# 2026 PCRS ANNUAL MEETING

## REPRODUCTIVE FRONTIERS: BRIDGING BIOLOGY, PRACTICE, AND POSSIBILITY

## MARCH 18-22 | RANCHO MIRAGE, CA

PACIFIC COAST REPRODUCTIVE SOCIETY

# The future of Early Pregnancy Viability Prediction


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# Disclosure Slide

- Neither I nor members of my immediate family have any actual or potential financial interests to disclose relating to the content of this presentation.

# Needs Assessment Statement and Expected Learning Outcomes

- The diagnosis of early pregnancy loss inducing mischarge and ectopic pregnancy has not changed in decades.  This presentation will explore how to incorporate biomarkers into the assessment of women with an early pregnancy.  Novel use in different clinical situations will be explored as well as the pitfalls of their development and validation.

- Assess how to identify novel markers of prediction of pregnancy viability.
- Demonstrate the importance of validation of a new companion diagnostic test.
- Integrate how to use novel biomarkers in different clinical situations.

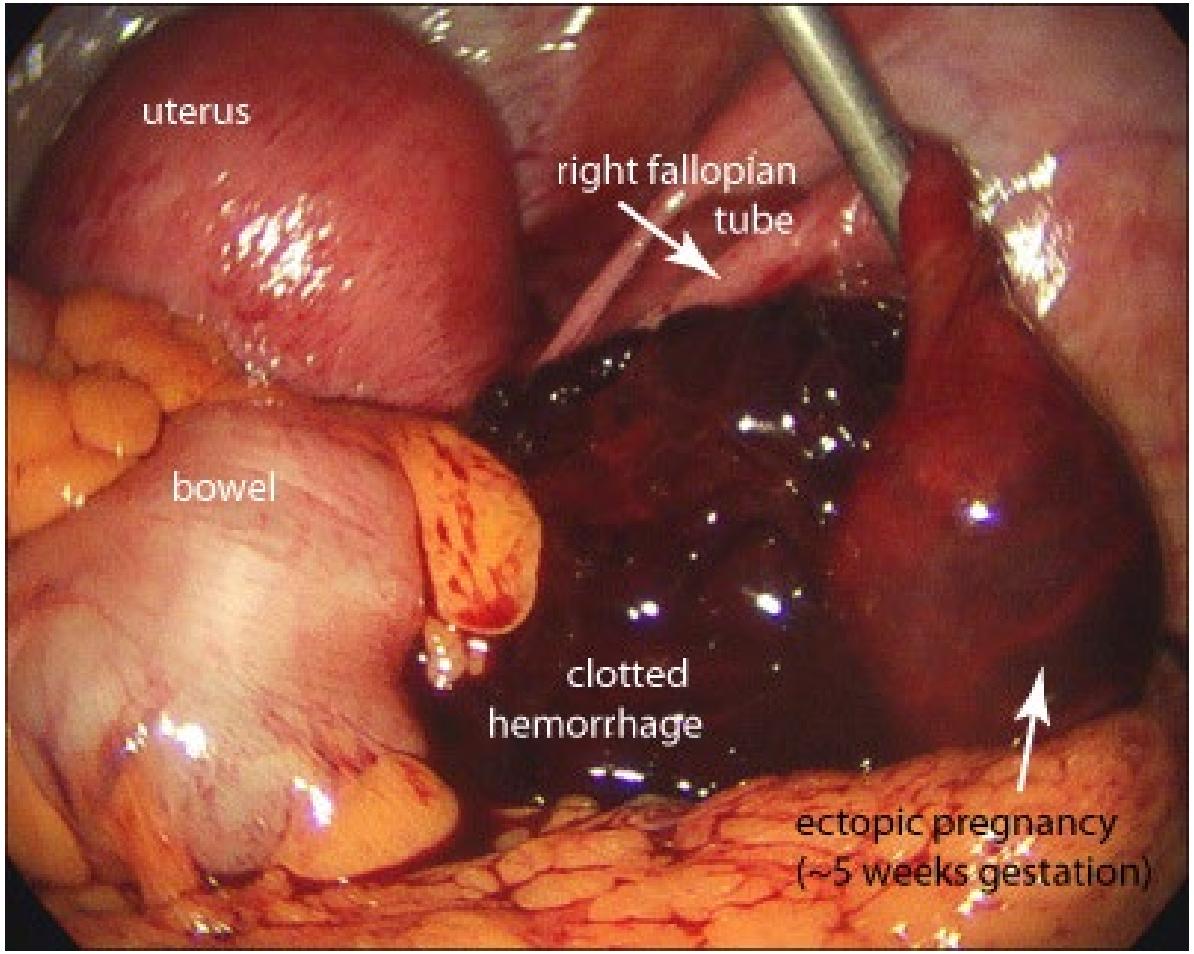
# Early Pregnancy Loss

- **Early pregnancy loss is 15% - 33% of all conceptions**
- **Ectopic Pregnancy is 1-2% of all pregnancies**
- **"Frustrating clinical conundrum for patients and health care teams"**
  - **Diagnosis with TVUS at first presentation with estimates ranging from 60 – 80%.**
  - **TVUS is indeterminate and ranges from 20 – 40%**
- **Follow up for women not diagnosed initially with TVUS is cumbersome, includes multiple office visits, serial hCG measurements, repeat TVUS and at times uterine curettage and/or laparoscopy. This process is arduous and stressful for women given the uncertainty, long duration, and lack of clarity**

# Modern Management

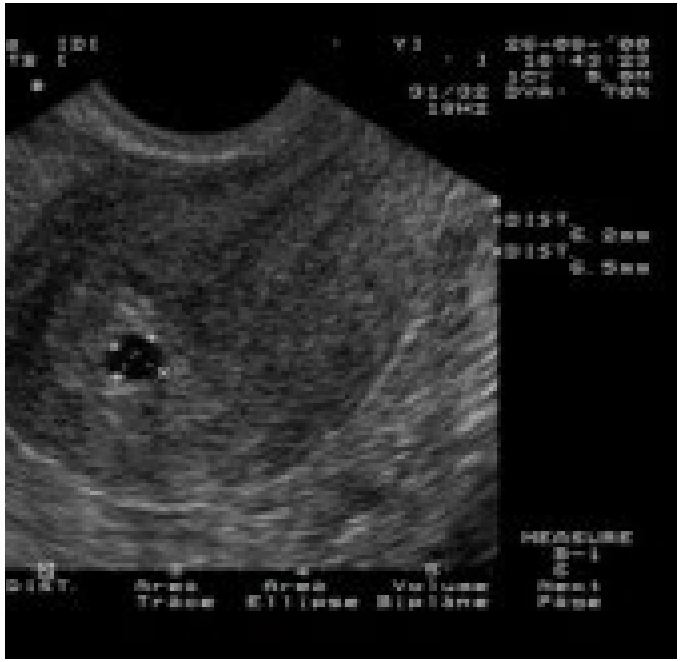
- Ultrasound needs clinical context
- Premature hCG surveillance can result in error
- The Discriminatory Zone is too low
- Pregnancy of unknown location  (PUL)
  - Active management reduces unscheduled surgery
  - Uterine evacuation reduces time to resolution
  - Women prefer expectant management

