), and trisomy 13(T13), with or without prior Down syndrome screening result. Participants were treated with ART at our hospital, and once twin pregnancy was confirmed, pregnant women were offered the choice of receiving NIPS. While a patient with any of the following factors was not recommended to perform the NIPS test: (1)Transplantation or stem cells therapy performed before; (2) Xenogenous blood transfusion within one year; xenogenous DNA-based cell immunotherapy within 4 weeks; (3) Pregnancy combined with any malignant tumor. All participants accepted the pre-test counseling and signed informed written consents before blood sampling.

2.2.2. Maternal plasma DNA sequencing and bioinformatic analysis.

After pre-testing counseling, we collected blood samples from twin pregnancy women after ART for NIPS. For each pregnant woman, 5 mL of peripheral blood was obtained in an ethylene diamine tetraacetic acid-anticoagulated tube. The plasma was separated within 8 hours and used to extract cfDNA. All subsequent procedures including DNA extraction, library preparation for sequencing, and NIPS using massively parallel sequencing have been performed. Z-score testing methods were used to identify fetal autosomal aneuploidy for trisomy as described in Liao's paper.[21] Z score range from –3 to 3 was considered to indicate a low risk for a trisomy chromosome, and if the Z score were >3, the sample was in the high-risk zone. The depth of chromosome Y (% chrY) was used to deduce the fatal DNA fraction of male foetus and the method of seqFF for females.[22]

2.3. Validation and follow-up

The NIPS results were further validated by G-banded karyotyping. For NIPS positive results, amniocentesis was followed by karyotyping. For NIPT negative results, routine healthcare procedures were provided. Clinical outcomes of the NIPT negative cases were obtained by telephone interview one month after the expected date of confinement.

2.4. Statistical methods

The SPSS statistical software package (version 25.0) was used for statistical analysis. For the analysis of sensitivity, specificity, PPV, NPV, and the corresponding 95% confidence intervals (CI), nonparametric test of one sample and the Clopper–Pearson method was used.

3. Results

3.1. Participants

A total of 484 twin pregnancies were screened by NIPS in a single medical center in Hangzhou, China from January 2019 to December 2020. 10 cases were excluded for the excluding reasons in this study. The flow diagram is shown in Figure 1. As was betrayed in Table 1, 6 pregnancies (about 1.266%) were affected with any one of the trisomy 21 and trisomy 18 according to the NIPS results. The characteristics also include, but are not limited to, maternal age, gestational age at the sample collection date, fetal fraction, ART, and chorionicity. The average maternal age is 32 years ranging from 22 to 43 years, and the group of 30–34 years dominates a significant proportion (50.21%). The average gestational age at the sample collection date is 15 weeks, and the second trimester gestational age group (82.2%) is the most predominant one. Among all pregnancies tested by NIPS, 474 (100%) were twin pregnancies undergoing ART; 464 (about 97.9%) were IVF-ET twin fetuses; 474 (100%) were dichorionic diamniotic (DCDA) twin fetuses.

3.2. Test results

Table 2 shows the observed performance of NIPS in twin pregnancies undergoing ART. As is shown in Table 1a total of 6 NIPS positive aneuploidies cases were detected among 474 twin pregnancies; however, 1 NIPS false positive case (Table 3) was validated by G-banded karyotyping and the clinical outcomes (two normal fetuses delivered) were followed up. For the other 5 true positive NIPS aneuploidies cases (Table 3), the fetal reduction surgeries were accepted. The other normal fetus A or B were delivered. The other clinical characteristics for NIPS true positive aneuploidies cases were also provided in Table 3, the maternal ages of 4 cases were higher than 35 years, while the fetal fraction is equal to or higher than 10% for 5 out of 6 cases. The reasons that the parents accepted the IVF-ET were conditions such as the male oligoasthenospermia, salpingitis, or endometriosis. There is a preponderance of the number affected among the maternal age more than 35 years groups (3 out of 5 true positive cases, 60%) over that among the maternal age less than 35 years groups (2 out of 5 true positive cases, 40%) observed consistent with the higher incidence after 35 years old (Table 3).

Table 4 presented the obstetrical outcomes of NIPT negative cases. Among the 468 cases with NIPT negative results, 446 cases(95.3%) gave birth to twins with apparently normal phenotypes at a term birth rate of 40.2%(188/468) and preterm birth rate of 55.1%(258/468). A total of 21 cases (4.5%) were reported to have adverse pregnancy outcomes, which were not consistent with typical phenotypes of T21, T18, or T13 by follow-up. Another 15 cases (3.2%) were reported to have developmental defects (including atrial deficiency, hemangioma, cleft lip, hypospadias, duplicate kidney, etc). One fetus intrauterine death happened in 5 cases (1.1%), including 2 cases that underwent selectivity reduction for a heartless fetus or osteogenic dysplasia

Table 1

Demographic and Characteristics of the Patients screened (2019–2020).

