Diagnostic Evaluation of Recurrent Pregnancy Loss
Agenda & Learning Objectives

Learning Objectives

- Understand prevalence of Recurrent Pregnancy Loss
- Current definitions of Recurrent Pregnancy Loss
- Describe the primary causes of miscarriage
- Society guidelines that exist for fetal and parental evaluation
- Summarize the testing options for RPL
Pregnancy loss is common occurring in an estimated 15% to 25% of recognized pregnancies\textsuperscript{1}.

Recurrent pregnancy loss, defined as \( \geq 2 \) failed pregnancies, occurs in about 5% of reproductive age women\textsuperscript{1}.

\~60\% of early pregnancy losses are associated with chromosomal abnormalities\textsuperscript{1}.

85\% chance of a successful pregnancy for those with 1 miscarriage
75\% for those who have had 2-3 losses
60\% success rate for those with 4 losses

\textsuperscript{1}. Practice Committee of ASRM. Fertil Steril. 2012;98:1103-1111.
Clinical RPL Definition

- Historically, RPL was defined as ≥3 spontaneous consecutive pregnancy losses\(^1\)
- The American Society of Reproductive Medicine (ASRM) and the American College of Obstetricians and Gynecologists (ACOG) now recommend that a physical exam and testing be performed after 2 first-trimester pregnancy losses, whether or not they are consecutive\(^2,3\)

<table>
<thead>
<tr>
<th>Medical Society</th>
<th>Year</th>
<th>RPL Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACOG(^2)</td>
<td>2016</td>
<td>≥2 miscarriages</td>
</tr>
<tr>
<td>ASRM(^3)</td>
<td>2012</td>
<td>≥2 miscarriages</td>
</tr>
</tbody>
</table>

- Most insurance companies will agree to pay for complete evaluation of RPL after 2 consecutive losses\(^1\)

Pregnancy Loss and Recurrent Miscarriage: More Couples Sharing Their Stories and Struggles

Former Olympian Shawn Johnson shares heartbreaking story of miscarriage

"It's a lonely experience," Mark Zuckerberg says, writing about his wife Priscilla Chan's three miscarriages
Miscarriages Impact Patients’ Lives

- In a survey of 1147 US men and women,¹
  - Most felt extremely or very upset about the thought of a miscarriage (whether or not they had previously had one)
  - Significantly more women (41%) than men (29%) were extremely upset
  - 47% felt guilty and 41% felt alone after a miscarriage
- Women with ≥3 miscarriages are significantly more likely to suffer from²
  - Moderate-to-severe depression (5.5x)
  - High stress (1.6x)

Most Would Like to Know the Cause of Miscarriages

- 78% of participants would like to know the cause of the miscarriage
  - Only 9% would not like to know
  - 14% are unsure
- Similar results when categorized by
  - Sex
  - Prior miscarriages
  - Religious affiliations

Would you like to know the cause of the miscarriage even if it cannot prevent a future miscarriage?

Participants (%)

<table>
<thead>
<tr>
<th></th>
<th>Participants (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongly like to know</td>
<td>47</td>
</tr>
<tr>
<td>Like to know</td>
<td>31</td>
</tr>
<tr>
<td>Unsure</td>
<td>14</td>
</tr>
<tr>
<td>Not like to know</td>
<td>5</td>
</tr>
<tr>
<td>Strongly not like to know</td>
<td>4</td>
</tr>
</tbody>
</table>

(n=1084)

When Pregnancy Loss Occurs, Diagnostic Testing Can Help Clinicians and Families Find Answers

Recurrent Miscarriage evaluation

Products of Conception analysis

Opportunity to explore potential etiology
- Identify events unlikely to re-occur
- Evaluate underlying immunologic, hematologic or structural issues that can be addressed
Most Frequent Causes of RPL

