An accurate and reliable screening test for ectopic pregnancy typically involves assaying for β-core fragment in a urine sample obtained from a pregnant woman during the month following her missed menstrual period, identifying her as at risk by observation of decreased β-core fragment levels in the sample in comparison to control sample levels, and then performing a transvaginal ultra-sound to determine the absence of a fetal sac. The test correspondingly provides a method for identifying a pregnant woman at risk for spontaneous abortion in cases where the ultrasound identifies intrinsic defects in the fetus.
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UG Uganda
US United States of America
UZ Uzbekistan
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YU Yugoslavia
ZW Zimbabwe
EARLY DETECTION OF ECTOPIC PREGNANCY

Related Application Data

This application claims priority benefit of U.S. provisional application serial number 60/020,309 filed June 19, 1996.

Technical Field of the Invention

This invention relates to a diagnostic test for ectopic pregnancy which can also be used to predict the likelihood of a spontaneous abortion.

Ectopic pregnancy remains one of the primary causes of maternal morbidity and mortality throughout the world (1). In recent years maternal morbidity and mortality have steadily declined, but that due to ectopic pregnancy is significantly increasing (3-5). In the last 3 decades, the incidence of ectopic pregnancy in the United States has risen from 17,800 to 109,000 cases per year (2,3). It now accounts for approximately 2% of reported pregnancies. Known risk factors for ectopic pregnancy include history of pelvic inflammatory disease, previous therapeutic abortion, use of intrauterine device and previous sterilization procedure. Risk is also significantly enhanced by in vitro fertilization and other assisted reproductive methods (1 in 30 births) (4-6).

Many pregnancies also result in spontaneous abortions during the first trimester. Though reports vary between 6% and 12%, in the United States this occurs in about 12% of natural, viable first trimester pregnancies (8-10). These spontaneous abortions are mainly due to intrinsic defects in the developing conceptus, abnormal germ cells or defective implantation. The incidence of spontaneous
abortion is significantly higher in viable in vitro fertilized pregnancies (22%) (11). Spontaneously aborting pregnancies can be complicated by infection or septic shock, sometimes leading to maternal death. Early identification and treatment of ectopic pregnancy and other types of potential pregnancy failure is important.

5 Background of the Invention

Significant advances in the diagnosis and treatment of ectopic pregnancy have been made over the last three decades. Among such advances are the sensitive immunoassay for detection of human chorionic gonadotropin (hCG). Human chorionic gonadotropin is a glycoprotein composed of two dissimilar subunits, α (92 amino acids) and β (145 amino acids), joined noncovalently, and is detected in the serum and urine of pregnant women, in those with trophoblast disease, and in some with malignancies. Free α- and free β-subunits, and degraded hCG and free β-subunit molecules are also detected in serum and urine samples. β-core fragment is the terminal degradation product of the β-subunit of hCG (molecular weight ~9,000, versus 36,700 for hCG), and is the principal hCGβ-related molecule in pregnancy urine (12,13).

Diagnostic ultrasound coupled with assessment of hCG level, or hCG level doubling rate, have become the standard for the detection of ectopic pregnancy (1,5). Detection of ectopic pregnancy prior to rupture has been important in decreasing maternal morbidity and mortality and in preserving maternal organs such as fallopian tubes and ovaries. Major developments have been made in the treatment of ectopic pregnancy. Laparoscopic techniques are now used to remove all but the most acute cases of tubal ectopic pregnancy involving rupture and acute hemorrhage (16,17). This has decreased morbidity. Linear salpingostomy permits removal of tubal ectopic pregnancies while preserving the fallopian tube for future child-bearing (17). Significant advances have also been made in the medical management of ectopic pregnancy. Single or multiple-dose therapy with the anti-
folic acid agent methotrexate has proven useful in the non-operative management of ectopic pregnancy.

Given these advances in diagnosis and treatment, the importance of early detection of ectopic pregnancy becomes apparent, together with timely non-operative or laproscopic therapy. Simple chemical tests identifying early in pregnancy women likely to have ectopic pregnancy could avoid the morbidity and mortality and fertility risks associated with advanced or ruptured extrauterine pregnancy or with late presentation in the emergency room. In 1993, hCG and β-core fragment levels in urine samples from patients coming to an emergency room, 36 with normal and 12 with ectopic pregnancies, were measured (14). While only a 48-fold lower hCG level was detected (median), an 800-fold lower β-core fragment concentration was measured (median) in those with ectopic pregnancies. While 8.3% overlap was evident in hCG levels, 2.3% overlap was detected in β-core fragment levels in normal and ectopic pregnancies (14).