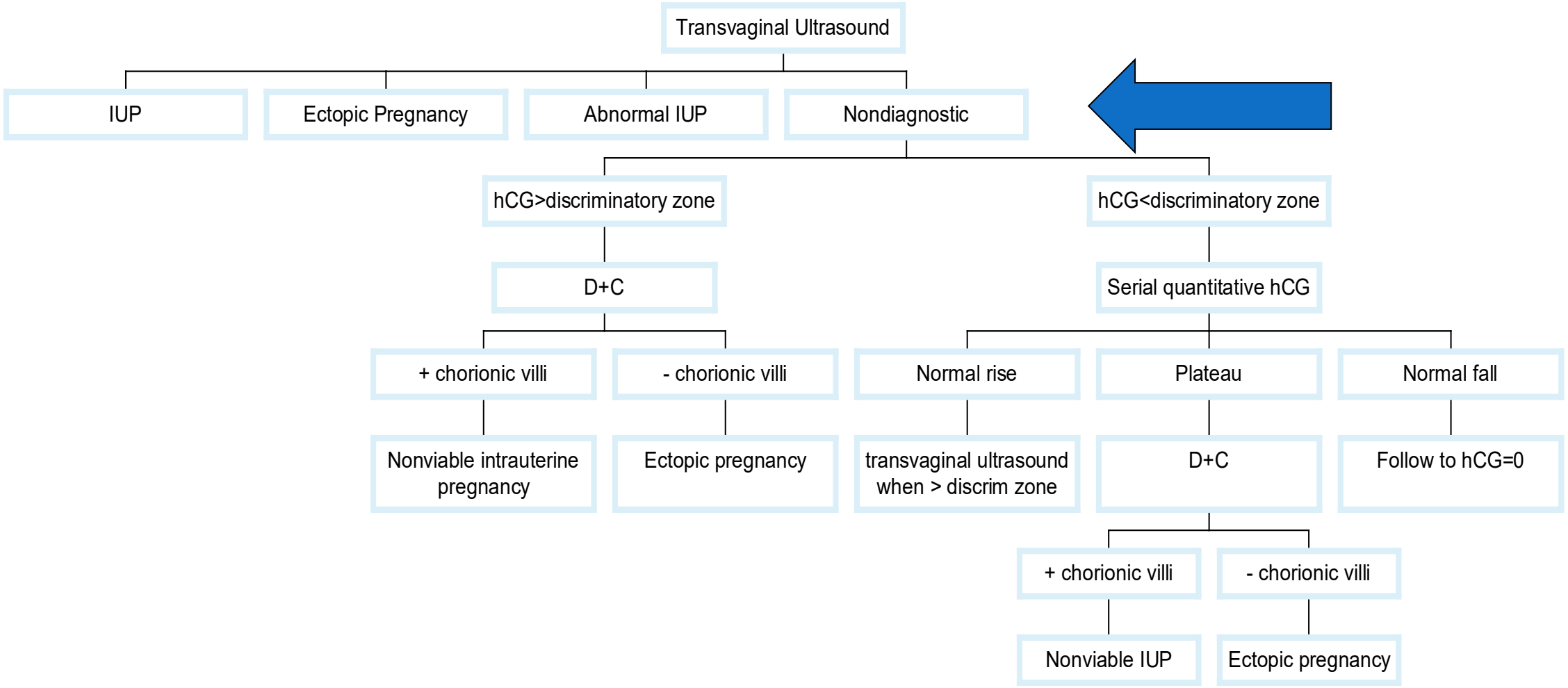
# Too late:
# Ruptured EP



# Too Early

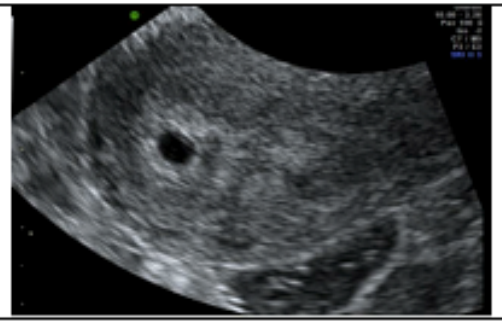
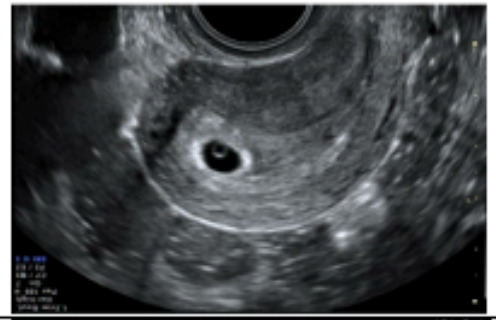
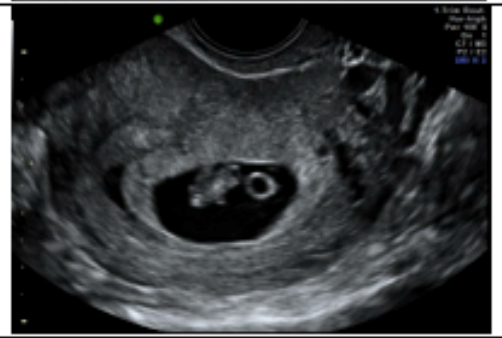
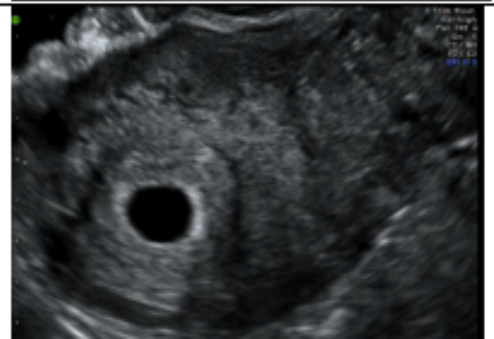
## Making a definitive dx based on non definitive information

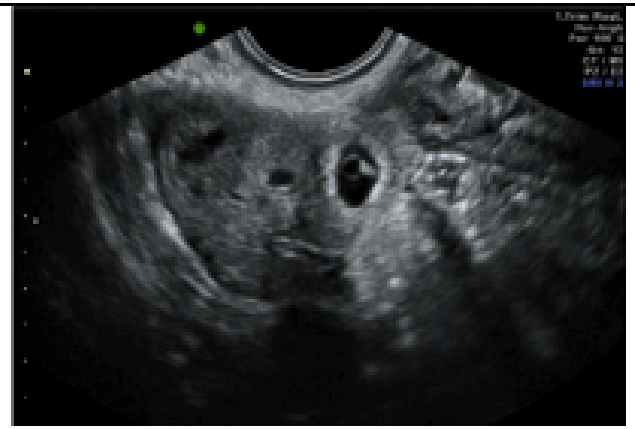
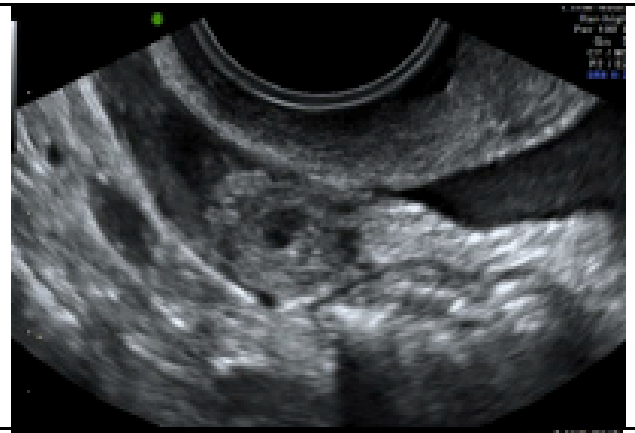
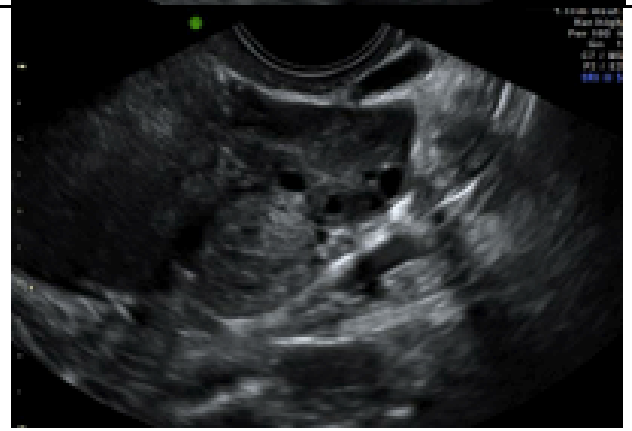






# Figure 1. Algorithm for the diagnosis of ectopic pregnancy in a hemodynamically stable patient

**Barnhart et al Obstet Gynecol 1994; 84:1010-5**
**Gracia C, Barnhart KT. Obstet Gynecol, 97(3):464-470, 2001.**

| Features visualised on TVS to suggest an IUP | | Narrow | Broad |
|---|---|---|---|
| Gestational sac only |  | Yes | No |
| Gestation sac with yolk sac |  | Yes | Yes |
| Gestation sac with CRL |  | Yes | Yes |
| Empty gestation sac (anembryonic) |  | Yes | No |

| | | | |
|---|---|---|---|
| Features visualised on TVS to diagnose EP | | narrow | broad |
| Gestation sac with a YS/embryo |  | Yes | Yes |
| Empty gestation sac |  | Yes | No |
| Inhomogeneous mass |  | Yes | No |
| No chorionic villi on uterine curettage and rising hCG level | | Persisting PUL | Yes |

# Accuracy of Ultrasound

- TVUS is very accurate when hCG is high
  - **Sensitivity 80 - 98%, PPV 90 – 96%**
- TVUS not accurate when hCG is low
  - **Sensitivity 25 – 33%,  PPV 60 – 80%**
- Definition of EP matters
  - EP “definitive”  Sens 13%, PPV 98%
  - EP “suspicious”  Sens 42%, PPV 80%

**Barnhart KT. Obstet Gynecol 1999; 94(4):583-587**
**Barnhart KT, Obstet Gynecol 2010; 117: 299-306.**

# Rise in hCG Depends on Value

- **The hCG rise in IUP differs by patient factors, and level at presentation.**
- **The 2-day (1st percentile) rise: faster when hCG values are low slower when hCG values are high.**

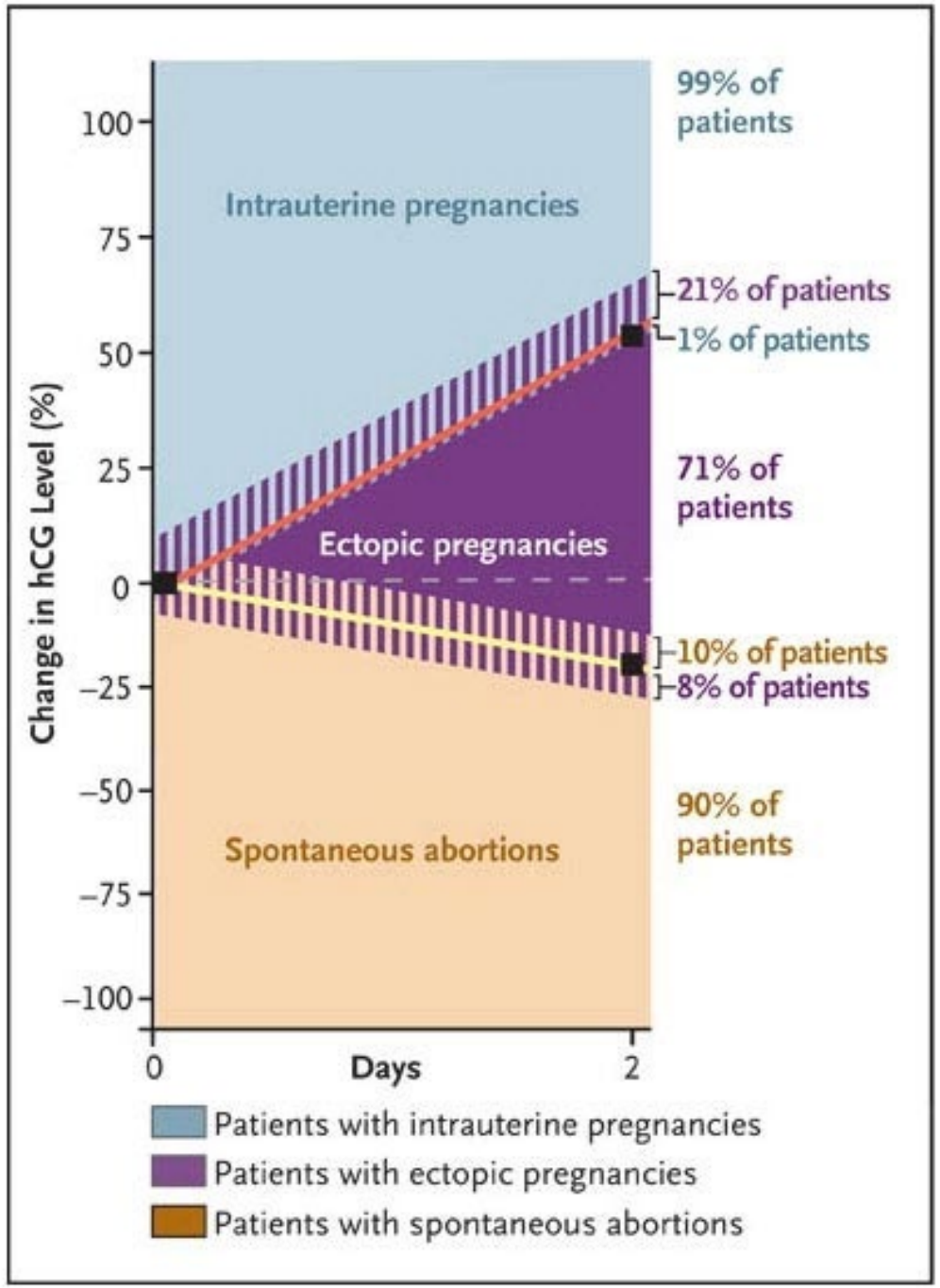
| Initial hCG | <1500 | 1500-3000 | > 3000 |
|---|---|---|---|
| 1% rise | 49% | 40% | 33% |

