Diagnostic Evaluation of Recurrent Pregnancy Loss

Quest Diagnostics™

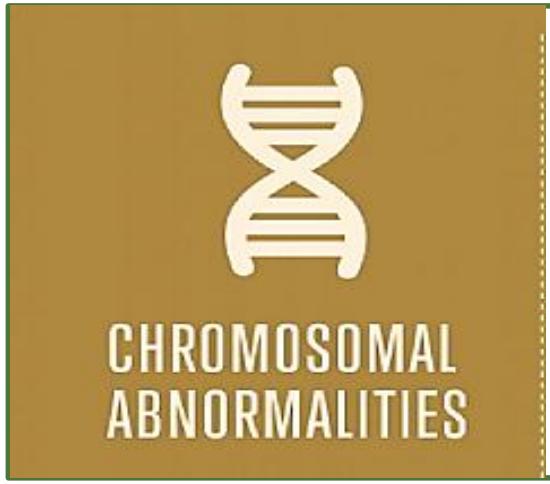
Agenda & Learning Objectives

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

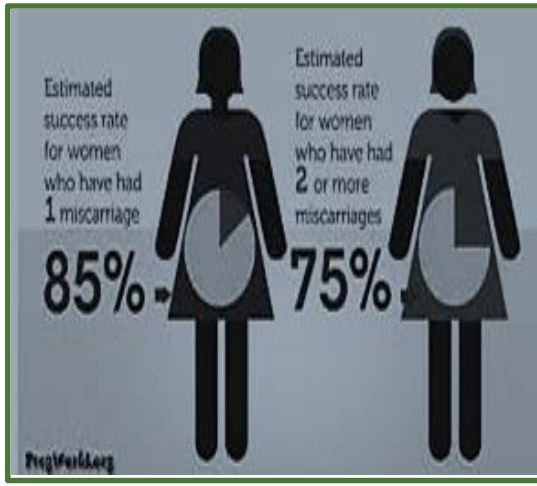
Pregnancy Loss and Recurrent Miscarriage By the Numbers

Pregnancy loss is common occurring in an estimated 15% to 25% of recognized pregnancies[1]



~60% of early pregnancy losses are associated with chromosomal abnormalities[1]

Recurrent pregnancy loss, defined as ≥2 failed pregnancies, occurs in about 5% of reproductive age women[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

1. Practice Committee of ASRM. *Fertil Steril.* 2012;98:1103-1111.

Quest Diagnostics™

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]

Medical Society	Year	RPL Definition
ACOG[2]	2016	≥2 miscarriages
ASRM[3]	2012	≥2 miscarriages

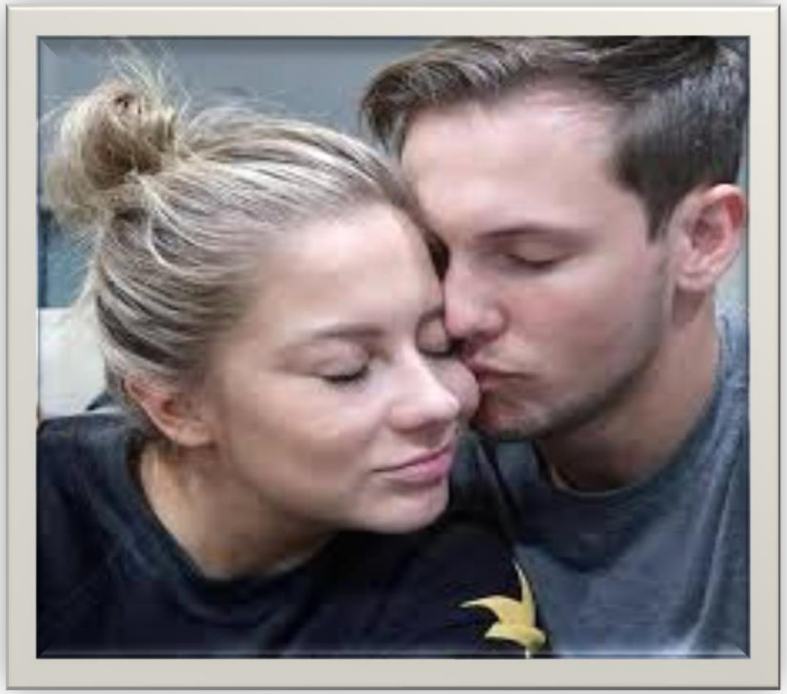
- Most insurance companies will agree to pay for complete evaluation of RPL after 2 consecutive losses[1]

1. Kutteh WH, et al. *Semin Reprod Med.* 2015;33:161-168. **2.** ACOG FAQ. Available at www.acog.org/-/media/For-Patients/faq100.pdf?dmc=1&ts=20180410T1515123241. Accessed on 10Apr2018. **3.** Practice Committee of ASRM. *Fertil Steril.* 2012;98:1103-1111.

