

Fetal cells from maternal blood – a powerful tool for prenatal diagnosis (non-invasive prenatal diagnosis – from theory to practice)

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Introduction

It has been known for more than a century that fetal cells circulate in the maternal bloodstream during pregnancy. The ability to isolate these rare cells from maternal blood would provide considerable prenatal diagnostic potential, and would avoid the need for current invasive procedures, such as chorionic villus sampling (CVS) and amniocentesis, which carry a risk of abortion.

Fetal diagnostics based on a blood sample from the mother bear no risk – for the mother or the child.

However, it has been difficult to isolate fetal cells – due to their rarity in the maternal blood, and to the absence of fetal cell specific markers. These issues have hindered research in this area and have held the diagnostic potential at bay.

We, at FCMB, have developed a method to isolate rare fetal cells from maternal blood suitable for subsequent genetic analyses. This fetal cell isolation method aims to provide an alternative low-risk, and relatively non-invasive, technique which could provide pregnant women with accurate genetic information for their unborn child.

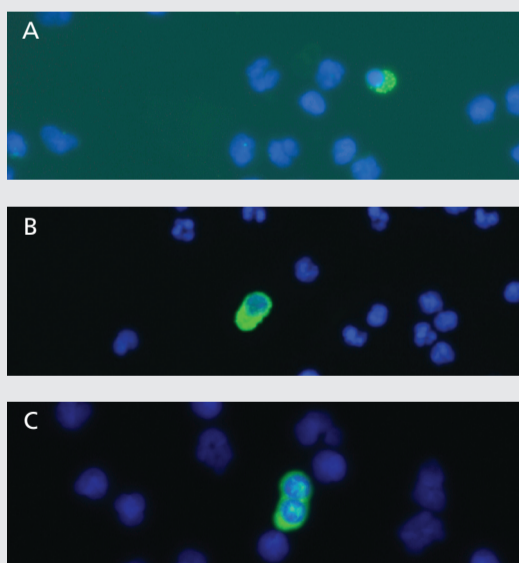
Isolation of male fetal cells and preparation of fetal cell specific cDNA libraries

- Fully preserved male fetal cells were isolated. The fetal origin of the cells was confirmed by XY-FISH.
- Information about the fetal cell specific markers was generated by creating a cDNA library from 100 of these male fetal cells (in a biological replicate of two), and microarray analysis was used to reveal a set of marker candidates expressed in fetal, but not maternal, cells. From this information, antibodies specific for fetal cells were identified and tested. For details, please refer to www.fcmb.dk

Validation of novel fetal cell enrichment technique and specificity of antibodies targeting fetal cells

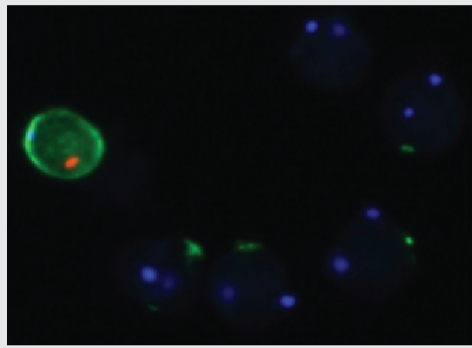
- Fetal cell enrichment was achieved by magnetic cell sorting using fetal cell specific antibodies in a magnetic cell sorting column.
- Samples enriched for fetal cells were stained using fluorescent dye labeled cocktails of fetal cell specific antibodies.
- The analysis showed that the gender-independent technique had successfully enriched for fetal cells.
- Figures 1 and 2 show a series of enriched samples labeled with fetal cell specific antibody cocktails.
- In both Figures 1 and 2, the absence of cross-reactivity with maternal cells indicates the specificity of the antibody cocktails for fetal cells.

Figure 1: Fetal cell enriched samples labeled with antibody cocktail-2



Fetal and maternal nuclei are stained blue with DAPI, but only fetal cells are stained green with the fetal-specific antibody cocktail. In C, two fetal cells close to each other are labeled.

Figure 2: Fetal cell enriched sample labeled with antibody cocktail-3

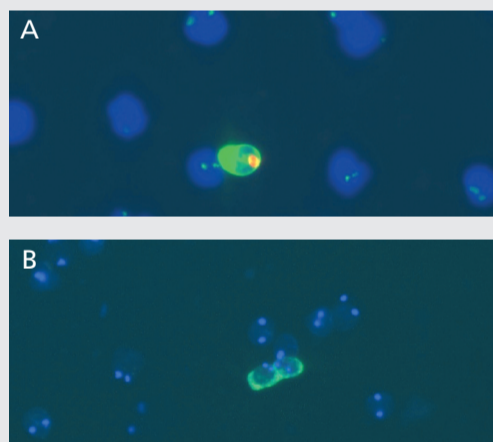


The fetal cell is stained green with the fetal-specific antibody cocktail. Also, X chromosomes are shown as blue dots and the Y chromosome in the fetal cell is shown as a red dot.

