http:// ijp.mums.ac.ir
*(Original Article (Pages: 15901-15909*

# When Is Non-Invasive Prenatal Testing Reliable in Pregnancies with a Vanishing Twin? - A Systematic Review of Case Reports

Somayeh Khanjani [1], Sedigheh Hantushzadeh [2], Leyla Sahebi [3], Mohsen Vigeh [4], Mamak Shariat [5], *Mahdieh Saeri [6]

[1] Department of Obstetrics and Gynecology, Faculty of Medicine, Isfahan University of medical science, Isfahan, Iran.
[2] Maternal-Fetal Neonatal Research Center Tehran University of medical sciences, Tehran, Iran.
[3] Family Health Research Institute, Maternal-Fetal and neonatal research center, Tehran University of Medical Science, Tehran, Iran.
[4] Maternal-Fetal Neonatal Research Center Tehran University of medical sciences, Tehran, Iran.
[5] Maternal-Fetal Neonatal Research Center Tehran University of medical sciences, Tehran, Iran.
[6] Medical Student, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

***Abstract***

***Background:*** Fetal demise can complicate aneuploidy screening in a multi fetal pregnancy. The cell-free DNA (CF-DNA) from a non-viable conception may be discordant with the viable fetuses. The Aim of study was to review the waiting period, follow-on single fetal demise in a twin gestation before performing NIPT (Non-Invasive Prenatal Testing).

***Methods:*** In this review article we searched through online databases of CINAHL, Cochrane, Database of Abstracts of Reviews of Effects (DARE), PubMed, Medical Library, and Google Scholar for English literature between 2011 to 2020, with the following keywords: "NIPT", "non-invasive prenatal screening testing", "cell-free DNA", "vanishing twin" and "co-twin demise". We included the studies regarding the duration between the twin vanishing or reduction and NIPT false results.

***Results:*** 201 studies across the eight scientific websites were detected; 178 of which were excluded for duplication or being irrelevant. And 29 studies were fully read. 4 case series, finally, met the criteria for systematic review. The findings suggested that the NIPT screening test can be falsely-positive several weeks after vanishing twins although the live fetus is normal. Therefore, the time duration in which the placenta can release CF-DNA of the vanished twin is unknown. In addition, several weeks after reduction, the fetal CF-DNA increases and then decreases, thus CF-DNA analyzing in multifetal pregnancies with reduction can be challenging as well.

***Conclusion:*** In pregnancies with vanishing twin or reduction, evaluating NIPT results is more complex than single fetal pregnancy. According to the reviewed studies, after a fetal demise, the cytotrophoblast continues to release to the CF-DNA in the maternal circulation for a variable time, which may cause a false-positive result if the demised twin is aneuploidy.

***Key Words:*** Cell-free DNA, Fetal demise, Multifetal pregnancy, Non-invasive prenatal testing, Vanishing twin.

* Please cite this article as: Khanjani S, Hantushzadeh S, Sahebi L, Vigeh M, Shariat M, Saeri M. When Is Non-Invasive Prenatal Testing Reliable in Pregnancies with a Vanishing Twin? - A Systematic Review of Case Reports. Int J Pediatr 2022; 10 (4):15901-15909. DOI: **10.22038/IJP.2021.56056.4410**

**** Corresponding Author:***

Mahdieh Saeri, Medical Student, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. Email: mh.saeri@gmail.com

