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Cell-Free DNA Screening in Twin Pregnancies using the Harmony® Prenatal Test

Introduction

Pregnancies with more than one fetus are not uncommon. According to 2016 data, almost 1 in 30 babies born are of a twin pregnancy.[1] Because infants born from a pregnancy with multiples are at a higher risk for adverse outcomes, professional societies around the world recommend increased surveillance and screening.[2–7]

Cell-free DNA (cfDNA) analysis for aneuploidy risk assessment has been used in twin pregnancies since 2013; however, published data regarding the performance of different test methodologies in twin pregnancies is less abundant as compared to singleton pregnancies.[8] cfDNA analysis of twin pregnancies is confounded by unique technical and biological factors. Proper interpretation and clinical implementation of cfDNA analysis in twin pregnancies requires evidence supporting this specific use of the technology.[9–11]

The purpose of this paper is to review the published data for the Harmony prenatal test and highlight the technical and clinical considerations involved in cfDNA testing of twin pregnancies.

Clinical scenarios included in published Harmony test twin studies
• Monochorionic Twins
• Dichorionic Twins
• Twins conceived with *in vitro* fertilisation
• Twins conceived naturally
• Pregnancies where both twins have aneuploidy
• Pregnancies where only one twin has aneuploidy
• First trimester testing
• Second trimester testing

Robust Validation Studies Support the Harmony Test Performance in Twin Pregnancies

The Harmony prenatal test was used in two publications where 22 of 24 twin pregnancies with trisomy 21 were given a high probability result.[12,13] No false positive results were reported among 564 euploid fetuses in twin pregnancies (Table 1). In contrast, traditional first trimester combined screening for trisomy 21 in twin pregnancies has detection rates of 75-90% with false positive rates of 5-9%.[14–17]

Experiences with the Harmony test in twin pregnancies have been reported in six publications with >1300 analyses, as of December 2017 (Table 2).

Technical Complexities in Twin Pregnancies

Accurate Fetal Fraction Assessment

The major difference between cfDNA analysis of singleton and twin pregnancies is the assessment of fetal fraction. The obstetrics community has come to recognise that accurate measurement of fetal fraction is critical for the performance of all NIPT methodologies.[21] The Harmony prenatal test uses single-nucleotide polymorphisms (SNPs) to assess the contribution of cfDNA from the pregnancy. This methodology has demonstrated accuracy and has been utilised in the validation studies for singletons, twins, sex chromosome aneuploidies and 22q11.2 deletion, as well as in the commercial laboratory. [22–25]

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Table 1.

The Harmony Test Results in Twin Validation Studies

Study	Trisomy 21	Trisomy 18	Trisomy 13	Euploid	Fetal Sex
Bevilacqua et al[13]	11 of 12	5 of 5	-	323 of 323	
*Gil et al[12]	9 of 10		1 of 1	181 of 181	
*Gil et al[12]	2 of 2	1 of 1		60 of 60	
Jones et al[18]					39 of 39
Totals	22 of 24	6 of 6	1 of 1	564 of 564	

*Retrospective and prospective data sets reported separately within publication

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Technical Complexities in Twin Pregnancies *continued from page 1*

Accurate Fetal Fraction Assessment

Twin fetuses may contribute differing amounts of cfDNA into maternal circulation.[9] Insufficient fetal fraction from one twin may lead to discordant cfDNA analysis results. The FORTE algorithm takes into account the twin pregnancy to estimate the fetal fraction contribution from each twin and to use the contribution of one twin as the basis to determine the probability score.[9,12,13,19,20]

Fetal Sex and Sex Chromosome Aneuploidy

The Harmony prenatal test can evaluate fetal sex in a twin pregnancy. A "female" result indicates the absence of Y chromosome and a "male" result indicates the presence of Y chromosome. For twin pregnancies, at least one male result indicates one or two male fetuses. The Harmony prenatal test has published validation data for fetal sex analysis in twin pregnancies.[18] Fetal sex assessment in 39 twin pregnancies correctly reported 18 of 18 twins with two female fetuses and 21 of 21 twins with at least one male fetus.

cfDNA analysis of the X and Y chromosomes requires a rigorous and advanced algorithm. The Harmony test evaluates the probability of five different sex chromosome aneuploidy conditions (monosomy X, XXX, XYY, XXY and XXYY) in singleton pregnancies.[18,26] The presence of more than one fetus exponentially increases the complexity of the analysis. The Harmony prenatal test is not validated to assess for sex chromosome aneuploidy in twin pregnancies.

