CHARACTERISTIC METABOLITES IN MISCARRIAGES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of SG provisional application No. 10201703502T, filed 28 April 2017, the contents of it being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates generally to the field of molecular biology. In particular, the present invention relates to the use of biomarkers for the detection of miscarriages and identification of women at risk of miscarriages.

BACKGROUND OF THE INVENTION

[0003] Threatened miscarriage is the most common gynaecological emergency, occurring in about 20% of pregnant women. Approximately one in four of these women go on to have spontaneous miscarriage, whereby the aetiology of miscarriage remains elusive. In a bid to identify possible biomarkers and novel treatment targets, many studies have been undertaken to elucidate the pathways that lead to a miscarriage.

[0004] No study on urine metabolites associated with spontaneous miscarriage has been performed to date. With existing pre-natal care unable to accurately identify women at high risk of spontaneous miscarriage with sufficient accuracy, there is a need to further understand the underlying causes so as to better predict and eventually prevent spontaneous miscarriage. Thus, there is an unmet need for a method of detecting and determining the risk of miscarriage in pregnant women.

SUMMARY

[0005] In one aspect, the present disclosure refers to a method of identifying the risk of a miscarriage comprising: measuring the concentration of at least one metabolite in a serum or a urine sample obtained from a subject, and comparing the concentration of the at least one metabolite with the concentration of the respective metabolite in a control group; wherein the metabolite is selected from the group consisting of tetrahydrocortisone, propionylcarnitine, isovalerylcarnitine, 3-methylglutarylcarnitine, and hexanoylcarnitine, and wherein the control group consists of subjects having undergone a full-term birth later on. In one example, the present disclosure refers to a method of identifying the risk of a miscarriage comprising detecting and measuring the concentration of at least one metabolite in a sample obtained from a subject, wherein absence or presence of the metabolite, compared to the control group identifies an increased risk of miscarriage, wherein the metabolite is selected from the group consisting of tetrahydrocortisone, propionylcarnitine, isovalerylcarnitine, 3-methylglutarylcarnitine, hexanoylcarnitine and 3α,20α -dihydroxy-5β-pregnane-3-glucuronide.

[0006] In another aspect, the present disclosure refers to a method of identifying the risk of a miscarriage comprising: measuring the concentration of at least one metabolite in a serum or a urine sample obtained from a subject; and comparing the concentration of the at least one metabolite with the concentration of the respective metabolite in a control group; wherein the metabolite is selected from the

group consisting of tetrahydrocortisone, propionylcarnitine, isovalerylcarnitine, 3 methylglutarylcarnitine, hexanoylcarnitine, 3α,20α -dihydroxy-5β-pregnane-3-glucuronide and combinations thereof, wherein at least one of the metabolite selected is tetrahydrocortisone, propionylcarnitine, isovalerylcarnitine, 3-methylglutarylcarnitine or hexanoylcarnitine, and wherein the control group consists of subjects having undergone a full-term birth later on. In another example, the present disclosure refers to a method of identifying the risk of a miscarriage comprising measuring the concentration of at least one metabolite in a sample obtained from a subject; and comparing the concentration of the at least one metabolite with the concentration of the respective metabolite in a control group; wherein the metabolite is selected from the group consisting of tetrahydrocortisone, propionylcarnitine, isovalerylcarnitine, 3-methylglutarylcarnitine, hexanoylcarnitine and 3α,20α -dihydroxy-5β-pregnane-3-glucuronide and combinations thereof.

[0007] In a further example, the present disclosure refers to a kit for identifying the risk of a spontaneous miscarriage according to the method as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0009] **FIG. 1** shows a diagram of an exemplary workflow for, in this example, urine metabolite analysis. Samples from both cohorts were randomly injected into the system for separation by ultra-performance liquid chromatography (UPLC) before analysis by mass spectrometry (MS).

[0010] **FIG. 2** shows a Schematic of set-up for the identification of metabolites between case and control groups. Quality Control (QC) samples were interspersed between the 80 samples to ensure that the resolution of the column is not compromised.

[0011] **FIG. 3** shows scatter plots depicting the results of principle component analysis (PCA) when applied to the metabolomics data after pre-processing. Visual inspection of the clustering of the quality control (QC) samples and drift of the run order QCs in the PCA scored plots were performed to assess the data integrity by tight clustering of the QC samples on the PCA score plots. The QC samples were found to be well clustered near the center of the scores plot for both (A) positive and (B) negative ionization mode. Case and control samples, however, do not exhibit any significant separations. Quality of the dataset is first assessed using principal component analysis (PCA).

[0012] **FIG. 4** shows scatter plots depicting the results of a multivariate analysis performed on urine metabolomics data. (A) Scores plot of ESI+ measurements. The urine samples from case and control subjects were found to be well separated along the predictive component axis with an explained variance R2Y = 0.67 and predictability Q2Y = 0.22. (B) Scores plot of the ESI- experiment, with a R2Y = 0.70 and Q2Y = 0.19.