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

Former Olympian Shawn Johnson shares heartbreaking story of miscarriage

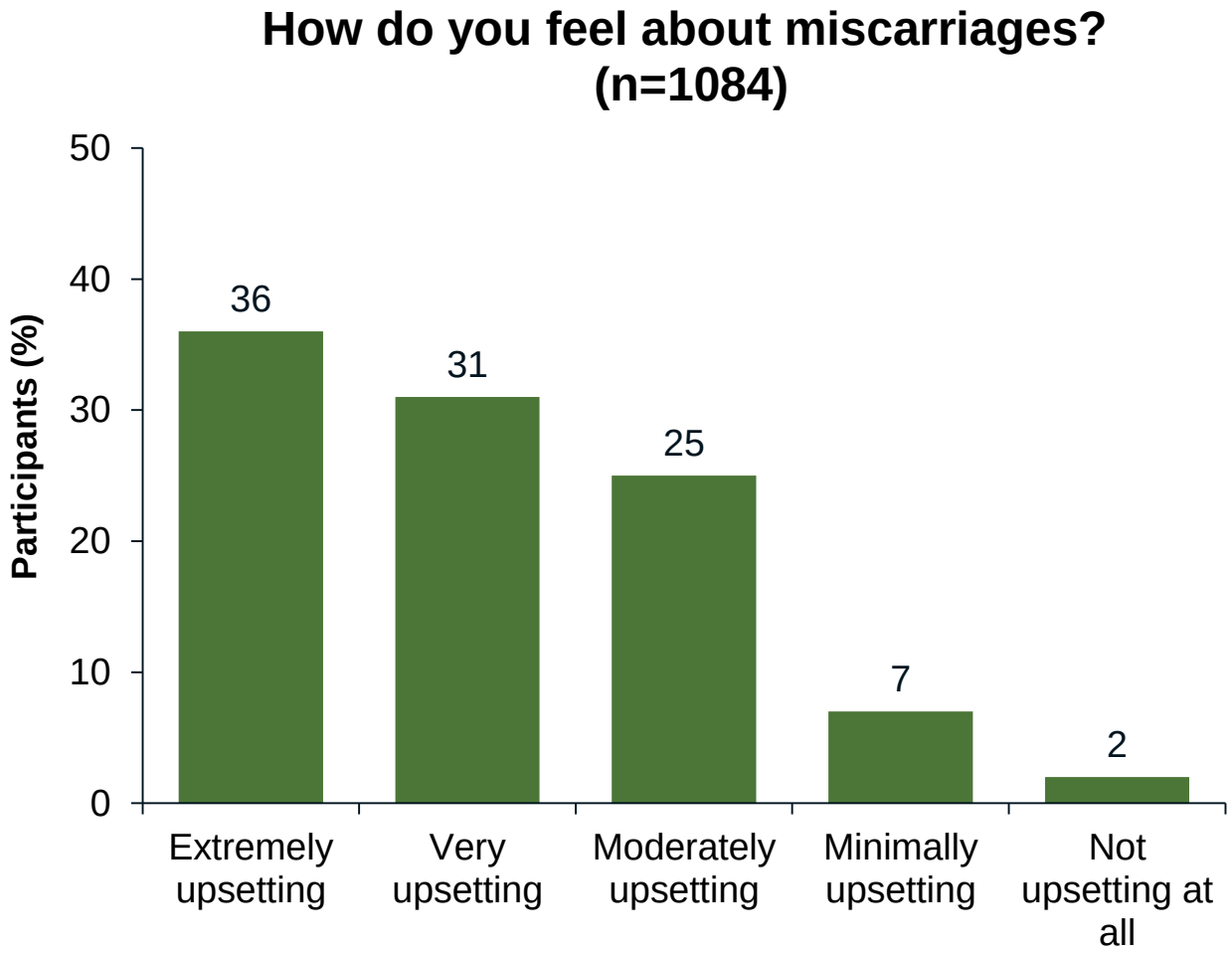
SCOTT GLEESON | USA TODAY SPORTS
Updated 5:06 p.m. EDT Oct. 21, 2017



"It's a lonely experience," Mark Zuckerberg says, writing about his wife Priscilla Chan's three miscarriages

Miscarriages Impact Patients' Lives

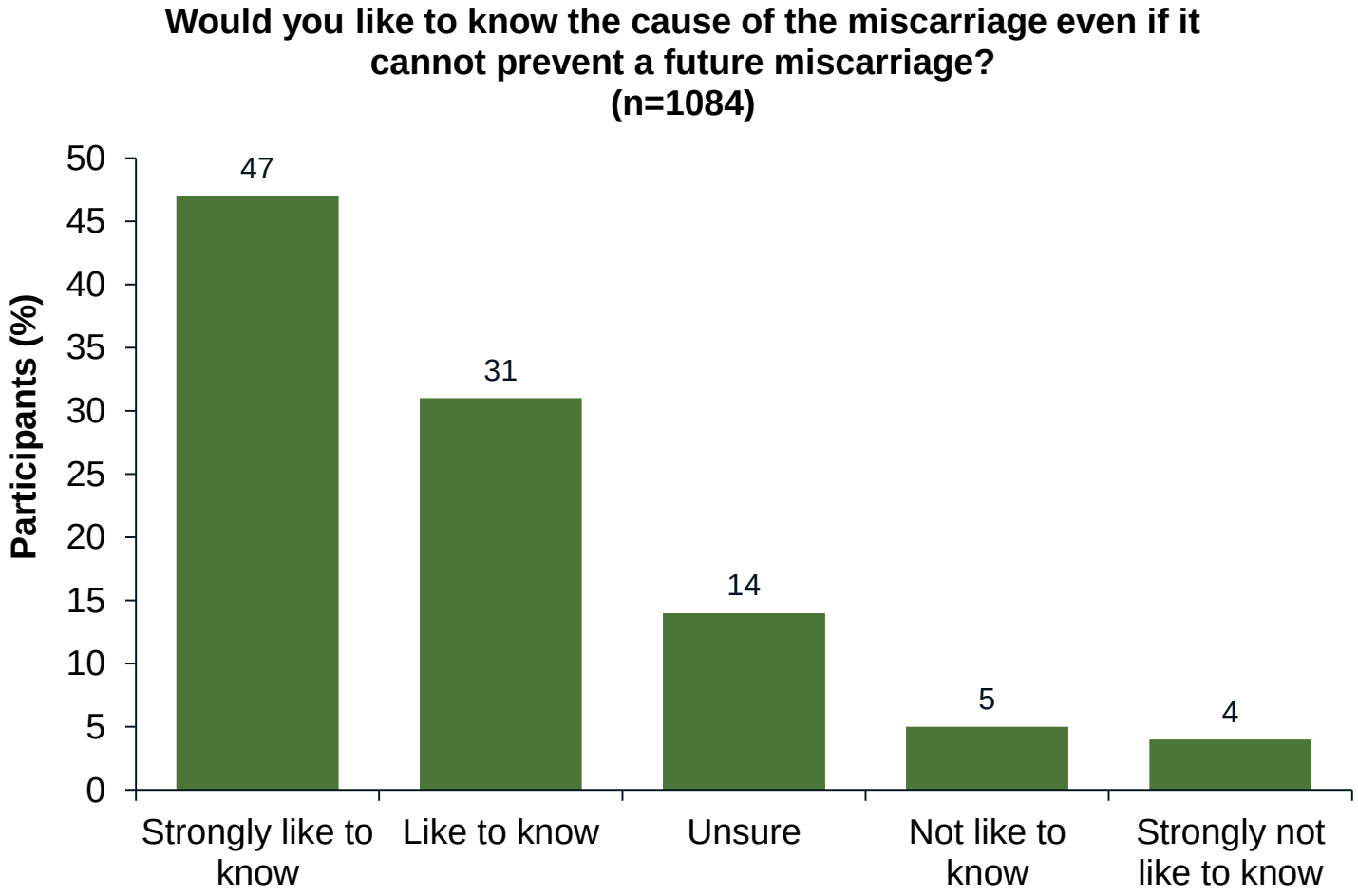
- In a survey of 1147 US men and women,[1]
  - Most felt extremely or very upset about the thought of a miscarriage (whether or not they had previously had one)
  - Significantly more women (41%) than men (29%) were extremely upset
  - 47% felt guilty and 41% felt alone after a miscarriage
- Women with ≥3 miscarriages are significantly more likely to suffer from[2]
  - Moderate-to-severe depression (5.5x)
  - High stress (1.6x)



1. Bardos J, et al. *Obstet Gynecol.* 2015;125:1313-1320. **2.** Kolte AM, et al. *Hum Reprod. 2*015;30:777-782.

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



Bardos J, et al. *Obstet Gynecol.* 2015;125:1313-1320.

