SciMedCentral

Journal of Embryology & Developmental Biology

Review Article

# Early Diagnosis of Ectopic Pregnancy Based on Algorithmic Approaches and New Biomarkers: A Narrative Review

**Maral Janan[1], Firouzeh Ghaffari[2], Poopak Eftekhari-yazdi[3]*, Hamidreza Bolhasani[4] and Fatemeh Hassani[5]***

[1]*Department of Genetics, Faculty of Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran*
[2]*Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for reproductive biomedicine, ACECR, Tehran, Iran*
[3,5]*Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran*
[4]*Islamic Azad University Science and Research Branch, Tehran, Iran*

***Corresponding author**
Fatemeh Hassani, Poopak Eftekhari Yazdi, Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, PO Box 19395-4644, Tehran, Iran; Email: Fatima1737@yahoo.com, eftekhari@royaninstitute.org

**Submitted:** 13 October 2021

**Accepted:** 23 November 2021

**Published:** 25 November 2021

**ISSN:** 2378-9409



**Keywords**

- Ectopic pregnancy
- Diagnostic algorithm
- Diagnostic biomarkers
- Early diagnosis

**Abstract**

Ectopic pregnancy (EP) is a condition of incorrect implantation of the fertilized egg outside the uterus. It is one of the major reasons for maternal morbidity and mortality in the first trimester. There are several types of EP depending on the implantation site. Tubal EP is of the most common case of ectopic pregnancy but there is also rare incidence of EP in cervix, abdominal site, and ovaries. Research has shown that the rate of ectopic pregnancies all over the world is 1.9 - 2%. Women who undergo infertility treatments such as IVF or ICSI show higher incidence rates of ectopic pregnancy (2 - 5%). Ectopic pregnancy complicates infertility treatment and early detection is key to device an effective treatment strategy. Algorithmic approaches to diagnosis, exemplified by emerging artificial intelligence and machine learning models, can help in rapid screening and early diagnosis of EP, and are being considered for use by clinicians to make better decisions regarding treatment protocols in recent years.

In this study, we perform a survey of literature on different algorithmic approaches and biomarkers that have been used for early and reliable detection of ectopic pregnancy in order to identify the best methods among them. The advantages, disadvantages, and limitation of each study are discussed, and suggestions for further research are provided

## ABBREVIATIONS

**EP:** Ectopic pregnancy; **IVF:** In vitro fertilization; **ICSI:** Intracytoplasmic sperm injection

## INTRODUCTION

Ectopic pregnancy is a serious maternal problem in the first trimester of pregnancy because of the morbidity and mortality associated with fallopian tube rupture, intra-abdominal bleeding, and infertility problems[1]. Approximately 10 to 15% of maternal death in first trimester is caused by ruptured ectopic pregnancy [2]. Ectopic pregnancy is the first diagnosis for pregnant women who are presented at a hospital with abdominal pain and/or vaginal bleeding, syncope or hypotension, seven weeks after amenorrhea [1]. The incidence of ectopic pregnancy has increased in the past 25 years and now EP occurs in 2% of all pregnancies in the United States[3]. In the western world ectopic pregnancy is a growing problem and 4 to 10% of pregnancy-related deaths are due to EP because of poor medical facilities [4].

Ectopic pregnancy is a medical emergency that requires immediate detection and treatment. In the past, approximately 50% of ectopic pregnancies were detected at the shock level and after extensive hemorrhage and the patients had to be operated soon after the diagnosis was made[5].

Diagnosis methods for EP have changed dramatically over time. Today EP can be detected before the shock and hemorrhage stages using advanced diagnosis methods such as measuring serum human chorionic gonadotropin (urinary hCG or serum hCG), serum progesterone, diagnostic curettage and also transvaginal ultrasound (TVUS)[6], which simplifies the treatment of EP so that ectopic pregnancy is no longer life-threating as it was in the past. Thus, despite increasing incidence of EP, the mortality associated with ectopic pregnancy has decreased [7].

TransVaginal UltraSound (TVUS) and serum β hCG are efficient methods that do not miss ectopic pregnancy. TVUS can help clinicians detect intrauterine pregnancy or the location of EP. β hCG levels increase by at least 53% every two days in normal pregnancy and the maximum rate is 100,000 mIU per mL. Due to early diagnosis[8], the treatment methods for ectopic pregnancy have shifted from invasive surgical to conservative management

strategies to minimize the need for hysterectomy, and this helps in preserving the uterus and future fertility [9].

An algorithmic approach to diagnostics essentially comprises a logical, sequential, and organized array of steps used to find a pattern in a dataset that helps predict clinical implications [10]. The adoption of the algorithmic approach can help physicians diagnose the outcome faster and with higher accuracy by analyzing data efficiently. This inspires the authors to review the various algorithmic approaches reported in literature, that have been used to identify patterns in clinical data, for the detection EP [11].

## Traditional diagnosis of ectopic pregnancy based on algorithmic approaches

Gracia CR et al.[12] and Fernandez H et al.[13] compared six algorithmic approaches used in the detection of ectopic pregnancy. They compared the six approaches in terms of missed diagnosis of ectopic pregnancies and potentially interrupted intrauterine pregnancies:

**Ultrasound followed by quantitative hCG:** In this approach, transvaginal ultrasound was first carried out. If the gestation was normal, a viable intrauterine pregnancy was diagnosed and if an ectopic pregnancy was diagnosed, the clinicians had to prepare the patient for treatment. In the non-diagnostic situation, the second step was to measure hCG. Under high levels of hCG, dilation and curettage (D&C) was recommended. D&C negative results led clinicians to perform a laparoscopy and if the hCG level was under discriminatory range, the patient was discharged to be followed up with hCG measurement[14].