Received date: Mar.1,2021; Accepted date:Jan.30,2022

## 1- INTRODUCTION

The discovery of the presence of fetal-derived sequences of nucleic acid in the blood circulation of pregnant women has revolutionized the field of prenatal diagnosis. This method uses the free fetal DNA released into the circulation as a result of apoptosis of placental cells and can be detected in the maternal peripheral blood (1, 2). Fetal DNA is highly fragmented, represents only a small fraction of total extracellular DNA in plasma, and more importantly, the fetus shares half of the genetic information with the mother (2). Despite several apparent disadvantages, the complete fetal genome can be reconstructed from maternal plasma and, thus, the fragmented cell-free fetal DNA in maternal circulation is suitable for non-invasive prenatal genetic testing (3, 4). Cell-free fetal DNA based aneuploidy screening has provided a highly accurate, non-invasive screening test in pregnancy (5, 6). NIPT (Non-Invasive Prenatal Testing) is able to detect more than 99% of trisomy 21 and 98% of trisomy 18 and 99% of trisomy 13 with a false positive rate of 0.13% (7). This high detection rate supports the use of this test for singleton pregnancies (8). However, if the results show abnormality, invasive tests are taken before important decisions. NIPT can replace prenatal screening tests such as serum biomarkers and ultrasound measurements (9). In one study, it was shown that combining the NIPT with quantitative fluorescence polymerase chain reaction increased the accuracy of NIPT results (10). Cumulatively, non-invasive prenatal testing has approximately 1% false-positive rates for down syndrome (11), some of which have biological explanations such as a maternal copy number variant, maternal mosaicism, maternal malignancy, confined placental mosaicism, fetal mosaicism, and vanishing twin (4,12).

It is currently unknown how long CF-DNA of the vanished or reduced fetus can be detected in maternal circulation. Our main goal is to collect data about the time between the vanishing or reduction of the twins and the NIPT test that leads to false results, to evaluate how long NIPT can be falsely positive.

## 2- MATERIALS AND METHODS

### 2-1. Method and information resources

We considered non-invasive prenatal screening tests for vanishing twins in the present survey. The Medical Subject Heading (MeSH) database was used to identify the search keywords for the term of “non-invasive prenatal screen testing for vanishing twin”. The final search strategy and keywords have been agreed by all authors as follows: “NIPT”, “non-invasive prenatal screening testing”, “cell-free DNA”, “vanishing twin” and “co-twin demise”.

**Search details:**

1) "invasive Prenatal Diagnosis"[tiab] OR "Noninvasive Prenatal Screening"[tiab] OR "Prenatal Cell-Free DNA Screening"[mh] OR "Prenatal cf DNA Screening"[mh] OR "Cell-Free DNA"[mh] OR "Cell-Free Deoxyribonucleic Acid"[mh] OR "Cell-Free Nucleic Acid"[mh] OR "Cell-Free Ribonucleic Acid"[mh] OR "Circulating Cell-Free Nucleic Acid"[mh] OR "Circulating Cell-Free Nucleic Acids"[mh] OR "Circulating DNA"[mh] OR "Circulating Nucleic Acid"[mh] OR "Circulating Nucleic Acids"[mh] OR "cfDNA"[mh] OR "cirDNA"[mh]

2) "vanishing twin"[tiab] OR "co-twin demise"[tiab] OR "Fetal Death" [mh] OR "Fetal Demise" [mh] OR "Fetal Mummification"[mh]

3) 1 AND 2

The published literature has been searched in scientific databases including CINAHL, Cochrane, Database of Abstracts of Reviews of Effects (DARE), PubMed, and Medical Library (MedLib). We also used Google Scholar engine. The search was limited to English language publications between 2011 and 2020.

### 2-2. Data Collection Process and Eligibility Criteria

First, the papers' titles, abstracts, and keywords were read to identify potentially eligible works. Our study includes English publications. To decrease the risk of bias, these steps were separately done by two of the researchers, using the Cochrane Collaboration's Tool (Higgins & Green 2011) (13). In case of controversies, a third researcher rechecked the documents. In addition, we searched reference lists of original and review articles for finding more relevant papers. All peer-reviewed studies on maternal cell-free DNA screening tests for aneuploidy in vanishing twins were included. However, discordant sex chromosome cases and conference abstract were not included in this data collection, due to our emphasis on false positive results of abnormality in fetus. The selected studies must have reported false-positive results of NIPT for chromosomal aneuploidy showing aneuploidy when the remained twin doesn't have any genetic abnormality, detected by NIPT results due to the abnormal CF-DNA found in maternal blood caused by vanished twin or reduced twin. After the first round of selection, 29 articles passed the inclusion and exclusion criteria and we began full text reading. Our aim was to find articles which had data about the gestational age both at the time of confirming the vanished twin or reduction, and at the time of taking the NIPT, as well as the data regarding the NIPT results to allow us to recognize the time between the NIPT screening and NIPT false results, for identifying how long NIPT results can be falsely positive. This simple factor of time led us to only four case reports and case series because other studies we reviewed had not mentioned the details of when the test had been taken and how long later the vanished twin (missing heartbeat or other criteria) or reduction was confirmed. **Fig. 1** shows the flow chart for literature search.