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

Recurrent Miscarriage evaluation

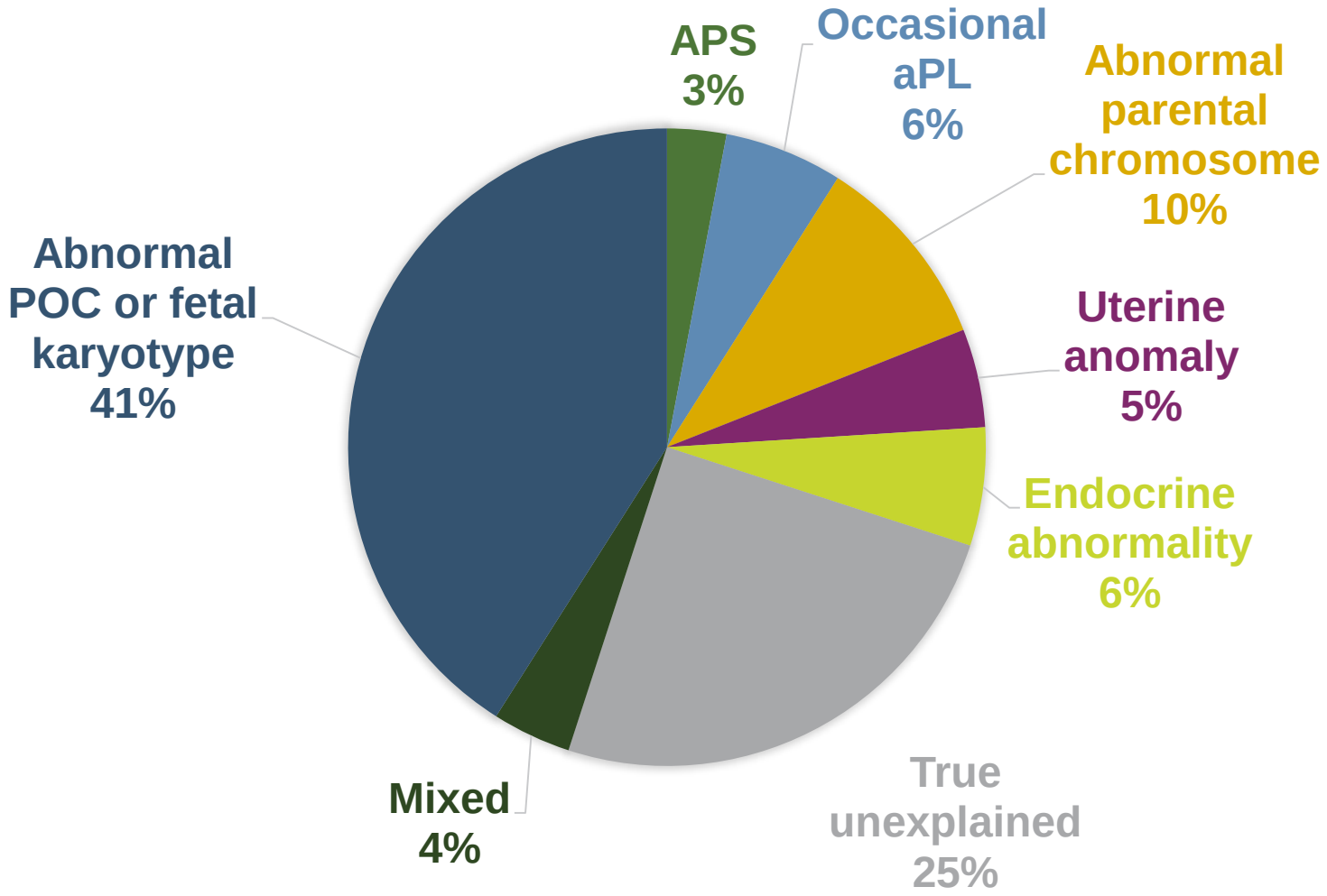
Products of Conception analysis

Opportunity to explore potential etiology

- Identify events unlikely to re-occur
- Evaluate underlying immunologic, hematologic or structural issues that can be addressed

Quest Diagnostics™

Most Frequent Causes of RPL



n=482

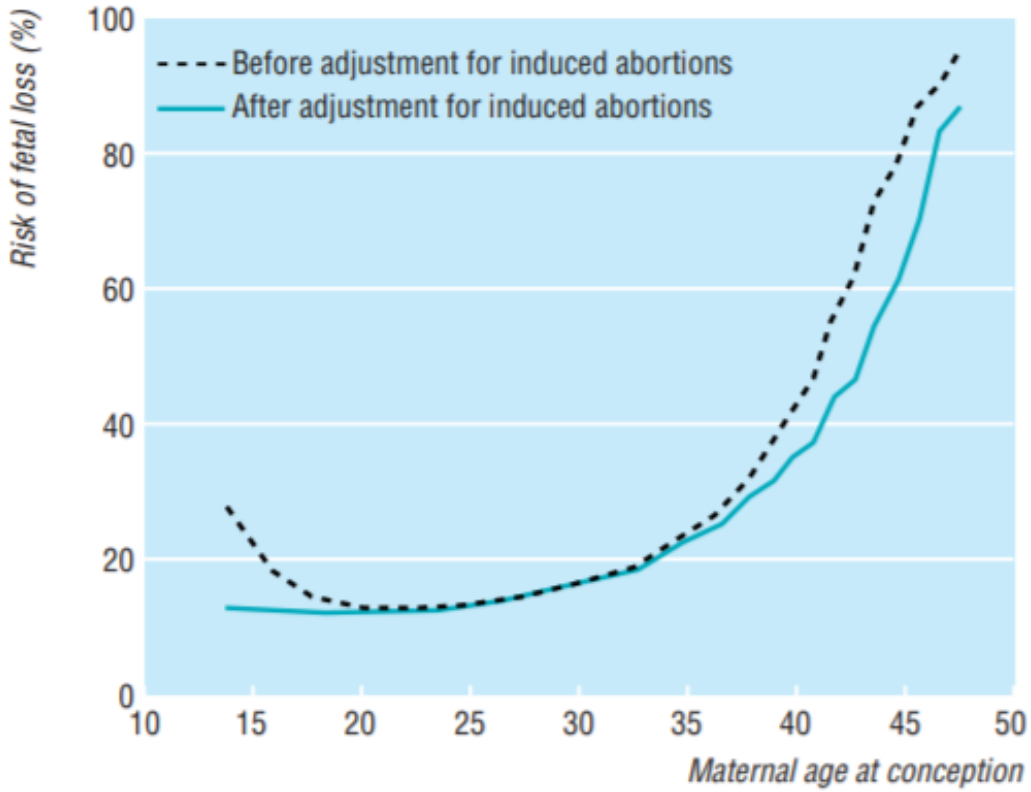
Sugiura-Ogasawara M, et al. *Hum Reprod.* 2012;27:2297-2303.

