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Biomarkers for Ectopic Pregnancy and Pregnancy of Unknown Location

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Abstract

Early pregnancy failure is the most common complication of pregnancy, and 1–2% of all pregnancies will be ectopic. As one of the leading causes of maternal morbidity and mortality, diagnosing ectopic pregnancy and determining the fate of a pregnancy of unknown location are of great clinical concern. Several serum and plasma biomarkers for ectopic pregnancy have been investigated independently and in combination. The following is a review of the state of biomarker discovery and development for ectopic pregnancy and pregnancy of unknown location.

Keywords

ectopic pregnancy; biomarker; pregnancy of unknown location; proteomics

Introduction

Symptoms of vaginal bleeding or abdominal pain can occur in 25–30% of viable pregnancies; however this clinical presentation also raises suspicion for early pregnancy failure (1). Early pregnancy failure is the most common complication of pregnancy, as roughly 25% of recognized pregnancies end in miscarriage and 1–2% will be ectopic pregnancies. Recent epidemiologic studies suggest that 6% of maternal deaths in the US are attributable to ectopic pregnancies (2). As one of the leading causes of morbidity and mortality worldwide, accurate and expeditious diagnosis of ectopic pregnancy is of particular concern. Of note, the diagnosis and management of women at risk for early pregnancy failure has not changed dramatically in decades. Novel biomarkers could change this paradigm.

Current standard of care for the diagnosis of ectopic pregnancy includes serial serum human chorionic gonadotropin (hCG) levels and pelvic ultrasound (3–8). Unfortunately, ultrasound at first presentation is inconclusive in up to 40% of women likely because a pregnancy has not progressed enough to be visualized by ultrasound, or it has failed before a gestational sac has formed (or has collapsed). Thus, ultrasound cannot determine location or viability of a gestation in a substantial number of women with an early pregnancy.

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Diagnosis of a women who initially present with a pregnancy of unknown location (PUL) (9–12), requires multiple visits for blood tests, ultrasounds, and possibly surgical procedures before a definitive diagnosis can be made. In the time required to make a diagnosis an ectopic pregnancy could rupture, leading to impaired future fertility and potentially life-threatening intra-abdominal hemorrhage. Not only is early diagnosis of ectopic pregnancy essential to avoid the added morbidity of delay in treatment, but also, differentiation of this state from that of an abnormal intrauterine gestation is crucial since optimal treatment strategies differ, and could impact management of future pregnancies(10). A biomarker may be able to aid in determining location or viability of an early gestation, or may aid in determining which patient is best treated urgently, surgically, medically or expectantly. As such, a number of investigators have focused efforts on the use of novel serum biomarkers for modeling early pregnancy outcomes.

There are numerous factors that are involved in the fate of a first trimester pregnancy, but due to a paucity of animal models of ectopic pregnancy, the relationships between these factors are incompletely understood (13). In developing a predictive model for early pregnancy outcome it is important to consider the implantation environment as well as the embryo itself. Factors can be categorized into subgroups based on physiology and include factors inherent to the embryo, which can be captured by markers of trophoblast function, factors reflective of corpus luteal function, those of endometrial function, and angiogenesis (figure 1). Furthermore, in tubal pregnancies, markers of inflammation, tubal muscle damage, and altered transport have been considered.

When considering biomarkers of EP and PUL, one must consider the characteristics of the ideal biomarker as well as the necessary steps to bring such a tool to the clinical arena. Since the fate of a pregnancy's location is determined prior to serum hCG detection, the ideal biomarker would be present in the early first trimester potentially before the pregnancy develops to the level of the ultrasound-based hCG discriminatory zone. Furthermore, the ideal marker would be consistent, accurate, inexpensive, and could be used at the point of care. As such, serum biomarkers are of particular interest.

Biomarker development for clinical use is generally divided into four phases: (I) preclinical exploration, (II) clinical assay development, (III) assessment of predictive ability in a retrospective cohort or case control study, and finally, (IV) validation in a prospective setting (14–18). The following reviews the most promising biomarkers for EP based on their role in the pathophysiology of abnormal gestation and their current state in biomarker development.

