Genetic workup of recurrent miscarriages
Disclosure

• Member of the Clinical Expert Panel of the Illumina Inc. Reproductive Genetic Health group, USA.
Terminology (1)

• Early pregnancy loss (EPL)
  • a nonviable intrauterine pregnancy with either an empty gestational sac or a gestational sac containing an embryo or fetus without fetal heart activity within the first 12 6/7 weeks of gestation.

• Late pregnancy loss (LPL) is between 13-20 weeks.

• In the first trimester, the terms miscarriage, spontaneous abortion and early pregnancy loss are used interchangeably.
• Recurrent miscarriages or RPL is defined as the loss of 3 or more consecutive pregnancies with or without previous live births, and this condition affects about 1% to 3% of normally conceiving women.

• Some working groups and other expert committees, especially in the USA define recurrent miscarriage as 2 or more failed normally conceived pregnancies.
  • Jaslow CR et al. Fertil Steril. 2010;93:1234-1243
<table>
<thead>
<tr>
<th></th>
<th>2 prior losses</th>
<th>3 prior losses</th>
<th>≥4 prior losses</th>
<th>p value (2 vs. 3 vs. ≥3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evidence-based test results</td>
<td>41%</td>
<td>40%</td>
<td>42%</td>
<td>NS</td>
</tr>
<tr>
<td>Investigative test results</td>
<td>20%</td>
<td>22%</td>
<td>21%</td>
<td>NS</td>
</tr>
<tr>
<td>Total abnormal test results</td>
<td>61%</td>
<td>62%</td>
<td>63%</td>
<td>NS</td>
</tr>
</tbody>
</table>

Notes: Frequency of abnormal test results in 1,020 women with recurrent pregnancy loss. See text for details about “Evidence-based tests” and “Investigative tests.”
Source: Data modified from Jaslow et al.8
Etiologies of RPL

<table>
<thead>
<tr>
<th>Control Frequency (%)</th>
<th>Abnormal test result</th>
<th>2 prior losses (n = 447)</th>
<th>3 prior losses (n = 343)</th>
<th>≥4 prior losses (n = 230)</th>
<th>p value (2 vs. 3 vs. &gt;3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>Parental genetics</td>
<td>2.8%</td>
<td>5.4%</td>
<td>5.2%</td>
<td>NS</td>
</tr>
<tr>
<td>7.5</td>
<td>Uterine anatomy</td>
<td>18.7%</td>
<td>18.2%</td>
<td>16.7%</td>
<td>NS</td>
</tr>
<tr>
<td>0.5</td>
<td>Lupus anticoagulant</td>
<td>5.0%</td>
<td>2.9%</td>
<td>1.9%</td>
<td>NS</td>
</tr>
<tr>
<td>6.7</td>
<td>Anticardiolipin antibodies</td>
<td>15.6%</td>
<td>13.1%</td>
<td>17.1%</td>
<td>NS</td>
</tr>
<tr>
<td>3.9</td>
<td>Thyroid-stimulating hormone</td>
<td>8.1%</td>
<td>6.5%</td>
<td>6.2%</td>
<td>NS</td>
</tr>
<tr>
<td>6.8</td>
<td>Factor V Leiden mutation</td>
<td>4.2%</td>
<td>8.1%</td>
<td>10.3%</td>
<td>NS</td>
</tr>
</tbody>
</table>

Notes: Frequency of abnormal test results in 1,020 women with recurrent pregnancy loss. Control frequency based on the percent of reproductive-aged, nonpregnant women without a history of miscarriage who had an abnormal test result. Source: Data modified from Jaslow et al.8

<table>
<thead>
<tr>
<th>Table 4 Spectrum of pregnancy loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Pregnancy of unknown location (PUL)</td>
</tr>
<tr>
<td>• Early embryonic Loss (&lt;6 wk)</td>
</tr>
<tr>
<td>• Embryonic loss (&gt;6–9 wk)</td>
</tr>
<tr>
<td>• Fetal loss (&gt;9–20 wk)</td>
</tr>
<tr>
<td>• Miscarriage (&lt;20 wk)</td>
</tr>
<tr>
<td>• Stillbirth (&gt;20 wk)</td>
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</tbody>
</table>
Incidence and main risk factors

Early pregnancy loss
- EPL is common, occurring in 10-15% of all recognized pregnancies.
- About 80% of all pregnancy loss occur in the first trimester,
- At least 50% (up to 70%) of all EPL are due to fetal chromosomal abnormalities.
- Most common risk factors identified for EPL is advanced maternal age (>35 yr) and previous EPL.