**Quantitative hCG followed by ultrasound:** hCG measurement was the first step in this approach and ultrasound was used only when the hCG level was higher than the discriminatory range. If quantitative hCG and ultrasound were non-diagnostic, D&C was recommended[15].

**Progesterone followed by ultrasound and quantitative hCG:** In this approach, the first step was to measure progesterone; patients with progesterone levels of 25ng/mL were considered as having intrauterine pregnancy (IUP). Those with less than 5 ng/Ml were recommended to have D&C. In D&C, if the endometrial curetting without chorionic villi was seen, laparoscopy treatment was provided. Patients with progesterone levels between 5 and 25 were referred to the first strategy with ultrasound and quantitative hCG[16].

**Progesterone followed by quantitative hCG measurement and Ultrasound:** This was similar to the third strategy. Progesterone was measured in the first step and the patient with a range of at least 25ng/mL have was considered to have normal IUP and for those with less than 5 ng/mL, D&C was recommended. For progesterone levels in the range 5 and 25 ng/mL, the second protocol was recommended[16].

**Ultrasound followed by repeat ultrasound:** TVUS was the first step and depending on the results or in the absence of a clear diagnosis, the ultrasound was repeated 24 hours later. If the results continued to be undiagnosable, D&C was recommended[17].

***Clinical examination*:** Clinicians examined the clinical symptoms of patient to diagnosis ectopic pregnancy and did not measure hCG, progesterone, etc. [15].

The results of comparison showed that the first and second strategies (TVUS and hCG measurement), did not fail to detect EP. Progesterone measurement strategy missed a few EP cases and therefore, the use of other measurement techniques such as hCG measurement or TVUS was recommended. Ultrasound followed by repeated ultrasound strategy was also accurate in EP diagnosis and had the shortest diagnosis time of all approaches. Ectopic pregnancies were consistently missed in the clinical examination approach. In conclusion, the best algorithmic approach to detecting EP based on time, cost, and sensitivity was that using transvaginal ultrasound and quantitative hCG values.

Lee R et al. [18] reported an algorithmic approach for early recognition of ectopic pregnancy. Every patient with symptoms of abdominal pain and vaginal bleeding presented to emergency department was required to first undergo a urinary hCG pregnancy test. Patients with positive test were considered as possibly having ectopic pregnancy and thus transvaginal ultrasound was recommended to confirm/negate EP and detect the location of the EP implant, if confirmed. The visualization of the gestational sac with yolk sac and/or embryo was considered reason to exclude ectopic pregnancy. If no IUP was detected, clinicians were to look for other symptoms such as the hCG level. In patients with hCG level above discriminatory zone, bilateral adnexa was to be carefully evaluated. Some other findings in ultrasonography could be related to ectopic pregnancy such as the presence of free intraperitoneal fluid. The limitation of this algorithmic approach was that it did not consider pregnant women with high risk of ectopic pregnancy. The advantage of this early recognition algorithmic approach was to consider normal and abnormal adnexa for better outcomes.

Anne-Marie Lozeau et al. [1] provided an algorithmic approach to initial diagnosis of suspected ectopic pregnancy. Clinical examination was the initial key of EP recognition. An enlarged uterus, vaginal bleeding, pelvic pain, and palpable adnexal mass were considered signs of ectopic pregnancy. This was not a suitable approach because up to 30% of patient have no clinical symptoms such as pelvic pain or vaginal bleeding. They suggested conservative methods for EP detection in their study such as: urine pregnancy test, ultrasonography, β hCG measurement diagnostic curettage and progesterone measurement. TVUS was mentioned as a first step recognition test of EP. If TVUS did not show intrauterine pregnancy with hCG levels greater than 1,500 mIU per mL (1,500 IU per L), ectopic pregnancy was considered highly probable. In this approach, βhCG was considered an assistive method in interpreting TVUS findings because β hCG measurement by itself could not be an accurate measure for EP recognition. The authors admitted to a βhCG sensitivity of 36% and specificity of 65%. For low-risk patients with negative diagnosis of intrauterine pregnancy in TVUS, hemodynamic stability and β hCG measurements less than 1,500 mIU per mL, another β hCG measurement was to be recommended after 48 hours. In cases of non-diagnostic TVUS with the same β hCG measure, in high-risk patients for EP and for patients under unstable conditions, surgical consultation

was deemed necessary. If D&C was done for a patient with no chorionic villi, EP was considered more likely. The authors of this work reported 96% sensitivity and 97% specificity for the combination of TVUS and β hCG measurement and concluded this algorithmic approach to be an optimal, cost-effective strategy for diagnosing ectopic pregnancy. The advantage of this approach was that it considered high-risk patients.

Murray H et al. [19] summarized recent advances in algorithmic approach to diagnosis and treatments of EP. β hCG measurement was the first step in their approach to confirm the pregnancy. Both serum and urine were accepted for measurement but serum can detect earlier gestational sac age. This step is usually used for patients in the first trimester who have symptoms of bleeding or pain or both. Measurement of βhCG alone could not identify the location of the gestational sac but low serum β-hCG levels (< 1000 IU/L) are associated with a high relative risk of ectopic pregnancy. Rising, falling or plateaued β-hCG levels are all EP signs, and therefore, following serial measures of β-hCG is more useful for the detection of fetal viability. The measurement of progesterone was reported to be a useful adjunct to β-hCG measurement. One of the advantages of serum progesterone measurement is the independence of gestational age. This measurement can identify 2 subgroups of patients with symptoms of ectopic pregnancy in the first trimester:1) patients with progesterone levels higher than 22 ng/mL are more likely to have viable intrauterine pregnancy and 2) patients with 5 ng/mL or less levels of progesterone are likely to have nonviable pregnancy.

