First-trimester screening based on cell-free DNA vs combined screening: a randomized clinical trial on women's experience

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Condensation: First-trimester risk assessment for trisomy 21 with a combination of ultrasound examination and cfDNA is associated with better maternal reassurance and better maternal satisfaction compared to the standard first-trimester combined screening

Why was this study conducted?

So far, there is lack of data regarding experience, emotional well-being, and satisfaction of women offered cell-free DNA (cfDNA) analysis plus detailed ultrasound examinationcompared to those offered first-trimester combined screening (FTCS) including detailed ultrasound examination as well.

What are the key findings?

Mean score for reassurance was significantly higher in the cfDNA group compared to the FTCS group. Women randomized to the cfDNA group had also higher satisfication and lower mean anxiety score.

What does this study add to what is already known?

First-trimester risk assessment for trisomy 21 with a combination of ultrasound examination and cfDNA is associated with better maternal reassurance and better maternal satisfaction compared to the standard first-trimester combined screening with nuchal trasclucency

Data Availability Statement:

Research Data not shared.

ABSTRACT

Objective: To compare women's experience of first-trimester combined screening (FTCS), with women's experience of an approach that uses the combination of a detailed early anatomy scan and cell-free DNA (cfDNA) analysis.

Methods: This was single-center, open label, parallel group, randomized clinical trial. Pregnant women were randomized at the time of their first prenatal visit to either a policy of first-trimester risk assessment based on FTCS, or to a policy of first-trimester risk assessment based on ultrasound findings and cfDNA. Control group included first-trimester risk assessment based on

FTCS. FTCS included ultrasound evaluation with crown-rump length, nuchal translucency (NT) measurement, and a detailed ultrasound scan, along with biochemistry (PAPP-A and free beta hCG). In this group, diagnostic testing was offered to patients with risk >1 in 100, or NT >3.5 mm, or any fetal abnormalities on ultrasound. Women randomized in the intervention group received an approach of first-trimester risk assessment based on ultrasound findings and cfDNA. cfDNA analysis included a simultaneous microarray-based assay of non-polymorphic (chromosomes 13, 18, 21, X and Y) and polymorphic loci to estimate chromosome proportion and fetal fraction. In the intervention group, diagnostic testing was offered to patients with abnormal cfDNA screening results, or NT >3.5 mm, or any fetal abnormalities on ultrasound. Participants received pre-test and post-test questionnaires regarding to measure reassurance, satisfaction, and anxiety. The primary outcome was the post-test reassurance, defined as mean score of reassurance post-test questionnaire. The effect of the assigned screening test on the cumulative incidence or on the mean of each outcome was quantified as the relative risk (RR) or mean difference (MD) with 95% confidence interval (CI).

Results: 40 women with singleton gestations were included in the trial. Mean score for reassurance was significantly higher in the cfDNA group compared to the FTCS group in the pre-test questionnaire (MD 0.80 points, 95% CI 0.27 to 1.33) and in the post-test questionnaire (MD 16.50 points, 95% CI 2.18 to 30.82). Women randomized to the cfDNA group had higher satisfication and lower mean anxiety score as assessed in the STAI pre-test questionnaire.

Conclusions: First-trimester risk assessment for trisomy 21 with a combination of a detailed ultrasound examination and cfDNA is associated with better maternal reassurance and better maternal satisfaction compared to the standard first-trimester combined screening with nuchal trasclucency, and maternal serum free beta-human chorionic gonadotrophin (FbetahCG) and pregnancy-associated plasma protein A (PAPP-A).

Trial registration: Clinicaltrials.gov NCT04077060.

INTRODUCTION

There is an ongoing debate regarding how cell-free DNA (cfDNA) screening can best be incorporated into current prenatal screening algorithms for chromosomal abnormalities (1).

Test performance of cfDNA has been shown to be better than first-trimester combined screening (FTCS) (2). However, the cost of the cfDNA testing is considered higher (3). Moreover, FTCS includes a detailed ultrasound examination of the fetus with nuchal translucency (NT) measurement that allows for early detection of fetal abnormalities (4).