It would be desirable to have an improved method for detecting ectopic pregnancy that had a superior diagnostic performance.

Summary of the Invention

It is an object of the invention to provide a screening method for ectopic pregnancy that is more accurate and reliable than previously described methods.

It is another object of the invention to provide a method for predicting substantial risk of spontaneous abortion in early pregnancy.

These and other objects of the invention are accomplished by the present invention, which provides a method for screening for an ectopic pregnancy in a pregnant woman by first identifying that she is at substantial risk of an ectopic
pregnancy by observation that the level of $\beta$-core fragment in a biological sample, preferably urine typically obtained during the month following her missed period, *e.g.*, at the time of a pregnancy test, is lower than the level in a corresponding control sample. If she appears at risk, then an ultrasound of uterine contents such as a transvaginal ultrasound is performed to determine the absence of a fetal sac. Preferred assays for $\beta$-core fragment are immunoassays, *e.g.*, ELISAs, and the levels determined are normalized, *e.g.*, to creatinine concentrations.

Pregnant women at risk for spontaneous abortion can also be identified using the method of the invention because a transvaginal ultrasound performed on a woman identified as at substantial risk for an ectopic pregnancy can identify intrinsic defects in the fetus.

**Brief Description of the Figure**

Fig. 1 is a line graph illustrating data obtained related to screening for ectopic pregnancy using $\beta$-core fragment. Thirteen urine samples were collected at 2½ to 5 weeks after embryo transfer from women with impending ectopic pregnancy ($\blacktriangle$), 15 from those with impending spontaneous abortion ($\blacklozenge$), and 50 from women with normal term intrauterine pregnancies ($\square$). Discrimination curve indicates 5% false positive rate.

**Detailed Description of the Invention**

This invention is based on the surprising finding that a combination of a $\beta$-core fragment assay followed by transvaginal ultrasound can be used together to identify ectopic pregnancy much more accurately and reliably than either procedure alone, or what would be predicted from their false positive rates if used together. Transvaginal ultrasound typically has a 95% detection (absence of
gestational sac) of suspected ectopic pregnancies, with 2% false positive rate (19). Other reports claim 84% detection of ectopic pregnancy (20,21), with 1% false positive rate, as early as early as 4 weeks after last menstrual period (20). The combination of β-core fragment immunoassay and ultrasound, however, predicts 87% of ectopic pregnancies with a 0.2% false positive rate (both tests false positive).

In the practice of the method of the invention, ectopic pregnancy or substantial risk of spontaneous abortion are screened in two stages. In the first stage, the level of β-core fragment in a biological sample is obtained from a pregnant woman is assessed. Any biological sample containing β-core fragment can be employed, including urine, serum, and saliva. Urine is particularly preferred, especially urine collected during the first month after her missed menstrual period. In one embodiment, the sample is obtained when she has a pregnancy test.

The level of β-core fragment is determined in the sample. As described in the specification and claims, β-core fragment level determinations include the direct measurements described herein, as well as enhanced versions of the assays employing progesterone and the like. The determined level is then compared to the level of β-core fragment in a corresponding normal control. Corresponding control levels are determined by plotting median levels of β-core fragment at each week of pregnancy against gestational age, and mathematically ascertaining the best fit to obtain normal control levels. Using this Gaussian distribution of the control group adjusted for gestation period, level decreases of about 5% or more are considered significant.

By "β-core fragment" is meant any population of β-core proteins and fragments thereof. The term encompasses chorionic gonadotropin, β-subunits, β-core fragments, and mixtures thereof, and specifically includes urinary gonadotropin fragment (UGF), urinary gonadotropin peptide (UGP), "β-core" and the like.
names used for β-core fragment, and variants of these species that have additional N-acetylgalcosamine observed in Down's syndrome β-core populations. The term further encompasses β-core precursors, such as the nicked free β-subunits and the like. β-core fragments are employed in many embodiments. It is an advantage of the invention that pregnancy urine contains large quantities of hCG, free β-subunit, and a population of β-core fragments. Indeed, the β-core population can account for as much as 70% of the total β-immunoreactivity in pregnancy urine.