Markers of Trophoblast Function

Human Chorionic Gonadotropin

Human Chorionic Gonadotropin (hCG) is the most widely studied biomarker of early pregnancy outcome, and is the only biomarker that is used routinely in clinical practice. As a single value it is non-diagnostic, however serial serum hCG levels are helpful in identifying patients who require closer surveillance for early pregnancy failure. The expected hCG rise in 48 hours for a viable IUP is at least 53% (19), although a recent study suggests that the optimal accuracy for correctly identifying a viable IUP may require a more conservative threshold of a 35% rise in 48 hours (20). A model incorporating hCG ratio (serum hCG at 48 hours/serum hCG at 0 hours) suggested a ratio<0.21 would predict EP with 91.7% sensitivity and 84.2% specificity in a cohort of patients with pregnancy of unknown location (21). An extension of this model did not validate well in a US population, with low sensitivity for EP (22, 23). Thus, while not sensitive for EP alone, the use of hCG has reached Phase IV of development as a serial marker for diagnostic triage of PUL.

Hyperglycosylated hCG

Hyperglycosylated hCG (hCG-H) is produced by extravillous cytotrophoblasts and is secreted from the time of implantation (24). A single value of hCG-H and proportion of hCG-H/hCG have both been proposed as markers of early pregnancy failure. One study identified a cutpoint of 13ug/L, below which pregnancies were likely to fail, reporting 73% detection and 2.9% false positive rates, corresponding to 73% sensitivity and 98.1% specificity (25). However, this study did not demonstrate the ability of this marker to discriminate between EP and abnormal IUPs. Another study from the same group sought to use a hCG-H/hCG ratio >50% to differentiate viable pregnancies from early pregnancy failure (26). While this cutoff identified all patients with early pregnancy failure, at levels below 50% one could not differentiate viable IUP from pregnancy failure in 35% of patients. As a single marker hCG-H has insufficient accuracy; however, perhaps with validation of other cutpoints this marker may move beyond Phase III of biomarker development.

Activin A

Activin A is a dimeric glycoprotein in the TGF-beta superfamily and a placental product that promotes cytotrophoblast invasion (27); thus, abnormalities in Activin A may be a harbinger of abnormal implantation and viability. A study of patients with PUL found an Activin A level 0.37ng/mL had 100% sensitivity and 99.6% specificity for the diagnosis of EP (28). In one study Activin A had had 80% sensitivity and 72% specificity as a single marker in a cohort of patients with EP or IUP (29). However, two groups did not find a difference in Activin A levels when including patients with resolving PULs or abnormal IUPs (30, 31). Thus, while initial studies were promising for Activin A as a single marker of extrauterine location, subsequent case-control studies have failed to replicate these findings and Activin A remains in Phase III of development.

Pregnancy-associated plasma protein-A

Pregnancy-associated plasma protein-A (PAPP-A) is also produced by the trophoblast and has been extensively studied as a marker of early pregnancy failure and aneuploidy (32). An early case series of PAPP-A and hCG levels in women with viable IUP, EP, and non-pregnant women suggested that PAPP-A levels were lower in EP compared to viable IUP (33). Since then, PAPP-A has been evaluated in conjunction with other potential markers of abnormal pregnancy. PAPP-A has been assessed as a triple marker test with VEGF and progesterone (34). However, others have found PAPP-A to have 67% accuracy for the diagnosis of EP, but limited utility in a multiple marker setting (29). Neither study considered abnormal IUPs. One study of patients including abnormal IUPs demonstrated PAPP-A levels <14.3ng/mL to have 64.5% sensitivity and 99% specificity for pregnancy failure but stated there was no difference in levels between EPs and abnormal IUPs (35). Two groups confirmed PAPP-A may help identify abnormal pregnancies (36, 37). Thus, data from Phase III of biomarker development suggest a role as a marker of viability, but not extra-uterine location.