- APS: antiphospholipid syndrome; aPL: antiphospholipid antibodies

<table>
<thead>
<tr>
<th>Cause</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal POC or fetal karyotype</td>
<td>41%</td>
</tr>
<tr>
<td>Occasional aPL</td>
<td>6%</td>
</tr>
<tr>
<td>Abnormal parental chromosome</td>
<td>10%</td>
</tr>
<tr>
<td>Uterine anomaly</td>
<td>5%</td>
</tr>
<tr>
<td>Endocrine abnormality</td>
<td>6%</td>
</tr>
<tr>
<td>True unexplained</td>
<td>25%</td>
</tr>
<tr>
<td>Mixed</td>
<td>4%</td>
</tr>
<tr>
<td>Abnormal POC or fetal karyotype</td>
<td>41%</td>
</tr>
</tbody>
</table>

n=482

Miscarriage Risk Increases with Parental Age

- Miscarriage increases with advancing maternal age (Figure)\(^1\)
  - Poor oocyte quality
  - Decline in uterine and ovarian function
- Incidence of first trimester miscarriage\(^2\)
  - 9\%-12\% in women ≤35 years
  - 50\% in women ≥40 years
- Advanced paternal age is a risk factor for miscarriage\(^3\)
- Risk of miscarriage is highest in couples when the woman is ≥35 years and the man ≥40 years\(^3\)

RPL Work-Up
RPL Diagnostic Workup Typically has Multiple Components Including Bloodwork, Genetic Analysis and Imaging

<table>
<thead>
<tr>
<th>Test Category</th>
<th>Example Tests</th>
<th>Test Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombophilia (Inherited)</td>
<td>• Factor V Leiden&lt;br&gt;• Protein C, Protein S&lt;br&gt;• Antithrombin</td>
<td>• Testing for inherited blood clotting disorders, which show strong correlation to RPL (Not routine, based on genetic/family Hx)</td>
</tr>
<tr>
<td>Genetic</td>
<td>• Karyotyping&lt;br&gt;• Microarray</td>
<td>• Evaluation for genetic abnormalities in POC (e.g., trisomy) or parents (e.g., translocations)</td>
</tr>
<tr>
<td>Immunology</td>
<td>• Anticardiolipin&lt;br&gt;• Anti-β2 glycoprotein-I&lt;br&gt;• Lupus anticoagulant</td>
<td>• Evaluation for antiphospholipid antibody syndrome (to diagnose acquired thrombophilia) and other autoimmune disorders</td>
</tr>
<tr>
<td>Imaging/Anatomic</td>
<td>• Ultrasound&lt;br&gt;• Hysterosalpingogram (HSG)</td>
<td>• Evaluation of uterine anatomy to check for scarring, polyps, fibroids, or septa which may be causing the miscarriage</td>
</tr>
<tr>
<td>Hormone</td>
<td>• Prolactin&lt;br&gt;• TSH&lt;br&gt;• Progesterone</td>
<td>• Assessment of ovulatory function and possible endocrine-related disorders&lt;br&gt;• Thyroid hormones are also monitored during pregnancy</td>
</tr>
</tbody>
</table>
Proposed Initial Evaluation for Early RPL

Miscarriage #1
(No action unless clinically indicated)

2nd Miscarriage

Cytogenetic analysis of miscarriage

Aneuploid karyotype

No further evaluation

Euploid karyotype or no POC analysis

RPL Workup

Unbalanced chromosomal translocation or inversion

Perform parental karyotypes and offer preimplantation genetic diagnosis for future pregnancy attempts

Question 4

For products of conception, do you order:

A. Mostly karyotyping
B. Mostly chromosomal microarray
C. Both about equally
D. I don’t order products of conception
Karyotype Analysis

- The traditional method used to find chromosome abnormalities in products of conception (POC)
- However, it has limitations
  - Karyotyping gives you results in 2-3 weeks
  - No results in 10%-40% of cases
  - Limited resolution (>3–5 Mb)
  - Chance of culture failure or maternal contamination
- Quest offers both chromosome analysis and maternal cell contamination tests

Karyotype: Trisomy 16

ClariSure® Oligo-SNP POC Array

Evaluates tissue from a pregnancy loss to determine whether a chromosomal abnormality was the likely cause of the miscarriage

- DNA extracted directly from POC tissue (no cell culture required)
- Uses DNA probes on a slide to compare a patient’s DNA to control DNA
- High coverage available with over 2.67 million probes
- Can find deletions and duplications (CNV) throughout the genome
- 10- to 14-day TAT