Any method for assaying for β-core fragment may be employed. Assay methods for β-core fragment include any chemical or biological method employed by skilled workers for such purpose, particularly immunoassays, e.g., enzyme-linked immunosorbent/immunometric assays (ELISAs), Western blots, Northern blots, Northern dot blots, radioimmunoassays, or mixtures of these. Immunoassays useful in the practice of the invention employ either specific antibodies to β-core fragment generated by standard means, and assays employing nonspecificity defined antibodies obtained by blind injections of β-core fragment into test animals using standard methods. Any type of monoclonal or polyclonal antibodies can be used in immunoassays of the invention, so long as the antibodies can be used in a reproducible fashion as markers for β-core fragment. ELISAs previously described (12, 14, 15) are particularly preferred.

As is appreciated by the skilled worker, when urine is used as the biological sample, the concentration of components vary according to the time of day and can be influenced by other factors such as beverage consumption. Therefore, in preferred embodiments using a urine sample, the determined level of β-core fragment in the sample is normalized. Any normalization method can be employed for this purpose. In preferred embodiments, β-core fragment levels are normalized to creatinine concentration as described previously (14). Using this normalization, measures of β-core fragment are normalized to creatinine concentrations expressed as ng/mg.
A pregnant woman is determined to be at substantial risk for ectopic pregnancy or spontaneous abortion by observation of a decreased β-core fragment level in the sample (14). The β-core fragment test detects 92% of impending ectopic gestations at the time of the pregnancy test, 4½ to 7 weeks after last menstrual period (2½ to 5 weeks after embryo transfer). A 4% false positive rate has been observed. The test also detects other pregnancy failures, 67% of impending spontaneous abortions. Assuming a 2% occurrence of ectopic pregnancy (2,3) and a 12% incidence of spontaneous abortions in viable pregnancies (8-10), then 76% (predictive value positive) of those identified by this test are predicted to have impending pregnancy failure (14% ectopic pregnancy plus 62% spontaneous abortion). On the contrary, 95% (predictive value negative) of those with normal range values in this test are predicted to have a non-failing, or term pregnancies.

As illustrated in the Examples that follow, while normal pregnancy levels increase with gestational age, levels of those with impending failing pregnancies did not. Discrimination of normal and failing pregnancies increased with advancing weeks of pregnancy, with the least discrimination at 2½-3 weeks, and the greatest at 4-5 weeks after embryo transfer. Early β-core fragment tests (2½-3 weeks) may be repeated for accuracy in some embodiments.

In the second part of the diagnostic method of the invention, a woman identified as at risk is subjected to transvaginal or transuterine ultrasound. The procedure images uterine contents for the presence or absence of a fetal sac, and the fetus if present. Since the fetal sac is small in early pregnancy, a color doppler-enhanced transvaginal ultrasound is particularly preferred. A woman is diagnosed as having an ectopic pregnancy if her uterus appears empty, i.e., the absence of a fetal sac.
Alternatively, if the woman has an intrauterine pregnancy, the ultrasound may be used to identify any intrinsic defects such as abnormal size, abnormal yolk sac diameter, and the like, shown in the fetus.

The β-core fragment assay is useful as a screening test for failing pregnancies, whether to identify impending ectopic pregnancy, or to determine success or outcome of pregnancy (14, 23). Over three quarters of those with abnormal test levels are destined to ectopic pregnancy failure. The test is applicable to the general pregnancy population, and is of particular use for those at increased risk due previous pregnancy failure, pelvic inflammatory disease or the employment of assisted reproductive methods. When combined with transvaginal ultrasound according to the method of the invention, it provides a critical improvement for the early diagnosis of ectopic pregnancy and spontaneous abortion, or for prediction of term pregnancy.

An advantage of the invention is that after diagnosis of ectopic pregnancy, the pregnancy can be terminated early by non-surgical methods, with minimal mortality or fertility loss. Methotrexate therapy, for example, can be used to treat the early ectopic pregnancy with minimal complications.

Another advantage is that pregnancies at risk for spontaneous abortion can be identified in situations where ectopic pregnancy is not confirmed by transvaginal ultrasound because the procedure can identify major fetal defects seen in spontaneous abortions. In such cases, chorionic villous sampling or possibly termination may be considered.