Characteristic	Value
No. of NIPS tested patients	474
Twins pregnancies (%)	474 (100%)
Average maternal age (range), y	32 (22–43)
≤24 y (%)	13 (2.74%)
25-29 y (%)	150 (31.65%)
30-34 y (%)	238 (50.21%)
≥35 y (%)	73 (15.4%)
Average gestational age at sample collection (range), weeks	15 (12–21)
First trimester (12–13 weeks) (%)	56 (11.8%)
Second trimester (14–21 weeks) (%)	418 (88.2%)
Average maternal height (range), m	160 (156–170)
Average maternal weight (range), kg	57 (52–62)
Chorionicity	
DCDA (%)	474 (100%)
MCDA (%)	0 (0%)
MCMA (%)	0 (0%)
ART conception (%)	474 (100%)
Ovulation (%)	5 (1.05%)
Artificial fertilization (%)	5 (1.05%)
IVF-ET (%)	464 (97.9%)
Fetal fraction before enrichment (range), %	11.15 (5.08–22.98)
Fetal fraction afterenrichment (range), %	18.33 (5.01–49.89)
Trisomy 21	5
True positive (%)	4 (80%)
False positive (%)	1 (20%)
Trisomy 18	1
True positive (%)	1 (100%)
False positive (%)	0 (0%)

ART = artificial reproductive technology, DCDA = dichorionic diamniotic, MCDA = monochorionic diamniotic, MCMA = monochorionic monoamniotic, IVF-ET = in vitro fertilization and embryo transfer.

Table 2

Test performance of NIPS in twin pregnancies.

Trisomy	TP	TN	FP	FN	Sensitivity (95% CI), %	Specificity (95% CI), %	PPV (95% CI), %	NPV (95% CI), %
21	4	469	1	0	100 (39.76–100.00)	99.79 (98.82–99.99)	80 (36.09–96.59)	100
18	1	473	0	0	100 (2.5–100.00)	1100 (99.22–100.00)	100	100

CI = confidence interval, FP = false positive, FN = false negative, NPV = negative predictive value, PPV = positive predictive value, TN = true negative, TP = true positive.

and spontaneous fetus death happened in another 3 cases. One miscarriage case (0.2%) was owing to intrauterine infection.

3.3. Estimates

In this study, for trisomy 21 and 18, the sensitivity (detection rate) and specificity of NIPS shown on table2 are quite high, and the positive predictive value (PPV) of NIPS is 80% (95 CI, 36.09–96.59) and 100%, respectively. If hemolysis occurs, another blood sample should be advised to be re-collected 3 days later for these twin pregnancies.

4. Discussion

This study analyzes the impact of NIPS in twin pregnancies in a single medical center in Hangzhou, China. This report focuses on the experience in a single medical center, which may also have great significance for other hospitals for their reference. After the second child policy in China was launched in October 2015, and the third child policy was launched in 2021, it may have brought the older mother effect and a baby boom in the following years (consistent with the previous report in China[23]). Besides, the average maternal age of the twin pregnancies undergoing ART in our study is older than 30 as shown in the chart (Table 1), about 65.61% of pregnant women were greater than equal to 30 years old and about 15.4% of pregnant women were not less than 35 years old. In consistence with the positive correlation between trisomy prevalence and maternal age especially after 35 years old, in addition to the higher prevalence in twin pregnancies as reported previously,[18] we observed a high prevalence of 0.84% and 0.21% in twin pregnancies undergoing ART for trisomy 21 and 18, respectively.

Invasive prenatal tests (chorionic villus sampling, amniocentesis, or cordocentesis) have a certain risk of resulting in fetal loss, especially in twin pregnancies.[24] The application of NIPS may potentially decrease the amount of unnecessary invasive tests as the previous global report.[25,26] Additionally, the application of NIPS may have contributed significantly to decreasing the trisomy 21 livebirth prevalence. In this study, the PPV of NIPS for chromosome 21 and 18 aneuploidies is 80% (95CI, 36.09–96.59) and 100%, respectively; the sensitivity of NIPS for both chromosomes 21 and 18 aneuploidies is 100%. The excellent performance of NIPS in twin pregnancies may partially resulted from the relatively small population (474) enrolled in this study. The trisomy 18 case (left choroid plexus cyst, Ventricular septal defect) and 1 trisomy 21 cases (high nuchal translucency, 0.45 cm) as shown in Table 3 were identified as the abnormal ultrasound finding, while also identified by NIPS. This implies a

Table 3

Clinical details of positive NIPT results.