An approach in which every woman isoffered an early anatomy scan along with cfDNA may be a reasonable option. Recently a randomized controlled trial, including 1,518 women with singleton pregnancy undergoing first-trimester screening, compared the screening performance of FTCS with an approach that uses the combination of a detailed ultrasound examination and cfDNA analysis. The trial showed that first-trimester risk assessment for trisomy 21 with non invasive prenatal testing (NIPT) was associated with a significant reduction in the false-positive rate compared with FTCS (5). This approach obviates the need for maternal serum free beta-human chorionic gonadotropin (FbetahCG)and pregnancy-associated plasma protein-A (PAPP-A) in screening for fetal aneuploidy.

During pregnancy, women experience elevated levels of stress and anxiety associated with potential adverse obstetrical outcomes such as intrauterine fetal death or preterm birth (6), and fetal abnormalities (7). So far, there is lack of data regarding experience, emotional well-being, and satisfaction of women offered cfDNA compared to those offered FTCS.

Objective

In this randomized controlled trial, we set out to compare women's experience of FTCS, with women's experience of an approach that uses the combination of ultrasound examinationand cfDNA analysis.

METHODS

Study design

This was a single-center, open label, parallel group, randomized clinical trialconducted at University of Naples Federico II, Napoli, Italy, from September 10, 2019 to February 15, 2020.Pregnant women were randomized at the time of their first prenatal visit to either a first-trimester risk assessment based on FTCS (i.e. control group), or to an approachof first-trimester risk assessment based on detailed ultrasound examination and cfDNA (i.e. intervention group). Inclusion criteria were pregnant women with singleton gestations, ≤12 6/7 weeks of gestation, and with crown-rump length (CRL) <84 mm at the time of randomization. Women with multiple gestations, including vanishing twins, and those who have already planned for invasive prenatal testingwere excluded. Gestational age was assessed from the menstrual history and confirmed by measurement of fetal CRL at the time of first prenatal visit.

Randomization and masking

After written informed consented was obtained from the eligible participants, women were randomly allocated in a 1:1 ratio to either intervention or control group using a web-based system.The trial was open-label, but the data analysts were blinded to allocated treatment group, until the entire analysis was completed.

Intervention and control group

Control group included first-trimester risk assessment based on FTCS. FTCS included ultrasound scan with crown-rump length, NT measurements, and a detailed ultrasound examination, along with biochemistry (PAPP-A and free beta hCG). In this group specific risk for aneuploidy was calculated by using the alghoritm of the UK Fetal Medicine Foundation (FMF) (8). In the control group, invasive test was offered to patients with risk >1 in 100, or NT >3.5mm, or any fetal abnormalities at ultrasound.

Women randomized in the intervention group received an approachof first-trimester risk assessment based on ultrasound findings and cfDNA. cfDNA analysis included a simultaneous microarray-based assay of non-polymorphic (chromosomes 13, 18, 21, X and Y) and polymorphic loci to estimate chromosome proportion and fetal fraction. In the intervention group, diagnostic invasive testing was offered to patients with abnormal cfDNA screening results, or NT >3.5mm, or any fetal abnormalities at ultrasound.

All operators who perform the ultrasound examinations were experienced operators certified by the FMF.

Women in both groups weregiven face to facecounseling by obstetricians and were counseled about test procedures, reporting time, test sensitivity and specificity, and the necessity to confirm abnormal screening results with invasive testing. The miscarriage risk for amniocentesis and chorionic villus sampling (CVS) was mentioned to be 0.3% and 0.5%, respectively (9).

Questionnaires

All women enrolled in the trialwereasked by their counselor to fill out two questionnaires. The first pre-test questionnaire (Q1) assessed women's preferences and was filled out at the time of randomization (Appendix 1). The second post-test questionnaire (Q2) was filled out after the screening test (Appendix 2) was performed.

Questionnaires were used to measure reassurance, satisfaction, and anxiety (10).

Reassurance

In Q1, women were asked to respond to a statement: 'I am happy of the assigned screening test (FTCS/cfDNA)', with a score from 1 (disagree strongly) to 5 (agree strongly), as pre-test reassurance questionnaire (Appendix 1).

