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REVIEW

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Non-invasive prenatal testing (NIPT) in twin pregnancies affected by early single fetal demise: A systematic review of NIPT and vanishing twins

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Abstract

The screening performance of non-invasive prenatal testing (NIPT) in vanishing twin (VT) pregnancies is relatively unknown. To close this knowledge gap, we conducted a systematic review of the available literature. Studies describing the test performance of NIPT for trisomy 21, 18, 13, sex chromosomes and additional findings in pregnancies with a VT were retrieved from a literature search with a publication date until October 4, 2022. The methodological quality of the studies was assessed with the quality assessment tool for diagnostic accuracy studies-2 (QUADAS-2). The screen positive rate of the pooled data and the pooled positive predictive value (PPV) were calculated using a random effects model. Seven studies, with cohort sizes ranging from 5 to 767, were included. The screen positive rate of the pooled data for trisomy 21 was 35/1592 (2.2%), with a PPV of 20% (confirmation in 7/35 cases [95% CI 9.8%–36%]). For trisomy 18, the screen positive rate was 13/1592 (0.91%) and the pooled PPV 25% [95% CI 1.3%–90%]. The screen positive rate for trisomy 13 was 7/1592 (0.44%) and confirmed in 0/7 cases (pooled PPV 0% [95% CI 0%–100%]). The screen positive rate for additional findings was 23/767 (2.9%), of which none could be confirmed. No discordant negative results were reported. There is insufficient data to fully evaluate NIPT performance in pregnancies with a VT. However, existing studies suggest that NIPT can successfully detect common autosomal aneuploidies in pregnancies affected by a VT but with a higher false positive rate. Further studies are needed to determine the optimal timing of NIPT in VT pregnancies.

Key points

What is already known about this topic?

- Non-invasive prenatal screening testing (NIPT) is being offered worldwide to detect chromosomal aberrations by analyzing cell-free DNA.

- Aneuploidy is a major cause of first trimester loss/vanishing twins. Therefore, a vanishing twin (VT) can complicate the interpretation of NIPT.
- Guidelines and healthcare providers generally advise against the use of NIPT in VT pregnancies due to uncertain screening performance.

What does this review add?

- This is the first systematic review on the test performance of NIPT, creating an overview and interpretation of the existing data.
- There was insufficient data to fully evaluate NIPT performance in pregnancies with VT. However, existing studies suggest that NIPT can successfully detect common autosomal aneuploidies in pregnancies affected by a VT but with a higher false positive rate.
- Further studies are needed to determine the optimal timing of NIPT in VT pregnancies.

1 | INTRODUCTION

In multiple countries worldwide, non-invasive prenatal screening testing (NIPT) is offered to detect chromosomal aberrations by analyzing cell-free DNA (cfDNA) circulating in the maternal bloodstream. In singleton pregnancies, multiple large studies have shown that NIPT has higher detection and accuracy rate compared to the traditional first-trimester combined testing (FCT [ultrasound + first-trimester serum markers test]): 98%, 96% and 92% for trisomy 21, 18 and 13, respectively, with a false positive rate below 1%,[1–4] compared to a FCT detection rate of about 90% and a false positive rate of 5%.[5] This improvement in accuracy and detection has led to the widespread implementation of NIPT around the world and, subsequently in a reduction of the number of invasive procedures performed.[1,6–8] In countries where NIPT is offered as a first-tier screening test, such as Belgium and the Netherlands, the FCT has been virtually discontinued, with the latter not offering FCT since October 2021.

Data on the test performance in twin pregnancies are scarcer but indicate detection rates similar to singletons: 95%, 82%, 80% for trisomy 21, 18 and 13, respectively, with a false positive rate below 1%.[9,10] In vanishing twin (VT) pregnancies, however, the screening performance of NIPT is relatively unknown. As NIPT becomes more established in routine prenatal care, it is important to address this knowledge gap. A VT is the spontaneous reduction of one or more fetuses in case of a twin, or multiple pregnancy, during the first trimester.[11,12] Although the exact number is unknown, a VT pregnancy is a frequent occurrence with incidence numbers ranging from 0.6% up to 36%.[13,14] Two types of VT can be distinguished. In a type I, there is an additional empty gestational sac (GS) visible by ultrasound, and in a type II, there is an additional GS with a non-viable embryo. Characteristics of each type of VT are shown in Table 1.