### 2-3. Inclusion and exclusion criteria

When searching, data was collected via a checklist designed by the researchers. The first variables to be extracted were: study year, sample size, and study designs. The next variables to report on, included gestational age (GA) at the time of diagnosing the fetal demise, positive NIPT(weeks + days), GA at NIPT (weeks + days) at the first, second, third, and forth repetition, minimum time between fetal demise and positive results in the test, as well as the z-score of trisomy in the vanished twins.

### 2-4. Limitations of the study

All the selected studies were case reports and case series, because of the details we required. Case reports and case series can bring up the question of newly found side effects or causal associations, but there is the limitation of their potentiality for publication bias (14). The studies were conducted in different countries and years. For the NIPT test, although not all of the test procedures and kits used in the studies were mentioned, it is supposable that they are different; and such differences may have affected the results.

## 3- RESULTS

### 3-1. Search Result

The search yielded 201 articles across the eight scientific websites, 23 of which were duplicates. After excluding the duplicate articles, titles and abstracts of 178 papers were reviewed. Then, 29 papers were selected for full text reading. Finally, 4 case report studies, including

one to five samples, met the eligibility criteria for this systematic review. General information of the studies is listed in **Table 1**.

### 3-2. Results of individual studies

For the purpose of finding the timing of NIST test we could only find four case report and case series which their summary and intention of study is reviewed below:

The study by Grömminger et al. (2014) reported two cases (15). The first case was a demised twin with 47, XX, 21 trisomy karyotype; and the fetal heartbeat was absent at 10$^{th}$weeks of gestation. In week 11 the NT results were 3.1 for the abnormal twin and 2.5 for the normal twin. At 17 weeks + 2 days, the demised twin was still visible on ultrasound but NIPT was performed because of the advanced maternal age and it was positive for the 21 trisomy (z-score 13.5). So they did an amniocentesis and the results were the same. The contribution of the vanishing twin to the CF-DNA pool calculated according to the Y chromosome was about 9.2 to 9.3, but according to the twins' blood samples was about 20.7 to 24.8. The viable twin showed a normal phenotype at birth (38 weeks + 2 days; 46, XY) and the vanishing twin became papyraceous at birth (none contributed). There was no evidence for the presence of 21 trisomy cells in the mother, placenta, or the newborn boy but the sample of the unlived twin after birth showed an exact female with trisomy of chromosome 21. The second case was sampled for NIPT at 13 weeks + 2 days of gestation because of the concern of aneuploidy in first trimester screening. A z-score of 3.4 for chromosome 21 and 3.0 for the Y chromosome suggested a 47, XY, and 21 trisomy for the vanishing twin, but as they repeated the test it decreased to the borderline, so the probability of the vanishing twin was first revealed from the decrease in CF-DNA results, and then the ultrasounds were checked. Ultrasonography showed that at first there was a twin pregnancy that in the follow up there only a normal female was shown as the viable twin. The vanishing twin's contribution to the CF-DNA pool was estimated 13.5 and 10 percent. In case two, resumption of the 21 trisomy twin was complete at 13 weeks + 2 days (15).