Pregnancy-specific beta glycoprotein 1

Pregnancy-specific beta glycoprotein 1 (SP-1) is one the earliest proteins identified in trophoblast cultures and immunohistochemical techniques for biomarker discovery (38, 39), and its potential role in diagnosing ectopic pregnancy was further supported by unbiased proteomic discovery (40). It is thought to be involved in immunomodulation, and low levels are suggestive of EP(41). SP-1 was unable to differentiate EP from abnormal IUP in one study, while another showed only modest discriminatory capacity with 65% sensitivity and 74% specificity at a cutpoint of 103.3ug/mL (29, 42). Further Phase III studies incorporating all relevant outcomes are needed to assess prediction of EP.

Human placental lactogen

Human placental lactogen (hPL), also known as human somatomammotropin, is also produced by the trophoblast and has been studied as a possible marker of abnormal pregnancy. However, two independent investigators found no difference in hPL levels when comparing EPs to abnormal IUPs or viable IUPs, respectively (29, 37). hPL levels were lower in EPs but had strong correlation with hCG, suggesting limited utility as an independent marker of EP (34).

A Disintegrin and Metalloprotease-12

A Disintegrin and Metalloprotease-12 (ADAM-12), is a glycoprotein produced primarily by placental syncytiotrophoblasts and is thought to play a role in syncytial fusion (43). A case control study of patients with EP and viable IUPs demonstrated that a level 48.49ml/mL was 97% sensitivity and 37% specific for EP, suggesting promising utility as a marker of extra-uterine location. However, ADAM-12 has also been explored as a marker of aneuploidy, and as such, its performance amidst a cohort including abnormal IUPs must be considered (44, 45). Others have attempted to validate ADAM-12 and found that it did not discriminate EP from all other outcomes when including treated persisted PULs, spontaneously resolving PULs, and probable EPs (AUC 0.65, $p>0.05$) but when restricting the analysis to well defined PUL outcomes, it had slightly better diagnostic potential (AUC 0.66, $p<0.05$) (46). Further studies are needed to move it to Phase IV of biomarker development, as a single marker or in combination.

Nucleic Acid Markers

Placental mRNAs have altered secretion patterns by extravillous trophoblasts in abnormal pregnancies and thus, have been considered for their diagnostic potential. A recent case-control study of 12 women with EP and 13 women with viable IUP demonstrated that patients with EP have significantly lower hCG and human placental lactogen (hPL) plasma mRNA copy numbers compared to viable IUP (47). Several studies have suggested that placental mRNAs are lower in aneuploid pregnancies, thus levels in women with miscarriage need to be considered (48, 49). Thus, placental mRNAs may be ready for Phase III of biomarker development, particularly as potential markers of viability.

Placental micro-RNAs are 19–25 nucleotide single stranded non-coding RNAs that regulate gene expression and at least 31 micro-RNAs have been associated with pregnancy (50, 51). A study of women with symptomatic PUL found that serum placental miR-323-3p was significantly increased in patients who were ultimately diagnosed as EP compared to viable IUPs and abnormal IUPs; as a single marker it had a 30% sensitivity and 90% specificity for EP (52). While the diagnostic test characteristics of this marker alone are unacceptable for clinical use, in combination with other markers, it may serve as an acceptable marker of extra-uterine location. Further studies are needed to replicate these findings and move this promising marker from Phase III to Phase IV of development.

Markers of Corpus Luteal Function

Progesterone

As a readily available clinical assay, progesterone has been extensively studied as a potential marker of early pregnancy failure and ectopic pregnancy both as a single marker and in combination (29, 30, 34, 37, 42, 52–77). A meta-analysis of 26 studies suggested serum progesterone $<5\text{ng/mL}$ had good prediction for non-viable pregnancies, but was unable to differentiate EPs from abnormal IUPs (75). Furthermore, a high progesterone value did not rule out the possibility of an EP. Thus, progesterone can aid in identifying those at risk for

EP and is in Phase 4 of development, but its optimal utility for predicting EP will likely be in combination with other markers.

