

Summary

In one aspect, the present disclosure refers to a method of prenatal diagnostics comprising the steps of:

- 5 a) contacting cells comprised in a blood sample obtained from a woman carrying a fetus with at least one magnetic labelling agent directed against a fetal trophoblast marker,
- b) enriching said sample for fetal trophoblasts with magnetic activated cell sorting (MACS),
- 10 c) following the enrichment step, the single cell sorting by FACS is performed by contacting the enriched cells with at least one fluorescent labelling agent selected from each of the groups:
 - i. fluorescent labelling agent directed against the nucleus,
 - ii. fluorescent labelling agent directed against a maternal cell marker, and
 - 15 iii. fluorescent labelling agent directed against a fetal trophoblast marker
- d) single cell sorting the fluorescently labelled cells on a fluorescence activated cell sorter (FACS) from said enriched sample based on:
 - 20 i. positive selection of said fluorescent labelling agent directed against a fetal trophoblast marker,
 - ii. positive selection of said fluorescent labelling agent directed against the nucleus, and
 - iii. negative selection of said fluorescent labelling agent directed against a maternal cell marker
- 25 e) identifying fetal trophoblasts among said sorted cells, comprising a step of assigning a fetal origin classifier to individually sorted cells by obtaining a genotype from the single sorted cells, and
- f) diagnosing the phenotype of the fetus.

30 In another aspect, the present disclosure refers to a method of prenatal diagnostics comprising the steps of:

- a) contacting cells comprised in a blood sample obtained from a woman carrying a fetus with at least one fluorescent labelling agent selected from each of the groups:
 - i. fluorescent labelling agent directed against the nucleus,
 - ii. fluorescent labelling agent directed against a maternal cell marker, and
 - iii. fluorescent labelling agent directed against a fetal trophoblast marker
- b) enriching said sample for fetal trophoblasts on a fluorescence activated cell sorter (FACS) based on fluorescence from said fluorescent labelling agents,
- c) following the enrichment step, the single cell sorting by FACS is performed by single cell sorting the fluorescently labelled cells on a FACS from said enriched sample based on:
 - i. positive selection of said fluorescent labelling agent directed against a fetal trophoblast marker,
 - ii. positive selection of said fluorescent labelling agent directed against the nucleus, and
 - iii. negative selection of said fluorescent labelling agent directed against a maternal cell marker.
- d) identifying trophoblasts among said sorted cells, comprising a step of assigning a fetal origin classifier to individually sorted cells by obtaining a genotype from said fetal trophoblast, and
- e) diagnosing the phenotype of the fetus.

In one example the disclosure relates to a method of isolating fetal cells from a biological sample of a pregnant woman, said method comprising the steps of

- a. providing a biological sample from said pregnant woman, the biological sample comprising a cellular fraction,
- b. contacting cells comprised in said cellular fraction with one or more fluorescent labelling agents directed against at least one fetal cell epithelial marker and/or endothelial marker,
- c. sorting said cells by Fluorescence-activated cell sorting (FACS) based on detection of said one or more fluorescent labelling agents bound to cells, and

- d. identifying fetal cells among said sorted cells comprising a step of assigning a fetal origin classifier to individual sorted cells.

5 The inventors have made the surprising observation that it is possible to perform single cell sorting using FACS and subsequently identify those cells that have a high probability of being fetal by simply analysing the output from the FACS in terms of a number of parameters such as scatter and signals from one or more fluorescently labelled antibodies. The output can be combined to a classifier, which may be numeric score or binary. Using methods of the disclosure, the inventors have developed a
10 method with a very high likelihood of identifying fetal cells among the sorted cells.

The advantages over prior art methods are illustrated in Figure 1, the main advantage being that the process can be fully automated and that it requires no picking of cells identified as probable fetal cells.

15 The identified cells can be verified as fetal cells by genetic analysis and can be further used for identifying genetic abnormalities in the cells.

In a further example the disclosure relates to a method of determining a genetic abnormality in a fetus said method comprising the steps of:

- a. Obtaining one or more fetal cells isolated by a method of the disclosure, and
b. Detecting one or more genetic markers associated with said genetic abnormality in
20 the genome of said fetal cell.

Claims

1. A method of prenatal diagnostics comprising the steps of:

- 5 a) contacting cells comprised in a blood sample obtained from a woman carrying a fetus with at least one magnetic labelling agent directed against a fetal trophoblast marker,
- b) enriching said sample for fetal trophoblasts with magnetic activated cell sorting (MACS),
- 10 c) following the enrichment step, the single cell sorting by FACS is performed by contacting the enriched cells with at least one fluorescent labelling agent selected from each of the groups:
 - i. fluorescent labelling agent directed against the nucleus,
 - ii. fluorescent labelling agent directed against a maternal cell marker, and
 - 15 iii. fluorescent labelling agent directed against a fetal trophoblast marker
- d) single cell sorting the fluorescently labelled cells on a fluorescence activated cell sorter (FACS) from said enriched sample based on:
 - 20 i. positive selection of said fluorescent labelling agent directed against a fetal trophoblast marker,
 - ii. positive selection of said fluorescent labelling agent directed against the nucleus, and
 - iii. negative selection of said fluorescent labelling agent directed against a maternal cell marker
- 25 e) identifying fetal trophoblasts among said sorted cells, comprising a step of assigning a fetal origin classifier to individually sorted cells by obtaining a genotype from the single sorted cells, and
- f) diagnosing the phenotype of the fetus.

