

[0010] However, despite these advances, what is needed are robust methods and markers for identifying patients that are likely to develop complications during pregnancy.

## SUMMARY

[0011] The present disclosure relates to the above-described important issue of identifying pregnant women at increased risk of pre-term birth. This is crucial in order to either delay the birth or prevent the pre-term birth, or alternatively to provide the specialized care needed to limit the complications associated with pre-term birth.

[0012] Amniochorionic membranes play a major role during gestation and parturition. Microfractures in the fetal membranes are one of the processes that lead to human parturition. The inventors have surprisingly identified a correlation between specific amnion and/or chorion cells in the blood of pregnant women at increased risk of pre-term birth or that have a preterm or premature rupture of membranes. Microfractures in the fetal membranes may cause invasion into maternal blood with cells from these membranes. Hence, early detection of these cells in maternal blood can be used to identify pregnancies that are at risk of pre-term birth.

[0013] Based on this finding, the inventors have developed a method for determining pre-term birth or an increased risk of pre-term birth based on the presence and/or increased amount of said amnion and/or chorion cells in a blood sample of said pregnant woman.

[0013a] In one aspect, the present disclosure includes a method for identifying the presence of amnion and/or chorion cells in a blood sample obtained from a pregnant woman by determining the presence of one or more specific amnion and/or chorion cell biomarkers selected from IGF2, IGFBP3, SERPINB10, FBN1, CRYAB, KRT18, PRTG, COL1A2, GPX8, MUC16, KRT5, TSPAN1, UPK1B, CADPS2, LAMC2, AHNAK2, EMP1, FN1, MET, PVRL4, A2ML1, DSP, THSD4, KRT17, PDLIM4, COL17A1, DKK3, PLS3, DPYSL3, TPPP3, SHROOM3, THY1, DCN, DIO2, IGFBP2, SPARCL1, NNMT, LPHN3, PEG3, FLT1, NPR3, AOC1, ITGB8, RXFP1, SPOCK1, CYP11A1, COL4A2, CNR1, SEMA3A, SERPINE1, IL1R1, FBLN1, UCHL1, RAI2, TGM2, PRLR, FSTL3, BCAR1, THSD4, FERMT2, PKP2, P4HA2, TEAD1, or AQPEP, in amnion and/or chorion cells in the blood sample or fraction thereof obtained from the pregnant woman.

[0013b] In another aspect, the present disclosure includes a method of determining pre-term birth or an associated clinical condition or an increased risk of pre-term birth or an associated clinical

condition by determining the presence of amnion and/or chorion cells in a blood sample or fraction thereof obtained from a pregnant woman; wherein the presence of amnion and/or chorion cells is determined by detecting one or more specific amnion and/or chorion cell markers selected from the group consisting of IGF2, IGFBP3, SERPINB10, FBN1, CRYAB, KRT18, PRTG, COL1A2, GPX8, MUC16, KRT5, TSPAN1, UPK1B, CADPS2, LAMC2, AHNAK2, EMP1, FN1, MET, PVRL4, A2ML1, DSP, THSD4, KRT17, PDLIM4, COL17A1, DKK3, PLS3, DPYSL3, TPPP3, SHROOM3, THY1, DCN, DIO2, IGFBP2, SPARCL1, NNMT, LPHN3, PEG3, FLT1, NPR3, AOC1, ITGB8, RXFP1, SPOCK1, CYP11A1, COL4A2, CNR1, SEMA3A, SERPINE1, IL1R1, FBLN1, UCHL1, RAI2, TGM2, PRLR, FSTL3, BCAR1, THSD4, FERMT2, PKP2, P4HA2, TEAD1, and AQPEP, wherein the presence of amnion and/or chorion cells is indicative of pre-term birth or an associated clinical condition or an increased risk of pre-term birth or an associated clinical condition of the pregnant woman.

[0014] In one example, the present disclosure includes a method of determining pre-term birth or an associated clinical condition or an increased risk of pre-term birth or an associated clinical condition, the method comprising the steps of: (a) providing a blood sample or a fraction thereof isolated from a pregnant woman, and (b) determining the presence or characteristics of amnion and/or chorion cells in the blood sample or fraction thereof; wherein the presence or characteristics of amnion and/or chorion cells is indicative of pre-term birth or an associated clinical condition or an increased risk of pre-term birth or an associated clinical condition of the pregnant woman. In one example, the presence of amnion and/or chorion cells is determined by detecting one or more specific amnion and/or chorion cell markers. In another example, the specific amnion and/or chorion cell markers are differentially expressed in amnion and/or chorion cells compared to a blood sample or a fraction thereof isolated from a pregnant woman. In another example, the log2 fold difference between the expression of the amnion and/or chorion cell marker in amnion and/or chorion cells compared with the expression in a blood sample or fraction thereof is at least 5, at least 10, or at