Inhibin A

Inhibin A is a heterodimeric peptide that is predominately secreted by the corpus luteum (78). A small case control study suggested that serum levels of Inhibin A were lower in patients with EP compared to viable IUPs but did not report specific test characteristics (79). Another group characterized Inhibin A performance in a population including EPs, viable IUPs, and various stages of abnormal IUPs and reported that a cutpoint of 50 pg/mL had 100% sensitivity and specificity for EP compared to viable IUPs (80). However, those test characteristics were not maintained when comparing EPs to missed abortions (sensitivity 41% specificity 86%) (80). Inhibin A has been evaluated over a 48-hour period with a reported accuracy of 60% for the diagnosis of EP (31). Another group looked at serum hCG, progesterone, Inhibin A, inhibin pro-alpha C-related immunoreactivity and insulin-like growth factor binding protein-1 (IGFBP-1) in a cohort of 109 patients with PUL and found that Inhibin A levels were significantly lower in resolving PULs, but was unable to discriminate EP from viable IUPs in this cohort (81). In contrast, other have found that Inhibin A had 83% sensitivity and 79% specificity for predicting EP in a cohort of EPs and viable IUPs at cutpoint of 28.67 pg/mL, and had the highest accuracy of any single marker studied (29). Thus, while Inhibin A appears to have promise as a marker of viability and has reached Phase III of biomarker development, conflicting results regarding its utility as a marker of extra-uterine location suggest further studies are needed before it can be used clinically.

Markers of Angiogenesis

Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) is an important contributor to the vascular development the fetoplacental unit and can be influenced by hypoxia and various cytokines in the embryonic environment (82). Its use as a marker of EP was first investigated in a case control study of patients with EP, viable IUP and abnormal IUP, and suggested that levels 200 pg/mL had 60% sensitivity and 90% specificity for predicting EP (83). These findings were corroborated by another case-control study (84). VEGF has been assessed as part of a multiple marker and found that a level 174.5 pg/mL had 78% sensitivity and 100% specificity for EP (37). Others did not find a difference in VEGF levels amongst EPs, viIUPs, and abnormal IUPs while a third study found VEGF 28.24pg/mL to have 95% sensitivity and 50% specificity as a single marker (29, 36). VEGF may be a promising marker of extra-uterine location but requires further Phase III and IV development.

Placental like growth factor

Placenta like growth factor (PLGF) is a proangiogenic growth factor that is predominantly produced by trophoblast cells and has been identified at implantation sites (85). A small case series reported that PLGF mRNA expression is lower in trophoblast cells from EP compared to abnormal IUPs and viable IUPs (86). A screening test rule based on PLGF and soluble fms-like tyrosine kinase-1 (sflt-1) levels suggested that a PLGF level greater than 15.73 pg/mL could differentiate viable IUP from nonviable gestations with 86% sensitivity and 67% specificity, but was unable to differentiate EP from abnormal IUP with sufficient accuracy (87). PLGF has yet to be validated in another cohort, and as such remain in Phase III of development.

Markers of Endometrial Function

Leukemic Inhibitory Factor

Leukemia inhibitory factor is a cytokine in the interleukin 6 family that plays a role in inflammation and implantation. Studies of LIF mRNA expression and secretion in tubal stromal cell cultures led to the hypothesis that LIF level would be differential in those with intrauterine processes compared to tubal EPs (88). In a study of 40 patients with diagnoses of EP, IUP, spontaneous abortion and threatened abortion, LIF < 6.2pg/mL had 73% sensitivity and 89% specificity for EP (89). However, attempts at validation of LIF have yielded conflicting results. Two groups found no significant difference in serum LIF levels in patients with EP compared with viable IUP, while another group found increased LIF levels in patients with EP compared with viable IUP (37, 90, 91). Furthermore, LIF levels were undetectable in serum from EP and viable IUP (34). Inconsistency of results limits the role of this marker.