Case	Maternal age	Gestational age	BMI	Fetal fraction before enrichment (%)	Fetal fraction after enrichment (%)	Parental condition	Conception	Ultrasound	NT (cm)	NIPT result	Karyotyping	Outcome
1	32	15W+5D	21.64	15.36	Not performed	Oligoasthenospermia	IVF-ET	Normal	0.23/0.10	T21	T21/Normal	Fetal reduction, fetus B delivered
2	31	13W	17.72	5.89	Not performed	Oligoasthenospermia	IVF-ET	High NT(0.45cm)	0.45/0.10	T21	T21/Normal	Fetal reduction, fetus B delivered
3	38	15W	26.37	11.50	23.42	Primary ovarian insufficiency	IVF-ET	Normal	0.09/0.09	T21	T21/Normal	Fetal reduction, fetus B delivered
4	39	14W+3D	17.90	11.42	22.12	Salpingitis	IVF-ET	Normal	0.15/0.14	T21	Normal	Two normal fetuses delivered
5	38	14W+5D	21.08	10.00	22.19	Endometriosis	IVF-ET	Normal	0.13/0.12	T21	T21/Normal	Fetal reduction, fetus B delivered
6	37	15W	26.37	13.40	20.64	Oligoasthenospermia	IVF-ET	Left choroid plexus cyst, Ventricular septal defect (3.5mm)	0.1/0.1	T18	Normal/T18	Two normal fetuses delivered

D = day, NT = nuchal translucency, W = week.

Table 4

Obstetrical outcomes of twin pregnancies with NIPT negative results.

Obstetrical outcomes of NIPT negative cases(n=468)	Number of cases n(%)
Normal twin birth	446 (95.3)
Term delivery (≥37weeks)	188 (40.2)
Preterm delivery (28–37 weeks)	258 (55.1)
Abnormal twin birth with no typical phenotypes of T21, T18, or T13	21 (4.5)
Birth defects (atrial deficiency, hemangioma, cleft lip, hypospadias, duplicate kidney, etc.)	15 (3.2)
One fetus intrauterine death (selectivity reduction for heartless fetus or osteogenic dysplasia, spontaneous fetus death, etc.)	5 (1.1)
Miscarriage (intrauterine infection, twin-to-twin transfusion syndrome, etc.)	1 (0.2)
Birth defect, stillbirth, and miscarriage with unconfirmed reasons	1 (0.2)
Total	468 (100)

complementary role of ultrasound for NIPS to achieve a better prenatal screening performance.

There was one NIPS false positive case (case 4 in Table 3) by G-banded karyotyping and the clinical outcomes (2 normal fetuses delivered). The potential reason may lie in the maternal malignancies or the confined placental mosaicism.

One limitation of the study was the lack of karyotyping in NIPT negative results, particularly 15 cases were diagnosed as birth defects (atrial deficiency, hemangioma, cleft lip, hypospadias, duplicate kidney, etc.) (Table 4). However, performing karyotyping for each patient with NIPT negative results was impractical, especially in ART twin pregnancy. If fetal defects were found by prenatal ultrasound, invasive procedures for karyotyping even whole exome sequencing should be applied in these patients with NIPT negative results. Alongside the gradual reduction of the sequencing costs and the accuracy of the NIPS detection both in twin and singleton pregnancies, NIPS results in a relief of the financial burden brought to families, and encouragement of the preference as the primary prenatal screening.

5. Conclusion

Integrating all the information described above, NIPS is feasible to detect trisomy 21 and 18 in twins and ART fetuses, meanwhile, the interventional prenatal diagnosis is required for high-risk screening due to the existence of false positive cases. Generally, we observed that NIPS had a good performance and positive impact in twin pregnancies undergoing ART in Hangzhou, China.

Acknowledgments

We thank all medical centers, participants, and the families in this study for their cooperation and support.

Author contributions

Conceptualization: Cuiyu Yang, Songying Zhang, Fengbing Liang.
Data curation: Linhua Hu, Shudan Jiang.
Formal analysis: Cuiyu Yang, Linhua Hua
Methodology: Cuiyu Yang, Shudan Jiang.
Resources: Fengbing Liang
Supervision: Songying Zhang, Fengbing Liang
Writing – original draft: Cuiyu Yang
Writing – review & editing: Cuiyu Yang