least 15. In another example, the method comprises the steps of: (a) providing a blood sample or a fraction thereof isolated from a pregnant woman, (b) contacting the sample with (i) a ligand directed to an amnion and/or chorion cell marker or (ii) a hybridization probe comprising at least 10 contiguous nucleotides complementary to a gene encoding the amnion and/or chorion cell marker, and (c) detecting the amnion and/or chorion cell marker in the blood sample or fraction thereof of (a); wherein the presence of an amnion and/or chorion cell marker is indicative of pre-term birth or an associated clinical condition or an increased risk of pre-term birth or an associated clinical condition of the pregnant woman. In another example, the amnion and/or chorion cell marker is selected from the group consisting of IGF2, IGFBP3, SERPINB10, FBN1, CRYAB, KRT18, PRTG, COL1A2, GPX8, MUC16, KRT5, TSPAN1, UPK1B, CADPS2, LAMC2, AHNK2, EMP1, FN1, MET, PVRL4, A2ML1, DSP, THSD4, KRT17, PDLIM4, COL17A1, DKK3, PLS3, DPYSL3, TPPP3, SHROOM3, THY1, DCN, DIO2, IGFBP2, SPARCL1, NNMT, LPHN3, PEG3, FLT1, NPR3, AOC1, ITGB8, RXFP1, SPOCK1, CYP11A1, COL4A2, CNR1, SEMA3A, SERPINE1, IL1R1, FBLN1, UCHL1, RAI2, TGM2, PRLR, FSTL3, BCAR1, THSD4, FERMT2, PKP2, P4HA2, TEAD1, and AQPEP. In another example, the amnion and/or chorion cell marker is selected from the group consisting of MUC16, UPK1B, EMP1, PVRL4, THY1, DIO2, LPHN3, FLT1, NPR3, RXFP1, CNR1, PRLR, IGF2, IGFBP3, SERPINB10, FBN1, CRYAB, KRT18, PRTG, COL1A2 and GPX8. In another example, the amnion and/or chorion cell marker is selected from the group consisting of MUC16, UPK1B, EMP1, PVRL4, THY1, DIO2, LPHN3, FLT1, NPR3, RXFP1, CNR1 and PRLR. In another example, the amnion and/or chorion cell marker is selected from the group consisting of MUC16, UPK1B, EMP1 and PVRL4. In another example, the amnion and/or chorion cell marker is selected from the group consisting of THY1, DIO2, LPHN3, FLT1, NPR3, RXFP1, CNR1 and PRLR. In another example, the amnion and/or chorion cell marker is selected from the group consisting of IGF2, IGFBP3, SERPINB10, FBN1, CRYAB, KRT18, PRTG, COL1A2 and GPX8. In another example, the amnion and/or chorion cell marker is selected from the group consisting of MUC16, UPK1B, EMP1, GPX8, FLT1/VEGFR1, RXFP1, CNR1 and PRLR. In another example, the method further comprises detecting an epithelial marker, such as a marker selected from the group consisting of CK1, CK2, CK3, CK4, CK5, CK6, CK7, CK8, CK9, CK10, CK12, CK13, CK14, CK15, CK16, CK17, CK18, and CK19. In another example, the method further comprises detecting a maternal marker such as a marker selected from the group consisting of CD14 and CD45. In another example, the method further comprises detecting a mesenchymal

marker, such as a marker selected from at least one of Vimentin. In another example, the clinical condition associated with pre-term birth is preeclampsia. In another example, the method further comprises: determining the amount of amnion and/or chorion cells in the blood sample or fraction thereof, and comparing the amount of amnion and/or chorion cells to a control; wherein an amount of amnion and/or chorion cells in the blood sample or fraction thereof higher than the amount in a control is indicative of pre-term birth or an associated clinical condition or an increased risk of pre-term birth or an associated clinical condition of the pregnant woman. In another example, the amount of amnion and/or chorion cells in a control is a pre-determined value. In another example, the method further comprises: determining the amount of amnion and/or chorion cell markers in the blood sample or fraction thereof, and comparing the amount of amnion and/or chorion cell marker to a control; wherein an amount of amnion and/or chorion cell markers in the blood sample or fraction thereof higher than the amount in a control is indicative of pre-term birth or an associated clinical condition or an increased risk of pre-term birth or an associated clinical condition of the pregnant woman. In another example, the amount of amnion and/or chorion cell marker in a control is a pre-determined value. In another example, the amnion and/or chorion cell marker is detected at the protein level and/or the RNA level. In another example, the blood sample is isolated from the pregnant woman after 20 weeks of gestation. In another example, the amnion and/or chorion cell marker is present in the cell membrane and/or are intracellular. In another example, the blood sample from a pregnant woman is whole blood. In another example, wherein a treatment is provided to the pregnant woman for minimizing the risk of preterm birth or ameliorating the consequences of pre-term birth, such as hospitalizing the individual.

