P01.012-M
Fetal Fraction estimate in twin pregnancies using directed cell-free DNA analysis

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Objective: To estimate fetal fraction (FF) in monozygotic and dizygotic twin pregnancies.
Methods: Maternal plasma samples were obtained from 35 monochorionic twin pregnancies with male fetuses (monozygotic) and 35 dichorionic pregnancies discordant for fetal sex (dizygotic) at 11-13 weeks' gestation. Cell-free DNA was extracted and chromosome-selective sequencing with digital analysis of selected regions (DANSR TM) was carried out. The fetal-fraction optimized risk of trisomy evaluation (FORTE TM) algorithm was used to estimate FF using polymorphic alleles. In dizygotic twins the FORTE algorithm was modified to estimate the smallest FF contribution of the 2 fetuses. In both types of twins, FF was also determined by analysis of Y-chromosome sequences. Results: In monozygotic twins, the median total FF was 14.0% (range 8.2-27.0%) and in dizygotic twins the median smallest FF was 7.9% (4.9-14.0%). There were significant associations in FF between the methods using polymorphic alleles and Y-chromosome sequences for both monozygotic ($r = 0.951$, $p < 0.0001$) and dizygotic ($r = 0.743$, $p < 0.0001$) twins.
Conclusions: The study demonstrates the feasibility of an approach for cfDNA testing in twin pregnancies. This involves estimation of total FF in monozygotic twins and estimation of the lower FF of the 2 fetuses in dizygotic twins.

P01.013-S
Prenatal Diagnosis of Aneuploidy by Cell Free Fetal DNA in Maternal Plasma

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This study examined the methylation difference in AIRE and RASSF1A between maternal and fetal DNA, and the implication of this difference in the identification of free fetal DNA in maternal plasma and in prenatal diagnosis of trisomy 21. Maternal plasma and amniotic fluid samples were collected from 30 singleton pregnancies. Methylation-sensitive restriction enzymes in digestion of differential maternal-fetal methylation followed by fluorescent quantitative PCR (MSRE + PCR) were employed to detect trisomy 21. Diagnosis of trisomy 21 was established according to the ratio of fetal-specific AIRE to RASSF1A that are hypermethylated in maternal plasma and are not digested with methylation sensitive restriction enzymes. All of the results were approved with karyotype results. Based on the data from 22 euploidy pregnancies, the 95% reference interval of the fetal AIRE/RASSF1A ratio in maternal plasma was 0.33-1.77, which was taken as the reference value for determining the numbers of fetal chromosome 21 in 30 pregnancies. Firstly, 18 from 22 euploidy pregnancies were detected euploid correctly and 4 cases incorrectly so the early sensitivity rate was 81.81% (18/22). But by repeating the test with better digestion, the four cases made correct results so the final sensitivity rate was 100% (22/22). All of the eight trisomy 21 pregnancies were diagnosed with this method correctly so the specificity of this method was 100%. Also with performing STR-Typing and checking paternal alleles in maternal plasma and comparison with maternal alleles in 16 loci, the protocol of cell free fetal DNA extraction from plasma were confirmed.

P01.014-M
Non-invasive EXamination of Trisomy (NEXT) Study: Directedcell-free DNA analysis versus 1st trimester combined screening for Trisomy 21 risk assessment in a large Routine pregnancy population

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Non-invasive prenatal testing (NIPT) with cell-free DNA (cfDNA) is highly accurate for fetal trisomy evaluation in high-risk pregnancies. Routine pregnancy population NIPT performance has not been evaluated in a large prospective study. Our objective was to compare NIPT with directed cfDNA analysis to first trimester combined screening (FTS) for trisomy 21 risk assessment in a general pregnancy population.
This prospective multi-center blinded cohort study compared HarmonyTM Prenatal Test, a directed cfDNA test, with FTS using first trimester PAPP-A, hCG and nuchal translucency measurement. Women with a singleton fetus presenting in the first trimester for routine prenatal screening for fetal aneuploidy were eligible. Participants had both FTS and Harmony. FTS results were provided as part of routine care. Participants and care providers were blinded to Harmony results, calculated as probability scores. Pregnancies were followed for newborn outcomes. Invasive test results or neonatal phenotype,with karyotype confirmation in cases of suspected aneuploidy, were used for trisomy 21 identification. Harmony, FTS results and outcomes were reported to an independent data coordinating center. Primary outcome was comparison of the area under the ROC curve for trisomy 21 test performance of the Harmony and FTS.
18,955 women were enrolled across 38 centers in USA, Canada and Europe from March 2012 to April 2013. The mean maternal age was 30.6 (18-52) years. The mean gestational age was 12.4 (10-14.3) weeks. Follow-up is complete.
Study results will be presented. Implications for use of NIPT for trisomy 21 risk assessment in the general pregnancy population will be discussed.

P01.015-S
A case report of a high level 46,XX/46XY true chimerism without clinical effect in a healthy female who gave birth to healthy twins after IVF

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Chimerismis a rare event in humans when two or more genetically distinct cell lines occur in an individual. It is mostly connected with ovotesticular disorder of sexual develompent. We now present a case of high level 46,XX/46,XY
chimerism in a healthy woman who was karyoptyped prior to IVF. The fenotype of the proband was that of a normal woman with an unambiguously female external and internal genitalia.The XX/XY cell lines were detected in peripheral lymphocytes, buccal mucosa, urine sediment and also in all other subsequently biopted tissues: skin, both ovaries,
peritoneum and endometrium.The male cell line was detected by FISH technique(CEP X/Y SateliteIII DNA probes) in all samples at a propotion of 20-50%. The presence of SRY was confirmed using PCR. The chimerism was confirmed by STR multipexes
on chromosomes X, 13, 18, 21 and by STR markers kit (AmpFlSTR Identifiler PCR Amplification Kit). The
result of the analysis proved tetragametic origin of the chimerism.Our proband underwent three cycles of IVF with own oocytes and ICSI. The third cycle was successful. ET of 2 embryos was performed and after normal preganacy healthy female twins were born.

P01.016-M
Cell culture conditions of chorionic villous samples do not modify the genomic imprinting pattern at locus 11p15.5

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Methylation of the CpG islands is a common epigenetic marker of gene repression. Monoallelic and parental-specific DNA methylation pattern at the Imprinting Control Region 1 (ICR1) and 2 (ICR2) regulates the expression of IGF2/H19 and KCNQ1/CDKN1C domains at the imprinted locus 11p15.5. Alterations of ICR1 and ICR2 methylation state are common in Beckwith-Wiedemann (BWS) and Silver-Russell (SRS) syndromes and a robust molecular investigation is crucial to support phenotypic evidences, particularly in prenatal diagnosis for BWS. Since it is known that cell culture conditions could per se modify the epigenetic signature of the cells, we aimed to compare ICR1 and ICR2 methylation profile in fresh chorionic villus samples (CVS) with the corresponding cell cultures (CVC) to verify whether methylation at ICRs is stable after cell culture. By pyrosequencing we analyzed 9 CVS and their relative CVC from healthy pregnancies that underwent prenatal