Glycodelin

Glycodelin is a protein found in the endometrium and fallopian tube that is associated with immunomodulation during implantation (92). In a study of 169 women in the first trimester of pregnancy, glycodelin levels were found to be significantly lower in patients with EP compared to incomplete abortion and viable IUPs (93). Three groups looked at the performance of serum glycodelin in a multiple marker setting: two found that levels were significantly lower in patients with EP but underperformed other markers, while another found no difference in levels between EP and abnormal IUP (29, 34, 37). None of these studies examined EP with both viable IUP and abnormal IUP. Thus, as a marker of extra-uterine location, glycodelin warrants further investigation at the Phase III level before prospective studies may be considered.

Mucin-1

Mucin-1 (MUC1) is an epithelial apical surface glycoprotein expressed in human endometrium and fallopian tube epithelium that is involved in blastocyst-endometrial interactions during implantation (94). MUC1 expression in fallopian tube tissue is reduced in EPs compared to pseudo-pregnant and nonpregnant states suggesting a greater receptivity for extra-uterine implantation; however its use as a serum protein biomarker has not yet been explored (Phase I–II) (95–97).

Adrenomedullin

Adrenomedullin is peptide hormone in the calcitonin/calitonin gene-related peptide (CGRP)/ amylin family that promotes endometrial angiogenesis. Studies of adrenomedullin expression in fallopian tube tissue suggest that plasma levels are lower in those with EP compared to viable IUP (98). Adrenomedullin expression needs further exploration in the abnormal IUP state, as well as assay development to assess its utility in the clinical setting (Phase I).

Markers of Inflammation and Muscle Damage

Several markers of peritoneal inflammation have been proposed including IL-6, IL-8, IL-10, IL-11, CA-125, and TNF-alpha. One study of 72 women suggested that concentrations of IL-6, IL-8 and TNF-alpha were significantly higher in women with EP compared to both viable IUPs and abnormal IUPs, and reported IL-8 > 40 pg/ml has 82.4% sensitivity and 81.8% specificity for EP (99). In another study, IL-6 showed no difference between EP and viable IUP and while IL-8 and TNF-alpha were significantly lower in EP compared to viable IUP, their accuracy for EP was less than 60% (29). IL-10 and IL-11 had no significant

differences in levels in a cohort of women with EPs and viable IUPs (90). Studies of CA-125 have had conflicting data with some suggesting levels are increased in EP (100), others suggesting decreased levels (42, 101), and some suggesting no difference in both retrospective and prospective settings (102–104). Thus, while inflammatory markers have theoretical promise, the inherent variability in inflammatory response limits the accuracy and consistency needed for clinical use.

Markers of muscle damage including creatine kinase, smooth muscle heavy chain myosin, myoglobin have also been explored for prediction of EP. While some studies have suggested that creatine kinase levels are significantly increased in EP compared to viable IUP, (105–107) it has failed to validate as a marker of EP in subsequent studies (57, 108–112). Both myoglobin and smooth muscle heavy-chain myosin have failed to show differences in serum levels amongst EP compared to other pregnancy outcomes (105). Markers of muscle damage may be more likely to detect impending rupture, as opposed to extrauterine location; thus their use for diagnostic triage in a stable PUL is likely limited.

Markers of Impaired Tubal Transport

Prior tubal disease is a known risk factor for ectopic pregnancy, but exact mechanisms of this predisposition are not fully understood at the molecular level. Alterations in paracrine signaling, abnormal tubal smooth muscle contractility, attenuated cytokine signals, and pathologic angiogenesis are all associated with EP (113). Abnormalities in the endocannabinoid system have also been implicated. Two studies of protein expression in fallopian tubes from women with ectopic pregnancy have suggested that high anandamide levels and reduced receptor expression (CB1) are associated with EP (114, 115). Further preclinical and clinical studies including development of an appropriate assay, are needed (Phase I).

Multiple marker tests

Given the complex processes involved in the establishment and maintenance of a viable pregnancy, it is not surprising that no single marker has been able to consistently predict PUL outcome with sufficient accuracy (Table 1). Thus, prediction modeling has expanded to incorporate various pathways in a multiple marker test (Table 2) (30, 34, 35, 52, 60). While the concept of exploring multiple markers simultaneously is not new, statistical analyses that allow for complex regression modeling have enabled investigators to explore the interactions, or synergies, of a multiple marker test (116). A case control study of 200 patients with EP or viable IUP demonstrated that a four-marker test including Progesterone, VEGF, Inhibin A, and Activin A derived by this methodology could predict EP with 100% accuracy in those with an hCG <1500 mIU/mL (29). Further studies incorporating abnormal IUPs are necessary to fully assess the discriminatory capacity of such a test.