[0015] In another example, the present disclosure includes an assay for identifying fetal markers in a blood sample obtained from a pregnant woman comprising: obtaining the blood sample from the pregnant woman; and determining the presence of one or more biomarkers selected from IGF2, IGFBP3, SERPINB10, FBN1, CRYAB, KRT18, PRTG, COL1A2, GPX8, MUC16, KRT5, TSPAN1, UPK1B, CADPS2, LAMC2, AHNAK2, EMP1, FN1, MET, PVRL4, A2ML1, DSP, THSD4, KRT17, PDLIM4, COL17A1, DKK3, PLS3, DPYSL3, TPPP3, SHROOM3, THY1, DCN, DIO2, IGFBP2, SPARCL1, NNMT, LPHN3, PEG3, FLT1, NPR3, AOC1, ITGB8, RXFP1, SPOCK1, CYP11A1, COL4A2, CNR1, SEMA3A, SERPINE1, IL1R1, FBLN1, UCHL1, RAI2, TGM2, PRLR, FSTL3, BCAR1, THSD4, FERMT2, PKP2, P4HA2, TEAD1, or AQPEP, in amnion and/or chorion cells in the blood sample or fraction thereof. In another example, the present

disclosure includes an assay for identifying fetal markers in a blood sample obtained from a pregnant woman comprising: determining the presence of one or more biomarkers in a blood sample obtained from the pregnant woman, wherein the one or more biomarkers are selected from IGF2, IGFBP3, SERPINB10, FBN1, CRYAB, KRT18, PRTG, COL1A2, GPX8, MUC16, KRT5, TSPAN1, UPK1B, CADPS2, LAMC2, AHNAK2, EMP1, FN1, MET, PVRL4, A2ML1, DSP, THSD4, KRT17, PDLIM4, COL17A1, DKK3, PLS3, DPYSL3, TPPP3, SHROOM3, THY1, DCN, DIO2, IGFBP2, SPARCL1, NNMT, LPHN3, PEG3, FLT1, NPR3, AOC1, ITGB8, RXFP1, SPOCK1, CYP11A1, COL4A2, CNR1, SEMA3A, SERPINE1, IL1R1, FBLN1, UCHL1, RAI2, TGM2, PRLR, FSTL3, BCAR1, THSD4, FERMT2, PKP2, P4HA2, TEAD1, or AQPEP, in amnion and/or chorion cells in the blood sample or fraction thereof. In one example, the presence of amnion and/or chorion cells is indicative of pre-term birth or an associated clinical condition or an increased risk of pre-term birth or an associated clinical condition of the pregnant woman.

[0016] In another example, the present disclosure includes a method of identifying a patient in need of decreased physical activity and/or bed rest during pregnancy comprising: performing an assay for identifying fetal markers in a blood sample obtained from the patient comprising: obtaining the blood sample from the patient; and determining the presence of one or more biomarkers selected from IGF2, IGFBP3, SERPINB10, FBN1, CRYAB, KRT18, PRTG, COL1A2, GPX8, MUC16, KRT5, TSPAN1, UPK1B, CADPS2, LAMC2, AHNAK2, EMP1, FN1, MET, PVRL4, A2ML1, DSP, THSD4, KRT17, PDLIM4, COL17A1, DKK3, PLS3, DPYSL3, TPPP3, SHROOM3, THY1, DCN, DIO2, IGFBP2, SPARCL1, NNMT, LPHN3, PEG3, FLT1, NPR3, AOC1, ITGB8, RXFP1, SPOCK1, CYP11A1, COL4A2, CNR1, SEMA3A, SERPINE1, IL1R1, FBLN1, UCHL1, RAI2, TGM2, PRLR, FSTL3, BCAR1, THSD4, FERMT2, PKP2, P4HA2, TEAD1, or AQPEP, in amnion and/or chorion cells in the blood sample or fraction thereof, wherein the presence of amnion and/or chorion cells with one or more of the biomarkers is indicative of pre-term birth or an associated clinical condition or an increased risk of pre-term birth or an associated clinical condition of the pregnant woman; and directing the patient to reduce physical activities and/or bed rest. In another example, the present disclosure includes a method of identifying a patient in need of decreased physical activity and/or bed rest during pregnancy comprising: performing an assay for identifying fetal markers in a blood sample obtained from the patient comprising: determining the presence of one or more biomarkers in a blood sample obtained sample from the patient, wherein the one or more biomarkers are selected from IGF2, IGFBP3,