## Ectopic Pregnancy

Kurt T. Barnhart, M.D., M.S.C.E

N Engl J Med 2009; 361:379-387

**Change in the hCG Level in Intrauterine Pregnancy, Ectopic Pregnancy, and Spontaneous Abortion.**



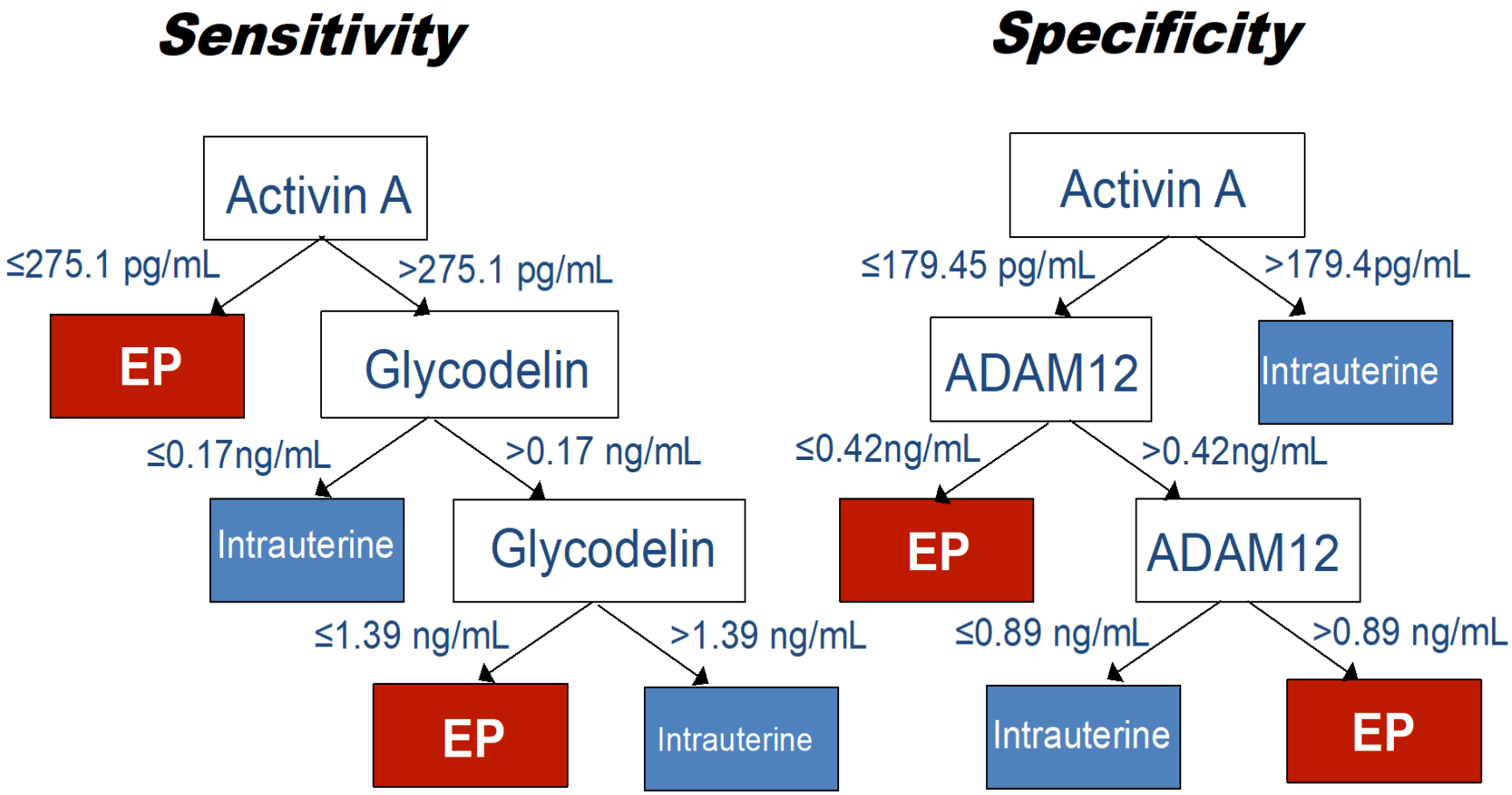
# Biomarkers

- **Molecules produced by affected individuals that signal specific exposures or disease states**
- **Used for**
  - **early diagnosis of disease**
  - **disease prevention**
  - **drug target identification**
  - **drug response**

# Diagnosis of a Women at Risk for Early Pregnancy Loss

- **Accurate diagnosis is paramount when evaluating a pregnancy women at risk for miscarriage or ectopic pregnancy (EP).**
- **Trade off between sensitivity and specificity**
  - **Maximize SENSITIVITY (find all ectopic pregnancies)**
    - **Error will be to diagnose some IUP as EP: Interrupt desired IUP**
  - **Maximize SPECIFICITY (find all intrauterine pregnancies)**
    - **Error will be to diagnose some EP as IUP: Miss EP diagnosis at risk of rupture**
  - **We have a <u>two step process to maximize accuracy</u>. Those not classified follow routine care to avoid diagnostic errors**

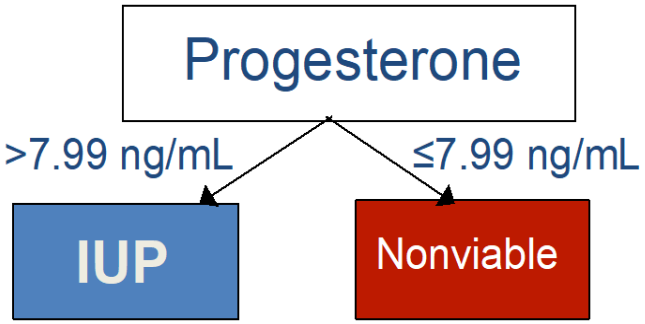
# Sensitivity and Specificity Trees for Pregnancy Location



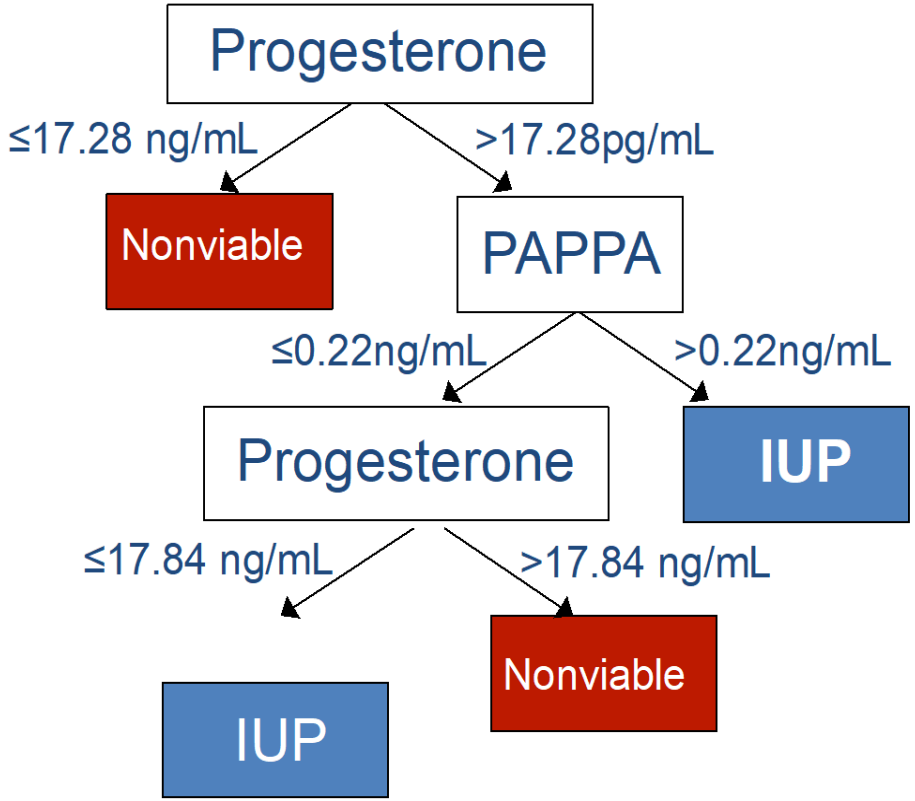

Cut offs and order of use of markers determined by Classification Tree Analysis (CART).
Separate models were used maximize sensitivity and specificity