In Q2, we measured post-test reassurance (Appendix 2). Three items were evaluated:

- I was reassured by the test-result
- I am confident that the test-result is correct

- The test-result offers me sufficient certainty whether my child has a disorder

Women could respond to each item with four scores:

- Not all applicable (5 points)
- Hardly applicable (10 points)
- Somewhat applicable (20 points)
- Very much applicable (35 points)

Satisfaction

During the Q2, women will be asked if retrospectively they were happy with the assigned screening test as post-test satisfaction questionnaire (Appendix 2):

- Not all applicable (5 points)
- Hardly applicable (10 points)
- Somewhat applicable (20 points)
- Very much applicable (35 points)

Women were also asked if they had diagnostic invasive testing after the normal screening test, or if were considering doing (Appendix 2) it.

Anxiety

Anxiety was measured by an Italian version of the six-item short form of the state scale of the Spielberger State-Trait Anxiety Inventory (STAI), as described by Marteau et al (11). Women were scored on a scale of 20-80, where higher scores mean higher levels of anxiety. A STAI score of 34-36 was considered normal anxiety. Child-related anxiety was measured by the 11-item Pregnancy-Related Anxiety Questionnaire-Revised Regardless of Parity (PRAQ-R2 scale), with a score from 1 to 5 for each of the 11 items (12, 13). Women were scored on a scale of 11-55, where higher scores mean higher levels of child-related anxiety. PRAQ-R2 was also assessed for the fear of bearing a physically or mentally handicapped child considering only four-items: item 4, 9, 10, and 11 with a total score ranging from 4 to 20 (13).

STAI and PRAQ-R2 were assessed at the time of randomization (pre-test questionnaire Q1), and after test results (post-test questionnaire Q2).

Primary and secondary outcomes

The primary outcome was the post-test reassurance, defined as mean score of reassurance post-test questionnaire.

The secondary outcomes were:

- Pre-test reassurance
    - Mean score of reassurance pre-test questionnaire
- Anxiety

    - Mean score of STAI in Q1
    - Mean score of STAI in Q2
    - Rate of STAI >36 in Q1
    - Rate of STAI >36 in Q2
    - Mean score of PRAQ-R2 in Q1
    - Mean score of PRAQ-R2 in Q2
    - Mean score of the subscale 4-item PRAQ-R2 in Q1
    - Mean score of the subscale 4-item PRAQ-R2 in Q2
- Satisfaction
    - Mean score of satisfaction post-test questionnaire
- Overall rate of invasive testing
- Rate of invasive testing for maternal request despite normal screening test
- False positive rate for trisomy 21
- False positive rate for any trisomy

Sample size calculation

The sample size calculation was based on detecting an effect that would produce a difference in the mean score of post-test reassurance questionnaire from about 60 points with standard deviation (sigma value) of 17 points in the control group to 75 points in the intervention group (10, 14).

We determined that a sample size of 40 (20 per group) patients would provide a power of 80% with a 2-sided type 1 error of 5%.

Statistical analysis

Data are shown as means with standard deviation, or as number (percentage). Univariate comparisons of dichotomous data were performed with the use of the chi-square test with continuity correction. Comparisons between groups were performed with the use of the T-test to test group means by assuming equal within-group variances. The primary analysis was an intention to treat comparison of the treatment assigned at randomization. The effect of the assigned screening test on the cumulative incidence or on the mean of each outcome was quantified as the relative risk (RR) or mean difference (MD) with 95% confidence interval (CI). A 2-sided P value less than .05 was considered significant.

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) v. 19.0 (IBM Inc).

RESULTS

Trial population

40 women with singleton pregnancies agreed to participate in the study, underwent randomization, and were enrolled and followed up (Figure 1). No women were excluded after

randomization or lost to follow up. Table 1 shows the baseline demographic and clinical characteristics for each group. Mean maternal age was 31 in the cfDNA group, and 29 years in the FTCS group. There were two women, one in each group, with history of chromosomal abnormalities in a prior pregnancy.

Primary and secondary outcomes

Mean score for test reassurance was significantly higher in the cfDNA group compared to the FTCS group in Q1 pre-test questionnaire (MD 0.80 points, 95% CI 0.27 to 1.33) and in Q2 post-test questionnaire (MD 16.50 points, 95% CI 2.18 to 30.82). Women randomized in the cfDNA group had higher satisfication as assessed in the Q2 questionnaire (MD 9.80 points, 95% CI 4.81 to 14.79) and lower mean anxiety score as assessed in the STAI Q1 pre-test questionnaire (MD -9.60 points, 95% CI -16.13 to -3.07). There was no significant between-group difference in the other secondary outcomes for maternal anxiety, but the trial was not powered for these outcomes (Table 2).

