Recurrent Early Pregnancy Loss

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Introduction

Recurrent Abortion (RAB)

- Only 2% of pregnant women experience 2 consecutive pregnancy losses
- Only 0.4 experience 3 consecutive losses
- Population-based probabilities of 2 and 3 consecutive losses are 4% and 0.4%, respectively!
- After 2 or 3 SABs, the subsequent miscarriage rate is 26% and 32%, respectively

Introduction

Reported prevalence of aneuploidy amongst isolated SABs is 64% to 88%:

» 62-70% autosomal trisomies
» 8-20% triploidy/tetraploidy
» 6% structural abnormalities

Definition

- ASRM defines RPL as 2 or more failed pregnancies (documented by US or histological exam) and suggests some assessment after each loss with a thorough evaluation after three or more losses.

- Need to consider whether losses are embryonic or fetal, primary or secondary, and whether they are related to AMA.
Recurrent Early Pregnancy Loss

Genetic Abnormalities
Infectious Diseases
Mullerian Tract Anomalies
Endocrine Causes
Alloimmune Losses
Inherited Thrombophilias
Antiphospholipid antibodies (APA)
Work-up and Treatment
Genetic Abnormalities: Chromosomal Abnormalities

Prevalence with recurrent SABs: 50%
Usually maternal age related

<table>
<thead>
<tr>
<th>Maternal Age</th>
<th>SAB rate</th>
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<tbody>
<tr>
<td>&lt; 30 years</td>
<td>&lt;12%</td>
</tr>
<tr>
<td>30-34</td>
<td>15%</td>
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<tr>
<td>35-39</td>
<td>25%</td>
</tr>
<tr>
<td>40-44</td>
<td>50%</td>
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<tr>
<td>&gt;44</td>
<td>&gt;90%</td>
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</tbody>
</table>

Preimplantation karotyping of embryos from RAB patients demonstrates a 6-fold increase rate in monosomies.

Chromosomal Abnormalities

Etiology: Parental genetic factors
- Fragile sites on chrom.
- Inversions (9) p11q12.
- Balanced translocations (3.5 - 4.4%).

Chromosomal Abnormalities

Etiology: Metabolic factors

- Women with low (≤2.19 ng/mL) folate levels are at increased risk of SAB (OR of 1.47; 95% CI: 1.01-2.14)
- Low folate levels associated with a significantly increased risk of SAB due to aneuploidy (OR of 1.95; 95% CI: 1.09-3.48) but not euploid losses (OR of 1.11; 95% CI: 0.55-2.24).
- Elevated homocysteine levels > 18 μmol/l; lower RBC folate (< 160 ng/ml); and low vitamin B₆ (< 49 nmol/L) linked to RAB.

Chromosomal Abnormalities

Etiology: Gamete aging

- Decreased Telomerase activity leads to shorter telomeres causing abnormal chiasma formation, in turn, leading to abnormal meiotic synapsis and/or non-disjunction

Chromosomal Abnormalities

Etiology: Gamete aging

- Transcriptional silencing begins with oocyte maturation and persists during initial mitotic divisions of the embryo. Gene expression during this period largely depends on the translational activation of maternal mRNAs by cytoplasmic polyadenylation and requires an embryonic poly(A) binding protein (EPAB).
Chromosomal Abnormalities

Etiology: Gamete aging

- EPAB plays a central role in the regulation of maternal mRNA activation by preventing deadenylation and promoting translation.
- Decreased EPAB levels may lead to decreased readenylation of dormant oocyte mRNA and thus, failure of translation of key meiotic proteins with resultant non-disjunction.

(Identification and characterization of human embryonic poly(A) binding protein (EPAB). Mol Hum Reprod. 2008;14:581-8; and E. Seli, Yale University, personal communication)
Chromosomal Abnormalities

Potential Treatments

• Folate and $B_6$
• Donor Egg IVF
Chromosomal Abnormalities

Potential Treatments

- IVF with PGD – conflicting results with both AMA-associated RAB and those related to parental balanced translocations

Platteau et al. Fertil Steril. 2005; 83:393-5;
Genetic Abnormalities: Mendelian disorders

1) Fetal single gene defects may promote RAB.
   - Lethal multiple pterygium syndromes (AR/X-LR) associated with fetal death at 14 to 20 weeks & arthrogryposis, hydrocephaleus, hydrops & cystic hygromas *(Am J Obstet Gynecol. 1988; 159:474-6).*
   - Incontinentia pigmenti (X-LR) lethal in males who develop hydrops and cystic hygromas; affected females have epidermal anomalies *(J Gynecol Obstet Biol Reprod. 1997; 26:633-6).*
   - Homozygosity for AD polycystic kidney disease (PKD1 and PKD2) is an embryonic lethal. *(Patterson et al, Am J Kidney Dis. 2002; 40:16-20)*
Mendelian disorders

2) Maternal single gene defects may promote RAB due to endometrial abnormalities.

