Fetal Cells in Maternal Blood
For Non-Invasive Prenatal Diagnosis

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SRI Orlando

15 March 2017
Cell-based Noninvasive Prenatal Diagnosis (cbNIPD)
ARCEDI GOAL

- Current Prenatal Testing/Diagnosis Technology Scenario

CVS/Amniocentesis
ARCEDI GOAL

- Current Prenatal Testing/Diagnosis Technology Scenario

Cell-based
ARCEDI METHOD - OVERVIEW

- Enrichment, Detection and Analyses of Circulating Fetal Cells

1. Pregnant women GA: 10 to 13 weeks
2. Blood Processing (30mL of whole blood)
3. Selection and Staining using ARCEDI markers
4. Scanning and Identification of Fetal Cells
5. ‘Picking’ the Fetal Cell
6. Whole Genome Amplification (WGA)
7. CBNIPT using CMA/NGS
8. Positively Identified Fetal Cell
FETAL CELLS IN MATERNAL BLOOD

• It’s known that Fetal Cells do circulate in Pregnant women’s blood

• Alternative to Invasive Prenatal Diagnosis was envisaged – Focus on Fetal Cells. Reasons:
  • Mitigate the risk of intervention associated with invasive methods
  • Make prenatal diagnostics ‘simpler’ and cost-effective

• Attempts to isolate Fetal Cells from Maternal Circulation consistently and in good numbers were not very successful
FETAL CELLS IN MATERNAL BLOOD

- Challenges

Fetal Cell Type  Markers

Rarity of the Fetal Cells
FETAL CELLS IN MATERNAL BLOOD
- A Love story rekindled
FETAL CELLS IN MATERNAL BLOOD
- A Love story rekindled

FÆTAL ERYTHROCYTES
IN THE MATERNAL CIRCULATION
Alvin Zipursky

Aarhus University Hospital
FETAL CELLS IN MATERNAL BLOOD
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PRACTICAL AND THEORETICAL IMPLICATIONS OF FETAL/MATERNAL LYMPHOCYTE TRANSFER
Janina Walkowska, Felix A. Conte, Melvin M. Grumbach

Fetal cells in the blood of pregnant women: Detection and enrichment by fluorescence-activated cell sorting
(Y chromatin/HLA/prenatal diagnosis/chromosome abnormalities)
Leonard A. Herzenberg*, Diana W. Bianchi*, Jim Schroeder†, Howard M. Cann*, and G. Michael Iverson*
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Intact fetal cells in maternal plasma: are they really there?
Ferdinand Z Blaschke, Sinouhe Hahn, Kirby L. Johnson, Joe Leigh Simpson, Diana W Bianchi, Dorothy C Lewis, William D Wieler, Katherine Kfouri, Sherman Flower, Laird G Jackson, Mark I Evans, Wolfgang Holzgreve, Felis do le Cruz

Rare fetal cells can be recovered from maternal blood, which suggests that non-invasive prenatal diagnosis is possible. However, recovery and analysis of fetal cells from blood is complex, and sensitivity is low because of the rarity of these cells in the maternal circulation. An alternative strategy, which suggested that intact fetal cells can be found in maternal plasma by use of simple enrichment methods, has been reported. We aimed to replicate this technique. However, five independent laboratories were unable to identify any intact male cells from the plasma of 38 women known to be carrying male fetuses. Although apoptotic intact fetal cells could contribute to the detection of fetal DNA in maternal plasma, we believe that recovery of these cells is difficult and not clinically practical.

Lancet 2003; 361: 139–40
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FETAL CELLS IN MATERNAL BLOOD

- A Love story rekindled
CHECKLIST

- Technology to be approved as a cbNIPT/D should fulfil the following criteria

- **IDENTIFICATION OF FETAL CELL TYPE**
- **MARKERS SENSITIVE AND SPECIFIC FOR FETAL CELLS**
- **ROBUST METHOD TO ENRICH AND IDENTIFY FETAL CELLS**
- **ACCESSIBILITY OF FETAL CELLS FOR DOWNSTREAM ANALYSIS**
- **VIABILITY OF FETAL CELLS FOR PRENATAL TESTING/DIAGNOSIS**
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FETAL CELLS IN MATERNAL BLOOD

Fetal Cell Type
FETAL CELL TYPE

- Establishing the Fetal Cell gene expression profile

Characterization of Fetal Cells from the Maternal Circulation by Microarray Gene Expression Analysis – Could the Extravillous Trophoblasts Be a Target for Future Cell-Based Non-Invasive Prenatal Diagnosis?