Chromosomal abnormalities and neonatal outcomes

In the FTCS group one women underwent CVS for NT of 3.6 mm. The CVS performed with arrayCGHand with RASopathypanel was normal.

One woman in the intervention group after a regular NIPT developed fetal cystic hygroma with NT 4.3 mm at 13 weeks of gestation, and underwent CVS with arrayCGH and panel for

RASopathy, that showed Noonan syndrome. This woman opted for induced termination of pregnancy.

No other cases of fetal chromosomal abnormalities were reported in either group. In the FTCS group one woman underwent spontaneous abortion due to cervical insufficiency at 20 weeks of gestation. The other participants had no complications.

Adverse events

No cases of maternal death, or serious injuries were reported during the study period.

DISCUSSION

Main findings

This randomized clinical trial aimed to compare the women's experience of FTCS (with NT measurement and serum screen), with women's experience of an approach based oncfDNA analysis.Participants in both groups received a detailed ultrasound examination for anomalies.

The trial showed that first-trimester risk assessment with a combination of ultrasound examination and cfDNA was associated with better maternal reassurance and better maternal satisfaction compared to the standard first-trimester combined screening based on nuchal trasclucency and maternal FbetahCG and PAPP-A. Internal validity was the major strenght of the study, with low risk of selection bias (a biased allocation to comparison groups was unlikely and

the concealment of allocation was adequate because a web-based system was used), performance and detection biases, with data analysts blinded to the allocated treatment group, and attritition bias, with no lost to follow-up. We used questionnaires validated in the literature as primary and secondary outcomes, including STAI and PRAQ-R2. The primary outcome of the trial was the post-test reassurance, defined as mean score of reassurance post-test questionnaire. This questionnaire was already validated by van Schendel et al. (15) In the study the authors performed a questionnaire study among pregnant women with elevated risk for fetal aneuploidy based on first trimester combined test or medical history who were offered NIPT. However, results from this trial were limited by the small sample size, and by the single center study design that raises the question of the external generalizability of the findings. Furthermore, women were given oral counseling by obstetricians, and the way information was given mayhave influenced women's preferences.In this trial, both groups received a detailed ultrasound examination. This approach is not routine for many centers worldwide in the first trimester. Pretest and posttest questionnaries had different multiple choise responses; this may be confusing for some participants.

Implication

Genetic counseling is an important step during pregnancy (16). Experts recommend that all pregnant women, regardless of age or circumstance, are offered genetic counseling and screening for Down'ssyndrome (17). The traditional method of screening for Down's syndrome has been

maternal age with amniocentesis or CVS offered to women aged 35 years or more. A more effective method of screening for Down's syndrome is the combined screening, which include maternal age, maternal blood sample for FbetahCG and PAPP-A, and an ultrasound evaluation at 11-13 weeks of gestation to measure the nuchal translucency (18,19). During the last decade, cfDNA has demonstrated a much higher sensitivity and specificity for trisomies 21, 18, and 13 compared to the FTCS (20). However, the test performance of cfDNA is not higher among patients who are low risk for aneuploidy (<35 years old, no prior aneuploidy). In this group, while the negative predictive values is high, the positive predictive values is significantly lower than in the high risk population since the prevalence of aneuploidy in this group is low. So far, cfDNA screening has usually been adopted in public health programsas a second line testmostly due to economic reason. Nonetheless, cost-effective studies showed that universal application of NIPT will increase fetal aneuploidy detection rates, reduce invasive procedure rate, and therefore can be economically justified in some settings (21). Recently, Ben et al. in an economic analysis of cfDNA, showed that replacing conventional screening with NIPTwould reduce healthcare costs if it can be provided for up to $744 (21).

Regarding couple's experience, the literature is lacking of robust and prospective data on women's satisfaction with cfDNA as first-tier screening test. Our study may be the first randomized trial comparing women's experience with combined screening, with women's experience with cfDNA analysis.