- Frequency of homozygosity of the VEGF -1154 A/A gene associated with recurrent implantation failure (19% versus 5%, P = 0.02). *(Goodman et al., Reprod Biomed Online. 2008; 16:720-3)*

Recurrent Early Pregnancy Loss

Genetic Abnormalities

Infectious Diseases

Mullerian Tract Anomalies

Endocrine Causes

Alloimmune Losses

Inherited Thrombophilias

APA

Work-up and Treatment
Infectious Disease

• No consistent association between Recurrent SAB and either chlamydia or mycoplasma species

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Mullerian Tract Anomalies

- Women with $\geq 3$ consecutive unexplained SABs have a higher rate of major mullerian anomalies than controls 23.8% vs. 5.3% using 3-D US (Hum Reprod. 2003; 18: 162-65).

- However, in both groups the most common anomalies were arcuate and subseptate uteri, which may not be associated with higher rates of recurrent SAB (Hum Reprod. 1997; 12:2277-81).
Mullerian Tract Anomalies

- Submucous myomas which distort the uterine cavity have been posited as causes of RAB and reduced IVF success rates (*Hum. Reprod.* 2000; 6: 614-20).

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Infectious Diseases
Mullerian Tract Anomalies
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Work-up and Treatment
Endocrine Causes: DM and Thyroid disease

- No association between sporadic or recurrent SABs and: well-controlled diabetes and subclinical hypothyroidism
- Possible link with anti-thyroid antibodies but uncertain treatment options.

Endocrine Causes: PCOS


• Among PCOS patients randomized to clomid, metformin or both, rates of SABs did not differ significantly among the groups. (*Legro et al. N Engl J Med.* 2007; 356:551-66)
Endocrine Causes: LPD

• Prevalence of LPD among RAB patients reported to be between 10 and 30% (Curr Opin Obstet Gynecol. 2005; 17:424-8).

• No definitive diagnostic criteria since the condition is intermittent (Curr Opin Obstet Gynecol. 1994; 6:121-7).

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Alloimmune losses (?)

Maternal tolerance of fetal allograft is mediated by trophoblast expression of

- non-immunogenic HLA-G
- Fas ligand
- immunosuppressive agents: hCG, PAPP-A, progesterone, and cortisol


Alloimmune losses (?)

Theories and Diagnostic tests
• Absence of maternal-anti-paternal leukocyte “blocking” antibodies.
• Excess circulating activated Natural Killer cells (CD57+ NK)

Alloimmune losses (?)

Diagnostic tests (cont.)

- Patients without maternal-anti-paternal leukocyte antibodies have normal pregnancies and patients with antibodies can miscarry.
- We have shown decidual NK cells express a very different repertoire of genes than those in the circulation.
- There is growing evidence that NK cell activity actually PROMOTES normal placentation.

Alloimmune losses

Treatments: Meta-analyses of Paternal/3rd Party leukocyte immunization (ratio of live births in Tx/Ctr groups) show no consistent benefit:

- 1.16 (95% CI 1.01-1.34, P=0.03)
- 1.21 (95% CI 1.04-1.37, P=0.02)
- 1.3 (95% CI 0.77-2.3)
- 1.3 (95% CI 0.44-3.8)

Alloimmune losses

Design: Double-blind, multi-centered RCT of Paternal WBC immunization (n=91) vs. saline placebo (n=92).

Outcome: Success rate was 31/86 (36%) in treatment group and 41/85 (48%) in the control group (OR 0.60, 95% CI: 0.33-1.12; p=0.108).