The following examples are presented to further illustrate and explain the present invention and should not be taken as limiting in any regard.

Examples
This section reports studies employing urine β-core fragment as a screening test for ectopic pregnancy and spontaneous abortion.

**Samples.** Urine samples were collected at an *in vitro* fertilization center 2½ to 5 weeks after embryo transfer. Samples were collected from 13 women with impeding ectopic pregnancies (shown by surgery at 5 to 9.3 weeks after embryo transfer). Fifteen samples were collected from those with impeding spontaneous abortions (> 5 weeks after embryo transfer), and 50 samples from normal singleton intrauterine pregnancies, that went to term. All urine samples were frozen at -20°C until assayed.

**Assay.** β-core fragment was determined in spot urines by an enzyme-linked immunometric assay as described previously (12,14,15). Briefly described, monoclonal antibody B210 is generated against β-core fragment and used with a polyclonal to the anti-β subunit of hCG labelled with horseradish peroxidase. To adjust for urine dilution, β-core fragment levels were normalized to creatinine concentration as described previously (14).

**Data Analysis.** Results and clinical information were stored in, and discrimination lines determined in a Microsoft Excel 7.0 spreadsheet. Data were plotted using Micrografx Charisma 4.0 software.

**Results.** Fig. 1 shows β-core fragment levels in those with normal pregnancies, and impeding ectopic pregnancy and spontaneous abortion, 2½ to 5 weeks after embryo transfer. While normal pregnancy levels increase with gestational age, levels of those with impeding failing pregnancies did not. Median β-core fragment levels at 2½ to 3, 3 to 4, and 4 to 5 after embryo transfer, were 6.7, 91 and 737 μg/g for unaffected pregnancies, 1.0, 5.9 and 0.6 μg/g for impending ectopic pregnancies (0.15, 0.065 and 0.0008, multiples of the unaffected pregnancy median, MoM), and 0.75, 6.8 and 12 μg/g for impending spontaneous abortions (0.11, 0.07 and 0.016 MoM). Increasing discrimination of unaffected and failing pregnancies was indicated with advancing weeks of pregnancy.

A gestation-linked curve was modeled to discriminate unaffected pregnancy from impending ectopic gestation or spontaneous abortion, at an
approximate 5% false positive rate. The equation for the curve was \( y = (x-1.9)^5 \), where \( y = \) concentration and \( x = \) weeks after embryo transfer. Plotted \( \beta \)-core fragment levels were below this curve in 12 of 13 impending ectopic pregnancies, and 10 of 15 impending spontaneous abortions. This was consistent with 92% detection of patients with impending ectopic pregnancy, and 66% detection of impending spontaneous abortion. Plotted \( \beta \)-core fragment levels were also below this curve in 2 of 50 (4%) normal pregnancies.

The above description is for the purpose of teaching the person of ordinary skill in the art how to practice the present invention, and it is not intended to detail all those obvious modifications and variations of it which will become apparent to the skilled worker upon reading the description. It is intended, however, that all such obvious modifications and variations be included within the scope of the present invention, which is defined by the following claims. The claims are intended to cover the claimed components and steps in any sequence which is effective to meet the objectives there intended, unless the context specifically indicates the contrary.

References


The papers cited herein are expressly incorporated in their entireties by reference.
CLAIMS

1. A method for screening for an ectopic pregnancy in a pregnant woman comprising:
   A) first identifying that a pregnant woman has a substantial risk of an ectopic pregnancy by the steps of
      1) obtaining a biological sample from the woman;
      2) determining the level of β-core fragment in the sample;
      3) comparing the determined level with the level of β-core fragment in a corresponding control sample;
      4) identifying a substantial risk by observation of a decreased β-core fragment level; and then
   B) performing an ultrasound of uterine contents on the woman to determine the absence of a fetal sac.

2. A method according to claim 1 wherein the sample is selected from the group consisting of urine, serum, and saliva.

3. A method according to claim 2 wherein sample is urine.

4. A method according to claim 1 wherein the determination of the level of β-core fragment is normalized.

5. A method according to claim 4 wherein the determination of the level of β-core fragment is normalized to creatinine concentration.

6. A method according to claim 1 wherein the β-core fragment level is determined using an immunoassay.

7. A method according to claim 6 wherein the β-core fragment level is determined using an ELISA.
8. A method according to claim 1 wherein the ultrasound is a transvaginal ultrasound employing color doppler enhancement.

