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(54) **METHODS FOR IDENTIFICATION OF PREGNANCY FAILURE**

(71) Applicant: **Amir Mor**, New Haven, CT (US)

(72) Inventor: **Amir Mor**, New Haven, CT (US)

(73) Assignee: **Amir Mor**, New haven, CT (US)

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G01N 33/53 (2006.01)
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CPC **G01N 33/689** (2013.01); **A61B 5/4343** (2013.01); **C12Q 2600/158** (2013.01); **G01N 2800/368** (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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(57) **ABSTRACT**

Provided is a method for identifying pregnancy failure in a subject, the method comprising determining a concentration of at least one element of fetal or embryonic origin in the vaginal fluid of the subject; and comparing the concentration of the at least one element of fetal or embryonic origin to a reference value, wherein when the concentration of the at least one element of fetal or embryonic origin in the vaginal fluid is higher than the reference value, pregnancy failure is indicated.

13 Claims, 4 Drawing Sheets

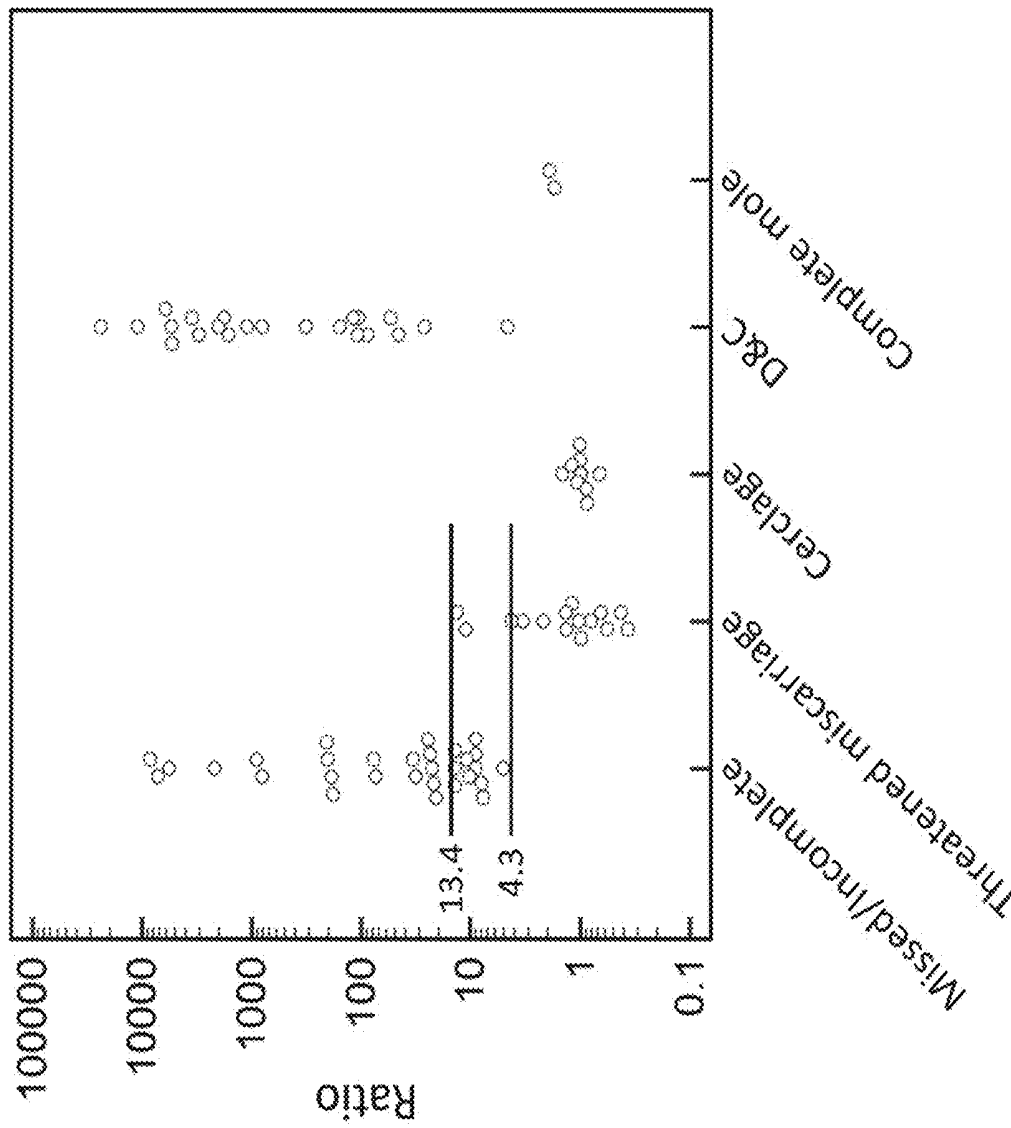


FIG. 1

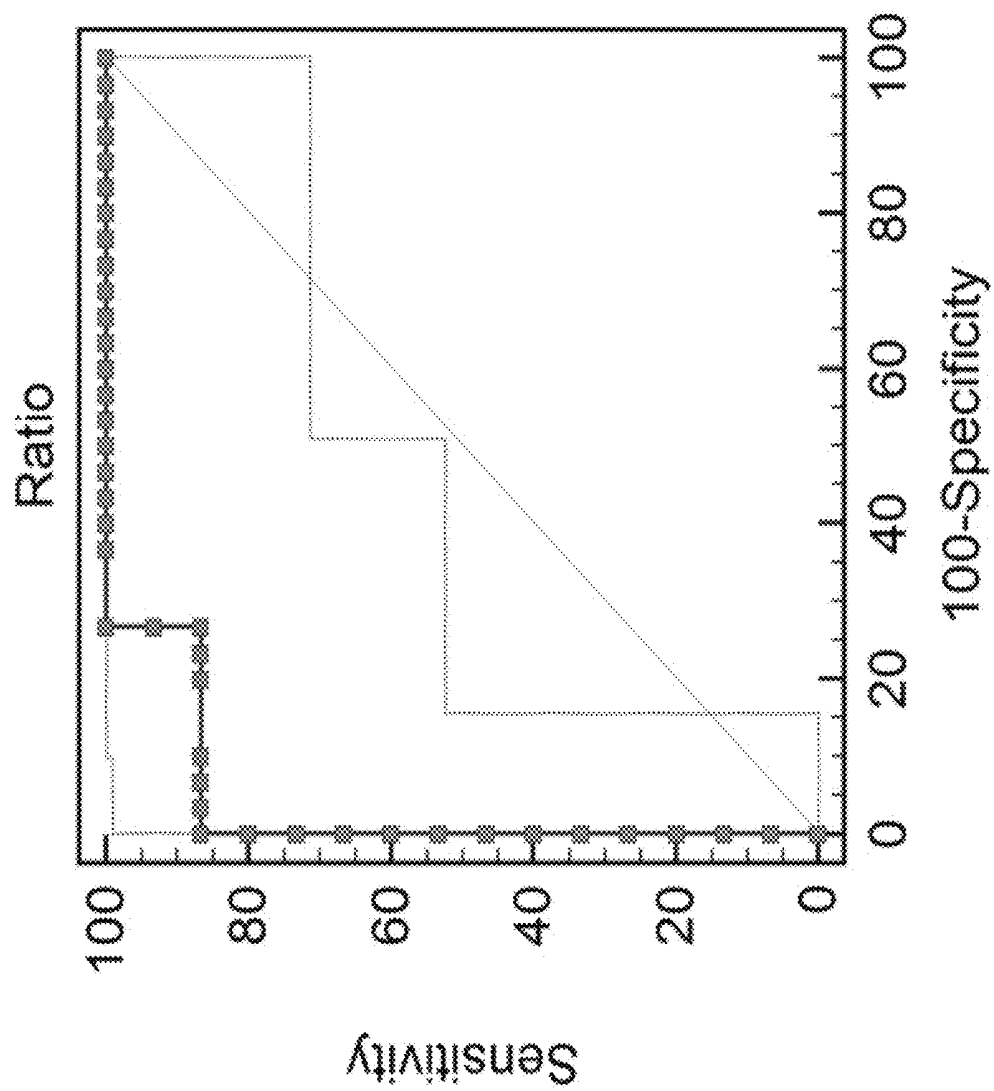


FIG. 2

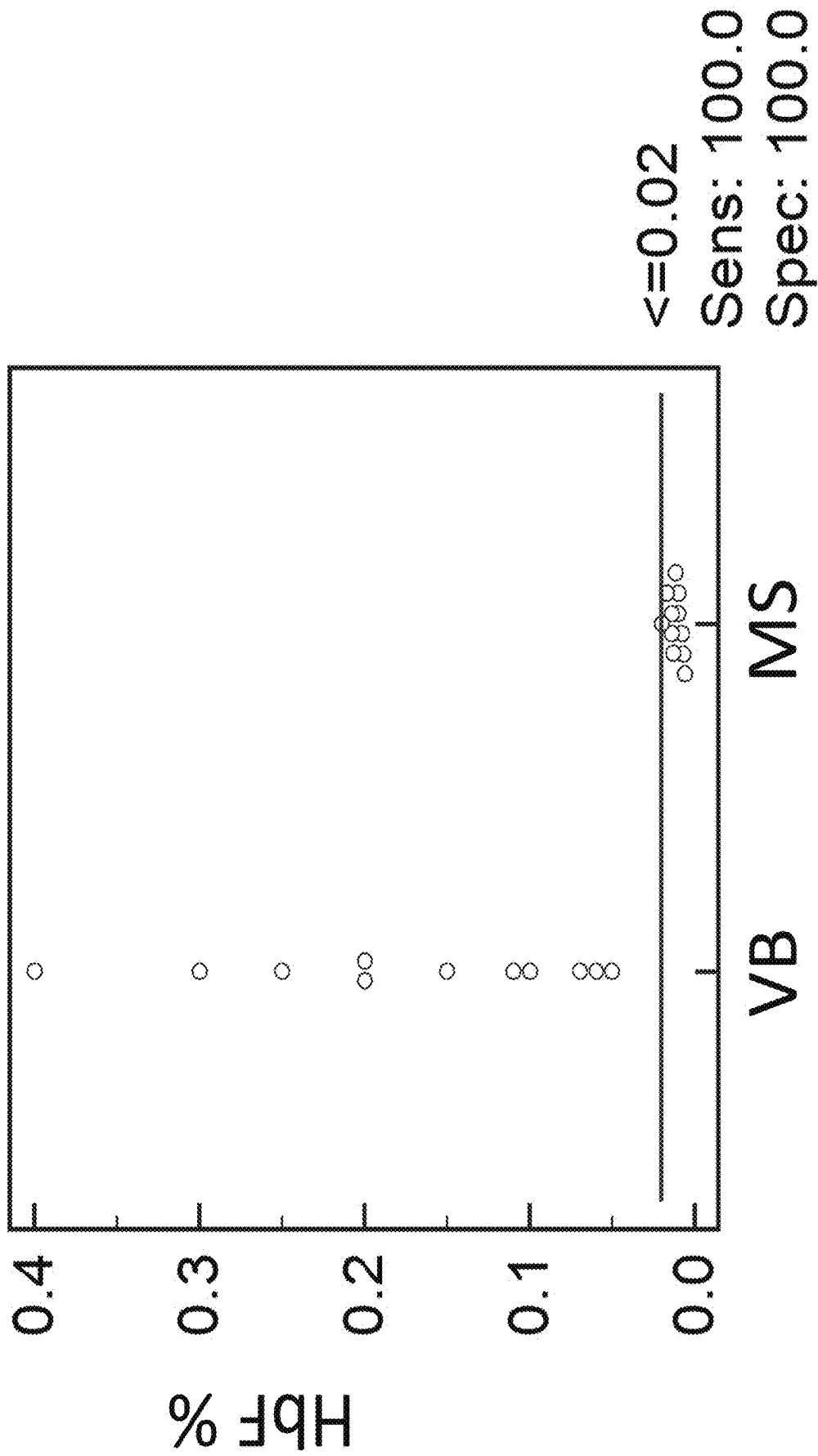


FIG. 3

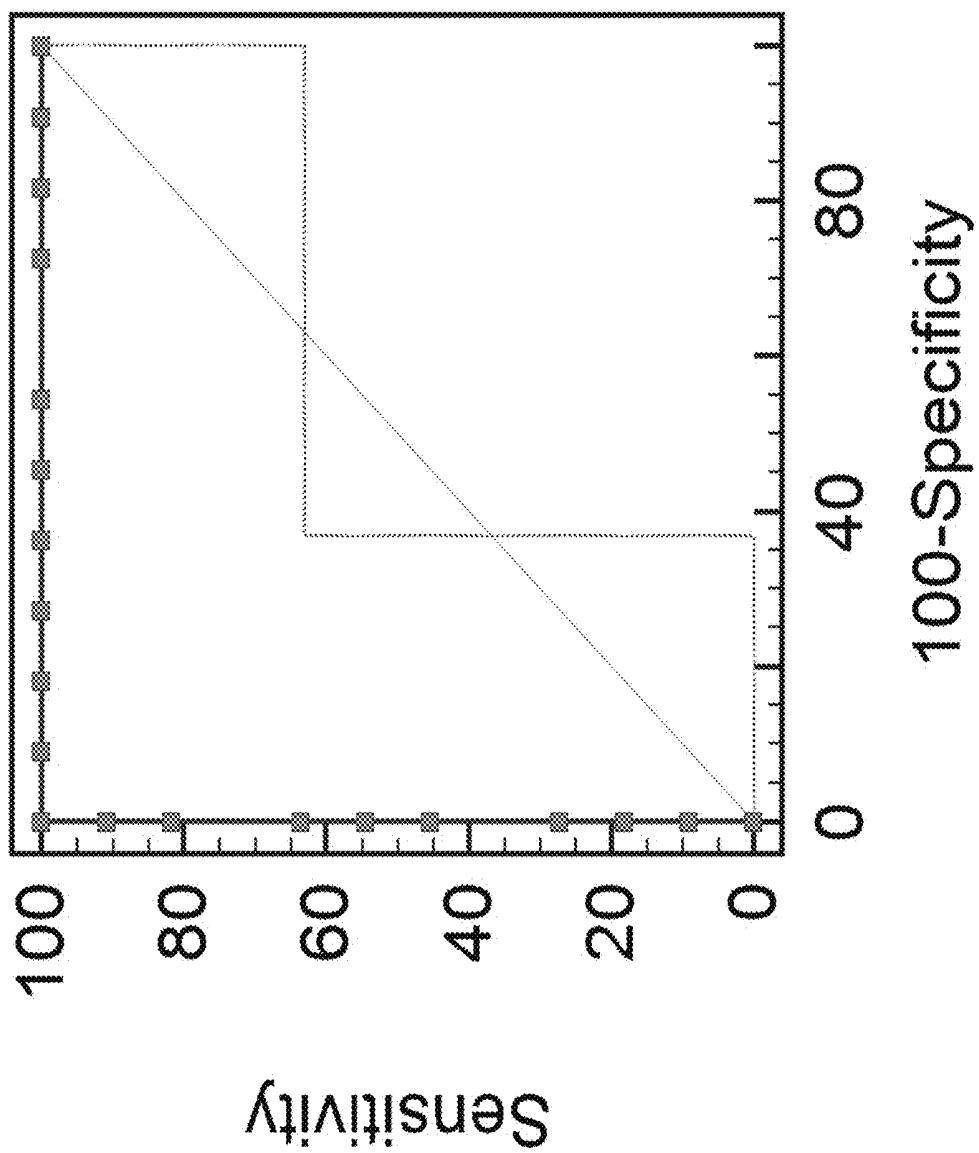


FIG. 4

METHODS FOR IDENTIFICATION OF PREGNANCY FAILURE

FIELD OF THE INVENTION

The invention relates to the field of pregnancy failure, and more particularly, to a method for identification of pregnancy failure comprising comparing a concentration of at least one element of fetal or embryonic origin in the vaginal fluid of a subject to a reference concentration of the same element of fetal or embryonic origin.