APS: antiphospholipid syndrome; aPL: antiphospholipid antibodies

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]

Incidence of Miscarriage by Maternal Age[1]



Adapted from Nybo Andersen AM. *BMJ.* 2000;320:1708-1712.

1. Nybo Andersen AM. *BMJ.* 2000;320:1708-1712. **2.** Shahine L and Lathi R. *Obstet Gynecol Clin N Am.* 2015;42:117-134.
3. De la Rochebrochard E, and Thonneau P. *Hum Reprod.* 2002;17:1649-1656.

RPL Work-Up

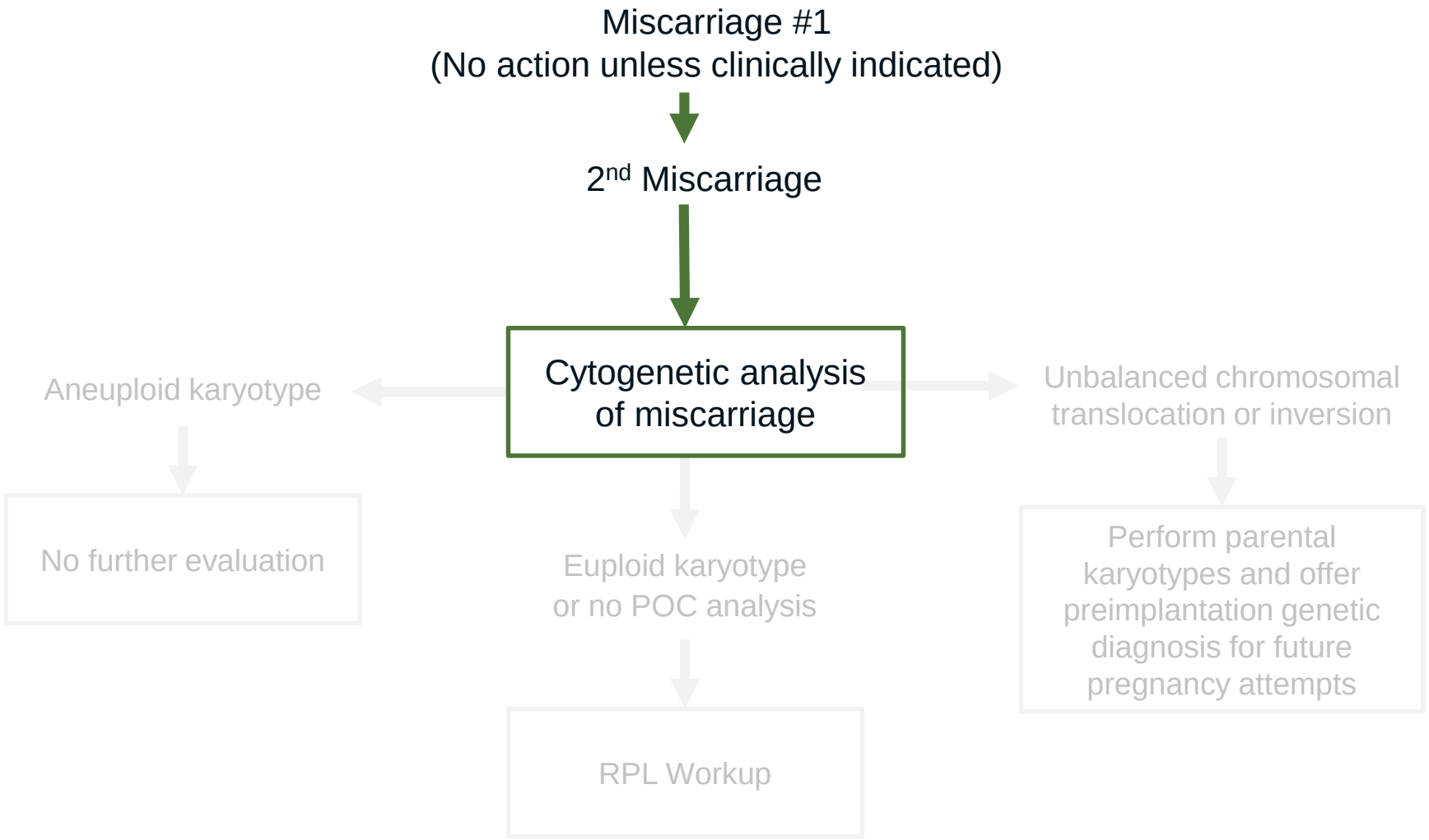
Quest Diagnostics™

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

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

Quest Diagnostics™

Proposed Initial Evaluation for Early RPL



Kutteh WH. *Semin Reprod Med.* 2015;33:161-168.

Question 4

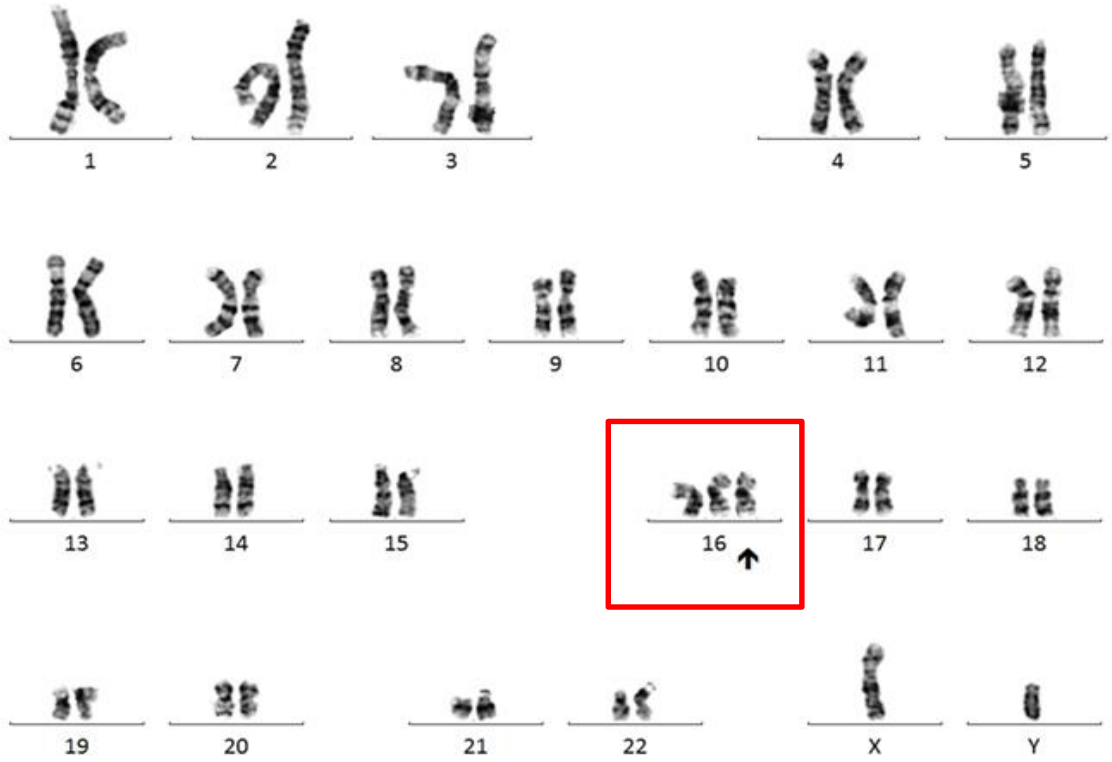
For products of conception, do you order:

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



van den Berg, et al. *Biochim Biophys Acta.* 2012;1822:1951-1959.