SNP – Single nucleotide polymorphisms are the most common type of genetic variation

Samples Required
- 2 x 3 mm POC tissue in transport media at room temperature or refrigerated
- FFPE tissue acceptable

Quest can perform parental follow up genetic testing
Proposed Initial Evaluation for Early RPL

- Miscarriage #1
  (No action unless clinically indicated)

- 2nd Miscarriage

  - Aneuploid karyotype
    - No further evaluation

  - Cytogenetic analysis of miscarriage
    - Euploid karyotype or no POC analysis
      - RPL Workup
    - Unbalanced chromosomal translocation or inversion
      - Perform parental karyotypes and offer preimplantation genetic diagnosis for future pregnancy attempts
Parental Genetic Abnormalities

- Incidence of parental genetic abnormalities increase from 0.4% with ≤1 miscarriage to 5% with 3 miscarriages\(^1\)

<table>
<thead>
<tr>
<th>Prior loss</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Tests for Diagnosis\(^2\)

Parental karyotype
- Chromosome analysis (detects balanced rearrangements)

Treatment Options
- Genetic counseling
- Preimplantation genetic diagnosis for balanced translocation

---

Initial Evaluation for Early RPL

- **Miscarriage #1**
  - (No action unless clinically indicated)

- **2nd Miscarriage**

- **Aneuploid karyotype**
  - No further evaluation

- **Cytogenetic analysis of miscarriage**

- **Euploid karyotype or no POC analysis**

- **Unbalanced chromosomal translocation or inversion**
  - Perform parental karyotypes and offer preimplantation genetic diagnosis for future pregnancy attempts

- **RPL Workup**
Antiphospholipid Syndrome

- Between 5% and 20% of patients with RPL test positive for antiphospholipid antibodies\(^1\)
  - Incidence could be as high as 42%

### Tests for Diagnosis\(^1\)
- Anticardiolipin (aCL)
- Anti-\(\beta\)2 glycoprotein-I (Anti-\(\beta\)2GPI)
- Lupus anticoagulant

### Treatment Options
- Heparin + aspirin

Syphilis infection should also be excluded as it can give a false-positive test for APS\(^4\)

---

Endocrine Factors

- Luteal phase deficiency, elevated thyroid-stimulating hormone (TSH) levels, uncontrolled diabetes, and hyperprolactinemia have been found associated with RPL\textsuperscript{1,2}

### Tests for Diagnosis\textsuperscript{2}
- Midluteal progesterone
- Thyroid-stimulating hormone
- Prolactin
- Fasting glucose or Hemoglobin A1c

### Treatment Options
- Progesterone
- Levothyroxine
- Bromocriptine
- Diabetes control (weight loss, nutrition, metformin)

Cervical incompetence evaluation is not recommended\textsuperscript{1}

Inherited Thrombophilias

- Screening may be clinically justified with a personal history of venous thromboembolism such as
  - Non-recurrent risk factor (such as with surgery)
  - First-degree relative with a known or suspected high-risk thrombophilia
- Routine testing of women with RPL for inherited thrombophilias is not currently recommended

Tests for Diagnosis

- Factor V Leiden mutation (FVL)
- Prothrombin G20210A gene mutation (PGM)
- Protein S deficiency
- Protein C deficiency
- Antithrombin deficiency

Anatomical Causes

- 16%-23% of patients with RPL have anatomical anomalies\(^1\)

### Tests for Diagnosis\(^2\)
- 3-D ultrasonography
- Sonohysterography (SHG)
- Hysterosalpingography (HSG)
- Hysteroscopy
- MRI

### Treatment Options
- Targeted surgical correction
  - Hysteroscopic resection of septum
  - Myomectomy
  - Hysteroscopic removal of polyps
  - Adhesiolysis

---

Live Births after ≥3 Miscarriages

- 67% of women achieved a live birth 5 years after a first consultation
  - Increased to 71% after 15 years
- A live birth was achieved after 5 years in
  - 72% of women with 3 miscarriages
  - 50% of women with ≥6 miscarriages

And Often There Is a Rainbow After the Storm…
Why Quest Diagnostics?
## Why Quest Diagnostics for Genetic Testing?