# Sensitivity and Specificity Trees for Pregnancy Viability

***Sensitivity***




***Specificity***




Cut offs and order of use of markers determined by Classification Tree Analysis (CART).
Separate models were used maximize sensitivity and specificity

# Two Part Classification

**Run the Max sensitivity tree**
**Then run Max specificity tree**

| Max sensitivity | Max specificity | | Classification |
|---|---|---|---|
| Positive | Negative | | unclassified |
| Negative | Positive | | unclassified |
| Negative | Negative | | No disease |
| Positive | Positive | | Disease |

**Only those in which classification agrees are classified with or without disease**

# Results of Validation of use of Multiple Markers to Determine Location and Viability of an Early Pregnancy

**Derivation study**
Senapati et al F&S 2016

**Validation Study**
Bollig et al JARG 2023

| | Outcome | Conclusive Classification | Accuracy Amongst Classified [95% CI] |
|---|---|---|---|
| | Location | 67/228 (29%) | 100% [95-100] |
| | Viability | 140/230 (61%) | 97% [93-99] |
| | Location | 94/175 (54%) | 78% [69 - 87] |
| | Viability | 112/192 (58%) | 89% [81 - 94] |

# 5 Phases of Biomarker Development (Pepe 2001)

| | | |
|---|---|---|
| Preclinical Exploratory | PHASE 1 | Promising directions identified |

**2 proteomic experiments and Olink (high thought put)**

| | | |
|---|---|---|
| Clinical Assay | PHASE 2 | Clinical assay detects established disease (case control) |

**Identified and validated Elisa assays. Proof of concept**

| | | |
|---|---|---|
| Retrospective<br>Longitudinal | PHASE 3 | Biomarker detects disease<br>early before becomes clinical |

**Three experiments to assess utility of marker alone and in combination**

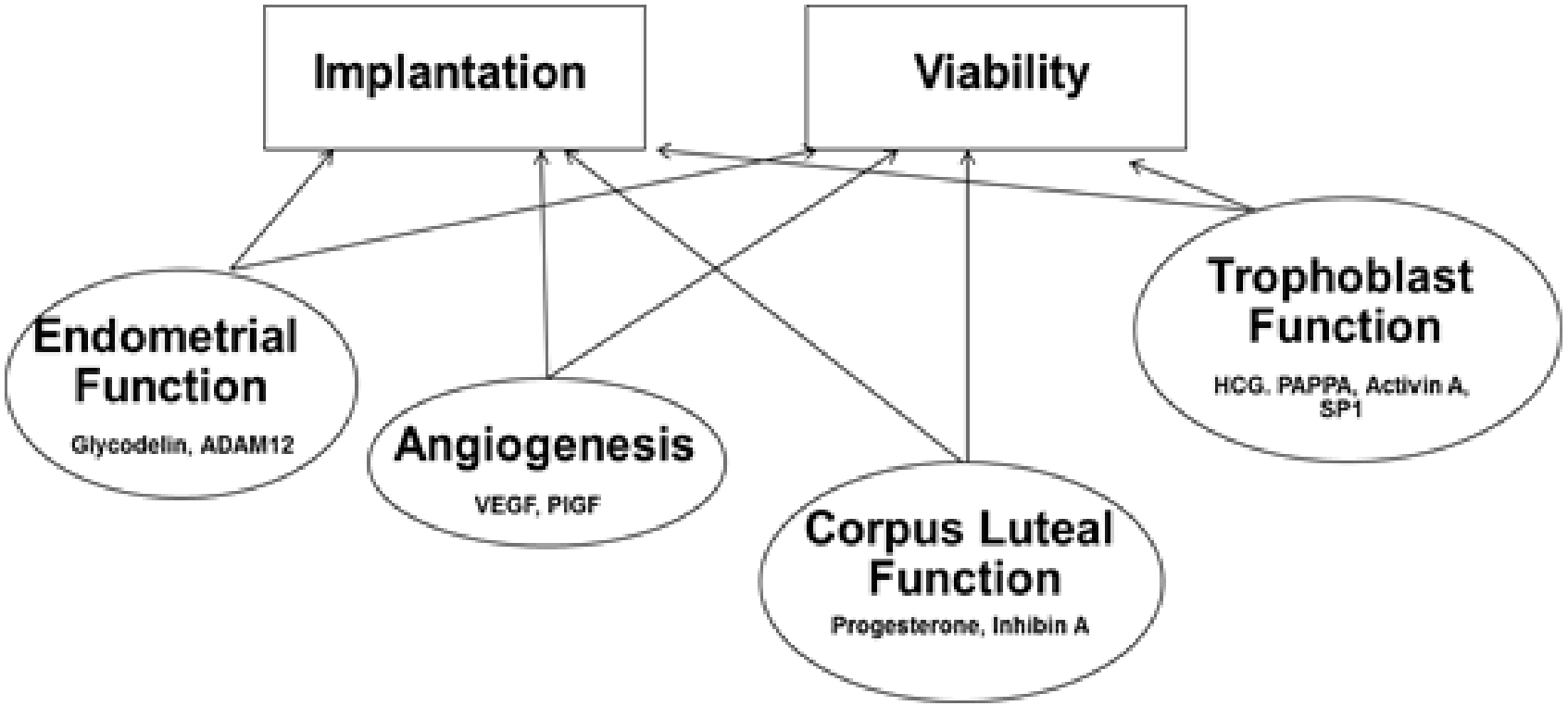
| | | |
|---|---|---|
| Prospective Screening | PHASE 4 | Extent and characteristics of<br>disease detected by test |

**Ongoing**

# Test of pool of markers in the same subject

"Picking the best ELISA candidates (2021)"

| Table 4<br>**Marker** | **Description** | **Viability/ Location** | **AUC** | **Biological Function/ Rationale** |
|---|---|---|---|---|
| Progesterone | Progestational steroid | Viability | 0.88 | Corpus luteal steroid hormone/ Validated putative maker |
| PSG9 | Pregnancy-specific beta-1-glycoprotein 9 | Location | 0.87 | Cell adhesion, hemostasis/ Discovered with proteomics |
| PSG1 | Pregnancy-specific beta-1-glycoprotein 1 | Location | 0.87 | Cell adhesion and hemostasis/ Discovered with proteomics |
| Tfpi-2 | Tissue factor pathway inhibitor 2 | Location | 0.85 | coagulation, extracellular matrix structural constituent/ Discovered with Olink |
| CGA | Glycoprotein hormones alpha chain | Location | 0.84 | Female hormone signaling pathway, Discovered with proteomic and Olink |
| GDF-15 | Growth/differentiation factor 15 | Location | 0.84 | Cell signaling, cell development/ Discovered with Olink |
| Activin A | Inhibin beta A chain | Location | 0.79 | Cell adhesion and regulation/ Validated putative marker |
| SFlt | Vascular endothelial growth factor receptor 1 | Location | 0.79 | Tyrosine kinase/Validated putative marker |
| ANGPT-2 | Angiopoietin-2 | Location | 0.78 | Endothelial cell apoptosis, cell migration and proliferation/ Olink |
| IGFBP1 | Insulin-like growth factor-binding protein 1 | Location | 0.76 | Carrier protein/ Discovered with proteomics |
| PSG3 | Pregnancy-specific beta-1-glycoprotein 3 | Viability | 0.73 | Cell adhesion and hemostasis/ Discovered with proteomics |
| Siglec-6 | Sialic acid-binding Ig-like lectin 6 | Location | 0.73 | Cell adhesion and cell signaling/ Discovered with Olink |
| Kisspeptin | Metastasis-suppressor KISS-1 | Location | 0.71 | G-coupled protein receptor ligand/ Newly validated assay in serum |
| EHD3 | EH domain-containing protein 3 | Viability | 0.68 | Cell structure regulation/ Novel marker of ovarian/tubal cancer |
| Adam 12 | Disintegrin and metalloproteinase domain-containing protein 12 | Location | 0.67 | Metalloproteinase, Cell adhesion/ Discovered with proteomics and validated |
| CD9 | CD9 antigen | Location | 0.66 | Cell adhesion regulation/ Novel marker of ovarian/tubal cancer |
| PLGF | Placental Growth Factor | Location | 0.66 | Developmental protein, Cell signaling, angiogenesis, differentiation/ Validated putative marker |
| Fibronectin | Fibronectin | Location | 0.63 | Cell adhesion and heparin-binding/ Validated putative marker |
| PAPPA | Pappalysin-1 | Viability | 0.61 | Metalloproteinase and cell regulation/ Discovered with proteomics |