Since aneuploidy is a major cause of first trimester loss/VTs,[16–18] detection of cfDNA from a deceased fetus with aneuploidy could result in a discordant positive result leading to stress and unnecessary invasive procedures.[19] Various national guidelines regarding prenatal screening currently consist of conflicting recommendations in VT pregnancies. The National Health Service guidelines (UK), "Screening for Down's syndrome, Edwards' syndrome and Patau's syndrome: NIPT' does not recommend NIPT in these cases because persistent cfDNA from the VT could confound results. Alternatively, patients should be offered FCT. The American College of Obstetricians and Gynecologists states that there is a significant risk of an inaccurate test result if either the FCT or NIPT is used, and patients should instead be offered diagnostic testing.[20] These conflicting recommendations reflect the scarcity of evidence of NIPT's test performance in VT pregnancies. Therefore, we performed a systematic review of the existing literature to provide an overview and interpretation of all available data.

2 | METHODS

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement.[21]

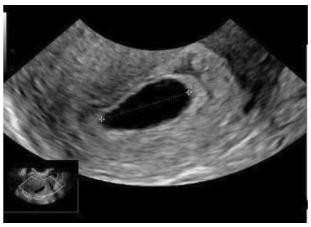
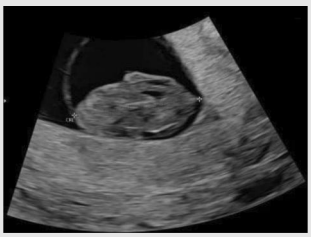
2.1 | Data sources and search

A literature search of Medline, Embase Web of Science Core Collection, Cochrane Library and Google Scholar was performed from 1997 to the 4th of October 2022. Variations of the following terms were used: "noninvasive prenatal testing," "cell-free DNA" and "vanishing twin pregnancy." The full detailed search is presented in Supporting Information S1.

2.2 | Study selection

Studies were eligible if they described the test performance of NIPT for trisomy 21, 18, 13, sex chromosomes and additional findings in VT pregnancies. Publication language was restricted to English or Dutch and case-reports, posters and conference abstracts were excluded. By definition, studies including selective feticide were excluded.

TABLE 1 Characteristics of the different types of vanishing twins.

Type of vanishing twin[a]	Description	Image
I	– Vital pregnancy and an additional empty gestational sac – First trimester combined screening performed as a singleton pregnancy[15]	
II	– Vital pregnancy and an additional gestational sac with a non-viable embryo – First trimester combined screening without PAPP-A[15]	

[a]A vanishing twin is the spontaneous reduction of one or more fetus in utero in case of a twin or multiple pregnancy in the first trimester.[11,12]

Two authors (JE and RJG) independently screened the titles and abstracts to identify all potentially relevant publications, after which a final selection was made based on full-text reviewing. Differences were resolved by consensus or through mediation with two other authors (MB and CB).

2.3 | Data extraction

Data on the number of VT pregnancies, type of VT, chorionicity, conception method, indication for NIPT, gestational age at NIPT, maternal age at NIPT, NIPT method, NIPT results, and results of follow-up testing were extracted from the included studies by one author (JE). If data were not described or unclear, the authors of the original studies were contacted for clarification.

2.4 | Definitions of NIPT test results

- True positive (TP): a high-risk NIPT result cytogenetically confirmed by invasive testing of the developing fetus or neonatal examination.
- Discordant positive: a high-risk NIPT result cytogenetically unconfirmed by invasive testing of the developing fetus or neonatal examination.
- Discordant negative: a low-risk NIPT result with a chromosomal aneuploidy cytogenetically confirmed in the developing fetus by invasive testing or in the infant after birth.
- True negative (TN): a low-risk NIPT result with no abnormal cytogenetic or clinical diagnosis in the developing fetus or infant after birth.

The screen positive rate of the pooled data and the pooled positive predictive value (PPV) were calculated using R software, v.4.1.1 (R Project for Statistical Computing).

2.5 | Quality assessment

The methodological quality of the studies was assessed by one author (JE) using the quality assessment tool for diagnostic accuracy studies-2 (QUADAS-2).[22] QUADAS-2 contains four domains: patient selection, index test, reference standard and flow and timing. Each one is assessed in terms of risk of bias and the first three also in terms of concerns regarding applicability.

2.6 | Risk of bias

The first domain refers to the patient selection. A study was considered to be at low risk of bias if NIPT was carried out in a consecutive or random sample of patients, and when a study avoided inappropriate exclusions.