2. A method of prenatal diagnostics comprising the steps of:

- 30 a) contacting cells comprised in a blood sample obtained from a woman carrying a fetus with at least one fluorescent labelling agent selected from each of the groups:

- i. fluorescent labelling agent directed against the nucleus,
 - ii. fluorescent labelling agent directed against a maternal cell marker, and
 - iii. fluorescent labelling agent directed against a fetal trophoblast marker
- b) enriching said sample for fetal trophoblasts on a fluorescence activated cell sorter (FACS) based on fluorescence from said fluorescent labelling agents,
- c) following the enrichment step, the single cell sorting by FACS is performed by single cell sorting the fluorescently labelled cells on a FACS from said enriched sample based on:
 - i. positive selection of said fluorescent labelling agent directed against a fetal trophoblast marker,
 - ii. positive selection of said fluorescent labelling agent directed against the nucleus, and
 - iii. negative selection of said fluorescent labelling agent directed against a maternal cell marker.
- d) identifying trophoblasts among said sorted cells, comprising a step of assigning a fetal origin classifier to individually sorted cells by obtaining a genotype from said fetal trophoblast, and
- e) diagnosing the phenotype of the fetus.

3. The method according to any of the preceding claims, wherein said magnetic- and/or fluorescent labelling agent directed against a fetal trophoblast marker is a magnetic- and/or fluorescent labelling agent directed against an endothelial or an epithelial marker.

4. The method according to any of the preceding claims, wherein said magnetic- and/or fluorescent labelling agent directed against a fetal trophoblast marker is a magnetic- and/or fluorescent labelling agent directed against an endothelial marker selected from Thy-1 CD90, Thrombomodulin CD141, Human Endoglin CD105, Human Vimentin (Vim), Vascular Cell Adhesion Molecule (VCAM), Intercellular Adhesion Molecule 1 (ICAM), Vascular Endothelial Growth Factor Receptor 1 (Flt-1) (VEGFR-1), Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2), Vascular Endothelial Growth Factor Receptor 3 (VEGFR-3), Plasminogen Activator Inhibitor 1 (PAI-1), Endothelial Protein C Receptor (EPCR), CD146, ITGA5, ITGB5, CDH11, CDH3 and/or CD59.

5. The method according to any of the preceding claims, wherein said magnetic- and/or fluorescent labelling agent directed against a fetal trophoblast marker is a magnetic- and/or fluorescent labelling agent directed against an epithelial marker.
6. The method according to claim 5, wherein said epithelial marker is a cytokeratin such as Human Cytokeratin 1 CK1, Human Cytokeratin 2 CK2, Human Cytokeratin 3 CK3, Human Cytokeratin 4 CK4, Human Cytokeratin 5 CK5, Human Cytokeratin 6 CK6, Human Cytokeratin 7 CK7, Human Cytokeratin 8 CK8, Human Cytokeratin 9 CK9, Human Cytokeratin 10 CK10, Human Cytokeratin 13 CK13, Human Cytokeratin 14 CK14, Human Cytokeratin 15 CK15, Human Cytokeratin 16 CK16, Human Cytokeratin 17 CK17, Human Cytokeratin 18 CK18, Human Cytokeratin 19 CK19.
7. The method according to any of the preceding claims, wherein said fluorescent labelling agents are antibodies, nucleotide probes, receptor ligands, and/or other specific binding molecules.
8. The method according to any of the preceding claims, wherein said fluorescent labelling agent directed against the nucleus is selected from any Hoechst dye, DAPI, propidium iodide, 7-AAD, Vybrant DyeCycle Stains, SYTOX stains, or SYTO stains.
9. The method according to any of the preceding claims, wherein said fluorescent labelling agent directed against a maternal cell marker is a fluorescent labelling agent directed against a leucocyte marker.
10. The method according to claim 9, wherein said leucocyte marker is selected from CD45, CD3, CD14, CD15, CD16, and/or CD19.
11. The method according to any of the preceding claims, wherein said genotype is obtained by STR- and/or SNP analysis.
12. The method according to any of the preceding claims, wherein said phenotype is diagnosed by detecting one or more markers associated with a genetic abnormality in the genome of the fetal cell.
13. The method according to claim 12, wherein said genetic abnormality is detected by one or more method selected from Microarray-based Comparative Genomic Hybridization(aCGH), Short Tandem Repeat analysis (STR analysis), whole genome amplification, whole genome scan, SNP array, Polony sequencing, Shotgun

sequencing, Massively parallel signature sequencing (MPSS), Sanger Sequencing, PCR-based methods and Next-Generation Sequencing methods.

14. The method according to claim 12 or 13, wherein said genetic abnormality is aneuploidy, monosomy, polysomy, trisomy, copy number variation (CNV), single nucleotide variation (SNV), or a monogenic disorder.
15. The method according to any of the preceding claims, wherein said blood sample is between 5-30 mL.
16. The method according to any of the preceding claims, wherein said blood sample comprises a cellular fraction.
17. The method according to any of the preceding claims, wherein a cellular fraction is separated from plasma of said blood sample.
- 18 The method according to claim **Error! Reference source not found.** or 17, wherein said cellular fraction comprises red blood cells, white blood cells and fetal trophoblasts, fetal extravillous trophoblasts, and fetal endovascular trophoblasts.
19. The method according to any of the preceding claims, said method further comprising a step of fixating the blood cells subsequent to being separated from said plasma fraction, wherein fixation is preferably a paraformaldehyde fixation.
20. The method according to any of the preceding claims, said method further comprising a step of selectively lysing red blood cells of said cellular fraction using a detergent subsequent to separation of said cellular fraction from said plasma fraction, wherein said lysing also permeabilizes the remaining cells in said cellular fraction.