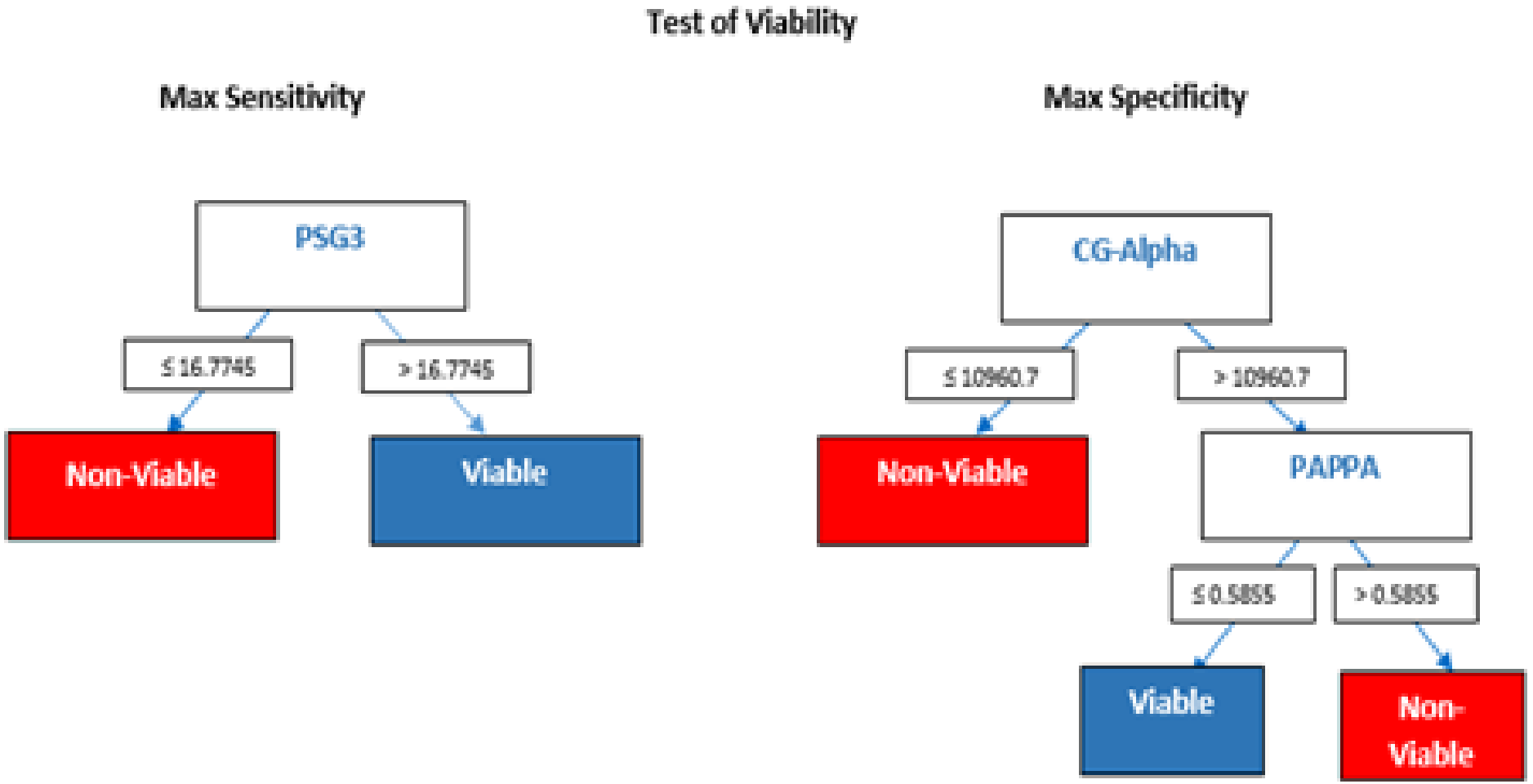

Implantation
Viability
Endometrial Function
Glycodelin, ADAM12
Angiogenesis
VEGF, PIGF
Corpus Luteal Function
Progesterone, Inhibin A
Trophoblast Function
HCG, PAPPA, Activin A, SP1

Top candidates used singly and together (2021-2)

# Ranking Based on AUC

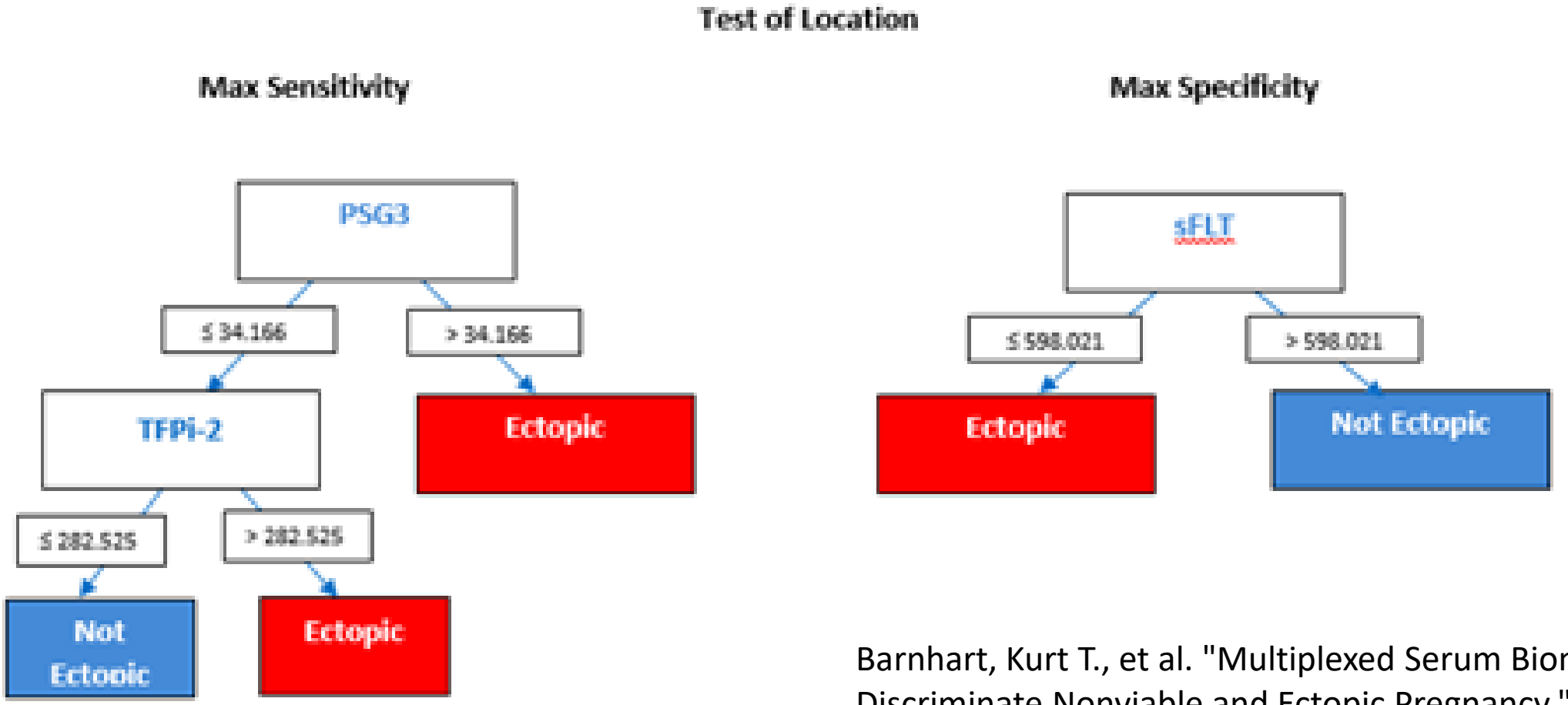
| | EP | | | Viability | | |
|---|---|---|---|---|---|---|
| | AUC | AUC Direction | Ttest p | AUC | AUC Direction | Ttest p |
| PSG1 | 0.110 | 0.890 | <0.001 | 0.656 | 0.656 | 0.034 |
| sFLT | 0.138 | 0.862 | <0.001 | 0.710 | 0.710 | 0.006 |
| GDF15 | 0.163 | 0.837 | <0.001 | 0.830 | 0.830 | <0.001 |
| PSG9 | 0.168 | 0.832 | <0.001 | 0.868 | 0.868 | <0.001 |
| hCG | 0.170 | 0.830 | <0.001 | 0.850 | 0.850 | <0.001 |
| CG-ALPHA | 0.192 | 0.808 | <0.001 | 0.865 | 0.865 | <0.001 |
| Adam12 | 0.261 | 0.739 | <0.001 | 0.474 | 0.526 | 0.222 |
| SIGLEC-6 | 0.262 | 0.738 | 0.899 | 0.629 | 0.629 | 0.453 |
| ANGPT2 | 0.266 | 0.734 | <0.001 | 0.491 | 0.509 | 0.886 |
| PSG3 | 0.272 | 0.728 | 0.001 | 0.902 | 0.902 | <0.001 |
| TFPI2 | 0.276 | 0.724 | <0.001 | 0.448 | 0.552 | 0.291 |
| AcivinA | 0.345 | 0.655 | <0.001 | 0.394 | 0.606 | 0.020 |
| PLGF | 0.352 | 0.648 | 0.025 | 0.498 | 0.502 | 0.669 |
| PAPPA | 0.363 | 0.637 | 0.060 | 0.385 | 0.615 | 0.996 |
| PRG | 0.382 | 0.618 | 0.147 | 0.817 | 0.817 | <0.001 |
| EHD3 | 0.618 | 0.618 | 0.085 | 0.392 | 0.608 | 0.088 |
| HAGH | 0.384 | 0.616 | 0.017 | 0.648 | 0.648 | 0.002 |
| ELAFIN | 0.385 | 0.615 | 0.201 | 0.554 | 0.554 | 0.455 |
| FIBRONECTIN | 0.396 | 0.604 | 0.023 | 0.555 | 0.555 | 0.125 |
| KISSPEPTIN | 0.413 | 0.587 | 0.019 | 0.544 | 0.544 | 0.248 |
| OPN | 0.578 | 0.578 | 0.064 | 0.357 | 0.643 | 0.004 |
| CD9 | 0.430 | 0.570 | 0.214 | 0.485 | 0.515 | 0.848 |
| IGFBP1 | 0.432 | 0.568 | 0.181 | 0.330 | 0.670 | <0.001 |
| NOTUM | 0.467 | 0.533 | 0.582 | 0.543 | 0.543 | 0.694 |

# Figure 2. Sensitivity and Specificity Trees for Pregnancy Viability




Barnhart, Kurt T., et al. "Multiplexed Serum Biomarkers to Discriminate Nonviable and Ectopic Pregnancy." Fertility and Sterility (2024).

# Figure 1. Sensitivity and Specificity Trees for Pregnancy Location




Barnhart, Kurt T., et al. "Multiplexed Serum Biomarkers to Discriminate Nonviable and Ectopic Pregnancy." Fertility and Sterility (2024).