ClariSure® Oligo-SNP POC Array

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

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

- 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

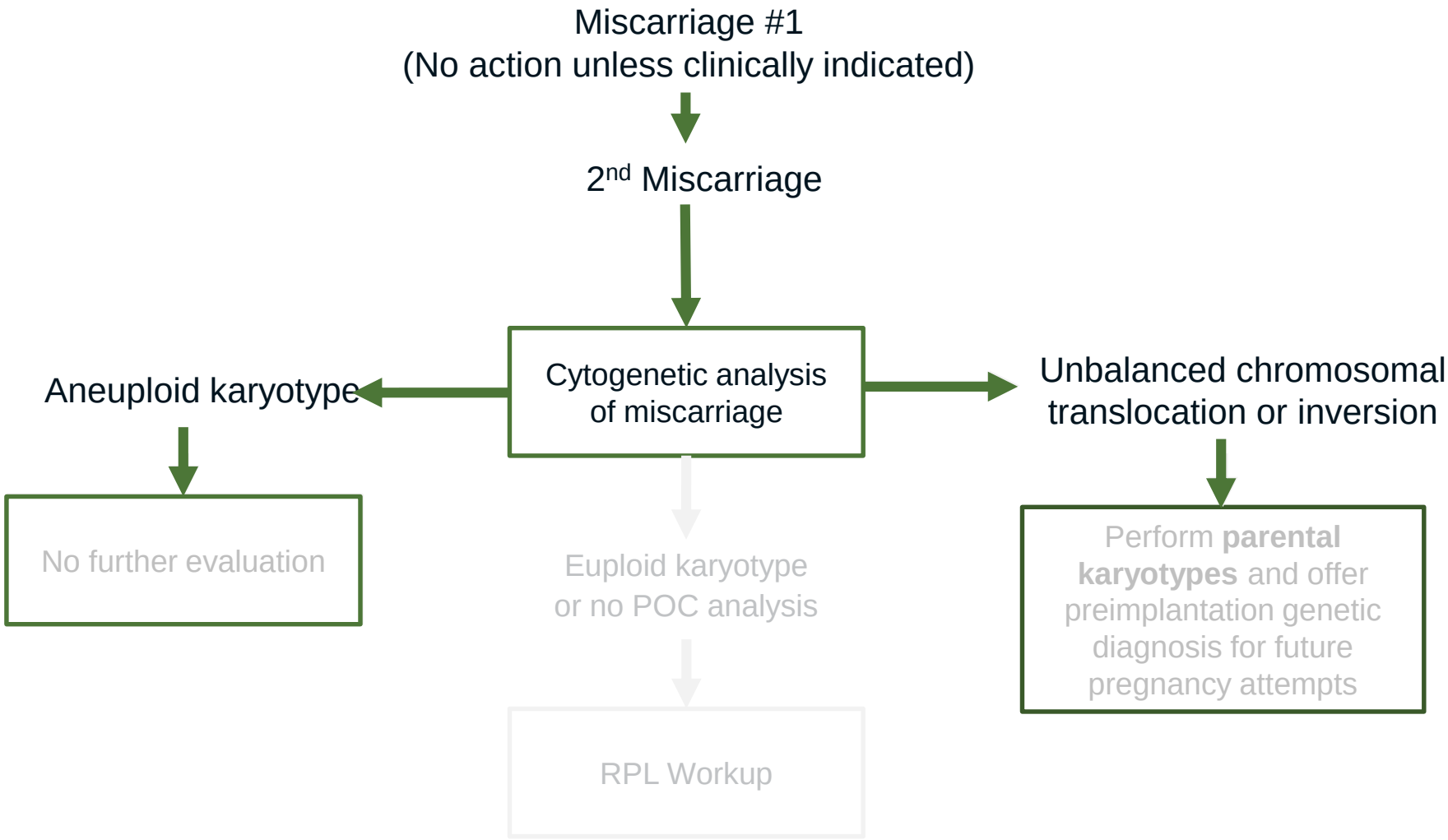
Samples Required
• **2 x 3 mm POC tissue in transport media at room temperature or refrigerated** • **FFPE tissue acceptable**

Quest can perform parental follow up genetic testing

Image from Affymetirix.com

Quest Diagnostics™

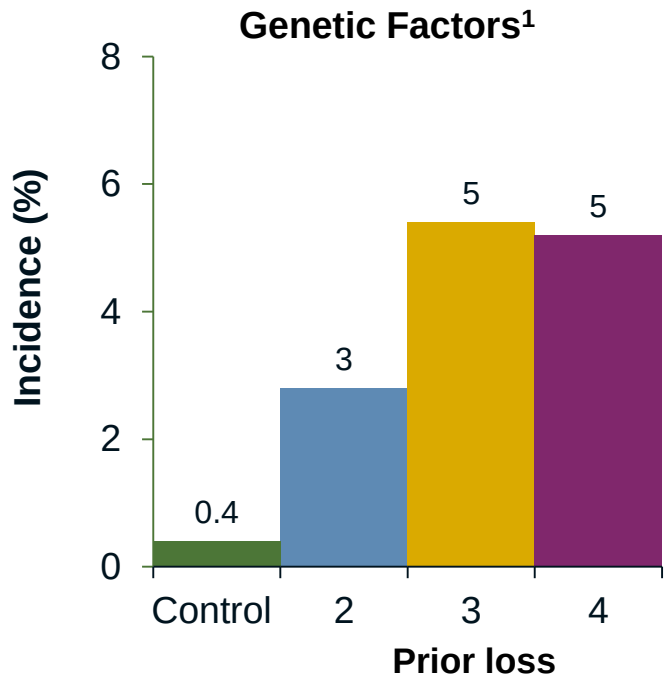
Proposed Initial Evaluation for Early RPL



Kutteh WH. *Semin Reprod Med.* 2015;33:161-168.