BACKGROUND OF THE INVENTION

Pregnancy failure is common, occurring in about 12 to 15% of all clinically recognized pregnancies, and up to 22% of all pregnancies in the United States. Common risk factors include chromosomal abnormalities.

Common symptoms of pregnancy failure include vaginal bleeding and intrauterine cramping. However, such symptoms may also occur during normal gestation, especially during the first trimester.

Sonographic findings in early pregnancy may be inconclusive, and differentiating between intrauterine and extrauterine pregnancies (IUP and EUP, respectively) is challenging.

Moreover, serial beta Human Chorionic Gonadotropin (β -HCG) measurements over time are oftentimes needed in order to differentiate between a normal and a failing pregnancy and therefore, may not be useful for same-day diagnosis.

It would therefore be desirable to have a simple, rapid and accurate method for identification of a pregnancy failure, that is devoid of at least some of the limitations of the prior art.

SUMMARY OF THE INVENTION

The invention, in some embodiments thereof, relates to a method for identification of pregnancy failure comprising comparing a concentration of at least one element of fetal or embryonic origin in the vaginal fluid of a subject to a reference concentration of the same element of fetal or embryonic origin.

The invention provides an effective, safe and non-invasive method for same-day detection of pregnancy failure, which may be used in early-stage pregnancy.