_Lancet 1999; 354: 365-9._
Alloimmune losses

Treatments: Meta-analyses of IVIG Rx
OR 1.48 (95% CI, 0.84-2.6)

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Rey at al., Lancet. 2003;361:901-8

<table>
<thead>
<tr>
<th>Study</th>
<th>FVL positive n/N</th>
<th>FVL negative n/N</th>
<th>Odds ratio (95% CI)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVL and recurrent fetal loss before 13 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Balasch⁸</td>
<td>1/2</td>
<td>54/103</td>
<td></td>
<td>0.91 (0.06–14.90)</td>
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<tr>
<td>Fatin⁹</td>
<td>6/8</td>
<td>53/121</td>
<td></td>
<td>3.85 (0.75–19.85)</td>
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<tr>
<td>Foka¹⁰</td>
<td>9/13</td>
<td>52/148</td>
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<td>4.15 (1.22–14.14)</td>
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<tr>
<td>Grandone¹¹</td>
<td>2/7</td>
<td>25/138</td>
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<td>1.81 (0.33–9.86)</td>
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<tr>
<td>Rai²⁰</td>
<td>59/71</td>
<td>845/983</td>
<td></td>
<td>0.80 (0.42–1.53)</td>
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<tr>
<td>Reznikoff²²</td>
<td>27/38</td>
<td>233/462</td>
<td></td>
<td>2.41 (1.17–4.98)</td>
</tr>
<tr>
<td>Younis²⁵</td>
<td>6/14</td>
<td>31/162</td>
<td></td>
<td>3.17 (1.03–9.80)</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>110/153</td>
<td>1293/2117</td>
<td></td>
<td>2.01 (1.13–3.58)</td>
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</tbody>
</table>

Test for heterogeneity p=0.11
Test for overall effect p=0.02
Rey at al., Lancet. 2003;361:901-8

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<tr>
<td>FVL and non-recurrent fetal loss after 19 weeks</td>
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<tr>
<td>Alfirevic30</td>
<td>0/3</td>
<td>18/59</td>
<td>0.32 (0.02–6.52)</td>
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<tr>
<td>Bare26</td>
<td>1/128</td>
<td>2/461</td>
<td>1.81 (0.16–20.09)</td>
</tr>
<tr>
<td>Gris27</td>
<td>15/2215</td>
<td>217/674</td>
<td>4.51 (1.81–11.23)</td>
</tr>
<tr>
<td>Kupferminc4</td>
<td>3/10</td>
<td>9/112</td>
<td>4.90 (1.08–22.30)</td>
</tr>
<tr>
<td>Many34</td>
<td>3/6</td>
<td>37/114</td>
<td>2.08 (0.40–10.81)</td>
</tr>
<tr>
<td>Martinelli35</td>
<td>5/11</td>
<td>62/288</td>
<td>3.04 (0.90–10.29)</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>27/180</td>
<td>345/1708</td>
<td>3.26 (1.82–5.83)</td>
</tr>
</tbody>
</table>

Test for heterogeneity p=0.6
Test for overall effect p<0.0001
Link Between Thrombophilias & SAB < 10 weeks

Large European retrospective cohort study involving 571 women with thrombophilia having 1524 pregnancies vs. 395 controls having 1019 pregnancies found a significant association between any inherited thrombophilia and stillbirth (OR 3.6; 95% CI: 1.4-9.4) but not SAB (OR 1.3; 0.9-1.7).

Link Between Thrombophilias & SAB < 10 weeks

Large case-control study nested in a 32,700 cohort revealed an association between FVL and pregnancy loss after 10 weeks (OR 3.46; 95% CI 2.53-4.72) but not for losses occurring between 3 and 9 weeks.

Link Between Thrombophilias & SAB < 10 weeks

Retrospective cohort study of 491 patients with a history of adverse pregnancy outcomes:

• >1 thrombophilia was protective of recurrent losses at <10 weeks with ORs of 0.55 (95% CI: 0.33-0.92) and 0.48 (0.29-0.78), respectively.

• >1 thrombophilia was modestly associated risk of losses >10 weeks [OR 1.76 (1.05-2.94) and 1.66 (1.03-2.68), respectively].

However Prospective Studies Generally Demonstrate No Association with APO

• FVL: Prevalence 2.7% (134/4885). Maternal FVL mutation carriage not associated with increased risks of pregnancy loss, preeclampsia, placental abruption, or SGA. *(Obstet Gynecol. 2005;106:517-24)*

• FVL: Prevalence of 3.61% (142/3,944) with no association found for PIH, PE-T, IUGR and fetal loss. However, FVL was associated with LGA (OR 1.81; 1.04-3.31) and mothers of 2 of 8 infants with neonatal deaths had FVL. *(Br J Haematol. 2008; 140:236-40)*
Prospective Studies Demonstrate
No Association with APO