Parental Genetic Abnormalities

- Incidence of parental genetic abnormalities increase from 0.4% with ≤1 miscarriage to 5% with 3 miscarriages[1]



Tests for Diagnosis[2]

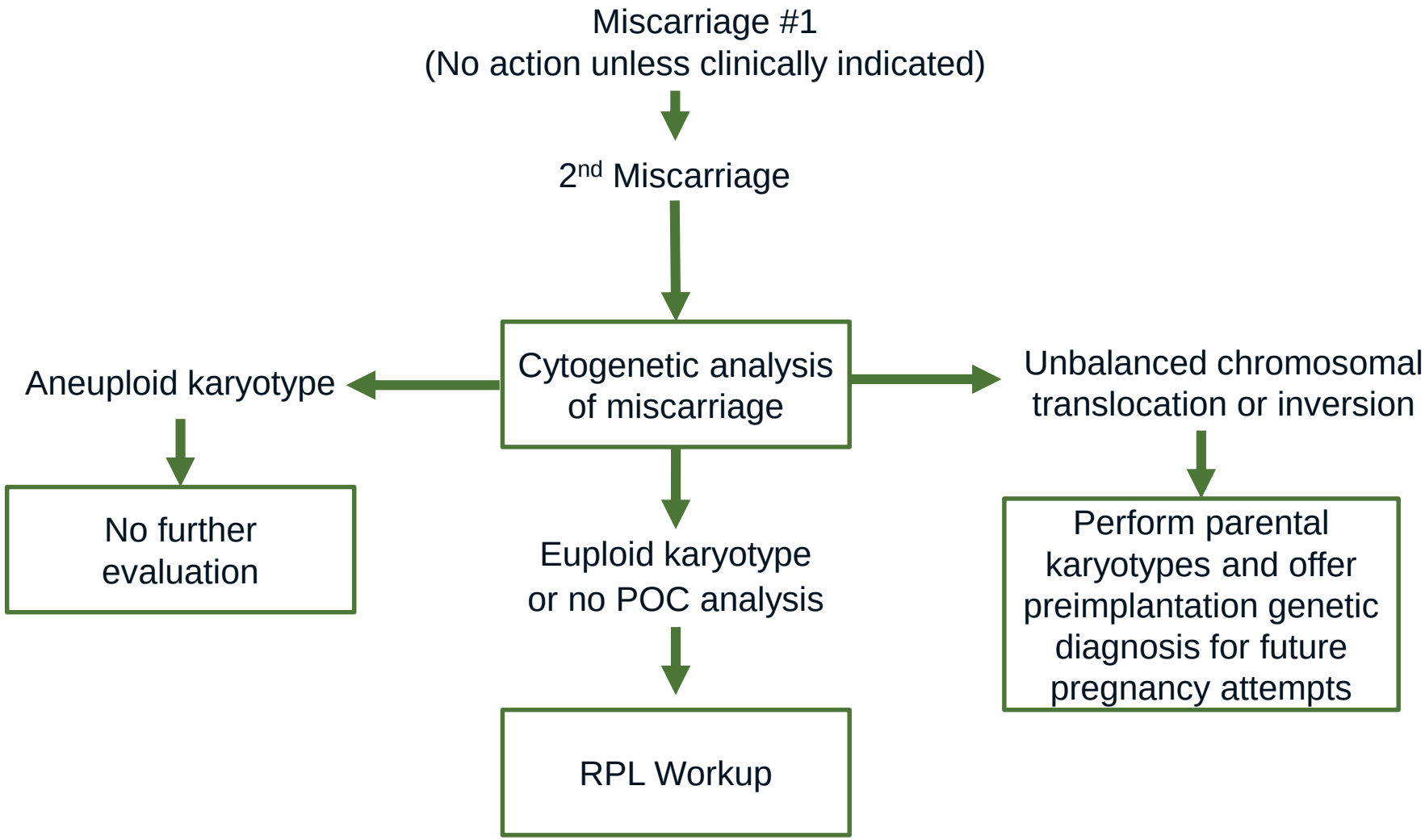
Parental karyotype
- Chromosome analysis (detects balanced rearrangements)

Treatment Options

- Genetic counseling
- Preimplantation genetic diagnosis for balanced translocation

1. Jaslow CR, et al. *Fertil Steril.* 2010;93:1234-1243. **2.** El Hachem H, et al. *Int J Womens Health.* 2017;9:331-345.

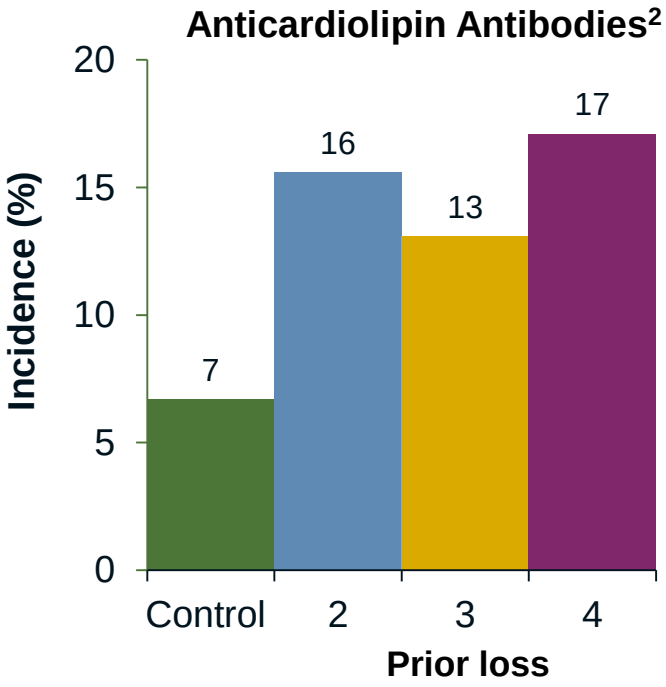
Initial Evaluation for Early RPL

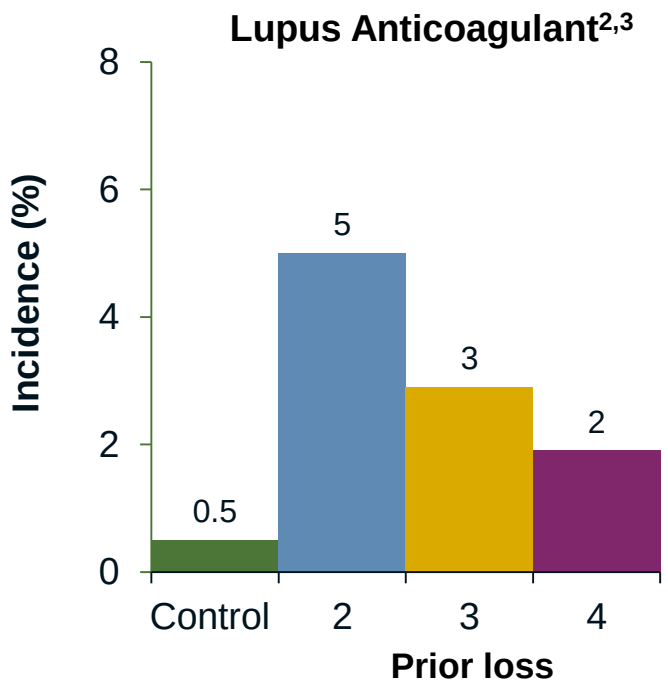


Kutteh WH. *Semin Reprod Med.* 2015;33:161-168.