Aspects and embodiments of the invention are described in the specification hereinbelow and in the appended claims.

Alpha-fetoprotein (AFP) is a glycoprotein produced by the embryonic yolk sac in early-stage pregnancy, and later on by the fetal liver.¹ In the first trimester, as early as the 5th week of gestation, AFP concentration can be measured from the embryonic tissue. It has been shown that AFP in the fetal serum is at least 1000 times higher than in the maternal serum at any gestational age.²

The present Inventor have surprisingly found that an AFP concentration in the vaginal blood of a subject, which is relatively high when compared to the AFP concentration in the serum of the same subject indicates the presence of a pregnancy failure, particularly a failure of an intrauterine pregnancy.

According to an aspect of some embodiments of the invention, there is provided a method for identifying pregnancy failure in a subject, the method comprising determining a concentration of at least one element of fetal or embryonic origin in the vaginal fluid of the subject; and

comparing the concentration of the at least one element of fetal or embryonic origin to a reference value, wherein when the concentration of the at least one element of fetal or embryonic origin in the vaginal fluid of the subject is higher than the reference value, pregnancy failure is indicated.

In some embodiments, the reference value is a concentration of said at least one element of fetal or embryonic origin in maternal serum of the same subject i.e. in the serum of the subject in whom pregnancy failure is to be identified.