Transvaginal ultrasound is more favorable way for EP detections. Many studies have shown that transvaginal ultrasound imaging can accurately confirm EP and intrauterine pregnancy. It can be used to have early and clear visualization of both normal or abnormal pregnancy at gestational age of about 5 weeks. Most protocols initiate the diagnosis with ultrasound imaging in Emergency Department (ED) patients or can be in the subgroup patients with β-hCG levels above threshold. Detection of ectopic pregnancy usually needs measurements of both TVUS and β-hCG levels. The strength of this article was the diagnosis of emergency patients.

Van Mello NM et al. [20] reported an algorithmic approach to ectopic pregnancy diagnosis. They reviewed historic changes of diagnosis methods and offered their own algorithm for EP detection. Laparoscopy was the first diagnosis method in 1937 and it remained the most reliable diagnosis method until 1980s but later, transvaginal ultrasound became the gold standard step of ectopic pregnancy recognition. Another important element of EP recognition has been β hCG measurement. In hemodynamically stable patients, non-diagnostic ultrasound leads clinicians to measure β hCG level to confirm IUP or EP. This strategy was not as reliable as TVUS and approximately 13% of ectopic pregnancies were missed. The authors posited that the best approach to EP recognition is to combine ultrasound findings and serum hCG concentrations. They admitted that if an intrauterine pregnancy was not detectable in TVUS, and serum β hCG is above the threshold 6,500 IU/l, ectopic pregnancy was more likely. Serum progesterone level was a conjunction tool with β hCG level for EP recognition but its discriminative capacity was deemed insufficient to detect EP from early normal pregnancy or miscarriage and therefore, it was recommended not to be used for this purpose.

A study conducted by Seeber BE et al. [21] posited that first step of EP recognition was to exclude intrauterine pregnancy. Transvaginal ultrasound can identify IUT for gestational ages greater than 5, $^{1/2}$ weeks but different sites of EP can complicate the recognition process. In cases with low accuracy of TVUS, β HCG measurement was to be used as surrogate for EP detection. In the absence of intrauterine pregnancy and β hCG measurement above the discriminatory zone, it was recommended to evacuate the uterus to recognize spontaneous abortion from ectopic pregnancy. Following evacuation of the uterus, in the absence of chorionic villi, EP treatment was to commence. In case of non-diagnosis in evacuating the uterus, β hCG measurement was to be repeated after 12-24 hours. For patients with β hCG levels below the discriminatory zone, they suggested either a growing pregnancy (early pregnancy age) or nonviable pregnancy. To confirm a viable pregnancy, the patient was to be followed up with serial β hCG. If the rise or decline of β hCG was not appropriate, it was considered nonviable pregnancy and for patients with level of β hCG greater than discriminatory zone, TVUS was to be administered to detect the presence or absence of intrauterine pregnancy. The strength of this article was that it showed the increase or decrease of β hCG measure as a factor in the recognition of ectopic pregnancy.

## Molecular diagnostics and therapeutics for ectopic pregnancy

In situation in which Transvaginal ultrasound could not detect pregnancy location (pregnancy of unknown location) biomarkers are suitable for early EP detection because when the blastocyst implants in an inappropriate site there are likely to be some biomarkers that release at different levels in the maternal blood compared to viable intrauterine pregnancy [figure 1]. Tong S et al. [22] ,Rausch ME et al. [23] and Reid S et al.[24] explored molecular diagnostic methods of ectopic pregnancy that can save time in EP detection and decrease tubal rupture. Although there are many biomarkers that are useful for EP diagnosis, many of them have not been approved after phase 2 over 5 phases of investigation [Table1]. Some biomarkers are able to diagnosis ectopic pregnancy from intrauterine pregnancy with high accuracy, but they could not distinguish ectopic pregnancy from spontaneous miscarriage although it is possible that combination of other biomarkers that have discovered yet, can distinguish different types of pregnancy. Biomarkers that have been evaluate for ectopic pregnancy diagnosis can be grouped into 5 categories: 1. Fallopian tube (dys)function, 2. embryo/trophoblast growth, 3. corpus luteum function, 4. inflammation, 5. uterine function and 6. Angiogenesis. In fallopian tube dysfunction, Creatine Kinase (CK)[25, 26] is an EP diagnosis biomarker which is released from damaged muscle and observed to be significantly high in EP patients rather than in cases of missed miscarriage or normal pregnancy; this biomarker has been reported to have 57% sensitivity and 67% specificity in EP diagnosis. Myoglobin, Smooth Muscle Myosin Heavy Chain (SMHC) and Adrenomedullin[27] are other biomarkers in this group but they are not discriminative enough for clinical use. In group of