Curnow et al. (2015) (16) interestingly used NIPT for detecting vanishing twins and undetected twins or triploids. They checked out the NIPT of 30,795 cases for aneuploidy and they performed ultrasounds and karyotype specifically for undetected pregnancies and vanishing twines in 76 cases. According to their results, 42.1 percent were vanishing twins. Thus, generally, they have reported five CF-DNA from vanished twins detected up to 8 weeks following co-twin demise (16).

The study of Hochstenbach et al. (2018)(17) reviewed the previous cases of false positive NIPT and stated that based on the previous cases, the vanishing twin releases DNA in maternal blood which can falsify the result and not much is known about the dynamic of this process. They added their case report with two gestational sacs at the 8$^{th}$-week sonography, both gestational sacs, one from a 47, XY, +14 and the other from a 47, XY, +21 fetus, with a z score of 3.00, continued to contribute to the CF-DNA in the maternal circulation for at least 2 weeks and 3 days after the demise of the trisomy-14 fetus (17).

Elisa Bevilacqua et al. (2020) (18) studied NIPT results after reduction. They investigated 7 pregnant women with reduction and in two cases they karyotyped the reduced fetuses. In the first case the reduced fetus was male with 21 aneuploidy and the birth child was a normal female.

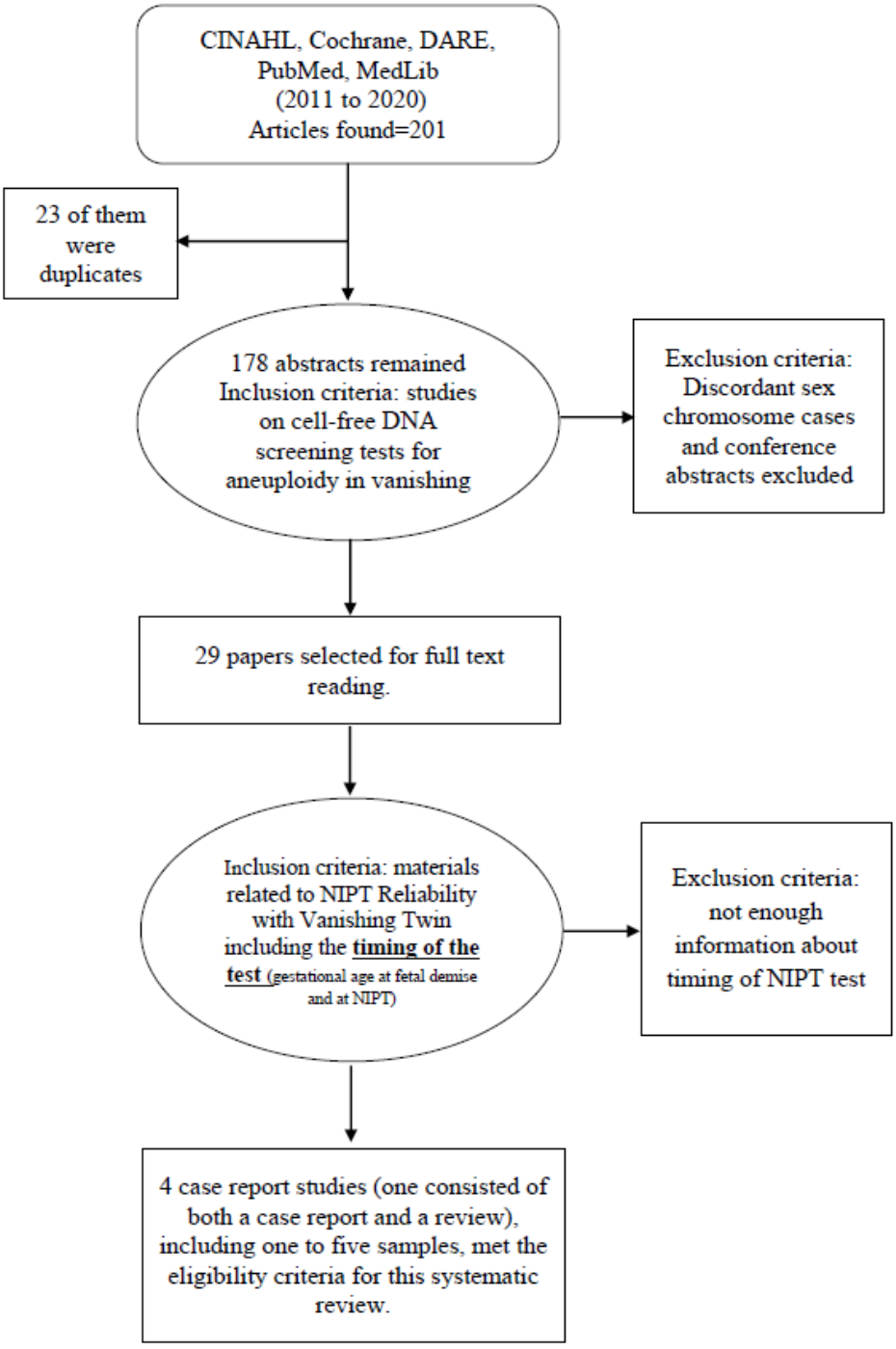


**Fig. 1:** flow chart for literature search

**Table-1:** Characteristics of the studies included in the systematic review

| Authors (year) | Country | Design | Number of cases |
|---|---|---|---|
| Gromminger et al. (2014) (15) | Hungary | Case Report | 2 |
| Curnow et al. (2015) (16) | United State | Case Report | 5 |
| Hochstenbach et al. (2018)(17) | Netherland | Review and Case Report | 1 |
| Bevilaqua et al. (2020) (18) | Belgium | Case Report | 7 |

Evaluation of pattern showed an increase in both Y and 21 aneuploidy values after reduction. In the second case, the reduced fetus was x aneuploidy and the birth child was normal, in both cases, the values transitorily returned to those expected for aneuploidy three to eight weeks following reduction. Their assessment of CF-DNA revealed that the fetal fraction increased and then reduced after reduction. Notably, the aneuploidy values never reached zero (the aneuploidy state) even months after the reduction. In five additional multifetal pregnancies undergoing reduction for other causes, the fetal fraction increased (n=3), decreased (n=1) or increased then decreased. Their data demonstrate the difficulty of CF-DNA study and decision making in multifetal pregnancies with reduction (18).