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-β2 glycoprotein-I (Anti-β2GPI)
- Lupus anticoagulant

Treatment Options

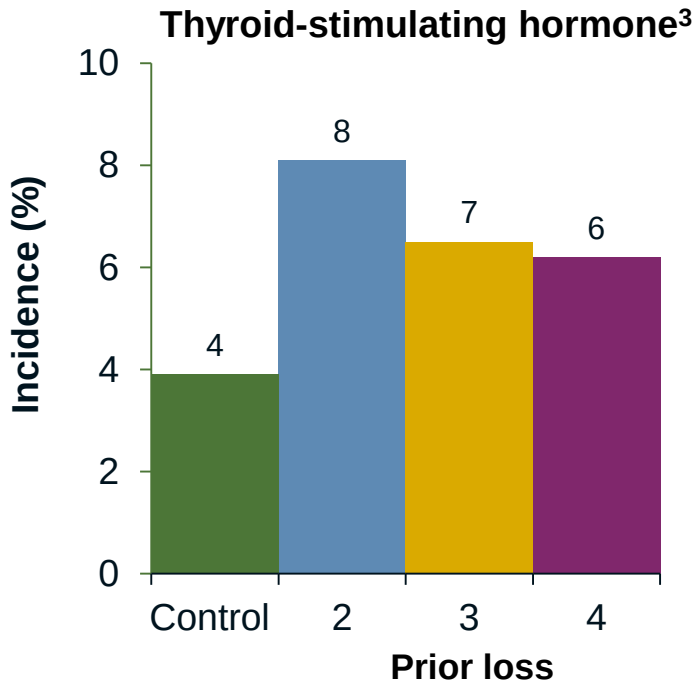
Heparin + aspirin

Syphilis infection should also be excluded as it can give a false-positive test for APS[4]

1. El Hachem H, et al. *Int J Womens Health.* 2017;9:331-345. **2.** Jaslow CR, et al. *Fertil Steril.* 2010;93:1234-1243.
3. Kutteh WH. *Semin Reprod Med.* 2015;33:161-168. **4.** Practice Committee of the ASRM. *Fertil Steril.* 2012;98:1103-1111.

Endocrine Factors

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



Tests for Diagnosis[2]

- Midluteal progesterone
- Thyroid-stimulating hormone
- Prolactin
- Fasting glucose or Hemoglobin A1c

Treatment Options

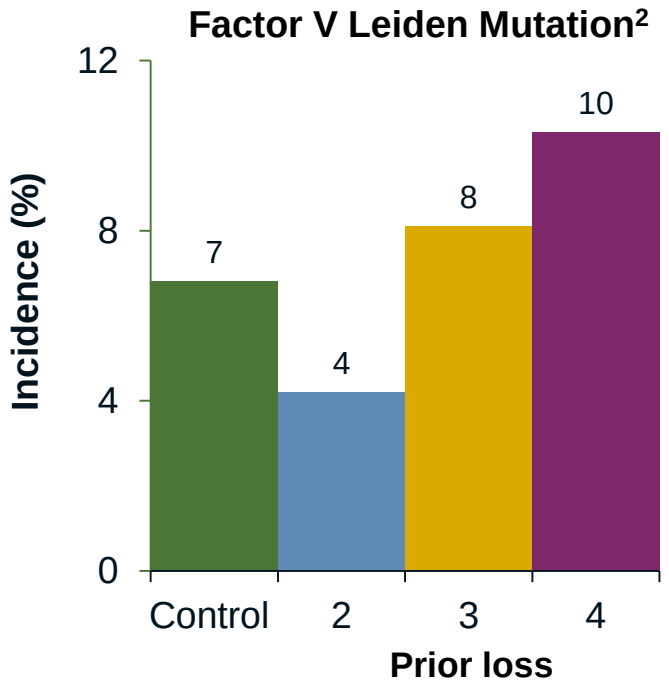
- Progesterone
- Levothyroxine
- Bromocriptine
- Diabetes control (weight loss, nutrition, metformin)

Cervical incompetence evaluation is not recommended[1]

1. Practice Committee of the ASRM. *Fertil Steril.* 2012;98:1103-1111. **2.** El Hachem H, et al. *Int J Womens Health.* 2017;9:331-345. **3.** Jaslow CR, et al. *Fertil Steril.* 2010;93:1234-1243.

Inherited Thrombophilias[1]