Conclusion

In summary, first-trimester risk assessment for trisomy 21 with a combination of ultrasound examination and cfDNA is associated with better maternal reassurance and better maternal satisfaction compared to the standard first-trimester combined screening

REFERENCES

1. Gil MM, Revello R, Poon LC, Akolekar R, Nicolaides KH. Clinical implementation of routine screening for fetal trisomies in the UK NHS: cell-free DNA test contingent on results from first-trimester combined test. Ultrasound Obstet Gynecol. 2016 Jan;47(1):45-52
2. Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol. 2019 Jun;53(6):734-742
3. García-Pérez L, Linertová R, Álvarez-de-la-Rosa M, Bayón JC, Imaz-Iglesia I, Ferrer-Rodríguez J, Serrano-Aguilar P. Cost-effectiveness of cell-free DNA in maternal blood

testing for prenatal detection of trisomy 21, 18 and 13: a systematic review. Eur J Health Econ. 2018 Sep;19(7):979-991

4. Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. Am J Obstet Gynecol. 2004 Jul;191(1):45-67
5. Kagan KO, Sroka F, Sonek J, Abele H, Lüthgens K, Schmid M, Wagner P, Brucker S, Wallwiener D, Hoopmann M. First-trimester risk assessment based on ultrasound and cell-free DNA vs combined screening: a randomized controlled trial. Ultrasound Obstet Gynecol. 2018 Apr;51(4):437-444
6. Mancuso RA, Schetter CD, Rini CM, Roesch SC, HobelCJ. Maternal prenatal anxiety and corticotropin-releasing hormone associated with timing of delivery. Psychosom Med. 2004 Sep-Oct;66(5):762-9
7. NakićRadoš S, Košec V, Gall V. The psychological effects of prenatal diagnostic procedures: maternal anxiety before and after invasive and noninvasive procedures. Prenat Diagn. 2013 Dec;33(12):1194-200
8. Wright D, Syngelaki A, Bradbury I, Akolekar R, Nicolaides KH. First-trimester screening for trisomies 21, 18 and 13 by ultrasound and biochemical testing. Fetal Diagn Ther. 2014;35(2):118-26
9. Akolekar R, Beta J, Picciarelli G, Ogilvie C, D'Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2015 Jan;45(1):16-26

10. van Schendel RV, Kater-Kuipers A, van Vliet-Lachotzki EH, DondorpWJ, Cornel MC, Henneman L. What Do Parents of Children with Down Syndrome Think about Non-Invasive Prenatal Testing (NIPT)? J Genet Couns. 2017 Jun;26(3):522-53
11. Marteau TM, Bekker H.The development of a six-item short-form of the state scale of the Spielberger State-Trait Anxiety Inventory (STAI).Br J Clin Psychol. 1992 Sep;31(3):301-6
12. Huizink AC, Mulder EJ, Robles de Medina PG, Visser GH, BuitelaarJK. Is pregnancy anxiety a distinctive syndrome? Early Hum Dev. 2004 Sep;79(2):81-91
13. Huizink AC, Delforterie MJ, Scheinin NM, Tolvanen M, Karlsson L, Karlsson H. Adaption of pregnancy anxiety questionnaire-revised for all pregnant women regardless of parity: PRAQ-R2.Arch Womens Ment Health. 2016 Feb;19(1):125-3
14. Seror V, L'Haridon O, Bussières L, Malan V, Fries N, Vekemans M, Salomon LJ, Ville Y; SAFE 21 Study Group.Women's Attitudes Toward Invasive and Noninvasive Testing When Facing a High Risk of Fetal Down Syndrome.JAMA Netw Open. 2019 Mar 1;2(3):e191062
15. van Schendel RV, Page-Christiaens GCML, Beulen L, et al. Women's Experience with Non-Invasive Prenatal Testing and Emotional Well-being and Satisfaction after Test-Results. J Genet Couns. 2017;26(6):1348-1356. doi:10.1007/s10897-017-0118-3
16. Mastantuoni E, Saccone G, Al-KouatlyHB, Paternoster M, D'Alessandro P, Arduino B, Carbone L, Esposito G, Raffone A, De Vivo V, Maruotti GM, Berghella V, Zullo