## 4- DISCUSSION

NIPT is able to detect more than 99% of trisomy 21 and 98% of trisomy 18 and 99% of trisomy 13 with a false positive rate of 0.13% (7). This high detection rate supports the use of this test for singleton pregnancies. Grömminger et al. reported a false positive case, where NIPT indicated trisomy 21, but amniocentesis, placenta, mother and the newborn baby were found to be euploid. The explanation for the discordant NIPT result originated from a vanished twin identified by ultrasound in the first trimester (15).

How long a placenta of a vanished twin can release CF-DNA to maternal blood is unidentified, but Curnow has reported on 5 studied pregnancies where CF-DNA from a vanished twin can be identified for up to 8 weeks following a co-twin demise (16). In the study by Ron Hochtenbach, the CF-DNA in the maternal circulation for at least 2 weeks and 3 days after the demise of the trisomy-14 fetus continued to contribute. In both cases of Elisa Bevilaqua, the values transiently returned to those expected in aneuploidy 3–8 weeks following reduction. **Fig. 2**, clarifies the timelines of each study, helping the comparison of the periods between vanished twin identification or reduction and the false positive NIPT result, for the remaining living twins. This graph is the summary of cases mentioned and gives an idea about the timeline of NIPT results.

Fetal fraction increased and then decreased following reduction. Notably, the aneuploidy values never reached 0 (i.e., the euploid state) even months after reduction (18).

Nevertheless, there is not adequate research on twin pregnancies. Despite high detection rates of NIPT in the diagnosis of trisomy 21 in twin pregnancies (11), most studies have suggested the need for more samples to confirm the use of NIPT for detection of trisomy 21, 18 and 13 in twin pregnancies(4). Since congenital twins are most expected to be aneuploidy, identifying these twin pregnancies is critical to preventing incorrect NIPT outcomes and unnecessary invasive procedures (7).