SERPINB10, FBN1, CRYAB, KRT18, PRTG, COL1A2, GPX8, MUC16, KRT5, TSPAN1, UPK1B, CADPS2, LAMC2, AHNAK2, EMP1, FN1, MET, PVRL4, A2ML1, DSP, THSD4, KRT17, PDLIM4, COL17A1, DKK3, PLS3, DPYSL3, TPPP3, SHROOM3, THY1, DCN, DIO2, IGFBP2, SPARCL1, NNMT, LPHN3, PEG3, FLT1, NPR3, AOC1, ITGB8, RXFP1, SPOCK1, CYP11A1, COL4A2, CNR1, SEMA3A, SERPINE1, IL1R1, FBLN1, UCHL1, RAI2, TGM2, PRLR, FSTL3, BCAR1, THSD4, FERMT2, PKP2, P4HA2, TEAD1, or AQPEP, in amnion and/or chorion cells in the blood sample or fraction thereof, wherein the presence of amnion and/or chorion cells with one or more of the biomarkers is indicative of pre-term birth or an associated clinical condition or an increased risk of pre-term birth or an associated clinical condition of the pregnant woman; and directing the patient to reduce physical activities and/or bed rest. In one example, the amnion and/or chorion cell marker is detected at the protein level and/or the RNA level. In another example, the blood sample is isolated from the pregnant woman after 20 weeks of gestation. In another example, the amnion and/or chorion cell marker is present in the cell membrane. In another example, the blood sample from a pregnant woman is whole blood.

[0017] A kit comprising one or more agents for the detection of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63 or all the markers in Table 1, on amnion and/or chorion cells in a blood sample or fraction thereof.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0018] For a more complete understanding of the features and advantages of the present invention, reference is now made to the detailed description of the invention along with the accompanying figures and in which:

[0019] FIG. 1 summarizes the steps for isolating fetal membrane cells from maternal blood samples of the present invention.

[0020] FIG. 2A shows fetal membrane candidate cells identified in maternal blood using antibodies specific for fetal membrane cells. FIG. 2B shows the same cells confirmed by X-Y FISH.

[0021] FIGS. 3A and 3B shows the expression of membrane specific markers in human primary amnion epithelia cells (AEC). Immunostaining shows AEC co-express epithelial marker cytokeratin-18 and mesenchymal marker vimentin, while “membrane” markers are seen on the cell

What is claimed is:

1. A method for identifying the presence of amnion and/or chorion cells in a blood sample obtained from a pregnant woman by

determining the presence of one or more specific amnion and/or chorion cell

5 biomarkers selected from IGF2, IGFBP3, SERPINB10, FBN1, CRYAB, KRT18, PRTG, COL1A2, GPX8, MUC16, KRT5, TSPAN1, UPK1B, CADPS2, LAMC2, AHNAK2, EMP1, FN1, MET, PVRL4, A2ML1, DSP, THSD4, KRT17, PDLIM4, COL17A1, DKK3, PLS3, DPYSL3, TPPP3, SHROOM3, THY1, DCN, DIO2, IGFBP2, SPARCL1, NNMT, LPHN3, PEG3, FLT1, NPR3, AOC1, ITGB8, RXFP1, SPOCK1, CYP11A1, COL4A2, CNR1, SEMA3A, SERPINE1, IL1R1, 10 FBLN1, UCHL1, RAI2, TGM2, PRLR, FSTL3, BCAR1, THSD4, FERMT2, PKP2, P4HA2, TEAD1, or AQPEP, in amnion and/or chorion cells in the blood sample or fraction thereof obtained from the pregnant woman.