In some embodiments, the reference value is a predetermined concentration value of said at least one element of fetal or embryonic origin and wherein pregnancy failure is indicated when a concentration of the element of fetal or embryonic origin in vaginal fluid of the subject is greater than the predetermined concentration value. Such a reference concentration is preferably determined as an average of maternal serum concentrations obtained from at least 30 reference subjects in the same trimester of pregnancy as the subject in whom pregnancy failure is to be identified.

In some embodiments, the vaginal fluid is vaginal blood.

In some embodiments, the vaginal fluid is physiological vaginal discharge

As used herein, the term "element of fetal or embryonic origin" refers to a biochemical element originating from a fetus or embryo, such as a component of a tissue, a fluid, a protein, a carbohydrate, electrolytes, fatty acids, or nucleic acids.

In some embodiments, the element of fetal or embryonic origin is selected from the group consisting of a protein (such as a glycoprotein, an antibody, an enzyme, a hemoglobin component, an amyloid, cytoskeleton component, muscle component); a nucleic acid (such as deoxyribonucleic acid or ribonucleic acid), a vitamin, an electrolyte, phospholipids, fatty acids, hormones, an antigen and a carbohydrate (such as a sugar molecule, an antigen, such as a cell surface antigen). In some embodiments, the element of fetal or embryonic origin is a glycoprotein. In some such embodiments, the glycoprotein comprises alpha-fetoprotein (AFP). In some embodiments, the element of fetal or embryonic origin is selected from the group consisting of carcinoembryonic antigen, squamous cell carcinoma antigen; prostate serum antigen; and hemoglobinA1C.

In some embodiments, the pregnancy failure is a failure of an intrauterine pregnancy.

In some embodiments, the reference value is a concentration of the element of fetal or embryonic origin in maternal serum of the same subject, and pregnancy failure is indicated when the ratio of the concentration of the element of fetal or embryonic origin in the vaginal fluid to the concentration of the element of fetal or embryonic origin in the maternal serum is greater than a predetermined threshold level.

In some embodiments, the predetermined threshold ratio level may be about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15 about 16, about 17, about 18, about 19, about 20, about 25, about 30.

In some such embodiments, wherein the element of fetal or embryonic origin is AFP, the predetermined threshold ratio level is about 13.4, such that pregnancy failure is indicated when the ratio of the concentration of AFP in vaginal fluid (such as vaginal blood) to the concentration of AFP in maternal serum of the same subject is greater than about 13.4.

In some embodiments, the reference value is a predetermined concentration value of the element of fetal or embryonic origin, such as a concentration value of from about 5 to about 20 ng/mL, such as, for example, about 5, about 6,

about 7, about 8, about 9, about 10, about 11, about 12, about 12, about 14, about 15, about 16, about 17, about 18, about 19 or about 20 ng/ml, such that pregnancy failure is indicated when the concentration of the element of fetal or embryonic origin in vaginal fluid of the subject is greater than the predetermined concentration value. In some such embodiments, the element of fetal or embryonic origin is AFP. In some such embodiments, when the element of fetal or embryonic origin is AFP, the predetermined concentration value is from about 9 to about 10 ng/ml, preferably about 9.2 ng/ml. In other such embodiments, the element of fetal or embryonic origin is an element other than AFP.

As used herein, the term “pregnancy failure” is intended to mean a non-viable pregnancy, including a failed intrauterine pregnancy; The terms ‘miscarriage’ and ‘abortion’ may also be used interchangeably to refer to a non-viable intrauterine pregnancy (IUP as above).

As used herein, the term “embryo” is intended to refer to a product of conception during the embryonic period. The embryonic period is defined as from conception until the end of 8 weeks gestation. As used herein, the term “fetus” is intended to refer to a product of conception during the fetal period. The fetal period is defined as beyond 8 weeks gestation. Abortion can occur during the embryonic or during the fetal periods, therefore, for simplification purposes, the terms “fetus” and “embryo” may also be used interchangeably.

In some embodiments, the pregnancy failure is a spontaneous abortion, which may be a complete abortion, an incomplete (partial) abortion or a missed abortion. In alternative embodiments, the pregnancy failure is an induced abortion.

In some embodiments, the pregnancy is a multiple pregnancy. In some such embodiments, the methods disclosed herein may identify the presence of at least one non-viable fetus or embryo, while one or more additional fetuses or embryos remain viable. In other such embodiments, all fetuses or embryos of the multiple pregnancy may be non-viable. In some embodiments, the multiple pregnancy is a heterotopic pregnancy (i.e. at least one intrauterine and at least one extra-uterine pregnancy occurring simultaneously). In some embodiments, all fetuses or embryos of the multiple pregnancy are intrauterine, and the concentration of the element of fetal or embryonic origin is proportional to the number of fetuses or embryos aborted, such that the ratio or concentration of the reference value is multiplied accordingly.

As used herein, the term “complete abortion” refers to an abortion in which all products of conception have been evacuated, including blood, tissue, embryo or fetus.

As used herein, the term “incomplete abortion” refers to an abortion in which bleeding has begun and the cervix is dilated, only some of the products of conception have been evacuated such that some fetal or embryonic tissue still remains in the uterus.

As used herein, the term “missed abortion” refers to an abortion in which the pregnancy is no longer viable, but no products of conception have been evacuated. The term “missed abortion” includes yolk sac miscarriage, embryonic miscarriage and fetal miscarriage, as defined by Kolte et al⁵, which is incorporated by reference as if fully set out herein. As used herein, the term “threatened abortion” refers to a condition in which vaginal bleeding of presumably uterine origin is identified, but in which a viable IUP is identified and no fetal or embryonic tissue has been passed.

In some embodiments, the pregnancy failure is a failure of a first or second trimester intrauterine pregnancy, i.e. a pregnancy prior to gestational week 28.

In some embodiments, the method disclosed herein is useful for identification of pregnancy failure in the absence of severe maternal hemorrhage.

The methods disclosed herein may also be useful in determining the location of a pregnancy, for example when an ectopic pregnancy is suspected.