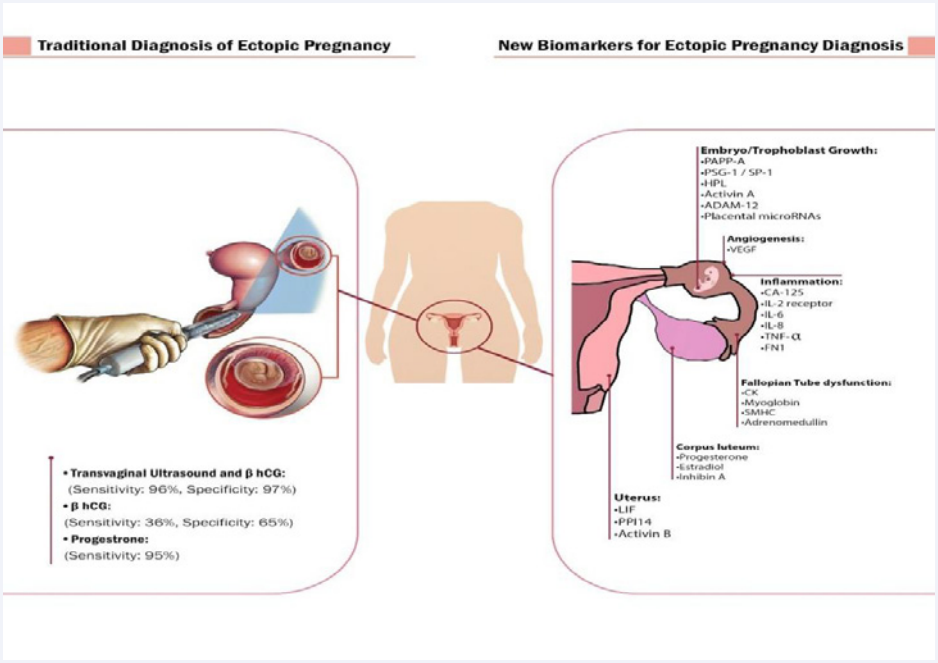


**Figure 1** Clustering of serum biomarkers of ectopic pregnancy according to biological themes vs traditional diagnosis.

**Table 1:** Phases of biomarkers development.

| phase | | |
|---|---|---|
| I | Preclinical exploration | Identification of promising markers |
| II | Developing and validating clinical assays | Diagnoses established diseases using clinical assays |
| III | Testing the efficiency of biomarkers with Retrospective/ longitudinal studies | It is possible to detect diseases early by using biomarkers and to define a screening positive rule |
| IV | Validation in a prospective screening | To evaluate clinical utility of biomarker |
| V | Clinical practice | Screening the effect of biomarker in decreasing burden of disease |

abnormal embryo/ trophoblast growth biomarkers, pregnancy-associated plasma protein-A (PAPP-A)[28] is significantly lower in ectopic pregnancy patients compared to viable pregnancy. Studies show that (PAPP-A) is age- dependent and its concentration is low up to 7 weeks of pregnancy and therefore, it is not discriminative enough to differentiate ectopic pregnancy from spontaneous miscarriage. Pregnancy-specific-glycoprotein 1 (PSG-1 or SP-1) [29]is a biomarker that seems to be lower in EP patients; however, it increases continuously and reaches a plateau in normal pregnancies. Human placental lactogen (HPL) [30] is another biomarker released from placenta and studies show that it is lower in patients with EP than in patients with normal pregnancy but its concentration is discriminative only after 7 weeks. Activin A[31] is a biomarker with significant decrease in ectopic pregnancy patients compared to intrauterine pregnancy or spontaneous miscarriage. A disintegrin and metalloprotease-12 (ADAM-12)[32] is other biomarker that belongs to this group. A case control study shows 97% sensitivity and 37% specificity for EP detection when this biomarker level is ≤48.49 ng/ml. Placental microRNAs[31] that regulate gene expression in pregnancy can be used as EP detection biomarkers. Serum placental miR-323-3p is one of them that increases in ectopic pregnancy. In the group of markers of abnormal corpus luteum function, Progesterone, Estradiol and inhibine A[33-35] are some valid biomarkers that have high sensitivity and specificity in EP diagnosis. Another group includes markers of inflammation such as Cancer Antigen-125 (CA-125)[36, 37], interleukin (IL)6, IL-8, IL receptor 2, tumor necrosis factor-a (TNF-a)[38] and glycoprotein fibronectin (FN1)[39]. Uterine markers of abnormal implantation (Leukaemia inhibitory factor (LIF)[40], placental protein-14 (PP14)[41], Activin B and markers of abnormal angiogenic response are the last groups of biomarkers, of which, Vascular Endothelial Growth Factor (VEGF)[42, 43] has the highest sensitivity and specificity. The strength of these articles is that a large number of biomarkers are identified and their validities are discussed. This study also discussed the use of proteomics in identifying novel biomarkers. All the biomarkers reviewed in this paper are not reliable enough for clinical use and more studies are needed to confirm their accuracy.

Rausch ME et al. [44] assessed biomarkers in ectopic pregnancy diagnosis. In this study a large set of biomarkers that have discriminative ability were evaluated and validated. This paper surveyed 100 ectopic pregnancy patients and 100 patients with intrauterine pregnancy that presented to their centers with abdominal pain and vaginal bleeding in their first trimester.

Twelve biomarkers were evaluated with the classification tree analysis. Single biomarkers were found to be inadequate to differentiate ectopic pregnancy from intrauterine pregnancy but a combination of two or three of them in different pathways was found to improve their detection ability. They developed four biomarker sets containing progesterone, inhibin A, Activin A and VEGF (Vascular endothelial growth factor) for use in a two-step approach for EP recognition with 99% (96 – 100%) diagnosis accuracy. The strengths of this study were the number of biomarkers that were evaluated and the sample size of study.