Lotte Hatt a  Marie Brinch a  Ripudaman Singh a  Kristine Møller b
Rune Hoff Lauridsen a  Niels Uldbjerg c  Berthold Huppertz d  Britta Christensen a
Steen Kølvraa a
FETAL CELL TYPE

- Trophoblast mediated Uterine Vessel Remodelling

Moser et al. 2016
(Histochem Cell Biol)
FETAL CELL TYPE

- Trophoblast mediated Uterine Vessel Remodelling

_Moser et al. 2016 (Histochem Cell Biol)_
FETAL CELL TYPE

- Trophoblast mediated Uterine Vessel Remodelling

Routes of extravillous trophoblast invasion (6-11 weeks)

Moser et al. 2016
(Histochem Cell Biol)
FETAL CELL TYPE

- Trophoblast mediated Uterine Vessel Remodelling

• EMT (Epithelial – Mesenchymal Transition)

• Fetal Cells that shed in the Maternal Circulation express markers for both:
  • EPITHELIAL CELLS
  • ENDOTHELIAL CELLS

• ARCEDI MARKER COMBINATION!
  • 8 Markers for Enrichment and Staining of Fetal Cells

Moser et al. 2016
(Histochem Cell Biol)
Enrichment and identification of fetal cells in maternal blood and ligands for such use

Nov 9, 2011

The present invention relates to enrichment and/or identification of fetal cells of a maternal blood sample using fetal cell specific ligands and/or fetal cell specific hybridization probes wherein the ligand or probes are directed to an endothelial/mesenchymal marker, e.g. CD105, CD146 or CD141, in a first round of enrichment and the ligand or probes, in a second round of enrichment, are directed to an epithelial marker, e.g. a cytokeratin, such as CK7, CK8, CK18 or CK19. Enriched or identified fetal cells may be subjected to steps of detection or diagnosis, wherefore the present invention enables non-invasive 5 prenatal diagnostics.
FETAL CELLS IN MATERNAL BLOOD

Fetal Cell Markers
ARCEDI METHOD – MARKER SENSITIVITY

- Performance - Retrieval of Fetal Cells

- 190 PREGNANT WOMEN at NT SCAN - 30ml Blood.
  - 99 SAMPLES FROM ‘LOW RISK’ GROUP
  - 91 SAMPLES FROM ‘HIGH RISK’ GROUP (offered CVS)
ARCEDI METHOD – MARKER SENSITIVITY

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MATERNAL AGE
PAPP-A, beta-hCG
NUCHAL TRANSLUCENCY SCAN

1:300
ARCEDI METHOD – MARKER SENSITIVITY
- Performance - Retrieval of Fetal Cells

• 190 PREGNANT WOMEN at NT SCAN - 30ml Blood.
  • 99 SAMPLES FROM ‘LOW RISK’ GROUP
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<td>No of Fetal Cells</td>
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<td>Mean (per sample)</td>
<td>12.8/30ml blood</td>
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<td>Median</td>
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ARCEDI METHOD – MARKER SENSITIVITY

- Frequency Distribution (‘High Risk’ vs ‘Low Risk’)

![Frequency Distribution Graph]
ARCEDI METHOD – MARKER SENSITIVITY

- Frequency Distribution (‘High Risk’ vs ‘Low Risk’)

Fetal Cell from Every Sample
FETAL CELLS IN MATERNAL BLOOD

Fetal Cell Morphology
ARCEDI METHOD – FETAL CELLS

- Gallery
ARCEDI METHOD – FETAL CELLS

- Gallery
FETAL CELLS IN MATERNAL BLOOD

Method
Robustness
ARCEDI METHOD – ROBUSTNESS
- Turnaround time/sample

ARCEDI method: Processing Time per sample in Hours (continuous)
ARCEDI METHOD – ROBUSTNESS

- Sample Stability (Parameter/Platform Independant)

Fetal Cell Number and Distribution unaffected by:

• Blood collection **tubes** (BD vs Streck Tubes)

• **Time** before blood processed – 24 hrs and 48 hrs

• **Transportation** – air and road and processed after 48 hrs

• Fetal cells from **every sample**!
FETAL CELLS IN MATERNAL BLOOD

Genetic Information from Fetal Cells
ARCEDI METHOD – VIABILITY
- Deciphering Genetic Information from Fetal Cells

WGA
Picoplex / Ampli1

CMA (180K) / NGS
Genome-wide copy number analysis on DNA from fetal cells isolated from the blood of pregnant women