According to some embodiments, the method disclosed herein may be used to identify an ectopic or molar pregnancy, which may be a continuing or terminated pregnancy (wherein the termination may be spontaneous or induced). In some such embodiments, the method further comprises determining a concentration of a hormonal marker of pregnancy, such as β -HCG, in the blood or urine of the subject and identifying the lack of a gestational sac within the uterus, such as by ultrasound scan. The presence of an ectopic or molar pregnancy is then identified when the level of a hormonal marker of pregnancy is above a predetermined threshold value, such as serum β -HCG of at least 1 mIU/mL, such that pregnancy is confirmed, but the ratio of a concentration of the element of fetal or embryonic origin, such as AFP, in the vaginal fluid to the concentration of the element of fetal or embryonic origin in the maternal serum is about 4.3 or less.

According to an aspect of some embodiments disclosed herein, there is provided a method for identifying a threatened abortion in a subject suffering from vaginal bleeding, the method comprising determining the source of bleeding; and determining a concentration of at least one element of fetal or embryonic origin in the vaginal fluid of the subject and comparing said concentration of said at least one element of fetal or embryonic origin to a reference value, wherein when said source of bleeding is uterine bleeding and said concentration of said at least one element of fetal or embryonic origin in said vaginal fluid is lower than said reference value. For example, in embodiments wherein the element of fetal or embryonic origin is AFP, the ratio of the concentration of AFP in vaginal blood to the concentration of AFP in maternal serum is less than 4.3. It should be noted that when vaginal bleeding is determined to originate from cervical bleeding, vaginal laceration, or other non-uterine sources, threatened abortion is not considered.

A non-limiting example of a situation in which the methods disclosed herein may be useful for identification of an ectopic pregnancy or other abnormal pregnancy (such as molar pregnancy or blighted ovum) is one in which a woman in whom pregnancy has previously been determined, based on the concentration of a hormonal marker of pregnancy, such as β -HCG, in serum or urine (e.g. serum β -HCG concentration of 10,000 mIU/mL) but no conclusive sonographic findings (such that the pregnancy is of unknown location), experiences first trimester bleeding. The inconclusive sonographic findings may be due to a failure of an IUP with intrauterine gestational sac that cannot be distinguished from the surrounding tissue or, alternatively, may be due to an ectopic pregnancy not visualized by ultrasound scan). In such a case, if the ratio of $\frac{[\text{AFP}]_{\text{vaginal blood}}}{[\text{AFP}]_{\text{maternal serum}}}$ is above 13.4, failure of an IUP is indicated. However, if the ratio of $\frac{[\text{AFP}]_{\text{vaginal blood}}}{[\text{AFP}]_{\text{maternal serum}}}$ is 4.3 or less, the pregnancy might be an ectopic pregnancy, a blighted ovum, or a complete mole (such that no intrauterine embryonic/fetal tissue is present). In the case of a molar pregnancy, since a complete mole does not contain embryonic/fetal tissue, the absolute concentrations of AFP are very low i.e. less than about 20 ng/mL.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. In addition, the descriptions, materials, methods, and examples are illustrative only and not intended to be limiting. Methods and materials similar or equivalent to those described herein can be used in the practices of the present invention.

As used herein, the terms “comprising”, “including”, “having” and grammatical variants thereof are to be taken as specifying the stated features, integers, steps or components but do not preclude the addition of one or more additional features, integers, steps, components or groups thereof. These terms encompass the terms “consisting of” and “consisting essentially of”.

As used herein, the indefinite articles “a” and “an” mean “at least one” or “one or more” unless the context clearly dictates otherwise.

BRIEF DESCRIPTION OF THE DRAWINGS

Some embodiments of the invention are described herein with reference to the accompanying figures. The description, together with the figures, makes apparent to a person having ordinary skill in the art how some embodiments of the invention may be practiced. The figures are for the purpose of illustrative discussion and no attempt is made to show structural details of an embodiment in more detail than is necessary for a fundamental understanding of the invention. For the sake of clarity, some objects depicted in the figures are not to scale.

In the Figures:

FIG. 1 is a dot graph showing alpha-fetoprotein (AFP) concentration ratio distributions for four subject groups (missed/incomplete abortion (group 1), threatened abortion (group 2), cerclage (group 3), D&C (group 4); as well as for two subjects with a complete molar pregnancy. Each dot represents the ratio $\frac{[AFP]_{\text{vaginal blood}}}{[AFP]_{\text{maternal serum}}}$ or $\frac{[AFP]_{\text{POC}}}{[AFP]_{\text{maternal serum}}}$ in a single subject.

FIG. 2 is a graph showing receiver operating characteristic (ROC) analysis for subject groups (1) and (2) as defined in FIG. 1.

FIG. 3 is a dot graph showing fetal hemoglobin (HbF) percentage in vaginal blood (VB) and in maternal serum (MS). Each dot represents the fraction (%) of HbF of the total hemoglobin detected in the sample.

FIG. 4. is a graph showing ROC analyses of fetal hemoglobin fraction in the vaginal blood vs. its fraction in the maternal serum. The predetermined threshold is 0.02 (2%).

DESCRIPTION OF SOME EMBODIMENTS OF THE INVENTION

The invention, in some embodiments thereof, relates to a method for identification of pregnancy failure comprising comparing a concentration of at least one element of fetal or embryonic origin in the vaginal fluid of a subject to a reference concentration of the same element of fetal or embryonic origin.

The principles, uses and implementations of the teachings herein may be better understood with reference to the accompanying description and figures. Upon perusal of the description and figures present herein, one skilled in the art is able to implement the invention without undue effort or experimentation. In the figures, like reference numerals refer to like parts throughout.

Before explaining at least one embodiment in detail, it is to be understood that the invention is not necessarily limited in its application to the details of construction and the arrangement of the components and/or methods set forth in the following description and/or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. The phraseology and terminology employed herein are for descriptive purpose and should not be regarded as limiting.

EXAMPLES

Example 1: APF Concentration as an Indicator of Pregnancy Failure

Materials and Methods:
Subjects

A total of 78 pregnant women in their first or second trimesters were recruited at the Maimonides Medical Center Labor and Delivery Department, Brooklyn, N.Y.

Written consent was obtained from all study participants prior to participation.