9. A method for identifying a pregnant woman with a substantial risk of spontaneous abortion comprising:

   A) first identifying that a pregnant woman might have a substantial risk of a spontaneous abortion by the steps of

   1) obtaining a biological sample from the woman;
   2) determining the level of $\beta$-core fragment in the sample;
   3) comparing the determined level with the level of $\beta$-core fragment in a corresponding control sample;
   4) identifying she might have a substantial risk by observation of a decreased $\beta$-core fragment level; and then

   B) performing an ultrasound of uterine contents on the woman and identifying intrinsic defects in the fetus.

10. A method according to claim 10 wherein the ultrasound comprises a transvaginal ultrasound that employs color doppler enhancement.

11. A method according to claim 9 wherein the sample is selected from the group consisting of urine, serum, and saliva.

12. A method according to claim 11 wherein the sample is urine.

13. A method according to claim 10 wherein the determination of the level of $\beta$-core fragment is normalized.

14. A method according to claim 13 wherein the determination of the level of $\beta$-core fragment is normalized to creatinine concentration.
15. A method according to claim 9 wherein the $\beta$-core protein level is determined using an immunoassay.

16. A method according to claim 15 wherein the $\beta$-core protein level is determined using an ELISA.

17. A method for screening for an ectopic pregnancy in a pregnant woman comprising:

   A) first identifying that a pregnant woman has a substantial risk of an ectopic pregnancy by the steps of

   1) obtaining a urine sample from the woman during the month following the woman’s missed period;

   2) determining the normalized level of $\beta$-core fragment in the sample;

   3) comparing the determined level with the normalized level of $\beta$-core fragment in a corresponding control sample;

   4) identifying a substantial risk by observation of a decreased $\beta$-core fragment level; and then

   B) performing transvaginal ultrasound to determine the absence of a fetal sac.

18. A method according to claim 17 wherein the level of $\beta$-core fragment is normalized to the creatinine concentration.

19. A method according to claim 17 wherein the level of $\beta$-core fragment is determined using an ELISA.

20. A method according to claim 17 wherein the ultrasound employs color doppler enhancement.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

<table>
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<th>IPC(6)</th>
<th>US CL</th>
<th>According to International Patent Classification (IPC) or to both national classification and IPC</th>
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**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

- U.S. : 422/20; 435/7.1, 7.7, 7.92; 436/510, 814, 818

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
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<tr>
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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>Y</td>
<td>US 4,123,509 A (BANIK et al) 31 October 1978, see entire document.</td>
<td>1-8, 17-20</td>
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<td>A</td>
<td>US 4,851,356 A(CANFIELD et al) 25 July 1989, see entire document.</td>
<td>9-16</td>
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[X] Further documents are listed in the continuation of Box C.  
[ ] See patent family annex.

**Date of the actual completion of the international search**  
28 AUGUST 1997

**Date of mailing of the international search report**  
1 OCT 1997

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks

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Form PCT/ISA/210 (second sheet)(July 1992)
### INTERNATIONAL SEARCH REPORT

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<td>X</td>
<td>Biosis Number 88113785. RISS et al. Serum Progesterone and Human Chorionic Gonadotropin in Very Early Pregnancy</td>
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</table>
### Box I  Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II  Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)
B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG, MEDLINE, CAPLUS, CAB, WPIDS, BIOSIS, PASCAL, MEDLINE, LIFESCIENCE, EMBASE
search terms: beta or b core fragment, chorionic gonadotropin, color doppler, ultrasound, urine, ectopic pregnancy,
abortion, immunoassay or assay

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single
inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search
fees must be paid.

Group I, claim(s) 1-8 and 17-20, drawn to methods for screening for an ectopic pregnancy in a pregnant woman.
Group II, claim(s) 9-16, drawn to a method for identifying a pregnant woman with a substantial risk of a spontaneous
abortion.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under
PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
The method of Group I is separate and independent from the method of Group II as it is designed to screen
for a different problem than the method of Group II, i.e., ectopic pregnancy. The methods of Groups I and II are
designed to detect two completely different problems which may be encountered during the course of pregnancy. For
these reasons, the inventions of Groups I and II are shown to have different properties with no common link between
them.