Biomarker Discovery and Future Directions

Novel Biomarker Discovery

Recognizing that the pathways involved in early pregnancy failure are incompletely understood, unbiased proteomics has also been explored as a method of biomarker discovery. The established approach for proteomics utilizes panels of monoclonal antibodies to deplete serum proteins (117). A newer approach known as shotgun proteomics incorporates combinatorial ligand library pre-fractionation to normalize the dynamic range of serum proteins (40, 118). A recent study used a label-free 3-D serum proteome comparison of serum from patients with EPs and viable IUPs to identify 9 biomarker candidates including ADAM12, isoforms of the beta-1 glycoprotein family, PAPP-A,

progesterone-associated endometrial protein (PAEP or glycodeilin), and chorionic somatomammotropin precursor (CSH1). A case-control study later confirmed that ADAM-12 could differentiate EP from viable IUP (AUC=0.81), thus validating this method for novel biomarker discovery (119). Similar methodology was used to identify Serum fibronectin as a candidate biomarker (AUC=0.64 for total cohort) by another group, although further study is needed in a larger cohort of EPs (Society for Gynecologic Investigation Abstract 2012, publication pending).

Issues in Biomarker Development

As the search for markers to predict PUL outcome continues, there are several challenges to address. First of all, many of the biomarkers studied will vary with gestational age. Gestational age can be difficult to accurately ascertain in a prospective setting, and may be unknown in up to 10% of patients presenting with symptomatic early pregnancies. This variable may require adjustment for application in the clinical setting. Alternatively, including gestational age independent markers like certain microRNAs may be helpful (52). Second, one must consider the impact of other pathophysiologic processes of pregnancy such as hypertensive disorders and aneuploidy and how these may impact biomarker interpretation (24, 32, 44, 45, 120). In addition, some markers, such as progesterone, may have altered expression in a pregnancy resulting from assisted reproduction. Therefore, any clinical tool would need to be separately validated with this subpopulation.

Conclusion

The introduction of novel marker of early pregnancy failure could dramatically affect clinical care. There are a number of promising biomarker candidates for use in women at risk for EP or with a PUL. A successful panel of markers will likely be derived from a number of biologic pathways and supplemented with markers from unbiased discovery. It is also likely that the panel will contain some markers that assess viability, while other may assess the location of implantation. Thus, biomarkers may ultimately aid in the discrimination of gestational location, viability or identification of those best served with surgical, medical, or expectant management. However, markers in this review are not ready for clinical use and will need to be validated in prospective cohorts representative of EPs, viable IUPs, abnormal IUPs, and resolving PULs. While progress has been made in discovery and validation, much is still to be done before a marker test can be used independently at the point of presentation in clinical care.

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Figure 1.
Early Pregnancy Biologic Pathways