Four subject groups were evaluated:

- (1) Women with incomplete/missed abortion experiencing vaginal bleeding as identified according to the diagnostic criteria for a nonviable intrauterine pregnancy (IUP) provided by Doubilet et al.³, which is incorporated by reference as if fully set forth herein;
- (2) Women with threatened abortion i.e. not passing fetal or embryonic tissue, experiencing vaginal bleeding, with a confirmed IUP and visible fetal or embryonic heartbeat;
- (3) Women experiencing vaginal bleeding during cerclage placement, with visible fetal or embryonic heartbeat before and after the cerclage procedure (negative control group for passage of fetal or embryonic tissue into the vagina); and
- (4) Women undergoing dilation and curettage (D&C) and having confirmed IUP (positive control group for presence of fetal or embryonic tissue).

After recruitment and initial evaluation (that included history taking, physical examination, sonographic imaging, and laboratory testing), each subject was assigned to one of the four groups described above; 31, 15, 9, and 23 women were assigned to Groups (1) to (4), respectively. For subjects in Group (3), McDonald (n=4) or Modified Shirodkar (n=5) techniques were used.

Confirmation of IUP

Specimens of tissue were obtained from the vagina for subjects in Group (1) and from the uterus (products of conception, POC) from subjects in Group (4). IUP was confirmed by a histopathological review of the tissue specimens. All fetal or embryonic tissue was subsequently evacuated from subjects in Group (1), either spontaneously or actively with the aid of uterotonics, with or without a D&C.

IUP was confirmed by sonogram for subjects in Groups (2) and (3), and no solid tissue specimens were obtained from subjects in these groups.

Subjects already recruited to Groups (1) and (4) were excluded from analysis if the histopathological report did not confirm the presence of intrauterine embryonic/fetal tissue.

Measurement of AFP Concentrations

AFP concentrations were measured by a fully automated assay (a chemiluminescence immunoassay by Beckman Coulter Inc., USA; Cat. No. 33211).

For each subject group, AFP concentration in the vaginal blood (Groups (1) to (3)) or in the liquid component of the

evacuated POC (Group (4)) was compared to the AFP concentration in the maternal serum of the same subject. Values were expressed as the median with the corresponding ranges. Wilcoxon signed-rank test was used for paired samples.

Concentration ratios were calculated for each subject individually as follows: $[AFP]_{\text{vaginal blood}}/[AFP]_{\text{maternal serum}}$ (for subjects in Groups (1) to (3)); $[AFP]_{\text{POC}}/[AFP]_{\text{maternal serum}}$ (for subjects in Group (4)). Receiver operating characteristic (ROC) analysis for the detection of fetal or embryonic tissue in the vaginal blood according to AFP concentration ratios was performed (Group (1) vs. Group (2)).

It was assumed by the present Inventor that AFP concentration in the fetal or embryonic serum is at least 1000 times higher than in the maternal serum. Since this resulted in an extremely large difference and tiny sample size, the present Inventor chose to limit the effect size to 0.8 resulting in 12 patients per group for 80% power, with $\alpha=0.05$ (online sample size calculator: <https://www.anzmtg.org/stats>). Statistical data were analyzed and graphical illustrations were constructed using MedCalc software (MedCalc Software bvba, Ostend, Belgium).

Results for this embodiment are presented in Table 1 below and in FIGS. 1 and 2.

Results

Confirmation of IUP

Except for two women, all histopathological reports respectively confirmed the presence of an IUP with embryonic/fetal tissue in subjects from Groups (1) and (4). One subject who was initially assigned to Group (1), and later had a D&C, and one woman who was initially scheduled for D&C for “an abnormal pregnancy” and assigned to Group (4), had histopathological reports showing complete moles (i.e. no embryonic/fetal tissue). These two cases were excluded from the original group assignments but the AFP concentrations were measured in the maternal sera and in the obtained intrauterine specimens and are presented in FIG. 1 below.

Fetal or embryonic heartbeats were identified for all subjects assigned to Group (3), both pre- and post-cerclage procedure, and none ruptured their membranes in conjunction with the procedure. Therefore, no subjects originally assigned to Groups (2) and (3) were subsequently excluded.

AFP Concentrations
Table 1 below present alpha-fetoprotein (AFP) concentration ratio distributions for Groups (1) to (4). In FIG. 1, each dot represents the ratio $[AFP]_{\text{vaginal blood}}/[AFP]_{\text{maternal serum}}$ or $[AFP]_{\text{POC}}/[AFP]_{\text{maternal serum}}$ in a single subject. In FIG. 1 the four groups are represented as well as the two subjects with a complete molar pregnancy.

TABLE 1

Group no.	Gestational age range (weeks)	Median $[AFP]_{\text{vaginal blood}}$ (ng/mL) or Median $[AFP]_{\text{POC}}$ (ng/mL; D&C group only) ^a	Median $[AFP]_{\text{maternal serum}}$ (ng/mL)	p	Median ratio ^b
1 (n = 30)	7 to 15	192.2 (9.2 to 195,242)	6.2 (0.9 to 211)	<0.001	24.5 (5.1 to 8,620)
2 (n = 15)	6 to 22	109 (1.8 to 712)	73.1 (1.5 to 280)	0.389	1.2 (0.4 to 13.4)
3 (n = 9)	14 to 22	26.0 (14.8 to 108)	25.2 (18.7 to 73.3)	1	1.01 (0.7 to 1.5)
4 (n = 22)	5 to 20	16,783 (122 to 155,466)	18.3 (0.9 to 755)	<0.001	957 (4.6 to 24,216)

As presented in Table 1, AFP concentrations in vaginal blood (Group (1) and in POC (Group (4)) were significantly higher than AFP concentration in the maternal serum in all subjects (Table 1).

Specifically, for Group (1), the median AFP concentration was 192.2 ng/mL in the vaginal blood and 6.2 ng/mL in the maternal serum ($p<0.001$). The median ratio was 24.5 with a range of 5.1 to 8,620 (Table 1).

For Group (4), the median AFP was 16,783 ng/mL in the liquid component of the POC and 18.3 ng/mL in the maternal serum ($p<0.001$). The median ratio was 957 with a range of 4.6 to 24,216 (Table 1).

For Groups (3) and (4), the median AFP concentration in the vaginal blood was relatively low and did not differ significantly from the median concentration in the maternal serum (Table 1).

The ratio distribution for each group is presented in FIG. 1.