ASRM: Patient fact sheet RPL 2005
Incidence of RPL

- Recurrent miscarriage or RPL (3 or more) affects about 1% to 3% of pregnant women with a normal conception.
- Recurrent miscarriage or RPL (2 or more) affects about 5% of pregnant women with a normal conception.
Genetic and molecular abnormalities

• 2 and 4% of couples who experience RPL because one partner is affected with a balanced structural chromosomal rearrangement, usually a balanced translocation.

• The incidence of balanced translocations is 2-fold higher among females than males.

• 40% are balanced Robertsonian and 60% reciprocal translocations.
Chromosomal inversions

- Chromosomal inversions have also been linked to RPL.
- Heterozygous carriers of inversions are phenotypically normal.
- The risk of abnormal offspring depends on the size and location of the inversion and whether the carrier is male or female, 5% versus 7% for pericentric inversions resp.
- Paracentric inversion recombinants are universally lethal
Pericentric inversion

- Inversions of small portions of the total chromosomal length lead to large duplications and deletions and are generally lethal.
- Paradoxically, larger pericentric inversions are more likely to be compatible with survival.
Recurrent aneuploidy

• Approximately 50% of sporadic miscarriage is a result of aneuploidy.
• Some couples with RPL might have a propensity for recurrent embryonic aneuploidy despite normal parental karyotypes.
  • Some analyses have found that a first aneuploid miscarriage is associated with a 50-70% likelihood of recurrence in the next pregnancy
  • The concept of recurrent aneuploidy is further supported by preimplantation genetic studies in which aneuploidy was present in more than 50% of embryos from couples of women with RPL.
Recurrent aneuploidy

• The concept of recurrent aneuploidy has been questioned because when karyotypic results are adjusted by maternal age there appears to be no statistically significant relationship between aneuploidy and RPL.

• Investigators found that the incidence aneuploidy in abortus material was significantly lower in women with a history of RPL compared to controls suggesting that if recurrent fetal aneuploidy contributes to RPL, it does so only in a small proportion of the patients.

Skewed X-chromosome inactivation

- Candidate genes on the X chromosome have been particularly studied because of its unique properties of transcriptional silencing (X-Chromosome inactivation, XCI) in female cells.
- So-called skewed XCI is a pathological state in which the majority of cells preferentially express one parent’s X chromosome over the other.
- The hypothesis that a mutation on the X chromosome results in skewed XCI and ultimately lethality of male embryos inheriting this mutated X does not explain the increase in autosomal trisomies observed in the karyotyped losses from women with RPL and skewed XCI.
Skewed X-inactivation & RPL

Figure 2 The association between skewed X chromosome inactivation and idiopathic recurrent spontaneous abortion.

Figure 6  Association of extremely skewed X chromosome inactivation (≥95%) and idiopathic recurrent spontaneous abortion.

Figure 7  Association of extremely skewed X chromosome inactivation (>90% or ≥90%) and idiopathic recurrent spontaneous abortion when defined as three or more losses.

Skewed X-inactivation & RPL
Genetic workup

• It is not recommended to evaluate a couple following one miscarriage.
• For years, it was recommended to wait for three miscarriages, but several studies have shown that the risk of a future miscarriage after two successive losses (24%-29%) is similar to or slightly lower than the risk after three losses (31%-33%), and the findings are comparable.
• Therefore, it is acceptable to start a workup following two consecutive losses, especially in women aged >35 years.
Genetic workup

• For years, *genetic evaluation of the POC was not routinely ordered* because it was considered difficult and unreliable.

• Because the genetic workup was done by routine karyotype analysis, it was associated with a risk of maternal cell contamination, a risk of false-negative results, and a risk of failed cell culture.

• Use of new techniques, such as single-nucleotide polymorphism microarrays and comparative genomic hybridization arrays (aCGH), resolved these issues and allowed for a 23-chromosome pair analysis at even higher resolution than karyotyping.
Analysis of POC

• If POC is found euploid, a full RPL workup is ordered. If an unbalanced chromosomal translocation or inversion is found, a parental karyotype is ordered and PGD offered for future attempts.

• Aneuploidy in the POC confirms the diagnosis and no further tests are necessary. Some studies have reported such a strategy to be more cost-effective than the classic evaluation.
Initial Evaluation for Early RPL

- Miscarriage #1
  (No action unless clinically indicated)
- 2nd Miscarriage
  - Aneuploid karyotype
    - No further evaluation
  - Obtain Miscarriage Karyotype
    - Euploid karyotype
      - RPL Workup
    - Unbalanced chromosomal translocation or inversion
      - Perform parental karyotypes and offer preimplantation genetic diagnosis for future pregnancy attempts
Whole exome sequencing in recurrent early pregnancy loss

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Thank you for your attention!