# Performance of CART Models to Predict Viability (IUP vs. EPL + EP) with 95% CI

| Model | Markers | Sensitivity | Specificity | PPV | NPV | % Classified | Accuracy Amongst Classified |
|---|---|---|---|---|---|---|---|
| Balance of sensitivity and specificity | **PSG3** | **93.3%** **(87.7, 99.0)** | **76.2%** **(69.3, 83.2)** | 67.3% (58.3, 76.3) | 95.6% (91.9, 99.4) | 100% (100.0, 100.0) | 82.1% (77.0, 87.2) |
| [1]Maximum Sensitivity | **PSG3** | **93.3%** **(87.7, 99.0)** | 76.2% (69.3, 83.2) | 67.3% (58.3, 76.3) | 95.6% (91.9, 99.4) | 100% (100.0, 100.0) | 82.1% (77.0, 87.2) |
| Maximum Specificity | **PSG3** **PAPPA** | 57.3% (46.1, 68.5) | **97.9%** **(95.6, 100.0)** | 93.5% (86.3, 100.0) | 81.4% (75.6, 87.2) | 100% (100.0, 100.0) | 83.9% (79.1, 88.8) |
| **Maximum Accuracy** | **PSG3** **CG_Alpha** **PAPPA** | 54.7% (43.4, 65.9) | 75.5% (68.5, 82.6) | 97.6% (93.0, 100.0) | 97.3% (94.3, 100.0) | 70.2% (64.1, 76.3) | **97.4%** **(94.9, 99.9)** |

Barnhart, Kurt T., et al. "Multiplexed Serum Biomarkers to Discriminate Nonviable and Ectopic Pregnancy." Fertility and Sterility (2024).

## Performance of CART Models to Predict Location (EP vs. EPL + IUP) with 95% CI

| Model | Marker(s) | Sensitivity | Specificity | PPV | NPV | % Classified | Accuracy Amongst Classified |
|---|---|---|---|---|---|---|---|
| Balance of sensitivity and specificity | PSG1 | **82.4%** **(73.3, 91.5)** | *__84.0%__ **(78.1, 89.9)** | 70.0% (60.0, 80.0) | 91.3% (86.6, 96.0) | 100% (100.0, 100.0) | 83.5% (78.6, 88.4) |
| Maximum Sensitivity | PSG3 TFPI2 | **98.5%** **(95.7, 100.0)** | 61.3% (53.5, 69.1) | 53.6% (44.9, 62.3) | 98.9% (94.2, 99.9) | 100% (100.0, 100 .0) | 72.9% (67.0, 78.8) |
| Maximum Specificity | sFLT | 63.2% (51.8, 74.7) | **95.3%** **(92.0, 98.7)** | 86.0% (76.4, 95.6) | 85.1% (79.7, 90.5) | 100% (100.0, 100.0) | 85.3% (80.6, 90.0) |
| **Maximum Accuracy** | PSG3 sFLT TFP12 | 63.2% (51.8, 74.7) | 61.3% (53.5, 69.1) | 86.0% (76.4, 95.6) | 98.9% (96.8, 100.0) | 65.6% (59.3, 71.9) | **94.4%** **(90.6, 98.2)** |

Barnhart, Kurt T., et al. "Multiplexed Serum Biomarkers to Discriminate Nonviable and Ectopic Pregnancy." Fertility and Sterility (2024).

# Performance of Random Forest (RF) Model to Predict Viability (IUP vs. EPL + EP)

| *Model* | Markers | Sensitivity | Specificity | PPV | NPV | % Classified | Accuracy Amongst Classified |
|---|---|---|---|---|---|---|---|
| *Balance of sensitivity and specificity* | All 10 | **91.8% (6.7%)** | **73.1% (18.5%)** | 88.0% (6.7%) | 84.0% (11.7%) | 100% | 85.6% (5.4%) |
| *Maximum Sensitivity* | All 10 | **99.1% (1.9%)** | 38.8% (19.1%) | 76.8% (5.7%) | 95.4% (7.1%) | 100% | 79.0% (6.0%) |
| *Maximum Specificity* | All 10 | 74.7% (11.2%) | **94.9% (10.5%)** | 97.2% (4.5%) | 66.6% (10.5%) | 100% | 81.4% (6.6%) |
| *§Maximum Accuracy* | All 10 | 98.9% (2.3%) | 88.4% (16.4%) | 97.2% (4.5%) | 95.4% (7.1%) | 65.0% (11.8%) | **96.9% (4.4%)** |
| *Maximum Accuracy with 6 Markers* | **PSG3 PSG9 hCG CG_Alpha GDF15 ADAM12** | 97.2% (5.9%) | 84.1% (18.1%) | 95.2% (5.8%) | 93.5% (12.6%) | 69.6% (13.9%) | **94.2% (6.1%)** |
| *Maximum Accuracy with 2 Markers* | **PSG3 hCG** | 90.5% (7.5%) | 92.0% (12.8%) | 97.1% (4.7%) | 78.9% (13.3%) | 72.9% (14.4%) | **91.0% (6.1%)** |

- The performance was reported in terms of the mean and standard deviation of the 100 testing results.
- The bolded values highlight the test characteristics that were optimized in each model.
- The bolded model is the preferred model.
- **"All 10" refers to the markers: PSG3, PSG9, PSG1, hCG, CG_Alapha, GDF15, sFLT, PRG, PAPPA, and ADAM 12.**
- Maximum Accuracy refers to a cross of the Maximum Sensitivity and Maximum Specificity models.

# Performance of Random Forest (RF) Model to Predict Location (EP vs. EPL + IUP)

| *Model* | Markers | Sensitivity | Specificity | PPV | NPV | % Classified | Accuracy Amongst Classified |
|---|---|---|---|---|---|---|---|
| *Balance of sensitivity and specificity* | All 11 | **93.0% (6.0%)** | **62.2% (17.1%)** | 85.1% (5.6%) | 82.3% (12.0%) | 100% | 83.5% (5.0%) |
| *Maximum Sensitivity* | All 11 | **96.6% (4.1%)** | 41.1% (20.0%) | 79.0% (5.8%) | 86.7% (13.8%) | 100% | 79.4% (5.5%) |
| *Maximum Specificity* | All 11 | 78.4% (11.7%) | **81.4% (11.8%)** | 90.8% (5.1%) | 65.4% (12.6%) | 100% | 79.3% (7.4%) |
| *Maximum Accuracy* | All 11 | 95.8% (4.9%) | 67.5% (20.1%) | 90.8% (5.1%) | 86.7% (13.8%) | 75.0% (12.8%) | **89.4% (4.9%)** |
| *Maximum Accuracy with 6 Markers* | PSG3 PSG9 PSG1 hCG GDF15 sFLT | 96.0% (4.3%) | 62.2% (22.5%) | 89.6% (5.0%) | 85.8% (16.3%) | 75.7% (13.1%) | **88.6% (5.1%)** |
| *Maximum Accuracy with 2 Markers* | PSG3 hCG | 96.1% (4.9%) | 65.8% (18.8%) | 89.7% (5.2%) | 88.8% (12.6%) | 77.5% (12.4%) | **88.9% (5.5%)** |

- The performance was reported in terms of the mean and standard deviation of the 100 testing results.
- The bolded values highlight the test characteristics that were optimized in each model.
- The bolded model is the preferred model.
- **"All 11" refers to the markers: PSG3, PSG9, PSG1, hCG, CG_Alpha, GDF15, sFLT, PRG, PAPPA, ADAM 12, and TFP12.**

# Summary companion test for treatment of miscarriage and/or EP

- **A series of 8 experiments has evaluated more than 50 putative and novel candidates to predict early pregnancy failure (from divergent biological pathways)**
- **When used in combination:**
  - **CG-Alpha, PAPPA and PSG3 can be used to predict viability: 97.4% accuracy in 70% of population**
  - **sFLT, TPFI2 and PSG3 can be used to predict pregnancy location: 94.4% accuracy 66% of population**
  - **When used in simultaneously: 95.9% accuracy in 73% of population**
- **Currently collecting prospectively to assess utility in a population-based cohort.**

Barnhart, Kurt T., et al. "Multiplexed Serum Biomarkers to Discriminate Nonviable and Ectopic Pregnancy." Fertility and Sterility (2024).