[0013] **FIG. 5** shows a schematic pathway showing the interconversion of carnitine and acylcarnitine and their transport from the cytosol into the mitochondria. CPTI: carnitine palmitoyltransferase-1, CPTII: carnitine palmitoyltransferase-2, CACT: carnitine-acylcarnitine translocase.

[0014] **FIG. 6** shows the MS/MS spectra of each of the metabolites identified. MS/MS spectra from samples were compared with that from commercially purchased standards to confirm the identities of the features. 3α, 20α-Dihydroxy-5β-pregnane-3-glucuronide, tetrahyrdocortisone, propionylcarnitine and isovalerycarnitine matched the standards, thus confirming their identities. On the other hand, 3-methylglutarylcarntine and hexanoylcarnitine have additional features and may be structural isomers of the proposed metabolites.

[0015] **FIG. 7** shows a schematic of the superhydrophobic SERS chip (a; top) used in the present disclosure, and its analyte concentrating effect (a; bottom). (b) shows SERS spectra of 3α,20α-dihydroxy-5β-pregnane-3-glucuronide (pregnane) sample measured on the described chip. (c) Standard calibration

CLAIMS

1. A method of identifying the risk of a miscarriage comprising:
measuring the concentration of at least one metabolite in a serum or a urine sample obtained from a subject, and
comparing the concentration of the at least one metabolite with the concentration of the respective metabolite in a control group;
wherein the metabolite is selected from the group consisting of tetrahydrocortisone, propionylcarnitine, isovalerylcarnitine, 3-methylglutarylcarnitine and hexanoylcarnitine, and wherein the control group consists of subjects having undergone a full-term birth later on.

2. A method of identifying the risk of a miscarriage comprising:
measuring the concentration of at least one metabolite in a serum or a urine sample obtained from a subject; and
comparing the concentration of the at least one metabolite with the concentration of the respective metabolite in a control group;
wherein the metabolite is selected from the group consisting of tetrahydrocortisone, propionylcarnitine, isovalerylcarnitine, 3-methylglutarylcarnitine, hexanoylcarnitine, 3α,20α -dihydroxy-5β-pregnane-3-glucuronide and combinations thereof, wherein at least one of the metabolite selected is tetrahydrocortisone, propionylcarnitine, isovalerylcarnitine, 3-methylglutarylcarnitine or hexanoylcarnitine, and
wherein the control group consists of subjects having undergone a full-term birth later on.

3. The method of any of the preceding claims, wherein an increase or decrease in concentration of the measured values, compared to the control group identifies an increased risk of miscarriage.

4. The method of claims 2 or 3, wherein a decrease of 3α,20α -dihydroxy-5β-pregnane-3-glucuronide is indicative of an increased risk of miscarriage.

5. The method of claim 4, wherein the decrease of 3α,20α-dihydroxy-5β-pregnane-3-glucuronide compared to the control is a decrease of at least 0.30 fold change (-0.30) in concentration compared to the control.

6. The method of any of the preceding claims, wherein an increase of tetrahydrocortisone is indicative of an increased risk of miscarriage.

7. The method of claim 6, wherein the increase of tetrahydrocortisone compared to the control is an increase of at least 0.40 fold change in concentration compared to the control.

8. The method of any of the preceding claims, wherein a decrease of propionylcarnitine is indicative of an increased risk of miscarriage.

9. The method of claim 8, wherein the decrease of propionylcarnitine compared to the control is a decrease of at least 0.41 fold change (-0.41) in concentration compared to the control

10. The method of any of the preceding claims, wherein a decrease of isovalerylcarnitine is indicative of an increased risk of miscarriage.

11. The method of claim 10, wherein the decrease of isovalerylcarnitine compared to the control is a decrease of at least 0.32 fold change (-0.32) in concentration compared to the control

12. The method of any of the preceding claims, wherein a decrease of 3-methylglutarylcarnitine is indicative of an increased risk of miscarriage.

13. The method of claim 12, wherein the decrease of 3-methylglutarylcarnitine compared to the control is a decrease of at least 0.59 fold change (-0.59) in concentration compared to the control

14. The method of any of the preceding claims, wherein an increase of hexanoylcarnitine is indicative of an increased risk of miscarriage.

15. The method of claim 14, wherein the increase of hexanoylcarnitine compared to the control is an increase of at least 0.38 fold change in concentration compared to the control

16. The method of any of the preceding claims, wherein the miscarriage is selected from the group consisting of spontaneous miscarriage, first-trimester miscarriage, second-trimester miscarriage, blighted ovum, and missed miscarriage.

17. The method of any of the preceding claims, wherein the subject is to be administered dydrogesterone if identified to be at risk of miscarriage.