Barnhart K et al. [45] developed multiplexed serum biomarker tests for ectopic pregnancy diagnosis in cases of non-diagnostic transvaginal ultrasound. There are some distinct phases to discover a diagnosis biomarker (table 1) and studies have developed several biomarkers that are accurate in predicting ectopic pregnancy detection but none of them has been approved or have progressed to Phase IV yet. Barnhart and coworkers specified individual biomarkers based on different functions, but they also posited that panels of multiple biomarkers that combine several diagnosis biomarkers can have better detection capabilities. Their study assessed 12 ectopic pregnancy diagnosis biomarkers. The following findings were reported: 1) in developed biomarkers, none are associated to ectopic pregnancy directly and 2) individual biomarkers are not adequate to distinguish ectopic pregnancy from intrauterine pregnancy. Among individual biomarkers, inhibin A, progesterone, activin A, VEGF, pregnancy-specific b-1-glycoprotein and PAPP-A have different expression in ectopic pregnancy patients compared to women with intrauterine pregnancy. They proposed a strategy to maximize sensitivity and specificity of biomarkers panel using classification and regression tree analysis. They conducted a two-steps diagnostic algorithm with 4 biomarkers (progesterone, VEGF, inhibin A and activin A) that showed 100% specificity and 98% sensitivity. This diagnostic algorithm could perfectly identify EP even in patients that were not diagnosed with ultrasound tests. There are other diagnostic biomarker panels that can have a high accuracy in EP identification such as: inflammatory cytokines IL-6, IL-8 and TNF-a; PAPP-A, inhibin A, activin A; cancer antigen-125, CK and also individual biomarkers including: Progesterone, inhibin and human chorionic gonadotrophin that are able to distinguish ectopic pregnancy from intrauterine pregnancy. Discovering new biomarkers to aid clinicians in managing patients surgically or medically is complex and challenging due to the variety and low concentrations of proteins. This paper presented disintegrin and metalloprotease (ADAM)-12 and isthmin 2 as new diagnostic biomarkers. Validation assay tests on (ADAM)-12 resulted in 78% specificity and 100% sensitivity in differentiating ectopic pregnancy from intrauterine pregnancy. A combination of (ADAM)-12, progestogen-associated endometrial protein and chorionic somatomammotropin hormone-1 can significantly improve the discriminatory power.

Horne AW et al. [46] also reported tubal ectopic pregnancy to be a major cause of pregnancy-related death and maternal morbidity in the first trimester. The study reported that while many studies have discovered biomarkers that show discriminatory powers between EP and other forms of pregnancies, they have been found to have limitations for clinical use. According to Horne and coworkers, biomarkers such as Estradiol, Pregnancy associated plasma protein A and cancer antigen 125 have the ability to differentiate between EP and IUP but are unable to differentiate non-viable intrauterine pregnancy from EP. The authors posited that the limitations in the utility of these biomarkers could be because of variations in the study design. Many of the studies were small cohort examinations and ectopic pregnancy prevalence was not constant during the study. Many of the biomarkers have limitation due to conflicting results. Genomic technology is increasingly being used to identify new diagnosis biomarkers. For example, investigations show lower activin B concentrations in decidualized endometrium of women with tubal ectopic pregnancies[47]. These findings have led to the discovery of multiple serum biomarkers for EP diagnosis.

## DISCUSSION & CONCLUSION

Early EP diagnosis and treatment can decrease pregnancy-related mortality and preserve fertility in patients. Several investigations have presented algorithmic approaches to diagnosis of ectopic pregnancy, which includes clinical examination, transvaginal ultrasound, and serum biomarkers for EP prediction, but these algorithms must be customizable to any kind of patient. Some patients are at high risk of ectopic pregnancy due to their history (previous ectopic pregnancy, pelvic inflammation, infertility treatment) and are also more vulnerable to tubal rupture. Some patients have unstable situations and hemoperitoneum. In such cases, a sensitive and accurate diagnosis algorithm should be able to help clinicians to diagnose or exclude ectopic pregnancy in early stages. Investigations show that the algorithmic combination of ultrasound and β hCG is the best approach for EP diagnosis, compared to progesterone because approaches using progesterone have more missed EPs and interrupted intrauterine pregnancies. Algorithms that have ultrasound as the first step have high sensitivity and specificity to confirm or rule out ectopic pregnancy in women presented to the emergency departments with abdominal pain and vaginal bleeding. In situations of non-diagnostic ultrasound, β hCG measurement can help clinicians for EP detection and the patient is treated according to β level. After reviewing the diagnostic algorithms, we provide an optimal algorithm that is used by clinicians at Royan Institute for early EP diagnosis [Figure2].

In addition to development of diagnostic algorithms, there is a need to identify biomarkers for early detection or EP prediction [Table 2]. New biomarkers have been identified from different biological functions such as implantation and pregnancy stages with gene expression microarray technology. Investigations have presented several biomarkers for EP diagnosis and prediction, but there is no evidence that these biomarkers by themselves are discriminative enough. Investigations have shown that multiple candidate biomarkers are more efficient than any single biomarker. Although there is no biomarker that is directly related to EP, studies have offered a panel of biomarkers containing progesterone, inhibin A, activin A and VEGF that can differentiate intrauterine pregnancy from EP. More studies and clinical examinations are needed for clinical use of biomarkers for the purpose of EP predictions.

There are several algorithmic approaches based on transvaginal ultrasound and/or biomarkers for EP recognition,