The findings related to influencing the vanishing twin on interpretation of NIPT results were contradictory. The unpredictable patterns of aneuploidy values and after fetal reduction did not support CF-DNA testing at intervals following a fetal demise.

### 4-1. Study Limitation

Due to the small number of cases and articles related, we were not able to perform Meta-analyzes and we still need large systematic studies to fully understand the persistence of CF-DNA from a vanished twin in maternal circulation.

## 5- CONCLUSION

The placental territory of a demised fetus may continue to release CF-DNA into maternal blood for more than three months, which may cause a false-positive result if the demise twin was aneuploidy.


**Fig. 2:** comparing the period between vanished twin identification or reduction to the false positive NIPT result for the remaining living twin.

Changes in FF following single fetal demise are unpredictable, and not agreeable for assigning a safe waiting period before performing NIPT. However, as the demised twin DNA decreases in maternal blood, CF_DNA results can be beneficial in presuming the vanished twin and it can be supported by a follow up of sonography. Based on the reviewed cases there can be an 8-week interval or waiting period for CF-DNA results to be more reliable for the living twin, although after 8 weeks the vanished twin DNA can still be present and sometimes be detected. We

could only find these few case reports and studies on this topic. Therefore, making decisions based on CF-DNA may not be recommended for aneuploidy screening at least until more data is composed showing NIPT and CF-DNA changes in maternal blood. We suggest stating the exact number of CF_DNA in addition to the gestational age in future studies, especially if NIPT is repeated, so that calculating a waiting period can be possible.

## 6- CONFLICT OF INTEREST

None.

## 7- REFERENCES

1. Dennis Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CWG, et al. Presence of fetal DNA in maternal plasma and serum. Lancet [Internet]. 1997 Aug 16 [cited 2021 Feb 28]; 350(9076):485–7. Available from: https://pubmed.ncbi.nlm.nih.gov/9274585.

2. Banerjee M, Misra D. Fetal Nucleic Acids in Maternal Circulation: A Genetic Resource for Noninvasive Prenatal Diagnosis. ISRN Genet. 2013 Sep 20; 2013:1–10.

3. Wagner AJ, Mitchell ME, Tomita-Mitchell A. Use of cell-free fetal DNA in maternal plasma for noninvasive prenatal screening [Internet]. Vol. 41, Clinics in Perinatology. W.B. Saunders; 2014 [cited 2021 Feb 28]. p. 957–66. Available from: https://pubmed.ncbi.nlm.nih.gov/25459783

4. Hartwig TS, Ambye L, Sørensen S, Jørgensen FS. Discordant non-invasive prenatal testing (NIPT) - a systematic review. Prenat Diagn [Internet]. 2017 Jun 1 [cited 2021 Feb 28]; 37(6):527–39. Available from: http://doi.wiley.com/10.1002/pd.5049.

5. Samura O. Update on noninvasive prenatal testing: A review based on current worldwide research. J Obstet Gynaecol Res [Internet]. 2020 Aug 17 [cited 2021 Mar 20]; 46(8):1246–54. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/jog.14268.

6. Liao GJW, Chan KCA, Jiang P, Sun H, Leung TY, Chiu RWK, et al. Noninvasive prenatal diagnosis of fetal trisomy 21 by allelic ratio analysis using targeted massively parallel sequencing of maternal plasma DNA. PLoS One [Internet]. 2012 May 29 [cited 2021 Mar 24]; 7(5). Available from: https://pubmed.ncbi.nlm.nih.gov/22666469

7. Motevasselian M, Saleh Gargari S, Younesi S, Pooransari P, Saadati P, Mirzamoradi M, et al. Non-invasive prenatal test to screen common trisomies in twin pregnancies. Mol Cytogenet [Internet]. 2020 Feb 5 [cited 2021 Feb 24]; 13(1). Available from: /pmc/articles/PMC7003371.