The second domain relates to the index test. Studies in which NIPT results were interpreted without knowledge of the results of the reference test were considered to be at low risk of bias.

The third domain relates to the reference test. Studies describing invasive prenatal procedures (amniocentesis or chorion villus sampling [CVS]), postnatal karyotype, or neonatal examination as reference standard were considered to be at low risk of bias.

The fourth domain refers to the flow and timing. Studies were considered to be at low risk if (1) all patients received a NIPT result and a reference standard and (2) all patients recruited were included

in the analysis. The reference standard did not have to be the same in all patients because both amniocentesis, CVS, postpartum karyotype and postnatal examination were considered to be equally reliable.

2.7 | Concerns regarding applicability

The first three of the QUADAS-2 domains were also assessed in terms of concerns regarding applicability. In the first domain, studies assessing the test performance of NIPT in a general population were considered to be at low risk of concerns regarding its applicability. If a study used NIPT as an index test and karyotype or neonatal examination as a reference test, they were considered to be at low risk of concerns regarding applicability for the second and third domains.

3 | RESULTS

We identified 703 studies in literature as potentially relevant and, after removing duplicates, 392 remained. Based on titles and abstracts, another 334 studies were excluded, leaving 58 studies to be examined by reading the full text. Of these, seven met the inclusion criteria. A summary of the selection process is displayed in Figure S1.

Four studies were retrospective observational cohort studies,[10,23–25] and three were prospective observational cohort studies,[26–28] all published between 2018 and 2021. The cohort sizes ranged from 5 to 767 VT pregnancies. Four studies included only high risk pregnancies,[25–28] that is, advanced maternal age (>35 years at delivery), abnormal fetal findings on ultrasound examination, personal history of a child with aneuploidy, abnormal serum screening results, or a parent carrying a balanced Robertsonian translocation. Two studies included a mixed risk population,[23,24] and one included women of the general obstetric population.[10] One study described the chorionicity of the VTs and made a distinction between two types of VTs: type I with an additional empty GS and type II with a non-viable embryo.[27] None of the studies reported the gestational age of the presumed fetal demise. One study reported the fetal fraction.[26] All testing was performed in the first trimester or early in the second trimester. The main characteristics of each study are shown in Table 2.

Two NIPT methods were described. Four studies used whole genome sequencing,[10,24–26] two used rolling-circle-replication,[27,28] and in one study the NIPT method was unclear.[23] Confirmatory testing in all studies was done by either CVS, amniocentesis, postpartum genetic testing or in three cases of Down syndrome neonatal examination.

3.1 | Methodological quality of included studies

The methodological quality assessment of the seven cohort studies by the QUADAS-2 tool is illustrated in Figure 1. Risk of bias was considered high in five studies.[10,23,25,27,28] In six studies, there was a concern regarding applicability, five due to the inclusion of only high risk women[24–28] and one due to only including pregnancies that resulted from assisted reproductive technology.[23] The results of the assessment per study are given in Table S1.

3.2 | Common trisomies

Due to the heterogeneity between the included studies, it was not possible to perform a meta-analysis. However, to create an overview, we pooled the data for the common trisomies, using a random effects (generalized linear mixed model) model to take maximum account of the heterogeneity between studies. The study by Suzumori et al. was not included in this analysis as they only present the data for the common trisomies combined. The outcomes of each study per chromosomal aneuploidy are shown in Table 3 and described in more detail below.

The NIPT returned positive results for trisomy 21 (T21) in 35/1592 pregnancies with a VT (2.2% [95% confidence interval (CI), 1.6%–3%]). T21 was confirmed in 7/35 cases (pooled PPV 20% [95% CI 9.8%–36%]). The NIPT returned positive results for trisomy 18 (T18) in 13/1592 pregnancies with a VT (0.91% [95% CI 0.33%–2.46%]), and was confirmed in 5/13 cases (pooled PPV 25% [95% CI 1.3%–90%]). The NIPT returned positive results for trisomy 13 (T13) in 7/1592 pregnancies with a VT (0.44% [95% CI 0.22%–0.92%]), and was confirmed in 0/7 cases (pooled PPV 0% [95% CI 0%–100%). No discordant negative results were reported (0/1537; [0%]). The pooled negative predictive value was 100% (95% CI 100%–100%).