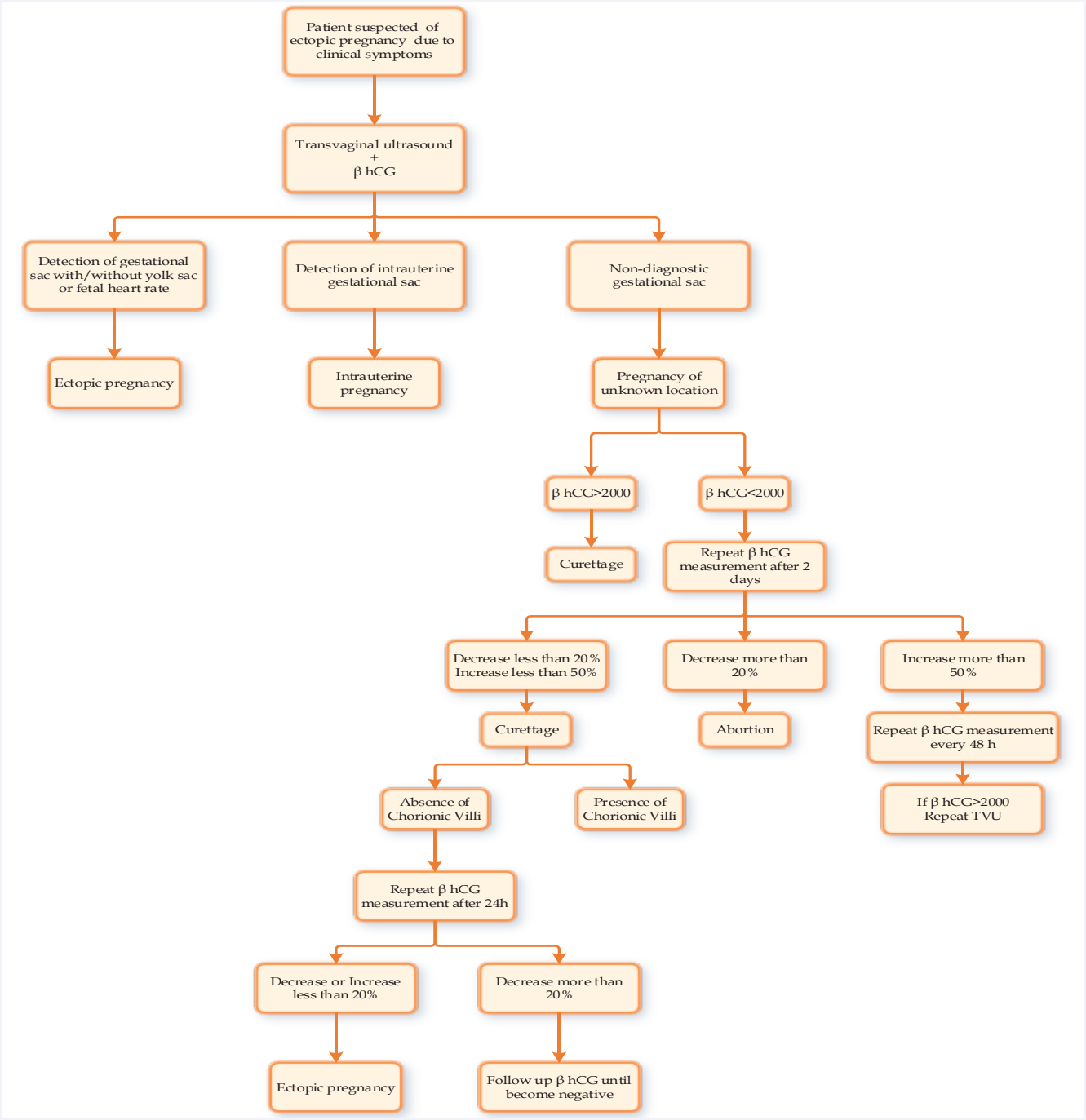


**Figure 2** Early ectopic pregnancy recognition based on serum β hCG and ultrasound Royan Institute.

**Table 2**: Categories of recent researches conducted in ectopic pregnancy diagnosis based on new biomarkers and algorithms.

| Research | Main context | Advantage | Weakness | New findings |
|---|---|---|---|---|
| Gracia CR et al [12] | - Six strategies of EP recognition | -Offered the most optimal algorithm | -Did not consider some factors which effect on results such as: maternal obesity | -EP recognition algorithm |
| Fernandez H et al[13] | -Analyzed six strategies for EP detection | - Considered high risk and suspected patients | -Did not offer new algorithmic approaches | -Introduced the most optimal algorithm |
| Lee R et al [18] | - Algorithm for early EP recognition | -Considered normal and abnormal adnexa for better outcome | - Did not consider patients in high risk such as: previous EP | - EP recognition algorithm |

| | | | | |
|---|---|---|---|---|
| Lozeau A-M et al [1] | - Conservative algorithms for suspected ectopic pregnancy | -Considered high risk and low risk patients | -High time complexity in diagnosis algorithm | - Algorithmic approaches to diagnosis suspected EP |
| Murray H et al [19] | - Algorithms for EP diagnosis | -Introduced serum progesterone measurement | -Did not consider some factors that exist in high risk patients | -Progesterone measurement can be useful beside the hCG measurement |
| Van Mello NM et al [20] | - Offered two diagnostic algorithms | -Measurement of Progesterone | - Did not compare strategies to find the optimal algorithm | - progesterone level as a conjunction tool with βhCG level |
| Seeber BE et al [21] | - EP recognition algorithms | - Evaluated the rise or fall of βhCG in EP patients | -Didn't consider high risk patients | -EP recognition algorithm |
| Tong S et al [22] | - Discovered biomarkers in EP diagnosis | -Evaluated a large set of biomarkers | - No clinical trials were conducted | -Superior accuracy in combination of biomarkers |
| Rausch ME et al.[23] | -Discovered new detective biomarkers | - Evaluated a large set of biomarkers | - No clinical trials were conducted | -Offered new set of biomarkers for EP detection |
| Reid S et al.[24] | - Discovered biomarkers in EP diagnosis | - Evaluated a large set of biomarkers | -More investigations need to prove biomarkers efficiency | Offered new set of biomarkers for EP detection |
| Rausch ME et al [44] | - Evaluated biomarkers efficiency in EP diagnosis | -Evaluated large scales of biomarkers | - No clinical trials were conducted | -Developed four biomarker sets that is efficient in EP detection |
| Barnhart K et al[45] | - New multiplexed serum biomarker | -Evaluated the known biomarkers efficiency | -More investigation needed to prove biomarkers utility | -Multiplex biomarkers have more utility for EP detection |
| Horne AW [46] | -Discovering new biomarkers for tubal EP | -Limitation of biomarkers utility | - No clinical trials were conducted | -Suggested activin B as a tubal EP diagnosis biomarker |