### Comprehensive Genetic Test Menu

<table>
<thead>
<tr>
<th>Molecular</th>
<th>Cytogenetic</th>
<th>Biochemical</th>
<th>Oncology</th>
<th>Women’s &amp; Reproductive Health</th>
<th>Neurology</th>
</tr>
</thead>
</table>

> 700 genetic tests

- Experience with Unusual and Rare Cases
- Innovative Test Menu that Spans Key Therapeutic Areas
- Clinically Appropriate Testing
- Continuum of Care
- Information & Analytics Capabilities, broad EMR integration
# Guidelines for Recurrent Pregnancy Loss

## Current Testing Guidelines for Recurrent Pregnancy Loss (RPL)

<table>
<thead>
<tr>
<th>Recurrent Pregnancy Loss Testing</th>
<th>ACOG</th>
<th>ASRM</th>
<th>ACG</th>
<th>ESHRE</th>
<th>Opinion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CRITERIA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 or 3 or more intrauterine pregnancy losses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid Stimulating Hormone</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Lupus anticoagulant (LA)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Anticardiolipin (aCL) IgG IgM</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Anti-β2GPI IgG IgM</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Karyotype X2 (both parents)</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

### Reference Sources
- ACOG: American College of Obstetrics & Gynecology
- ASRM: American Society of Reproductive Medicine
- ACG: Antiphospholipid Consensus Group
- ESHRE: European Society of Human Reproduction & Embryology
- Opinion: Expert Opinion Papers & Reviews

### References
Quest Diagnostics’ Testing Spans the Continuum of Care for Procreative Screening and Diagnostics

**Preconception**
- Carrier Testing
  - QHerit®
  - Cystic Fibrosis
  - Fragile X
  - SMA
  - AJ panel
  - Thalassemias
  - Fertility-related

**Prenatal**
- Screening
  - MSS:
    - First Trimester Screen
    - Quad, Penta
    - Integrated, Sequential Serum
  - QNatal® Advanced cfDNA noninvasive prenatal Screen

- Diagnostic
  - Amniocentesis
  - CVS
  - Chromosomal Microarray
    - Karyotyping
    - FISH
  - Products of Conception
    - ClariSure® Oligo-SNP

**Postnatal**
- Diagnostic
  - Identify inherited conditions via phenotype, or genotype
  - Chromosomal Microarray
    - Karyotyping
    - FISH
Expert Consultation: Genetic & Genomics Client Services for Providers

- Genetic Consultation Services for Providers
  - Genomic Client Services (1.866.GENE.INFO or 1.866.436.3463)
    - Available 8:00 AM – 8:00 PM ET always answered live
  - Over 35 genetic counselors board certified by the American Board of Genetic Counseling
  - Our genetic counselors assist clinicians with
    - Test selection alternatives
    - Result interpretation
    - VUS reclassification & verification
    - Review of order
  - Positive findings on fetal screening & diagnostic are proactively mentioned to ordering HCP
- Quest supports the Perinatal Quality Foundation (PQF)
  - Quest is the first commercial diagnostics laboratory to support the PQF with a grant for a national campaign to improve understanding of prenatal test results for patients and providers

Summary

- ASRM and ACOG medical societies defined recurrent pregnancy loss as ≥2 failed clinical pregnancies, whether or not consecutive
  - Majority of miscarriages are sporadic and are thought to result from genetic causes that are greatly influenced by maternal age
  - However, recurrent pregnancy loss occurs in up to 5% of women
  - Evaluation of recurrent pregnancy loss should proceed after 2 clinical pregnancy losses
- POC should be tested for genomic abnormalities by traditional karyotype or chromosomal microarray (e.g., ClariSure®-Oligo-SNP, POC)
  - Parental karyotypes should be performed if POC evaluation detects unbalanced chromosomal translocation or inversion
  - If POC genomic evaluation appear normal, a full RPL workup should be performed as described
- Quest Diagnostics offers:
  - Support for clinicians and families
  - Diagnostic tests including, but not limited to
    - Parental and POC karyotyping and chromosomal microarrays
    - APS, inherited thrombophilia, and endocrine factors
    - Parental follow up genetic testing