# Viability

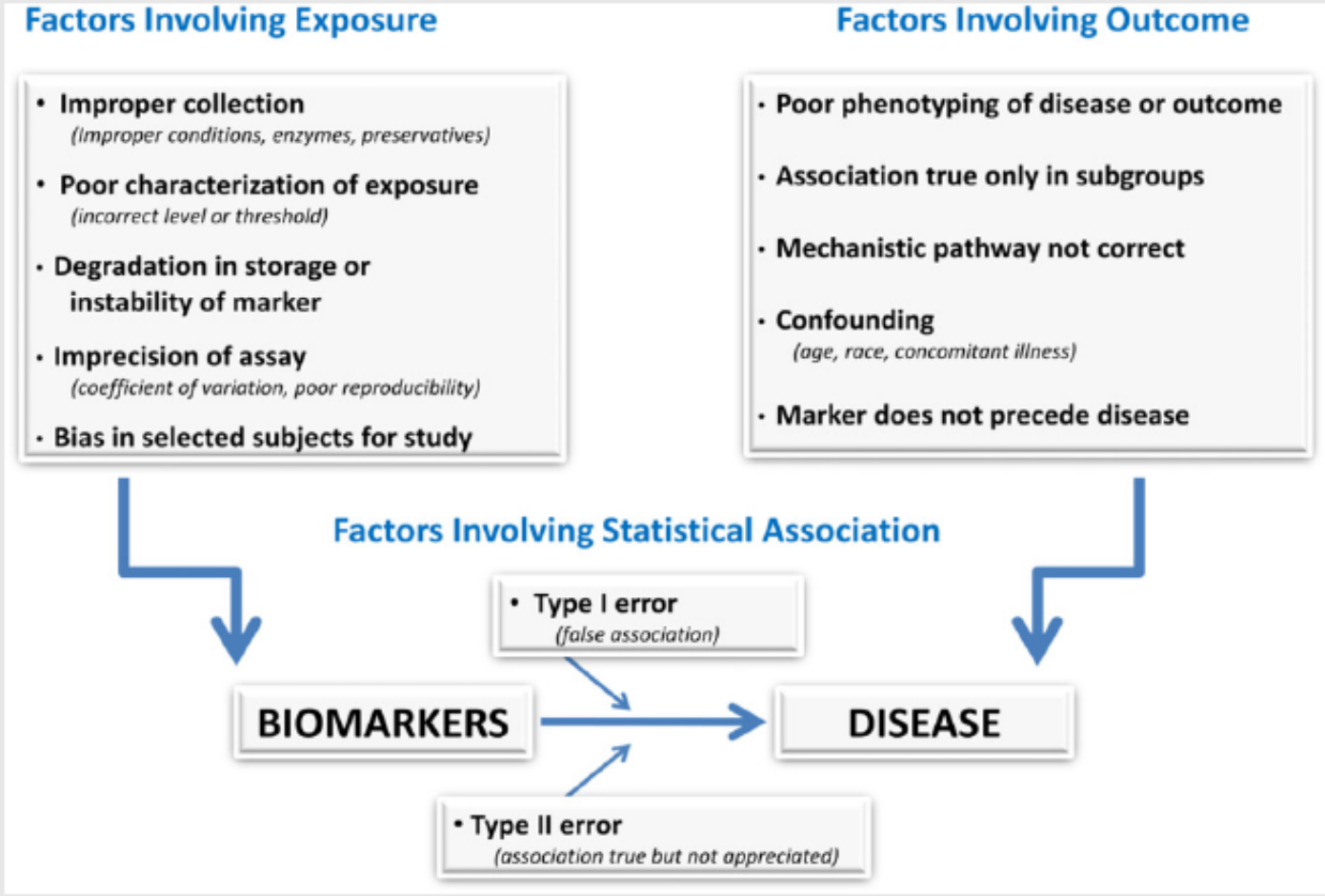
| | | |
|---|---|---|
| CG-Alpha | Glycoprotein hormones / chorionic gonadotropin- alpha subunit | Trophoblast function |
| PSG3 | Pregnancy-specific beta-1-glycoprotein 3 | Trophoblast function, cell adhesion, hemostasis |
| PAPPA | Pregnancy-associated plasma protein-A | Trophoblast function, metalloproteinase and cell regulation |

# Location

| | | |
|---|---|---|
| PSG3 | Pregnancy-specific beta-1-glycoprotein 3 | Trophoblast function, cell adhesion, hemostasis |
| sFLT | Soluble fms-like tyrosine kinase-1 | Abnormal angiogenesis, tyrosine kinase |
| TFPI2 | Tissue factor pathway inhibitor 2 | Trophoblast function, blood coagulation, extracellular matrix structural constituent |

# Why Do Biomarkers Fail to Reach Their Potential?

**FIGURE 1**




For a biomarker to be valid it must have a strong and valid association with the disease process of interest. Although initial studies of this association may be promising, there are many reasons that the association may not ultimately be demonstrated valid. Alternatively, there may be a true association that is overlooked or abandoned owing to methodologic factors. Factors can be categorized into issues involving the exposure, those regarding statistical associations, and those involving the outcome.

*Palmer. Promise of biomarkers in reproduction. Fertil Steril 2013.*

# How can these tests be used?

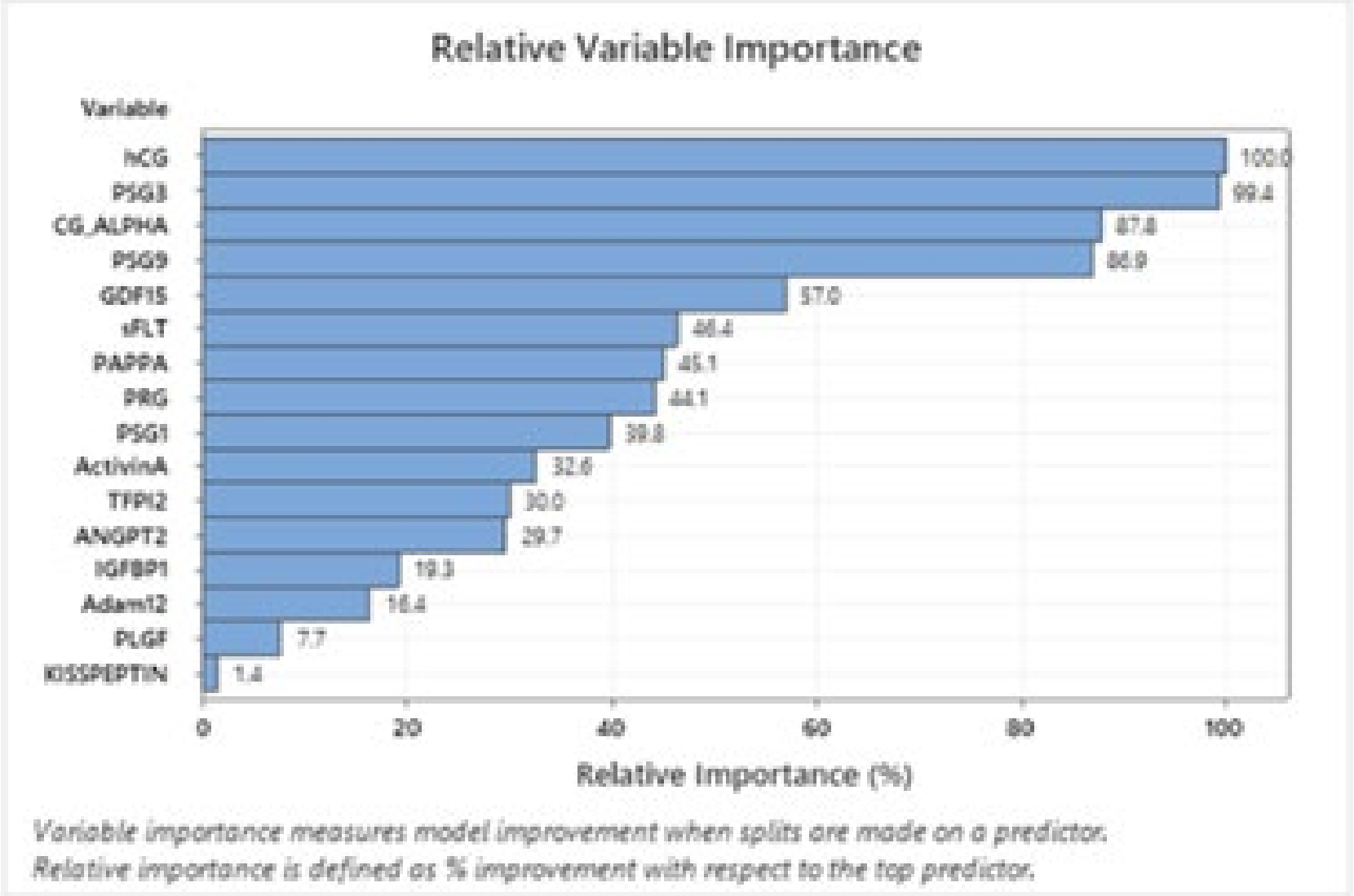
- **Companion diagnostic**
  - **Obtain when you get hCG and confirm equivocal ultrasound (or make you pause and take more time)**
  - **Help in "diagnostic dilemma", do not have normal ultrasound and biomarkers suggest EP**
  - **Some growth in US or rise in hCG but biomarker suggests nonviable (important in places that prohibit abortion)**
- **Obtain early in gestation before pregnancy can be visualized with US**
  - **Help tirage to high or low risk surveillance, patient reassurance**
- **Determine medical, surgical or expectant management for women at risk for EP or with PUL**
  - **May help predict who is at risk for rupture**