8. Heft H, Soulodre C, Cowan K, Laing A, Kaulback K, Mcdowell S, et al. Noninvasive Prenatal Testing for Trisomies 21, 18, and 13, Sex Chromosome Aneuploidies, and Microdeletions: A Health Technology Assessment. Ont Health Technol Assess Ser [Internet]. 2019 [cited 2021 Feb 28]; 19(4):1–166. Available from: http://www.hqontario.ca/evidence-to-improve-care/journal-ontario-health-technology-assessment-series.

9. Badeau M, Lindsay C, Blais J, Nshimyumukiza L, Takwoingi Y, Langlois S, et al. Genomics-based non-invasive prenatal testing for detection of fetal chromosomal aneuploidy in pregnant women. Vol. 2017, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2017.

10. Huo P, Luo Q, Li J, Jiao B, Rong L, Zhang J, et al. High accuracy of quantitative fluorescence polymerase chain reaction combined with non-invasive prenatal testing for mid-pregnancy diagnosis of common fetal aneuploidies: A single-center experience in China. Exp

Ther Med [Internet]. 2019 Jul 29 [cited 2021 Mar 20]; 18(1):711–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31281451.

11. Rafi I, Hill M, Hayward J, Chitty LS. Non-invasive prenatal testing: Use of cell-free fetal DNA in Down syndrome screening [Internet]. Vol. 67, British Journal of General Practice. Royal College of General Practitioners; 2017 [cited 2021 Feb 28]. p. 298–9. Available from: /pmc/articles/PMC5565870.

12. Wilkins-Haug L, Zhang C, Cerveira E, Ryan M, Mil-homens A, Zhu Q, et al. Biological explanations for discordant noninvasive prenatal test results: Preliminary data and lessons learned. Prenat Diagn [Internet]. 2018 May 1 [cited 2021 Mar 24]; 38(6):445–58. Available from: /pmc/articles/PMC6721610.

13. Higgins, J.P.T. and Green S. Cochrane Handbook for Systematic Reviews of Interventions. Version 5.1.0. [Internet]. The Cochrane Collaboration; 2011. Available from: http://handbook-5-1.cochrane.org.

14. Nissen T, Wynn R. The clinical case report: A review of its merits and limitations. BMC Res Notes [Internet]. 2014 Apr 23 [cited 2021 Mar 19]; 7(1):264. Available from: /pmc/articles/PMC4001358.

15. Grömminger S, Yagmur E, Erkan S, Nagy S, Schöck U, Bonnet J, et al. Fetal Aneuploidy Detection by Cell-Free DNA Sequencing for Multiple Pregnancies and Quality Issues with Vanishing Twins. J Clin Med [Internet]. 2014 Jun 25 [cited 2021 Feb 23]; 3(3):679–92. Available from: /pmc/articles/PMC4449645.

16. Curnow KJ, Wilkins-Haug L, Ryan A, Kirkizlar E, Stosic M, Hall MP, et al. Detection of triploid, molar, and vanishing twin pregnancies by a single-nucleotide polymorphism-based noninvasive prenatal test. Am J Obstet Gynecol. 2015 Jan 1; 212(1):79. e1-79. e9.

17. Hochstenbach R, Elferink MG, van Zon PHA, Lichtenbelt KD, van Harssel J, Schuring-Blom H, et al. Discordant NIPT result in a viable trisomy-21 pregnancy due to prolonged contribution to cfDNA by a demised trisomy-14 cotwin. Clin Case Reports [Internet]. 2018 May 1 [cited 2021 Feb 28]; 6(5):788–91. Available from: http://doi.wiley.com/10.1002/ccr3.1424.

18. Bevilacqua E, Chen K, Wang Y, Doshi J, White K, Marchin J, et al. Cell-free DNA analysis after reduction in multifetal pregnancy. Ultrasound Obstet Gynecol [Internet]. 2020 Jan 1 [cited 2021 Feb 23]; 55(1):132–3. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/uog.20366.