F.Expanded carrier screening: A currentperspective.Eur J Obstet Gynecol Reprod Biol. 2018 Nov;230:41-54

17. Spencer K, Nicolaides KH. Screening for trisomy 21 in twins using first trimester ultrasound and maternal serum biochemistry in a one-stop clinic: a review of three years experience. BJOG. 2003 Mar;110(3):276-80.
18. Nicolaides KH, Sebire NJ, Snijders RJ. Down's syndrome screening with nuchal translucency. Lancet. 1997 Feb 8;349(9049):438
19. Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10-14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. Lancet. 1998 Aug 1;352(9125):343-6
20. Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH.Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis.Ultrasound Obstet Gynecol. 2017 Sep;50(3):302-314
21. Benn P, Curnow KJ, Chapman S, Michalopoulos SN, Hornberger J, Rabinowitz M.An Economic Analysis of Cell-Free DNA Non-Invasive Prenatal Testing in the US General Pregnancy Population.PLoS One. 2015 Jul 9;10(7):e0132313

TABLES

Table 1. Characteristics of the included women

	cfDNA N = 20	**FTCS N = 20**	**p-value**
Age (year)	31.4±4.3	29.8±5.3	0.30
Race			0.31

Caucasian **Other**	20 (100%) -	19 (95.0%) 1 (5.0%)	
BMI **mean ± SD(Kg/m^2)**	24.6±4.0	25.9±6.3	0.44
Smoking	1 (5.0%)	2 (10.0%)	0.54
Nulliparous	4 (20.0%)	10 (50.0%)	0.09
Prior chromosomal abnormalities	1 (5.0%)	1 (5.0%)	0.99

Data are presented as number (percentage) or as mean ± standard deviation
FTCS, first-trimester combined screening; cfDNA, cell-free DNA; BMI, body mass index

Table 2. Primary and secondary outcomes

	cfDNA N = 20	FTCS N = 20	RR or MD (95% CI)
Reassurance			
Mean Reassurance Q1	4.9±0.2	4.1±1.2	**0.80 (0.27 to 1.33)**
Mean Reassurance Q2*	79.3±19.4	62.8±26.3	**16.50 (2.18 to 30.82)**
Anxiety			
Mean STAI Q1	37.2±9.1	46.8±11.8	**-9.60 (-16.13 to -3.07)**
Mean STAI Q2	39.6±12.9	45.7±12.9	-6.10 (-14.10 to 1.90)
STAI >36 Q1	11 (55.0%)	16 (80.0%)	0.31 (0.07 to 1.25)
STAI >36 Q2	13 (65.0%)	12 (60.0%)	1.24 (0.34 to 4.46)
Mean PRAQ-R2 Q1	24.5±6.6	29.4±9.8	-4.90 (-10.08 to 0.28)
Mean PRAQ-R2 Q2	25.0±9.0	27.3±9.3	-2.30 (-7.97 to 3.37)
Mean subscale 4-item PRAQ-R2 Q1	9.5±3.4	11.4±5.2	-1.90 (-4.62 to 0.82)
Mean subscale 4-item PRAQ-R2 Q2	9.3±5.5	10.3±5.6	-1.00 (-4.44 to 2.44)
Satisfaction			
Mean Satisfaction Q2	31.3±6.7	21.5±9.2	**9.80 (4.81 to 14.79)**
Invasive testing			
Overall rate of invasive testing	1 (5.0%)**	1 (5.0%)***	1.00 (0.06 to 17.18)
Invasive testing for maternal request despite normal screening test	0	0	Not applicable

Data are presented as number (percentage) or as mean ± standard deviation . Boldface data, statistically significant

RR, relative risk; MD, mean difference; CI, confidence interval; FTCS, first-trimester combined screening; cfDNA, cell-free DNA; STAI, Spielberger State-Trait Anxiety Inventory; PRAQ-R2, Pregnancy-Related Anxiety Questionnaire-Revised Regardless of Parity

**Primary outcome*

***For fetal cystic hygroma developed after regular cfDNA*

****For increased NT*

FIGURES
Figure 1. CONSORT Study flow-chart

APPENDICES
Appendix 1. Pre-test questionnaire
Appendix 2. Post-test questionnaire