As seen in Table 1 and FIG. 1, the median concentration ratio of AFP in vaginal blood to AFP in maternal serum was 24.5 (5.1-8,620) for group (1) (n=30), whereas they were only 1.2 (0.4-13.4) and 1.01 (0.7-1.5) for group (2) (n=15) and group (3) (n=9), respectively. Median concentration ratio of AFP in POC for group (4) (n=22) was 957 (4.6-24,216).

Receiver operating characteristic analyses (ROC) of Groups (1) and (2) are presented in FIG. 2. The determined cutoff levels for AFP concentration ratios was 4.3, for which the corresponding sensitivity was 100% (confidence interval [CI] 88.4-100%) and the corresponding specificity was 86.7% (CI 59.5-98.3%). The calculated area under the curve was 0.96 (CI 0.86-1.0).

Raising the cutoff ratio to 13.4 resulted in a 73.3% sensitivity for 100% specificity (FIG. 1).

A ratio of 4.3 or lower was thus found to be consistent with a threatened abortion, whereas a ratio of above 13.4 was found to be consistent with pregnancy failure.

As further seen in Table 1, for the two subjects initially assigned to Groups (1) and (4), respectively, but later found to have complete moles, the AFP concentration in vaginal blood was 2.3 ng/mL (for the subject assigned to Group (1)) and in the liquid component of the POC was 1.9 ng/mL (for the subject assigned to Group (4)), and the AFP concentrations in the maternal sera of the two subjects were 1.2 and 1.1 ng/mL, respectively. Each of these values was thus very low for the two subjects, and was within the respective ranges for non-pregnant women. The AFP concentration ratios were 1.92 and 1.73, respectively (AFP ratios for these two subjects are represented in FIG. 1).

Conclusion

The present Inventor have surprisingly found that a ratio of $[AFP]_{\text{vaginal blood}}/[AFP]_{\text{maternal serum}}$ of 4.3 or lower is

consistent with a threatened abortion in subjects suffering from bleeding of intrauterine origin, whereas a ratio of above 13.4 is consistent with pregnancy failure. A ratio that falls between 4.3 and 13.4 (including 13.4) should be interpreted as indeterminate.

It should be noted that the given values are those for AFP. Different indicators of pregnancy failure may be used, and may involve different concentration ratios.

The present Inventor thus disclose a novel approach for the diagnosis of pregnancy failure based on the passage of fetal or embryonic tissue, by quantification of AFP concentration in the vaginal blood of a subject. Excluding the relatively rare possibilities of multiple or heterotopic pregnancies, an $[AFP]_{\text{vaginal blood}}/[AFP]_{\text{maternal serum}}$ ratio above 13.4 is shown to indicate pregnancy failure. It is further considered that the higher the ratio, the higher the possibility of a pregnancy failure.

A limitation of this approach would be its reliability in the setting of severe maternal hemorrhage. Heavy bleeding could greatly dilute the AFP originating from the fetal or embryonic tissue and lead to a false negative result (i.e., a low ratio). Therefore, in cases of maternal hemorrhage, this technique should be used with caution or not used at all.

The Inventor conclude that sampling vaginal blood in the setting of first trimester (or early second trimester) bleeding or sampling the liquid component of POC following a diagnostic or a therapeutic D&C can serve as a confirmation of a dissolved/mixed fetal or embryonic tissue in the obtained specimen. A strength of this approach is that it is a same-day (in some cases, a result is available within 25-30 minutes from the time it is received in the lab), simple test that has the potential to provide a reliable diagnosis of an IUP failure.

Example 2: DNA Concentration as an Indicator of Pregnancy Failure

An additional example of an element of fetal or embryonic origin for use in the methods of the present invention is fetal or embryonic DNA/RNA. The presence of fetal or embryonic genetic material above a threshold level of about 2% of the total DNA/RNA sample obtained can confirm, for example, pregnancy failure. Moreover, laboratory or bedside tests for the sequence of these molecules can provide information about the etiology of the pregnancy failure (e.g., fetal or embryonic chromosomal abnormalities) without or prior to obtaining fetal or embryonic tissue.

For example, trisomy 13 and 18 are not rare and can result in early pregnancy loss. The sample of vaginal bleeding for these genetic abnormalities or other abnormalities such as mutations, deletions, duplications, and oligonucleotide repeats can provide the etiology of the failing pregnancy.

DNA/RNA levels may be detected by any method known in the art. As a non-limiting example, a bedside test for DNA detection can be done with a lateral flow assay employing DNA biorecognition molecules such as aptamers or molecular beacons' as disclosed by Sajid M et al., which is incorporated by reference as if fully set out herein. For example, the biorecognition molecules can bind specific DNA/RNA sequences which are associated with known mutations/excess repeats as well as abnormal sequences following deletions or additions of genetic material. Therefore, a lateral flow assay employing biorecognition molecules can be a powerful tool for detecting fetal or embryonic DNA/RNA and recognizing sequence abnormalities almost in or in real time (i.e., while vaginal bleeding occurs).

Subjects are recruited, evaluated, and an IUP confirmed as described for Example 1 above.

DNA concentration is measured by lateral flow assay.

For each subject group, DNA concentration in the vaginal blood (Groups (1) to (3)) or in the liquid component of the evacuated POC (Group (4)) is compared to the DNA concentration in the maternal serum of the same subject. Values are expressed as the median with the corresponding ranges. Wilcoxon signed-rank test is used for paired samples.

Concentration ratios are calculated for each subject individually as follows: $[DNA]_{\text{vaginal blood}}/[DNA]_{\text{maternal serum}}$ (for subjects in Groups (1) to (3)); $[DNA]_{\text{POC}}/[DNA]_{\text{maternal serum}}$ (for subjects in Group (4)). Receiver operating characteristic (ROC) analysis for the detection of fetal or embryonic tissue in the vaginal blood according to DNA concentration ratios is performed (Group (1) vs. Group (2)).

Statistical data are analyzed and graphical illustrations are constructed using MedCalc software (MedCalc Software bvba, Ostend, Belgium).

Example 3: Fetal/Embryonal Hemoglobin Concentration as an Indicator of Pregnancy Failure

An additional example of an element of fetal or embryonic origin for use in the methods of the present invention is fetal or embryonic hemoglobin (HbF). The presence of fetal or embryonic hemoglobin above a threshold level detected in the maternal serum can confirm pregnancy failure. Alternatively, since oftentimes HbF is not detected at all in the maternal serum or is present in relatively low levels, a detectable IMF in the vaginal blood above a determined threshold can confirm pregnancy failure, such that the threshold level is zero or a relatively low number/percentage. In general, HbF in the newborn is 60 to 80% of the total hemoglobin whereas it is only 0 to 2% in the maternal serum.