2. A method of determining pre-term birth or an associated clinical condition or an increased risk of pre-term birth or an associated clinical condition

15 by determining the presence of amnion and/or chorion cells in a blood sample or fraction thereof obtained from a pregnant woman;

wherein the presence of amnion and/or chorion cells is determined by detecting one or more specific amnion and/or chorion cell markers selected from the group consisting of IGF2, IGFBP3,

20 SERPINB10, FBN1, CRYAB, KRT18, PRTG, COL1A2, GPX8, MUC16, KRT5, TSPAN1, UPK1B, CADPS2, LAMC2, AHNAK2, EMP1, FN1, MET, PVRL4, A2ML1, DSP, THSD4, KRT17, PDLIM4, COL17A1, DKK3, PLS3, DPYSL3, TPPP3, SHROOM3, THY1, DCN, DIO2, IGFBP2, SPARCL1, NNMT, LPHN3, PEG3, FLT1, NPR3, AOC1, ITGB8, RXFP1, SPOCK1, CYP11A1, COL4A2, CNR1, SEMA3A, SERPINE1, IL1R1, FBLN1, UCHL1, RAI2, TGM2, PRLR, FSTL3, BCAR1, THSD4, FERMT2, PKP2, P4HA2, TEAD1, and AQPEP,

25 wherein the presence of amnion and/or chorion cells is indicative of pre-term birth or an associated clinical condition or an increased risk of pre-term birth or an associated clinical condition of the pregnant woman.

3. The method according to claim 2, wherein the method comprises the steps of:

a) contacting the sample with (i) a ligand directed to an amnion and/or chorion cell marker or (ii) a hybridization probe comprising at least 10 contiguous nucleotides complementary to a gene encoding the amnion and/or chorion cell marker, and

b) detecting the amnion and/or chorion cell marker in the blood sample or fraction thereof of a);

wherein the presence of an amnion and/or chorion cell marker is indicative of pre-term birth or an associated clinical condition or an increased risk of pre-term birth or an associated clinical condition of the pregnant woman.

4. The method according to any of the preceding claims, wherein the amnion and/or chorion cell marker is selected from the group consisting of MUC16, UPK1B, EMP1, PVRL4, THY1, DIO2, LPHN3, FLT1, NPR3, RXFP1, CNR1, PRLR, IGF2, IGFBP3, SERPINB10, FBN1, CRYAB, KRT18, PRTG, COL1A2 and GPX8.

5. The method according to any of the preceding claims, wherein the amnion and/or chorion cell markers are membrane cell markers selected from the group consisting of MUC16, UPK1B, EMP1, PVRL4, THY1, DIO2, LPHN3, FLT1, NPR3, RXFP1, CNR, PRLR, and GPX8.

6. The method according to any of the preceding claims, wherein the amnion cell markers are amnion cell membrane markers selected from the group consisting of MUC16, UPK1B, EMP1 and PVRL4.

7. The method according to any of the preceding claims, wherein the chorion cell markers are chorion cell membrane markers selected from the group consisting of THY1, DIO2, LPHN3, FLT1, NPR3, RXFP1, CNR1 and PRLR.

8. The method according to any of the preceding claims, wherein the amnion and/or chorion cell marker are cell markers common between amnion and chorion is selected from the group consisting of IGF2, IGFBP3, SERPINB10, FBN1, CRYAB, KRT18, PRTG, COL1A2 and GPX8.

9. The method according to any of the preceding claims, wherein the method further comprises detecting an epithelial marker, such as a marker selected from the group consisting of CK1, CK2, CK3, CK4, CK5, CK6, CK7, CK8, CK9, CK10, , CK12, CK13, CK14, CK15, CK16, CK17, CK18 and CK19 and/or a mesenchymal marker selected from at least one of: Vimentin.

10. The method according to any of the preceding claims, wherein the method further comprises:

- a) determining the amount of amnion and/or chorion cells in the blood sample or fraction thereof, and
- b) comparing the amount of amnion and/or chorion cells to a pregnant women who is at a same gestational age, but not at a high risk of pre-term birth;

5 wherein an amount of amnion and/or chorion cells in the blood sample or fraction thereof higher than the amount in a control is indicative of pre-term birth or an associated clinical condition or an increased risk of pre-term birth or an associated clinical condition of the pregnant woman.

11. The method according to claim 9, wherein the amount of amnion and/or chorion cells in a control is a pre-determined value.

10 12. The method according to any of the preceding claims, wherein the amnion and/or chorion cell marker is detected at the protein level and/or the RNA level.

13. The method according to any of the preceding claims, wherein the blood sample is isolated from the pregnant woman after 20 weeks of gestation.

14. The method according to any of the preceding claims, wherein the amnion and/or chorion  
15 cell marker is present on the cell membrane or intracellularly.

15. The method according to any of the preceding claims, wherein the blood sample from a pregnant woman is whole blood.

16. The method according to any of the preceding claims, wherein the clinical condition associated with pre-term birth is preeclampsia, pre-term birth, or a tear or rupture of the amniotic  
20 membrane.

17. The method according to any of the preceding claims, wherein the method further comprises detecting a maternal marker, such as a marker selected from the group consisting of CD14 and CD45.