References

[1] de Groot-van der Mooren MD, Tamminga S, Oepkes D, et al. Older mothers and increased impact of prenatal screening: stable livebirth prevalence of trisomy 21 in the Netherlands for the period 2000-2013. Eur J Hum Genet. 2018;26:157–65.
[2] Weijerman ME, de Winter JP. Clinical practice. The care of children with Down syndrome. Eur J Pediatr. 1452;169:1445.
[3] Greene MF, Phimister EG. Screening for trisomies in circulating DNA. N Engl J Med. 2014;370:874–5.
[4] Zhang H, Gao Y, Jiang F, et al. Non-invasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146,958 pregnancies. Ultrasound Obstet Gynecol. 2015;45:530–8.
[5] Gil MM, Accurti V, Santacruz B, et al. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol. 2017;50:302–14.
[6] Hui L, Hutchinson B, Poulton A, et al. Population-based impact of non-invasive prenatal screening on screening and diagnostic testing for fetal aneuploidy. Genet Med. 1345;19:1338.
[7] Wald NJ, et al. Prenatal reflex DNA screening for trisomies 21, 18, and 13. Genet Med. 2018;20:825–30.
[8] Norton ME, Wapner RJ. Cell-free DNA analysis for noninvasive examination of trisomy. N Engl J Med. 2015;373:2581–2.
[9] Gregg AR, Skotko BG, Benkendorf JL, et al. Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics. Genet Med. 2016;18:1056.
[10] Ewans LJ, Schofield D, Shrestha R, et al. Whole-exome sequencing reanalysis at 12 months boosts diagnosis and is cost-effective when applied early in Mendelian disorders. Genet Med. 2018;20:1564–74.
[11] Stark Z, Schofield D, Martyn M, et al. Does genomic sequencing early in the diagnostic trajectory make a difference? A follow-up study of clinical outcomes and cost-effectiveness. Genet Med. 2019;21:173–80.
[12] Oepkes D, Lieve Page-Christiaens GC, Bax CJ, et al. Trial by Dutch laboratories for evaluation of non-invasive prenatal testing. Part I-clinical impact. Prenat Diagn. 2016;36:1083.
[13] Johansen P, Richter SR, Balslev-Harder M, et al. Open source non-invasive prenatal testing platform and its performance in a public health laboratory. Prenat Diagn. 2016;36:530–6.
[14] Neyt M, Hulstaert F, Gyselaers W. Introducing the non-invasive prenatal test for trisomy 21 in Belgium: a cost-consequences analysis. BMJ Open. 2014;4:e005922.
[15] He Y, Wang Y, Li Z, et al. Clinical performance of non-invasive prenatal testing for trisomies 21, 18 and 13 in twin pregnancies: a cohort study and a systematic meta-analysis. Acta Obstet Gynecol Scand. 2020;99:731–43.
[16] Yang J, Qi Y, Hou Y, et al. Performance of non-invasive prenatal testing for trisomies 21 and 18 in twin pregnancies. Mol Cytogenet. 2018;11:47.
[17] Collins J. Global epidemiology of multiple birth. Reprod Biomed Online. 2007;15(suppl 3):45–52.
[18] Kahler C, Gembruch U, Heling K-S, et al. [DEGUM guidelines for amniocentesis and chorionic villus sampling]. Ultraschall Med. 2013;34:435–40.
[19] Bianchi DW, Parker RL, Wentworth J, et al. DNA sequencing versus standard prenatal aneuploidy screening. N Engl J Med. 2014;370:799–808.
[20] Fesahat F, Montazeri F, Sheikhha MH, et al. Frequency of chromosomal aneuploidy in high quality embryos from young couples using preimplantation genetic screening. Int J Reprod Biomed (Yazd). 2017;15:297–304.
[21] Liao C, Yin AH, Peng CF, et al. Noninvasive prenatal diagnosis of common aneuploidies by semiconductor sequencing. Proc Natl Acad Sci USA. 2014;111:7415–20.
[22] Chiu RW, Akolekar R, Zheng YW, et al. Non-invasive prenatal assessment of trisomy 21 by multiplexed plasma DNA sequencing: large scale validity study. BMJ. 2011;342:1756–833.
[23] Liang J, Mu Y, Li X, et al. Relaxation of the one child policy and trends in caesarean section rates and birth outcomes in China between 2012 and 2016: observational study of nearly seven million health facility births. BMJ. 2018;360:k817.
[24] Akolekar R, Beta J, Picciarelli G, et al. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2015;45:16–26.
[25] Chitty LS, Wright D, Hill M, et al. Uptake, outcomes, and costs of implementing non-invasive prenatal testing for Down's syndrome into NHS maternity care: prospective cohort study in eight diverse maternity units. BMJ. 2016;354:i3426.
[26] Beamon CJ, Hardisty EE, Harris SC, et al. A single center's experience with noninvasive prenatal testing. Genet Med. 2014;16:681–7.