Feasibility study: gender determination

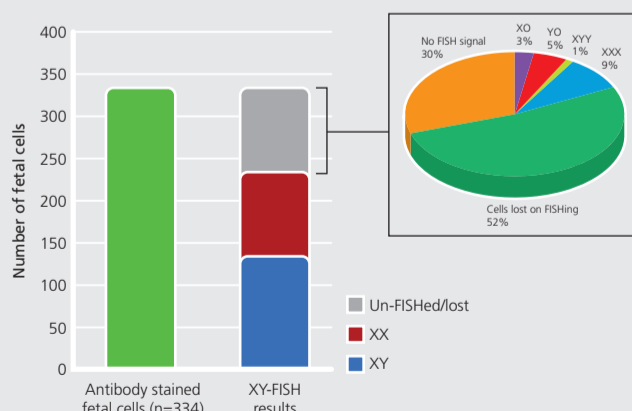
- A feasibility study was performed to assess whether our novel fetal cell enrichment and labeling technique could be applied to fetal gender determination.
- Intravenous blood (30 ml) was collected from 78 pregnant women (gestation age 11–14 weeks).
- On average, 4.2 fetal cells were detected per 30 ml blood sample. Fetal gender was determined by XY-FISH (Figure 3), and checked by performing Y chromosome-specific PCR on cell-free fetal DNA.
- A total of 334 fetal cells underwent XY-FISH testing, of which 234 (70%) gave clear gender results (Figure 4). It was not possible to determine gender in the remaining 100 (30%) cells for various reasons – cells lost during FISH procedure, no FISH signal, unclear gender (single X, single Y, XYY, XXX).

Figure 3: XY-FISHed fetal cell enriched sample



Fetal and maternal nuclei are stained blue with DAPI, but only the fetal cells are stained green with the fetal-specific antibody cocktail. The gender of the fetus was determined by XY-FISH. The X chromosome is depicted in blue and the Y chromosome is depicted in red. In A, the fetus is male; in B, the fetus is female.

Figure 4: Results of XY-FISH testing of fetal cells



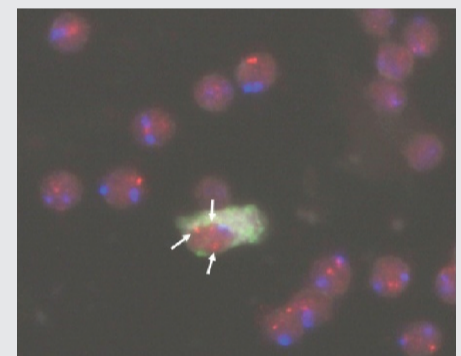
- Of the 234 cells with clear XY-FISH results, there was 98.5% concordance between fetal gender determined by XY-FISH and gender determined by PCR on cell-free fetal DNA. In the enriched samples with three or more fetal cells, gender concordance was 100%. There was one false positive.
- The gender feasibility study confirmed the successful enrichment and detection of fetal cells, and illustrated a potential application of this non-invasive approach for gender-independent fetal genetic testing.

Application of fetal cell enrichment procedure to non-invasive prenatal genetic testing

Feasibility study: detection of trisomy 21

- The ability to enrich fetal cells from maternal blood opens the possibility of using the technique to facilitate non-invasive prenatal diagnostic testing.
- The detection of trisomy 21, where three copies of chromosome 21 result in Down syndrome, is one of the most sought-after applications in prenatal diagnostics.
- During an ongoing feasibility study using our proprietary method described above, we have successfully diagnosed the outcome of a pregnancy affected by trisomy 21 (Figure 5), which was confirmed by CVS.

Figure 5: Non-invasive prenatal diagnosis of trisomy 21 (Down syndrome)



Fetal cell enriched sample stained with FISH probe for chromosome 21 (red) and the X chromosome (blue). Arrows point to the three copies of chromosome 21 in the fetal cell; background of maternal cells contain two copies each of chromosome 21

Conclusions and future perspectives

- We have developed a novel method of consistently and specifically separating rare fetal cells from maternal blood in a gender-independent manner, which has given us the unparalleled ability to perform non-invasive prenatal diagnosis.
- A comparison of cDNA array data from the fetal cells and control maternal blood has provided vital information on the unique markers expressed in fetal cells.
- A feasibility study, based on gender determination, has confirmed the application of the technique.
- In an ongoing feasibility study, our ground-breaking non-invasive technique has been used in the successful diagnosis of a Down syndrome (trisomy 21) pregnancy.

Abbreviations

FISH fluorescence *in situ* hybridization
CVS chorionic villus sampling
DAPI 4',6-diamidino-2-phenylindole, a fluorescent stain that binds strongly to DNA
DNA deoxyribonucleic acid
cDNA complementary deoxyribonucleic acid
PCR polymerase chain reaction

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Enrichment of fetal cells