but two diagnosing algorithms that combine transvaginal ultrasound and β hCG measurement (1. ultrasound followed β hCG and 2. β hCG followed ultrasound) have fewest missed diagnosis of ectopic pregnancy. Further investigations have shown that using transvaginal ultrasound as the first step has the fewest interrupted intrauterine pregnancies. This type of algorithmic approach is more accurate and sufficient for early EP diagnosis and can result in the best outcome. For the purpose of early EP detection or in a situation of non-diagnostic result in transvaginal ultrasound, new serum biomarkers can help and this necessitates identification of these biomarkers. To the date more than 20 biomarkers have been identified to have some level of accuracy and discriminatory value for early EP prediction, but none of them has hitherto passed all phases of investigations. Studies have shown that a set of diagnostic biomarkers has better EP detection than any single biomarker. A set comprising progesterone, inhibin A, activin A and VEGF is recommended for EP diagnosis with acceptable accuracy but this has not been subjected to clinical validation yet. More studies are needed to establish the efficacy in terms of sensitivity and specificity of new sets of biomarkers for ectopic pregnancy prediction.

## REFERENCES

1. Lozeau AM and Potter BJAfp. Diagnosis and management of ectopic pregnancy. Am Fam Physician. 2005; 72:1707-1714.
2. Shaikh NB, Shaikh S and Shaikh FJJoAMCA. A clinical study of ectopic pregnancy. J Ayub Med Coll Abbottabad. 2014; 26:178-181.
3. Goldner TE, Lawson HW, Xia Z, Atrash HK. Surveillance for ectopic pregnancy—United States, 1970–1989.
4. MMWR CDC Surveill Summ. 1993; 42: 73-85.
5. Valley VT, Mateer JR, Aiman EJ, Thoma ME, Phelan MB. Serum progesterone and endovaginal sonography by emergency physicians in the evaluation of ectopic pregnancy. Acad Emerg Med. 1998; 5: 309-313.
6. Taylor KJ and Meyer WR. New techniques in the diagnosis of ectopic pregnancy. Obstet Gynecol Clin North Am.1991;18:39-54.
7. Condous G, Emeka Okaro, Asma Khalid, Chuan Lu, Sabine Van Huffel, et al. The accuracy of transvaginal ultrasonography for the diagnosis of ectopic pregnancy prior to surgery. 2005; 20: 1404-1409.
8. Perveen F and Tayyab SJJOSP. Ruptured ectopic pregnancy: clinical presentation and management. 2007; 12: 47.
9. Cacciatore B, Stenman UH, Ylöstalo P. Diagnosis of ectopic pregnancy by vaginal ultrasonography in combination with a discriminatory serum hCG level of 1000 IU/1 (IRP). Br J Obstet Gynaecol. 1990; 97: 904-908.
10. Kung FT and Chang SY. Efficacy of methotrexate treatment in viable and nonviable cervical pregnancies. Am J Obstet Gynecol. 1999; 181: 1438-1444.
11. Das N, Topalovic M, WJCoipm Janssens. Artificial intelligence in diagnosis of obstructive lung disease: current status and future potential. Curr Opin Pulm Med. 2018; 24:117-123.
12. Gruber KJTLDH. Is the future of medical diagnosis in computer algorithms? 2019; 1: e15-e16.
13. Gracia CR, Barnhart KTJO. Diagnosing ectopic pregnancy: decision analysis comparing six strategies. Obstet Gynecol. 2001; 9: 464-470.
14. Fernandez H and Hru AJG. Ectopic pregnancies after infertility treatment: modern diagnosis and therapeutic strategy. Hum Reprod Update. 2004.10: 503-513.
15. Kaplan BC, Dart RG, Moskos M, Kuligowska E, Chun B, et.al. Ectopic pregnancy: prospective study with improved diagnostic accuracy. Ann Emerg Med. 1996; 28:10-17.
16. Barnhart K, Mennuti MT, Benjamin I, Jacobson S, Goodman D, et al. Prompt diagnosis of ectopic pregnancy in an emergency department setting.Obstet Gynecol. 1994; 84: 1010-1015.
17. Stovall TG, Ling FW, Cope BJ, Buster JE. Preventing ruptured ectopic pregnancy with a single serum progesterone. Am J Obestet Gynecol. 1989; 160:1425-1431.

18. Goldstein SR, Snyder JR, Watson C, Danon M. Very early pregnancy detection with endovaginal ultrasound. Obstet Gynecol. 1988; 72: 200-204.

19. Robert Lee, Carolyn Dupuis, Byron Chen, Andrew Smith, Kim YH .Diagnosing ectopic pregnancy in the emergency setting. Ultrasonography. 2018; 37:78.

20. Heather Murray, Hanadi Baakdah, Trevor Bardell, Togas Tulandi. Diagnosis and treatment of ectopic pregnancy. CMAJ. 2005; 173: 905-912.

21. van NMM, Femke Mol, Ankum WM, Willem BM, van FdV, et al. Ectopic pregnancy: how the diagnostic and therapeutic management has changed. Fertil Steril. 2012; 98: 1066-1073.

22. Seeber BE and Barnhart KT. Suspected ectopic pregnancy. Obstet Gynecol. 2006; 107: 399-413.

23. Tong S, Skubisz MM and Horne AW. Molecular diagnostics and therapeutics for ectopic pregnancy. Mol Hum Reprod. 2015; 21: 126-135.

24. Rausch ME, Barnhart KJCO. Serum biomarkers for detecting ectopic pregnancy. Clin Obstet Gynecol. 2012; 55:418.

25. Shannon Reid, Ishwari Casikar, Kurt Barnhart, George Condous. Serum biomarkers for ectopic pregnancy diagnosis. Expert Opin Med Diagn. 2012; 6:153-165.

26. Birkhahn RH, Gaeta TJ, Leo PJ, Bove JJ. The utility of maternal creatine kinase in the evaluation of ectopic pregnancy.Am J Emerg Med. 2000; 18: 695-697.