3.3 | Trisomy 21

The performance of NIPT for T21 was reported in five studies.[10,23,24,27,28] Van Riel et al. reported a NIPT positive test for T21 in 19/767 (2.5%) pregnancies with a VT and T21 was confirmed in 3/19 (PPV 15.8%) cases and discordant positive in 13/19 (68%). In the study of Zou et al., NIPT was positive for T21 in 9/579 (1.6%) pregnancies with a VT and was confirmed in 1/9 (PPV 11.1%), and discordant positive in 8/9 (88.9%) Balaguer et al. reported 4/206 (1.9%) NIPT positive tests, of which 2/4 (PPV 50%) cases were confirmed. Pooh et al. reported 2/20 (10%) NIPT positive results, none of which could be confirmed. Pavanello et al. reported 1/5 (20%) positive NIPT result for T21, which was confirmed in the developing fetus.

Due to the low number of aneuploidies, the studies were not able to calculate the test characteristics.

3.4 | Trisomy 18

Four studies described NIPT positive tests for T18.[10,23,24,27] NIPT returned positive results for T18 in 4/767 (0.5%),[10] 2/579 (0.3%),[23] 6/206 (2.9%)[24] and 1/20 (5%)[27] pregnancies with a VT, of which 0, 0, 4 (PPV 66.7%) and 1 were confirmed, respectively.

TABLE 2 Main characteristics of studies reporting on the test performance of the non-invasive prenatal test in vanishing twin pregnancies.

First author, year	Study description	Definition VT	Study population	Conception method	Inclusion number	Mean maternal age at NIPT (95% CI)	Mean GA at NIPT (95% CI)	NIPT method	Type of genetic testing	Diagnostic testing rate (%)[a]
Balaguer, 2020[24]	Retrospective observational	-	Mixed	89 spontaneous 117 ART	206	37.1 (36.6, 37.7)	14.9 (14.4, 15.3)	WGS	AC, CVS or after birth	100%
Pavanello, 2021[28]	Prospective	Spontaneous reduction to singleton	High risk	-	5	36[b]	-	Rolling-circle-replication	AC or CVS	100%
Pooh, 2021[27]	Prospective	-	High risk	-	20	36.2[b]	-	Rolling-circle-replication	AC or CVS	100%
Suzumori, 2020[25]	Retrospective cohort	IUFD confirmed in one of the two GS	High risk	-	87	38.5[b]	13.2[b]	WGS	AC or CVS	Unclear
Takeda, 2018[26]	Prospective observational	Spontaneous in utero reduction in fetuses, with either partial or complete dissipation occurring during pregnancy.	High risk	4 spontaneous 2 ovulation drugs 4 IVF-ET 5 ICSI	15	39.4	13.6	WGS	AC	100%
van Riel, 2021[10]	Retrospective cohort	Spontaneous reduction of a fetus in utero	General population	-	767	-	-	WGS	AC or CVS	82%
Zou, 2020[23]	Retrospective observational	Pregnancies that started with double fetal sacs and spontaneously reduced into one (two fetal sacs but one fetal heartbeat)	Mixed	428 IVF 145 ICSI 6 PGT	579	31.8	11.9	Not clear	AC or CVS	100%

Abbreviations: AC, amniocentesis; ART, assisted reproductive technology; CVS, chorion villus sampling; GA, gestational age; GS, gestational sac; ICSI, intracytoplasmic sperm injection; IUFD, intra-uterine fetal demise; IVF, in vitro fertilization; IVF-ET, in vitro fertilization-embryo transfer; NIPT, non-invasive prenatal testing; PGT, preimplantation genetic testing; VT, vanishing twin; WGS, whole genome sequencing.

[a]Number of patients with genetic testing divided by the total number of patients with an abnormal NIPT result.

[b]Mean of whole study population from included article not only of the VTs.