- 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



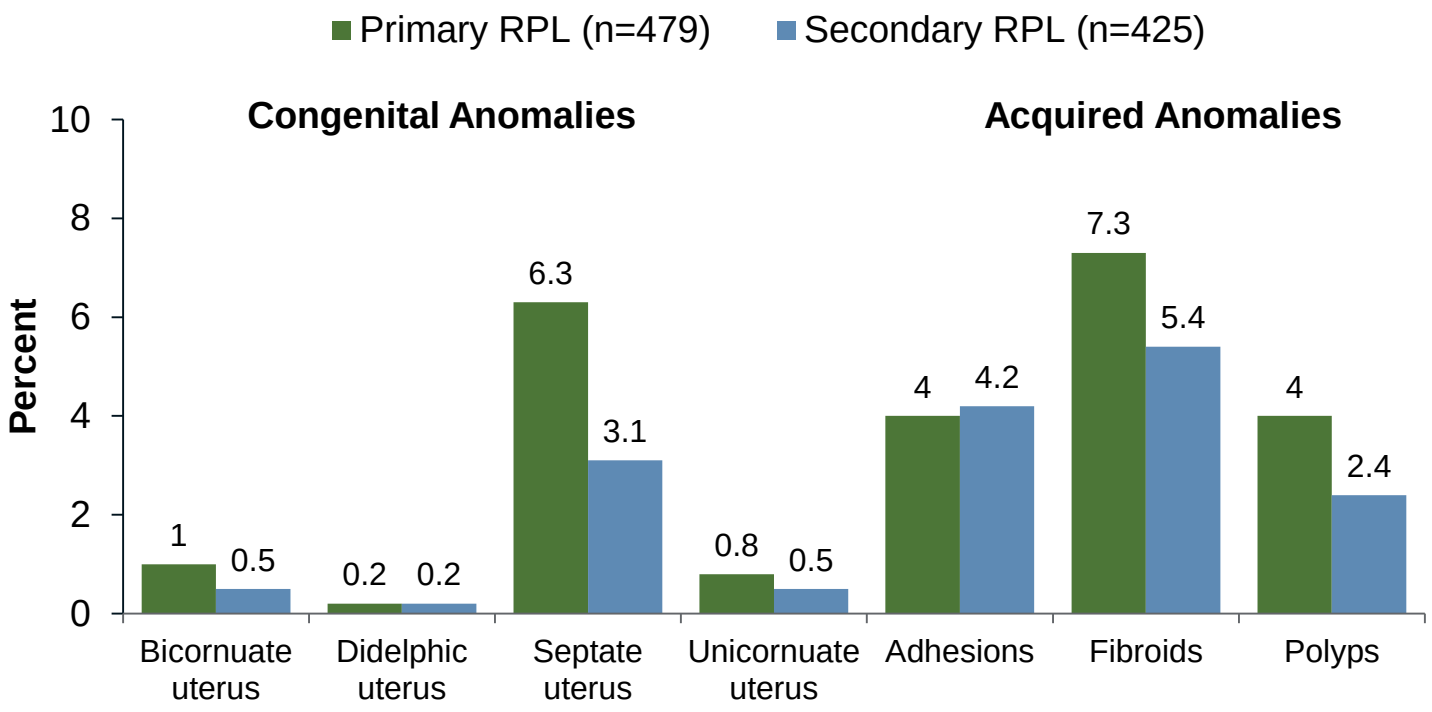
Tests for Diagnosis

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

1. Practice Committee of the ASRM. *Fertil Steril.* 2012;98:1103-1111. **2.** Jaslow CR, et al. *Fertil Steril.* 2010;93:1234-1243.

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

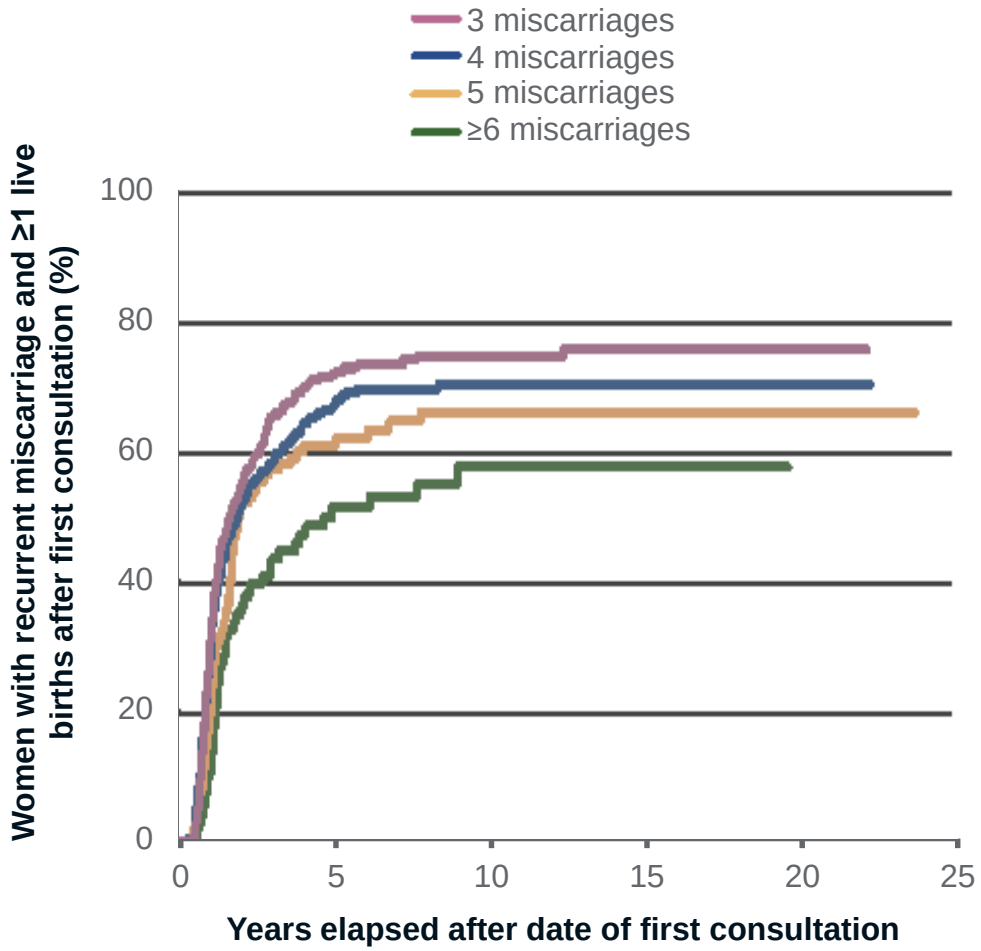
Targeted surgical correction

- Hysteroscopic resection of septum
- Myomectomy
- Hysteroscopic removal of polyps
- Adhesiolysis

1. Kutteh WH. *Semin Reprod Med.* 2015;33:161-168. **2.** El Hachem H, et al. *Int J Womens Health.* 2017;9:331-345.

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



Adapted from Lund M, et al. *Obstet Gynecol.* 2012;119:37-43.

Lund M, et al. *Obstet Gynecol.* 2012;119:37-43.

And Often There Is a Rainbow After the Storm…

Why Quest Diagnostics?

Why Quest Diagnostics for Genetic Testing?

Comprehensive Genetic Test Menu					
Molecular	**Cytogenetic**	**Biochemical**	**Oncology**	**Women's & Reproductive Health**	**Neurology**

> 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)

Recurrent Pregnancy Loss Testing		ACOG	ASRM	ACG	ESHRE	Opinion
Criteria	Tests					
2 or 3 or more intrauterine pregnancy losses	Thyroid Stimulating Hormone		Yes	Yes	Yes	Yes
	Lupus anticoagulant (LA)	Yes	Yes	Yes	Yes	Yes
	Anticardiolipin (aCL) IgG IgM	Yes	Yes	Yes	Yes	Yes
	Anti-β_2GPI IgG IgM	Yes	Yes	Yes	Yes	Yes
	Karyotype X2 (both parents)	Yes	Yes		Yes	Yes

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

1. ACOG Practice Bulletin 150 (2015) Early Pregnancy Loss
2. ACOG Practice Bulletin 132 (2012) Antiphospholipid Syndrome
3. ASRM Committee Opinion (2013) Evaluation and Treatment of RPL
4. ACG International Consensus Statement (2006) Antiphospholipid Syndrome (APS)Recommendations
5. ESHRE Practice Guidelines (2017) Recurrent Pregnancy Loss
6. Stephenson M. Clinical Obstetrics & Gynecology (2007) Evaluation and Management of Recurrent Early Pregnancy Loss
7. Kutteh W. Seminars in Reproductive Medicine (2015) Novel Strategies for the Management of Recurrent Pregnancy Loss

Quest Diagnostics' Testing Spans the Continuum of Care for Procreative Screening and Diagnostics

Preconception | **Prenatal** | **Postnatal**

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

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

Diagnostic
- Identify inherited conditions via phenotype, or genotype
  - Chromosomal Microarray
    - Karyotyping
    - FISH

Quest Diagnostics™

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

Quest press release, July 2015.

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