Subjects were recruited, evaluated, and an IUP confirmed as described for Example 1 above.

Fetal or embryonic hemoglobin concentration was measured using an immunoassay using antibodies reacting specifically with human hemoglobin F (Anti-fetal hemoglobin antibody ab19364 Abcam, Cambridge, UK).

HbF percentage of the total hemoglobin in the sample was calculated for each subject individually as follows: HbF % in the vaginal blood and HbF % in the maternal serum (FIG. 3). Receiver operating characteristic (ROC) analysis for the detection of fetal tissue in the vaginal blood according to hemoglobin concentrations was performed (FIG. 4). Statistical data were analyzed and graphical illustrations were constructed using MedCalc software (MedCalc Software bvba, Ostend, Belgium).

Devices Useful in the Method of the Present Invention

The concentration of an element of fetal or embryonic origin may be determined by any method known in the art, such as, but not limited to, quantification of a known marker of the element of fetal or embryonic origin.

Examples of such methods include a chemiluminescence immunoassay (for example, that produced by Beckman Coulter Inc., USA, as referred to above, for measurement of AFP concentration), enzyme-linked immunosorbent assay (ELISA), lateral flow assay, immunohistochemistry, or radioimmunoassay. Other methods can include use of a biorecognition molecule rather than an immunoassay.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the scope of the appended claims.

Citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the invention.

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What is claimed is:

1. A method for treating a subject suspected of pregnancy failure, the method comprising:
 determining a concentration of alpha fetoprotein in the vaginal fluid of the subject; and
 comparing said concentration of said alpha fetoprotein to a reference value,
 wherein when said concentration of said alpha fetoprotein in said vaginal fluid is higher than said reference value, pregnancy failure is indicated, and
 treating the subject in whom pregnancy failure is indicated by administration of uterotonics and/or a dilation and curettage procedure.

2. The method of claim 1, wherein said reference value is a concentration of said alpha fetoprotein in maternal serum of the same subject.

3. The method of claim 1, wherein said vaginal fluid is vaginal blood.

4. The method of claim 1, wherein the pregnancy failure is a failure of an intrauterine pregnancy.

5. The method of claim 4, wherein said reference value is a concentration of said alpha fetoprotein in maternal serum of the same subject, and wherein pregnancy failure is indicated when a ratio of a concentration of said alpha fetoprotein in said vaginal fluid to concentration of said alpha fetoprotein in said maternal serum is higher than a predetermined threshold ratio level.

6. The method of claim 5, wherein said predetermined threshold ratio level is 13.4.

7. The method of claim 4, wherein said reference value is a predetermined concentration value of said alpha fetoprotein, and wherein pregnancy failure is indicated when a concentration of said alpha fetoprotein in said vaginal fluid of the subject is higher than the predetermined concentration value.

8. The method of claim 7, wherein said predetermined reference value is 9.2 ng/mL.

9. The method of claim 1, wherein the pregnancy failure is a spontaneous abortion or an induced abortion.

10. The method of claim 9, wherein the pregnancy failure is selected from the group consisting of a complete abortion, an incomplete abortion and a missed abortion.

11. The method of claim 1, wherein the pregnancy failure is a failure of a first or second trimester pregnancy.

12. The method of claim 1, wherein said pregnancy failure is selected from the group consisting of an ectopic pregnancy, a molar pregnancy, a blighted ovum, and an anembryonic pregnancy

the method further comprising determining a concentration of a known hormonal marker of pregnancy in the blood or urine of the subject and identifying the absence of alpha fetoprotein,

wherein said reference value is a concentration of said alpha fetoprotein in maternal serum of the same subject,

and wherein ectopic pregnancy is indicated by

- i) a ratio of a concentration of said alpha fetoprotein in said vaginal fluid to concentration of said alpha fetoprotein in said maternal serum of about 4.3 or less,
- ii) absence of sonographic evidence of an intrauterine fetal or embryonic tissue; and
- iii) a concentration of said hormonal marker of pregnancy in the blood or urine of the subject of at least 1 mIU/mL.

13. The method of claim 12, wherein said hormonal marker of pregnancy comprises beta Human Chorionic gonadotropin (β -HCG).

* * * * *

专利名称(译)	识别妊娠失败的方法		
公开(公告)号	US10509043	公开(公告)日	2019-12-17
申请号	US15/951187	申请日	2018-04-12
[标]发明人	MOR AMIR		
发明人	MOR, AMIR		
IPC分类号	G01N33/68 G01N33/53 A61B5/00		
CPC分类号	G01N33/689 A61B5/4343 C12Q2600/158 G01N2800/368 C12Q1/6883		
优先权	62/486467 2017-04-18 US		
其他公开文献	US20180299459A1		
外部链接	Espacenet		

摘要(译)

提供了一种用于识别受试者的妊娠失败的方法，该方法包括确定受试者的阴道液中胎儿或胚胎来源的至少一种元素的浓度；将胎儿或胚胎来源的至少一种元素的浓度与参考值进行比较，其中当阴道液中胎儿或胚胎来源的至少一种元素的浓度高于参考值时，表明妊娠失败。

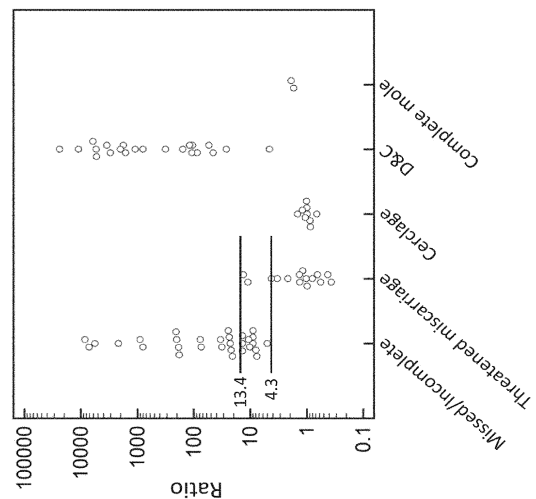


FIG. 1