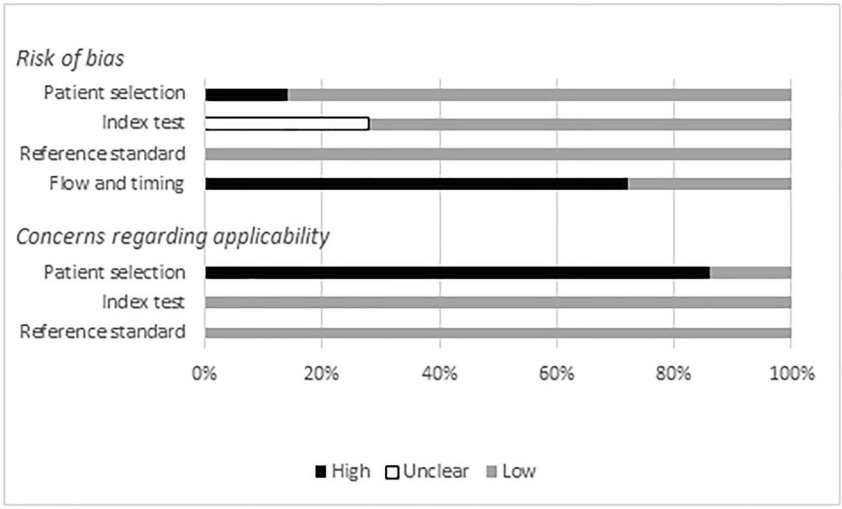


FIGURE 1 Quality assessment of the seven included cohort studies by quality assessment tool for diagnostic accuracy studies-2.

3.5 | Trisomy 13

Three studies reported the screening performance of NIPT for T13.[10,23,26] Van Riel et al. reported 5/767 (0.7%) positive NIPT tests for T13, Zou et al. 1/579 (0.2%) and Takeda et al. 1/15 (6.6%). None could be confirmed.

One study, Suzumori et al., solely reported the number of TPs, discordant positives, TNs, and false negatives for the common trisomies combined. They reported a NIPT positive test for one of the common trisomies in 11/87 (12.6%) pregnancies with a VT of which 4/11 (PPV 36%) cases were confirmed and 7/11 (64%) were discordant positive.

3.6 | Additional findings

Van Riel et al. reported additional findings by whole genome NIPT. Positive results were found in 23/767 (2.9%) pregnancies with a VT (one T4, 8, 9, 12, 20; four T7, six T15, four T16 and four T22). None were confirmed in the developing fetus.

3.7 | Sex chromosomal aneuploidies

Balaguer et al. reported on the screening performance of NIPT for Sex chromosomal aneuploidies (SCA). NIPT detected 1/206 (0.5%) 45, X (Turner syndrome) and 1/206 (0.5%) XXX (Triple X syndrome). Turner syndrome could be confirmed, but Triple X syndrome was discordant positive.

3.8 | Sex discrepancy

Two studies, van Riel et al. and Balaguer et al., used NIPT to predict the fetal sex. In 6/767 (0.8%) and 21/206 (10.2%) cases, respectively, the fetal sex detected by NIPT could not be confirmed by ultrasound. In all cases, a male fetus was predicted but the developing fetus was female.

3.9 | False negative NIPT results

None of the included studies reported false negative cases.

4 | DISCUSSION

We performed a systematic review of available literature and conclude that the NIPT can successfully detect common autosomal aneuploidies in conceptions affected by a VT, but with a higher false positive rate. This higher false positive rate is presumably due to the detection of cfDNA from the demised twin that was aneuploid.

Aneuploidy is a major cause of first trimester loss/vanishing twins.[16–18] In this review, the screen positive rate varied between 2% (mixed population) and 12% (high risk), excluding one study due to small sample size,[28] which is considerably higher than in singletons: 0.48% (low risk) and 3.03% (high risk).[2,3,7] cfDNA fragments, originating from the placenta of both the developing fetus as well as the VT, end up in the maternal bloodstream. Therefore, a VT can complicate the interpretation of NIPT, causing a discordant positive result and leading to more invasive procedures.

As expected, we found that the majority of NIPT positive results could not be confirmed in the developing fetus. Although the PPV of the NIPT for T21 in the two largest studies, conducted by van Riel et al. and Zou et al.—15.8% and 11.1%, respectively—is lower than reported in singleton studies—96%— it is comparable to the PPV of the FCT in singletons-5%.[2,29–31] Additionally, when including the number of TNs, the majority of the NIPT results in VT pregnancies was concordant.

FCT was historically offered to women with a VT despite a lack of evidence regarding the test's performance. We identified only five studies describing the effect of a VT on first-trimester serum

TABLE 3 Outcomes of studies reporting on the test performance of the non-invasive prenatal test in vanishing twin pregnancies.