Clinical Considerations in Twin Pregnancies

cfDNA Analysis Cannot Evaluate Chorionicity

cfDNA analysis cannot provide information about chorionicity and amnionicity of the twin pregnancy, as these are structural rather than genetic features. Optimal management of a twin pregnancy relies on understanding the placenta and membrane structures.[4,5,27] There is no substitute for ultrasound, which in the first trimester is an effective and reliable tool for determining these clinical characteristics and has been shown to improve outcomes for twin pregnancies.[5,28]

Twin fetuses who share a common placenta are at an increased risk for vascular abnormalities of the placenta such as twin-twin transfusion syndrome (TTTS).[29] Fewer than 15% of monochorionic twins may develop TTTS, which can be diagnosed with ultrasound alone. Optimal twin pregnancy outcomes rely on understanding chorionicity; however, knowledge of twin zygosity may not contribute to clinical care.

There is scant published data supporting the reliability of cfDNA analysis for twin zygosity assessment .[30–32] Zygosity screening may be possible through cfDNA analysis; however, performance remains unknown. Furthermore, rare instances of non-identical monozygotic twins have been reported and may be more common in pregnancies achieved with assisted reproduction.[33–35] Prenatal cfDNA analysis is a screening test and potential zygosity testing through noninvasive methods would not be equivalent to diagnostic tests.

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Table 2.

Peer-reviewed publications with twin pregnancy samples and the Harmony prenatal test

Publication Year	Study	Gestational Age (Median)	# Twin Samples	Dichorionic	Monochorionic
2014	Gil et al[12]	12.0* 10.6*	207 68	174	101
2014	Struble et al[9]		70	35	35
2015	Stokowski et al[19]		40		
2015	Bevilacqua et al[13]	13.0	515	301	67
2016	Sarno et al[20]	11.5	438		
2017	Jones et al[18]	16.7	51		
	TOTALS		**1,389**	**510**	**203**

*Retrospective and prospective data sets reported separately within publication

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Clinical Considerations in Twin Pregnancies *continued from page 2*

Redraw Request Rate May be Increased for Twin Pregnancies

Twin pregnancy samples may be more likely to not receive a probability score result on the first blood draw, depending on the gestational age of the sample draw, maternal weight and other unknown factors.[20] Twin pregnancies have lower fetal fraction levels as compared to singleton pregnancies.[20] Some studies suggest that *in vitro* fertilisation (IVF) is associated with lower fetal fraction although current data regarding this theory is very limited.[36] Test performance is based on sufficient fetal fraction from each fetus in the sample, and is critical for quality assurance.

The Harmony test and twin pregnancies
6 Published peer-reviewed studies
29 of 31 Trisomies detected in published data sets of twin pregnancies[12,13]
Accurate method of fetal fraction assessment, evaluated in over 148,000 published pregnancy samples (twins & singletons)
Available for donor egg pregnancies
Fetal sex analysis available

Vanishing Twin

The loss of a fetus in a multiple pregnancy is recognised to be a complicating factor when using maternal plasma for aneuploidy screening.[37] cfDNA from a non-viable embryo or fetus is released into the maternal bloodstream; however, the amount and duration of this biological process is not well understood.[11,38–40] Presence of this additional cfDNA may lead to an increased chance of a discordant NIPT result (both "false positives" and "false negatives"). For this reason, the Harmony prenatal test is not validated for use when a demised twin has been identified. Our validation data for twin pregnancies is based on the presence of two live fetuses in the uterus. Patients who have a demised fetus should consider other methods of evaluating the pregnancy for aneuploidy.

Twin pregnancy considerations
Two fetuses may contribute different amounts of cfDNA to maternal plasma
Redraw requests may be more frequent, as compared to singleton pregnancies, possibly due to low fetal fraction
Sex Chromosome Aneuploidy Panel not available
cfDNA analysis should not be used in pregnancies with a vanished or demised twin

Summary

- The Harmony prenatal test provides accurate aneuploidy risk assessment in twin pregnancies, with robust validation studies.[12,13] Traditional maternal serum screening has false positive rates of 5-9% in twin pregnancies.[14,16,17]
- Reliable evaluation of cfDNA contribution from each fetus is especially important when there are two fetuses contributing fetal cfDNA.
- Redraw requests may be more frequent in twin pregnancies, as compared to singletons, and reflect the quality control measures of the Harmony prenatal test.
- Chorionicity and amnionicity of twin pregnancies is critical information and can only be determined by ultrasound assessment. Zygosity assessment, by any means, cannot replace the need for this ultrasound assessment.
- Fetal sex may be reported from a twin pregnancy and reflects either one or both fetuses being the reported sex. The sex chromosome aneuploidy panel is not offered for twin pregnancies.
- The presence of a demised fetus within a pregnancy may increase the risk for a discordant cfDNA result. The Harmony prenatal test should not be ordered for a patient who is known to have a demised or vanished twin.

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Non-invasive prenatal testing (NIPT) based on cell-free DNA analysis is not diagnostic: results should be confirmed by diagnostic testing. Before making any treatment decisions, all women should discuss their results with their healthcare provider, who can recommend confirmatory, diagnostic testing where appropriate. The Harmony Prenatal Test was developed by Ariosa Diagnostics. The Harmony Prenatal Test is performed in Australia.

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