Steen Kalvraa1,2, Ripudaman Singh1,2,*, Elizabeth A. Normand2, Sadeem Qdaisa2, Ignatia B. van den Veyver2,3, Laird Jackson4, Lotte Hatt1, Palle Schelde1, Niels Uldbjerg5, Else Marie Vestergaard6, Li Zhao2, Rui Chen2, Chad A. Shaw2, Amy M. Breman2 and Arthur L. Beaudet2

1ARCEDI Biotech ApS, Vejle, Denmark
2Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA
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6Department of Clinical Genetics, Aarhus University Hospital, Aarhus N, Denmark
*Correspondence to: Ripudaman Singh. Email: rfs@arcedi.com
1Deceased.
ARCEDI METHOD – VIABILITY

- Two ‘High Risk’ cases

  • Two ‘High Risk’ Pregnancies offered CVS/Amniocentesis

  • Maternal Blood collected before CVS/Amniocentesis
ARCEDI METHOD – VIABILITY

- Two ‘High Risk’ cases

  • Two ‘High Risk’ Pregnancies offered CVS/Amniocentesis
  
  • Maternal Blood collected before CVS/Amniocentesis
  
  • Fetal Cells enriched and identified using ARCEDI Method
  
  • WGA and Array CGH (Agilent 180k microarray) performed at Baylor College of Medicine, Houston TX.
ARCEDI METHOD – VIABILITY

- Case I (HR33): Female Pregnancy (T21) – array CGH

Kølvraa et al. 2016
(Prenatal Diagnosis)
ARCEDI METHOD – VIABILITY

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Kølvraa et al. 2016 (Prenatal Diagnosis)

Array CGH on FC
ARCEDI METHOD – VIABILITY
- Case I (HR33): Female Pregnancy (T21) – array CGH

Kølvraa et al. 2016 (Prenatal Diagnosis)

Array CGH on FC
Array CGH on CVS

Aarhus University Hospital
ARCEDI METHOD – VIABILITY

- Case I (HR33): Female Pregnancy (T21) – array CGH

Kølvraa et al. 2016 (Prenatal Diagnosis)
ARCEDI METHOD – VIABILITY

- Case III (NIPT413): Mosaicism [45,X/46,X,r(X)] – array CGH

Kølvraa et al. 2016
(Prenatal Diagnosis)
ARCEDI METHOD – VIABILITY

- Case III (NIPT413): Mosaicism [45,X/46,X,r(X)] – array CGH

Kølvraa et al. 2016 (Prenatal Diagnosis)
ARCEDI METHOD – VIABILITY

- Case III (NIPT413): Mosaicism [45,X/46,X,r(X)] – array CGH

Cell 1

377

Cell 2

450

Amnio

Kølvraa et al. 2016
(Prenatal Diagnosis)
ARCEDI METHOD – VIABILITY

- Case III (NIPT413): Mosaicism [45,X/46,X,r(X)] - NGS

45,X: cell 377

46,X,r(X): cell 450
CVS vs cbNIPT

- HR146: Male Pregnancy (Trisomy 21; Risk 1:2)
CVS vs cbNIPT

- HR146: Male Pregnancy (Trisomy 21; Risk 1:2)
CVS vs cbNIPT

- HR146: Male Pregnancy (Trisomy 21; Risk 1:2)
CVS vs cbNIPT

Resolution of Detection
CVS vs cbNIPT

- HO10 46,XX,t(4;8)(p16;p23)

  • 2 Fetal Cells

Array CGH on CVS
CVS vs cbNIPT

- HO10 46,XX,t(4;8)(p16;p23)

  • 2 Fetal Cells
CVS vs cbNIPT

- HR308 46,XY,Partial T21
  • 4 Fetal Cells

Array CGH on CVS
CVS vs cbNIPT

- HR308 46,XY,Partial T21

• 4 Fetal Cells

Array CGH on CVS

Array CGH on FCs
WHAT NEXT?

- Validation Study (CVS vs cbNIPT)

  - Recruit ‘High Risk’ pregnancies from 6 different hospitals in Denmark (which are offered CVS)
  - Enrich Fetal Cells from the blood
  - Perform cell based fetal DNA analysis on the cells
  - Check whether the results from two tests correlate
WHAT NEXT?
- Supporting Studies

• Fetal cell numbers affected by BMI?

• Fetal cell numbers as predictor of pregnancy related complications? (Eg. Pre-eclampsia) or even Preterm Birth?

• Fetal cells persisting post-partum?
IN BRIEF
- We have..

• Identified the Circulating Fetal Cell Type

• Tested Markers which are highly sensitive and specific

• Robust method of enriching fetal cells

• Picked the cells and perform downstream analyses (WGA/array CGH/NGS)

• Detected Aneuploidies as well as CNVs using Fetal Cells
## ACKNOWLEDGEMENTS

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