27. Darai E, Vlastos G, Benifla JL, Sitbon D, Hassid J, et al. Is maternal serum creatine kinase actually a marker for early diagnosis of ectopic pregnancy? Eur J Obstet Gynecol Reprod Biol.1996; 68: 25-27.

28. Birkhahn RH, Gaeta TJ, Paraschiv D, Bove JJ, Suzuki T, et al. Serum levels of myoglobin, creatine phosphokinase, and smooth muscle heavy-chain myosin in patients with ectopic pregnancy. Ann Emerg Med. 2001; 38: 628-632.

29. Patrick Dumps , Arielle Meisser, Dominique Pons, Ange MM, Anguenot JL, et al. Accuracy of single measurements of pregnancy-associated plasma protein-A, human chorionic gonadotropin and progesterone in the diagnosis of early pregnancy failure. Eur J Obstet Gynecol Reprod Biol. 2002; 100: 174-180.

30. Horne CH, Towler CM, Pugh-Humphreys RG, Thomson AW, Bohn H. Pregnancy specific β 1—a product of the syncytiotrophoblast. Experientia. 1976; 32: 1197-1199.

31. Handwerger S, Freemark MJ. The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development. J Pediatr Endocrinol Metab. 2000; 13: 343-356.

32. Joshua Warrick, Ann Gronowski, Courtney Moffett, Quihong Zhao, Emily Bishop, et al. Serum activin A does not predict ectopic pregnancy as a single measurement test, alone or as part of a multi-marker panel including progesterone and hCG. Clin Chim Acta. 2012; 413: 707-711.

33. Huppertz B, Bartz C and Kokozidou MJM. Trophoblast fusion: fusogenic proteins, syncytins and ADAMs, and other prerequisites for syncytial fusion. Micron. 2006; 37: 509-517.

34. Jorine Verhaegen, Gallos ID, van NMM, Mohamed Abdel-Aziz, Yemisi Takwoingi, et al. Accuracy of single progesterone test to predict early pregnancy outcome in women with pain or bleeding: meta-analysis of cohort studies. BMJ. 2012; 345.

35. Guillaum JE, Benjamin F, Sicuranza BJ, Deutsch S, Seltzer VL, et al. Serum estradiol as an aid in the diagnosis of ectopic pregnancy. Obstet Gynecol. 1990; 76: 1126-1129.

36. Illingworth PJ, Groome NP, Duncan WC, Grant V, Tovanabutra S, et al. Measurement of circulating inhibin forms during the establishment of pregnancy. J Clin Endrocrinol Metab. 1996; 81: 1471-1475.

37. G Condous-G , Kirk E, Lu C, Van SH, Gevaert O, et al. Diagnostic accuracy of varying discriminatory zones for the prediction of ectopic pregnancy in women with a pregnancy of unknown location. Ultrasound Obstet Gynecol. 2005; 26: 770-775.

38. Ilias Katsikis, David Rousso, Dimitrios Farmakiotis, Anargyros Kourtis, Evanthia Diamanti-Kandarakis, et al. Receiver operator characteristics and diagnostic value of progesterone and CA-125 in the prediction of ectopic and abortive intrauterine gestations. Eur J Obstet Gynecol Reprod Biol. 2006; 125: 226-232.

39. David Soriano, Danielle Hugol, Nhuan Tran Quang, Emile Darai. Serum concentrations of interleukin-2R (IL-2R), IL-6, IL-8, and tumor necrosis factor alpha in patients with ectopic pregnancy. Fertil Steril. 2003; 79: 975-980.

40. Roumen Pankov, KM. Fibronectin at a glance. J Cell Sci. 2002; 115:3861-3863.

41. Daponte A, Pournaras S, Zintzaras E, Kallitsaris A, Lialios G, et al. The value of a single combined measurement of VEGF, glycodelin, progesterone, PAPP-A, HPL and LIF for differentiating between ectopic and abnormal intrauterine pregnancy. Hum Reprod. 2005; 20: 3163-3166.

42. Ruge S, Sørensen S, Vejtorp M, Vejerslev LO. The secretory endometrial protein, placental protein 14, in women with ectopic gestation. Fertil Steril. 1992; 57: 102-106.

43. Smith SKJHR. Angiogenesis and implantation. Hum Reprod. 2000; 15: 59-66.

44. Daniel Y, Geva E, Lerner-Geva L, Eshed-Englender T, Gamzu R, et al., Levels of vascular endothelial growth factor are elevated in patients with ectopic pregnancy: is this a novel marker? Fertil Steril. 1999; 72: 1013-1017.

45. Rausch EM, Sammel MD, Peter Takacs, Karine Chung, Alka Shaunik, et al. Development of a multiple marker test for ectopic pregnancy. Obstet Gynecol. 2011; 117: 573-582.

46. Barnhart K and DWJEromd Speicher. Molecular diagnosis of ectopic pregnancy. Expert Rev Mol Diagn. 2011; 11: 759-762.

47. Horne AW, Duncan WC, HOJAoegS Critchley. The need for serum biomarker development for diagnosing and excluding tubal ectopic pregnancy. Acta Obstet Gynecol Scand. 2010; 89: 299-301.

48. Horne AW, van SdD, King AE, Burgess S, Myers M, et al. Endometrial inhibin/activin β-B subunit expression is related to decidualization and is reduced in tubal ectopic pregnancy. J Clin Endocrinol Metab. 2008; 93: 2375-2382.

**Cite this article**

*Janan M, Ghaffari F, Eftekhari-Yazdi P, Bolhasani H, Hassani F (2021) Early Diagnosis of Ectopic Pregnancy Based on Algorithmic Approaches and New Biomarkers: A Narrative Review. J Embryol Develop Biol 2(1): 1004.*