# Next steps

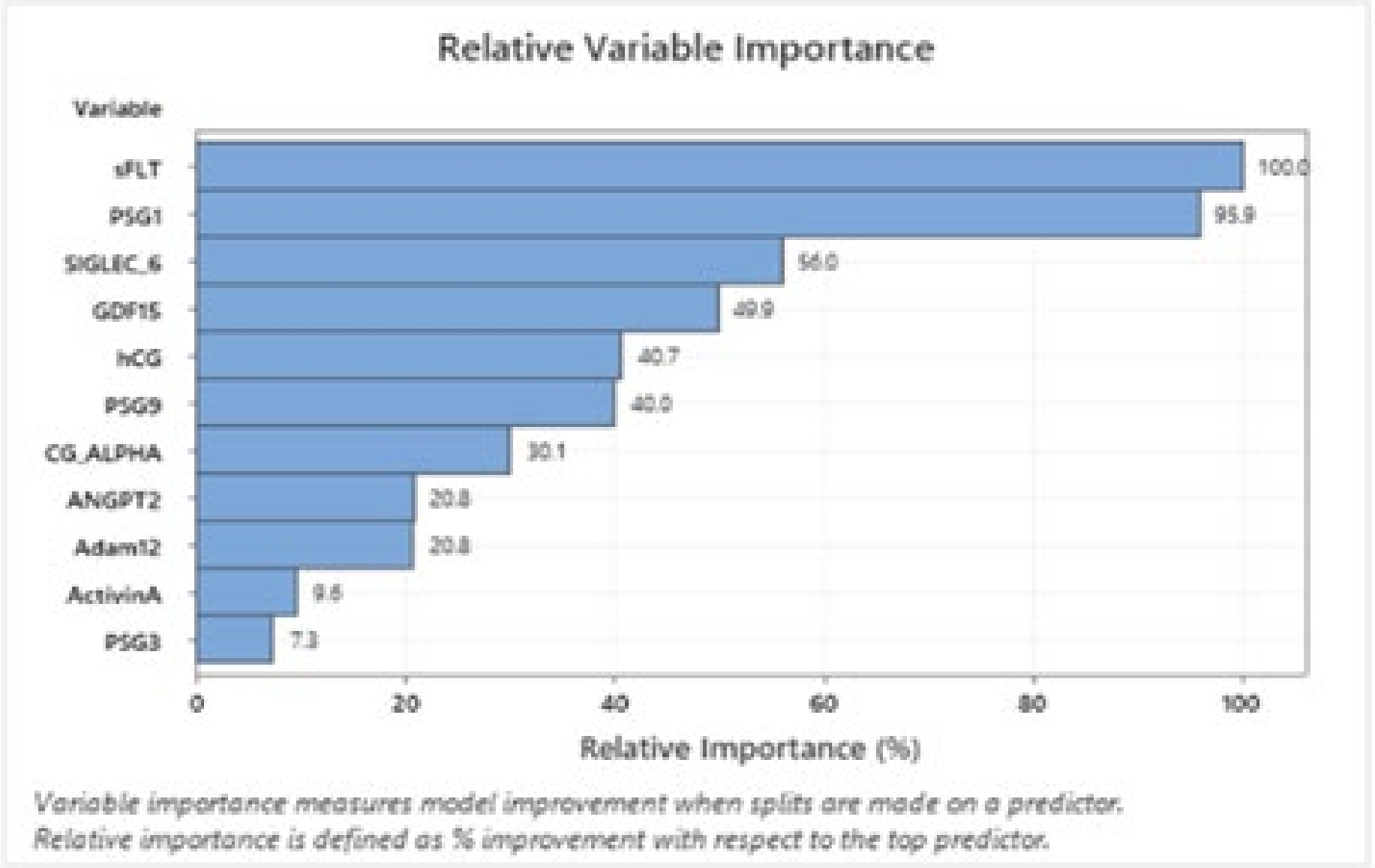
- **Validate our biomarker tests in populations with high and low risk of EP**
- **Assess accuracy when the Genetics of the pregnancy is known**
- **Serial levels for natural history (slope)**
  - **EP, SAB, IUP**
  - **How does delta compare to fall in hCG (improve?)**
  - **Use of the test(s) in special circumstances.**
- **Unusual EP (cervical, ovarian, interstitial)**
- **When can the test detect non-viability?**
  - **How far away from the diagnosis**

# Thank you

# Viability

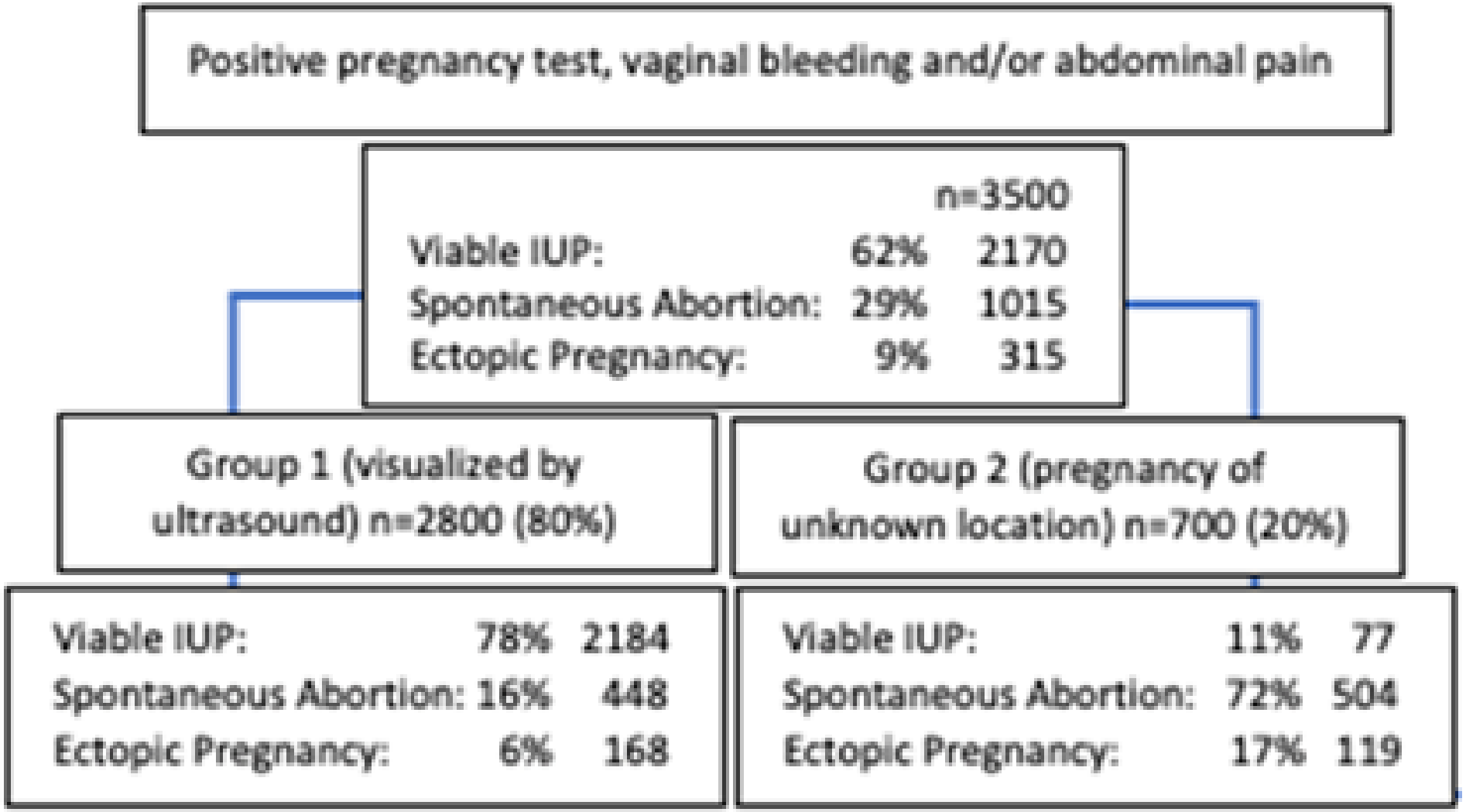

Relative Variable Importance
Variable
hCG
PSG3
CG_ALPHA
PSG9
GDF15
sFLT
PAPPA
PRG
PSG1
ActivinA
TFPI2
ANGPT2
IGFBP1
Adam12
PLGF
KISSPEPTIN
100.0
99.4
87.8
86.9
57.0
46.4
45.1
44.1
39.8
32.6
30.0
29.7
19.3
16.4
7.7
1.4
0
20
40
60
80
100
Relative Importance (%)
Variable importance measures model improvement when splits are made on a predictor.
Relative importance is defined as % improvement with respect to the top predictor.

# Location


Relative Variable Importance
Variable
sFLT
PSG1
SIGLEC_6
GDF15
hCG
PSG9
CG_ALPHA
ANGPT2
Adam12
ActivinA
PSG3
100.0
95.9
56.0
49.9
40.7
40.0
30.1
20.8
20.8
9.6
7.3
0
20
40
60
80
100
Relative Importance (%)
Variable importance measures model improvement when splits are made on a predictor.
Relative importance is defined as % improvement with respect to the top predictor.

# Correlation Amongst top markers

| | PSG1 | PSG3 | PSG9 | hCG | CG_Alpha | sFLT | PRG | Adam12 | TFPI2 | hCG_blood |
|---|---|---|---|---|---|---|---|---|---|---|
| PSG1 | **1** | | | | | | | | | |
| PSG3 | **0.395** | **1** | | | | | | | | |
| PSG9 | **0.579** | **0.45** | **1** | | | | | | | |
| hCG | **0.581** | **0.488** | **0.957** | **1** | | | | | | |
| CG_Alpha | **0.562** | **0.451** | **0.983** | **0.949** | **1** | | | | | |
| sFLT | **0.662** | **0.269** | **0.452** | **0.462** | **0.440** | **1** | | | | |
| PRG | **0.106** | **0.261** | **0.375** | **0.397** | **0.369** | 0.084 | **1** | | | |
| Adam12 | **0.459** | **0.222** | **0.169** | **0.177** | **0.156** | **0.390** | -0.043 | **1** | | |
| TFPI2 | **0.712** | **0.447** | **0.286** | **0.310** | **0.251** | **0.466** | 0.009 | **0.432** | **1** | |
| hCG_blood | **0.530** | **0.436** | **0.858** | **0.882** | **0.864** | **0.443** | **0.338** | **0.167** | **0.267** | **1** |


Positive pregnancy test, vaginal bleeding and/or abdominal pain
n=3500
Viable IUP: 62% 2170
Spontaneous Abortion: 29% 1015
Ectopic Pregnancy: 9% 315
Group 1 (visualized by ultrasound) n=2800 (80%)
Group 2 (pregnancy of unknown location) n=700 (20%)
Viable IUP: 78% 2184
Spontaneous Abortion: 16% 448
Ectopic Pregnancy: 6% 168
Viable IUP: 11% 77
Spontaneous Abortion: 72% 504
Ectopic Pregnancy: 17% 119

# Other questions

- Use of the test(s) in special circumstances.
  - Unusual EP (cervical, ovarian, interstitial)
- When can the test detect non-viability?
  - How far away from the diagnosis
  - What about in patient with genetic abnormalities.

# Ancillary studies

- Discovery of better biomarkers (Wistar Institute)
  - Mass spec assay of candidates
  - Metabolomics
  - Lipidomics

- Natural history of new markers (PSG family, TFPI2)
  - Serial levels in IUP, SAB, EP
  - Change in markers compared to change in hCG

# Future Directions

- Multiplex final panel into high though put assay.
- Explore use in urine
- Optimize clinical diagnostic and treatment algorithm based on accuracy and utility of test (i.e. focus on vitality, location, aggressive growth or all of the above)
- Consider “home test”

# Q&A