APC resistance: Prevalence 11% (270/2480). APCR subgroup had no higher rate of APO than non-APCR patients, but did have an 8-fold higher risk of VTE (3/270 vs. 3/2210), a lower rate of intrapartum hemorrhage (3.7% vs. 7.9%) (p = 0.02), and less intrapartum blood loss (340 ml vs. 361 ml) (p = 0.04). (Thromb Haemost. 1999; 81:532-7)
Prospective Studies Demonstrate No Association with APO

PGM: 157 carriers among 4,167 patients with 1st trimester samples available (3.8%). Carriers had similar rates of pregnancy loss, preeclampsia, SGA neonates, and abruption compared with noncarriers. *(Obstet Gynecol. 2010;115:14-20)*

Inherited Thrombophias as a class: Found in 2034 nulliparas. Only PGM linked to composite APO* (aOR 3.6; 1.2-10.6); only individual outcome linked to PGM was abruption (OR 12.2; 2.4–60.4) but there were only 9 patients had abruption. *(Obstet Gynecol. 2010;115:5-13)* * Fetal loss, severe preeclampsia, IUGR, abruption and NND
Association between MTHFR mutations
And recurrent pregnancy loss:

- MTHFR & RPL (OR = 0.98 (0.5-1.72))

Nelen et al., Fertil Steril. 2000;74:1196-9;
Conclusions: Thrombophilia and Adverse Obstetrical and Fetal Outcomes:

- Many contradictory studies

- Appears to be a modest association between thrombophilia and fetal loss after 10 weeks in retrospective but not most prospective studies.
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Work-up and Treatment
Antiphospholipid Antibodies

APA’s represent a general class of self-recognition Ig’s directed against proteins bound to negatively charged surfaces, usually anionic phospholipids. Must be present during two evaluations 12 weeks apart and associated with thrombosis or adverse pregnancy outcomes.

Antiphospholipid Antibodies

They can be identified by searching for antibodies that specifically bind protein epitopes:

- β-2-glycoprotein-1,
- prothrombin,
- annexin V, and
- others - activated protein C, S and X
Antiphospholipid Antibodies

They can be identified by searching for antibodies that bind proteins that bind anionic phospholipids – cardiolipin and phosphatidylserine.
Antiphospholipid Antibodies

They can be identified by searching for antibodies that exert downstream effects on prothrombin activation in a phospholipid milieu - lupus anticoagulants (LAC).
Antiphospholipid Antibodies

Reported ORs for LAC-associated fetal loss range from 3.0 to 4.8 while anti-cardiolipin antibodies (ACA) display a wider range of reported OR’s of 0.86 to 20.0

Antiphospholipid Antibodies

There is conflicting evidence whether APA are also associated with recurrent (≥ 3) early SAB in the absence of stillbirth:

- At least 50% of pregnancy losses in APA patients occur after the 10th week (Lupus 1996; 5:409-13).

- Compared to patients having unexplained early SAB without APA, those with APA more often display fetal cardiac activity (86% vs. 43%; P < 0.01) (Hum Reprod 1995; 10:3301-4).
Antiphospholipid Antibodies

There is conflicting evidence whether APA are also associated with recurrent (≥ 3) early SAB:

– Meta-analysis shows no association between APA and either IVF clinical pregnancy (OR 0.99; 0.64–1.53) or live birth rates (OR 1.07; 0.66–1.75) (*Fertil Steril.* 2000; 73:330–3).

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Work-up and Treatment
No real evidenced-based work-up and treatment but I recommend:

1) Parental karyotypes and/or assessment of abortus’ karyotype.

2) If parental chromosomal abnormalities present (e.g., translocation) consider IVF ± PID.
Recurrent Early Pregnancy Loss

3) When karyotypes of abortus’ specimens are euploid, the patients’ losses are intermittent and generally occur at the same gestation age analyze placentas of prior losses for trophoblast inclusions. (*J Theor Biol.* 2003; 225:143-5)

4) If single gene disorders are suspected, Yale offers whole genomic sequencing of POCs. Should this detect a developmentally lethal mutation present in both parents (due to AR inheritance or germ line loss of heterozygosity), offer IVF with PGD.

5) If maternal endometrial implantation disorders are suspected (*e.g.*, recurrent primary losses in a young patient) consider surrogacy (gestational carrier).
Recurrent Early Pregnancy Loss

7. Evaluate uterus with sonohysterography and 3-D US and with hysteroscopic repair of remediable defects prior to conception.

8. Rule-out APA (LAC, ACA, anti-ß2-glycoprotein-I). If positive x 2 at least 12 weeks apart, treat with LMWH, and LDA in subsequent pregnancy.
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