First author	Total (*n*)	T21				T18				T13				Additional findings			
		Screen positive (*n*)	True positive (*n*)	Discordant positive (*n*)	Lost to follow-up (*n*)	Screen positive (*n*)	True positive (*n*)	Discordant positive (*n*)	Lost to follow-up (*n*)	Screen positive (*n*)	True positive (*n*)	Discordant positive (*n*)	Lost to follow-up (*n*)	Screen positive (*n*)	True positive (*n*)	Discordant positive (*n*)	Lost to follow-up (*n*)
Balaguer[24]	206	4	2	2	0	6	4	2	0	-	-	-	-	-	-	-	-
Pavanello[28]	5	1	1	0	0	-	-	-	-	-	-	-	-	-	-	-	-
Pooh[27]	20	2	0	2	0	1	1	0	0	-	-	-	-	-	-	-	-
Suzumori[25]	87	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Takeda[26]	15	-	-	-	-	-	-	-	-	1	0	1	0	-	-	-	-
van Riel[10]	767	19	3	13	3	4	0	3	1	5	0	4	1	23	0	20	3
Zou[23]	579	9	1	8	0	2	0	2	0	1	0	1	0	-	-	-	-
Total	1679	35	7	25	3	13	5	7	1	7	0	6	1	23	0	20	3

markers. These studies demonstrated that PAPP-A may be increased, the extent of which was influenced by the timing of the FCT. This can lead to a false negative result as decreased levels of PAPP-A are associated with T21, T18 and T13.[5,32] However, due to the limited evidence and heterogeneity, the exact impact on the FCT performance is unknown. Therefore, the current evidence does not provide guidance on whether FCT performs better than NIPT in VT pregnancies.

Two factors might influence the test performance of NIPT: timing and the type of VT. The duration of cfDNA persistence from the VT in maternal blood is unclear, attributable to a scarcity of studies with serial NIPT measurements to track cfDNA clearance, and the challenge of establishing the precise date of fetal demise. Several studies reported that cfDNA was detectable up to 8 weeks after fetal demise,[33–37] but unfortunately did not repeat the NIPT to assess at which point in time cfDNA of the VT became undetectable.

Balaguer et al. assessed the timing of NIPT and concluded that the number of screen positives and discordant positives was lower if NIPT was performed after 14 weeks.[24] They recommend repeating the NIPT in case a positive result is returned before 14 weeks of gestation. In Zou et al., the NIPT was repeated in 16 cases of test failure and in three NIPT positive cases.[23] For tests repeated after 15 weeks, 92% (11/12) received a negative NIPT result compared to 57% (4/7) before 15 weeks.

Based on this review, we are unable to determine the optimal timing of NIPT in VT pregnancies. Larger studies, including repetition of the test, are necessary to identify the optimal timing of NIPT in the case of a VT.

We also propose that the type of VT, I with an additional empty GS or II with a non-viable embryo, can influence the performance of NIPT. The association between fetal percentage of cfDNA and gestational age has been established, showing a weekly increase as the pregnancy progresses.[38–40] As the fetal demise of a type I VT is earlier in the pregnancy compared to a type II, it is hypothesized that less cfDNA circulates in the maternal bloodstream, potentially resulting in fewer discordant positives. Only Pooh et al. made a distinction between the types, but this study was too small to draw conclusions.[27]

4.1 | Strengths and limitations

To our knowledge, this is the first systematic review of the test performance of NIPT in VT pregnancies. This is important considering the increasing uptake of NIPT worldwide.

The review has several limitations. Firstly, only seven studies were included with relatively small sample sizes and a low number of aneuploidies per study. Therefore, the test characteristics (sensitivity and specificity) of the NIPT could not be calculated with a high degree of confidence. Secondly, meta-analysis of the data was not possible due to considerable heterogeneity in the study populations: four involved high risk pregnancies, two had a mixed risk population and one represented the general obstetric population.

Thirdly, none of the studies reported a gestational age of the presumed fetal demise and only one study made a distinction between the two types of VTs. Therefore, we were unable to assess the influence of timing and type. Finally, because the majority of the studies were performed with a high risk population, most studies were considered at high risk of bias regarding applicability. The three largest studies were considered to be at high risk of bias in relation to flow and timing due to test failure and cases lost to follow-up. However, it should be noted that obtaining a low risk of bias in a large clinical trial is very difficult, as it requires complete follow-up on all participants.

5 | CONCLUSION

There is insufficient data to fully evaluate NIPT performance in pregnancies with a VT. However, the results from the included studies suggest that NIPT can successfully detect common autosomal aneuploidies in conceptions affected by a VT but with a higher false positive rate. This higher false positive rate is presumably due to the detection of cfDNA from the demised twin that was aneuploid. Further studies are needed to determine the optimal timing of NIPT in VT pregnancies.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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