Table 1

Individual Biomarker Performance for Prediction of EP

Biomarker	Study	Cut-Point	Sensitivity	Specificity	Phase of Development/Comments	
TROPHOBLAST FUNCTION						
hCG	48 hour hCG rise	Barnhart '04, Morse '12	<53% rise	91.1%	66.6%	III; insufficient specificity for extra-uterine location
	M1 model	Condous '04	n/a	91.7%	84.2%	IV (for failed PUL); not accurate for diagnosis of EP
	M4 model	Condous '07 Barnhart '11	n/a	80.8% 49.0%	88.9% 87.4%	III; did not validate in US population
	Activin A	Florio '07	0.37ng/mL	100%	99.6%	III; Conflicting results as marker of location; additional studies needed
Kirk '09		0.37ng/mL	93%	13%		
Rausch '11		0.38 ng/mL	80%	72%		
Warrick '12		0.26 ng/mL	59.6%	69%		
PAPP-A	Rausch '11	0.53 ng/mL	81%	54%	III; Promising marker of viability, but not location	
	Dumps '02, Mueller '04,* Daponte '05, Ugurlu '09					Test characteristics report PAPP-A as a marker of viability; characteristics as single marker of EP N/A
SP1	Rausch '11	103.3ug/mL	65%	79%	III; Possible marker of location, additional studies needed	
ADAM-12	Rausch '11	48.49 ng/mL	97%	37%	III; Additional studies needed including study of abnormal IUP	
	Home '12	Not reported: AUCs 0.65-066	N/A	N/A		
Placental mRNA	Takacs '12	N/A	N/A	N/A	III; need assessment in abnormal IUP	
miRNA 323-3p	Zhao '12	0.2 (concentration relative to 18S rRNA)	37%	90%	III; promising marker of location, but with insufficient sensitivity alone; needs validation	
CORPUS LUTEAL FUNCTION						
Progesterone	Mol '98	20ng/mL	95%	<40%	IV; Good discrimination for viability, not but location, consider in combination with other markers	
	Buckley '00	22ng/mL	100%	27%		
	Dart '02	5ng/mL	88%	40%		
	Katsikis '06	10.75ng/mL	85%	85%		

Biomarker	Study	Cut-Point	Sensitivity	Specificity	Phase of Development/Comments
	El Bishry '08	16ng/mL	44%	60%	
	Rausch '11	13.5 ng/mL	82%	70%	
	Warrick '12	10ng/mL	63%	61%	
	Zhao '12	23ng/mL	90%	44%	
Inhibin A	Segal '08	50pg/mL EP v IUP	100%	100%	III: promising marker of viability, conflicting data on location, further studies needed with all relevant outcomes
		50pg/mL EP v mAB	41%	86%	
	Kirk '09	N/A – AUC 0.55	N/A	N/A	
	Rausch '11	28.67 pg/mL	83%	79%	
ANGIOGENESIS					
VEGF	Daniel '99	200pg/mL	60%	90%	III: promising for location and viability; needs validation in cohort with all relevant early pregnancy outcomes
	Felemban '02	200pg/mL –vIUP v EP	88%	100%	
		200pg/mL –aIUP v EP	87.5%	75%	
	Daponte '05	174.5 pg/mL	78%	100%	
	Rausch '11	28.24pg/mL	95%	50%	
PIGF	Daponte '11	15.7pg/mL**	86%	73%	III: possible marker of viability, need further data on prediction of EP
		19.9 pg/mL***	30%	90%	
		*** viable IUP v EP			
		*** missed abortion v EP			
ENDOMETRIAL FUNCTION					
Glycodelin	Rausch '11 – AUC 0.57; Mueller '04, Daponte '05 – N/A				III: possible marker of location, needs assessment in a cohort with all relevant early pregnancy outcome
LIF	Wegner '01	6.2ng/mL	73%	89%	III: Conflicting results in other studies suggest not an appropriate marker of EP or PUL
	Mueller 2004, Daponte 2005- no significant difference in EP levels compared to other pregnancy outcomes				

* no clinical test characteristic data for MUC1, adrenomedullin

Table 2

Multiple Marker Test Performance for Prediction of Ectopic Pregnancy

Markers	Study	Sensitivity	Specificity	Phase of Development/Conclusions
Estradiol, Progesterone, CA-125	Witt 1990	N/A	N/A	III: Needs replication to assess test characteristics and prospective assessment
VEGF, PAPP-A, Progesterone	Mueller 2004	97.7%	92.4%	III: needs validation with abnormal IUP
Progesterone, Activin A, Inhibin A, VEGF	Rausch 2011	90–98%	100%	III; diagnostic in 42% of patients; needs validation with abnormal IUP
Activin A, Progesterone, HCG	Warrick 2012	70%	69%	III: no benefit over hCG alone
hCG, Progesterone, miR-323-3p	Zhao 2012	96.3%	72.6%	III; needs validation in other cohorts and prospective assessment