Thrombophillias, Antibodies, and Pregnancy Outcomes: Are We Overdiagnosing and Overtreating our Patients?

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The Roadmap

- What is recurrent pregnancy loss (RPL)?
- What are the potential causes of RPL?
- Thrombophilia testing – who, when, ever?
- Autoimmune issues/APL
- Empiric/alternative therapies: any evidence?
Case #1

- 43-yr-old G4P1112, hx lupus since age 18
- OB hx
  - 1989: term SVD; on prednisone for lupus
  - 2004: twins @ 34+ weeks for NR-FHR, no meds had PPTL with c/s
- No lupus sx or meds until 2008
- 2013: 2 IVF cycles
  - 1 loss 3 wks after transfer, 1 failed cycle
- Seen by reproductive immunologist
  - ACA, LAC, B2GP all (-), ANA, SS-A (+)
- Further workup in immunologist’s office
  - Monthly NK cell panels and cytokine assays
  - Monthly lymphocyte surface marker assays
- Immunologist’s “protocol” pre-IVF
  - Prednisone 10 mg BID
  - Lovenox 60 mg BID
  - Humira (adalizumab) q 2 weeks for 1-2 months
  - 3 cycles of paternal leukocyte immuniz. in Mexico
- Underwent IVF with local REI
- Currently 8 weeks pregnant
  - Still on Lovenox and Prednisone

Case # 1 (cont’d)
Case #2

- 38 yr-old G5 P1031
- OB history
  - 2009: full term SVD
  - 2012-13: 7 week Sab, 8 week Sab with documented aneuploidy, 6-7 week chemical pregnancy
- Seen by her naturopath
  - Workup there: (+) antiphosphatidylglycerol IgM, heterozygous (+) MTHFR mutation
  - Placed on Lovenox prior to IVF and continued
- Twin pregnancy post IVF/PGD
  - 1st visit @ 18 weeks: (+) integrated screen for T21, (-) NIPT
Pregnancy Loss: Statistics

- Properly categorizing pregnancy losses is critical both for patient management and for quality research into losses.
- Most pregnancy losses occur in pre-embryonic and embryonic periods.
  - Only 1.7% of losses in 1 series were after 9-10 weeks.
  - In U.S., 0.6% of losses after 20 weeks.
- Pregnancy loss reported to occur in almost 1/3 of all conceptions.
  - 2/3 are peri-implantation losses, often not symptomatic.

Goldstein SR, Obstet Gynecol 1994; MacDorman MF, Natl Health Ctr Stats 2009

Aneuploidy and Pregnancy Loss

- Aneuploidy reported in up to 90% of pre-embryonic losses.\(^1\)-\(^3\)
  - 50% of losses between 8-11 weeks are aneuploid.
  - Decreases to 30% for losses at 16-19 weeks.
  - Aneuploidy < 15% in stillbirths > 20 weeks.
    - Rates higher in more recent studies using SNP/microarray.\(^4\)

What is Recurrent Pregnancy Loss?

- **ASRM (2008)**
  - “A disease distinct from infertility, defined by 2 or more failed pregnancies” *(ASRM Practice Committee, Fertil Steril 2008)*

- **11th Intl. Congress on APL/APS (2006)**
  - One or more unexplained deaths of morphologically normal fetus at or beyond 10th week of gestation *(Miyakis S, J Thromb Haem 2006)*

- If RPL defined as loss of 3 or more consecutive pregnancies → 1% of couples *(Rai R, Lancet 2006)*

- Many experts consider 2 consecutive losses as sufficient for diagnosis of RPL *(Branch DW, NEJM 2010)*
  - *Pregnancy loss is NOT a failed IVF transfer cycle*

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34-year old with three 1st-trimester losses at 9-11 weeks

- Normal parental karyotypes
- Normal TSH, Hgb A1c
- Normal sonohysterogram

**Autoimmune testing?**

- YES, but focused

**Thrombophilia testing?**

- NO

*From: Kutteh WH; SRM, Feb 2012*
Thrombophilias in Pregnancy: VTE risks

- Pregnancy is marked by ↑ clotting potential, ↓ fibrinolysis, ↓ anticoagulant activity
  - Thrombotic potential of pregnancy exacerbated by: venous stasis in lower extremities, as well as hormone-mediated ↑s in insulin resistance and hyperlipidemia: **VTE in 1/1600 pregnancies**

- **Strong** association between thrombophilias & VTE
  - Recurrence risk ~10-12% for untreated pregnant women with such a personal hx of VTE and a thrombophilia *(Brill-Edwards P. NEJM 2000)*

- **Controversial** (at best) association with adverse pregnancy outcomes *(ACOG PB # 138, September 2013)*

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Prevalences and Risks of VTE with Thrombophilias

<table>
<thead>
<tr>
<th></th>
<th>Prev in Gen Pop %</th>
<th>Lifetime VTE Risk</th>
<th>% of all VTE</th>
<th>VTE Risk/Preg (No hx) %</th>
<th>VTE Risk/Preg (Prior VTE) %</th>
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<tbody>
<tr>
<td><strong>Low-risk thrombophilias</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVL heterozygote</td>
<td>1-15</td>
<td>3-8</td>
<td>40</td>
<td>&lt; 0.3</td>
<td>10</td>
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<tr>
<td>PTG heterozygote</td>
<td>2-5</td>
<td>3</td>
<td>17</td>
<td>&lt; 0.5</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Protein C activity (&lt;50%)</td>
<td>0.2-0.4</td>
<td>10-15</td>
<td>14</td>
<td>0.1-0.8</td>
<td>4-17</td>
</tr>
<tr>
<td>Protein S free Ag (&lt;55%)**</td>
<td>.03-0.1</td>
<td>2</td>
<td>3</td>
<td>0.1</td>
<td>0.22</td>
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<tr>
<td><strong>High-risk thrombophilias</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>FVL homozygote</td>
<td>&lt; 1</td>
<td>2</td>
<td>1.5</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>PTG homozygote</td>
<td>&lt; 1</td>
<td>0.5</td>
<td>2.8</td>
<td>&gt; 17</td>
<td></td>
</tr>
<tr>
<td>FVL/PTG compound</td>
<td>0.01</td>
<td>1-3</td>
<td>4.7</td>
<td>&gt; 20</td>
<td></td>
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<tr>
<td>Antithrombin III def (&lt;60%)</td>
<td>0.02</td>
<td>25-50</td>
<td>1</td>
<td>3-7</td>
<td>40</td>
</tr>
</tbody>
</table>

** Should not be tested in pregnancy or high estrogen states.
Can We Extrapolate from Thrombophilias and VTE?

- Diagnosis and prophylaxis against VTE is effective in lowering the risks of primary and recurrent disease
  - Chemoprophylaxis for thrombophilias (other than APS) is heparin or LMWH, not aspirin (clotting factors, not platelets)

- Regarding adverse pregnancy outcomes:
  - Thrombophilias have been associated with adverse outcomes, including RPL, in retrospective studies
  - Prospective intervention studies limited
  - Large prospective studies show no association

Thrombophilias and Pregnancy Outcomes

Since 1990s, venous prothrombotic factors explored to explain unexplained pregnancy loss and other uterine/placental problems

- First large meta-analysis: Rey R et al, Lancet 2003
  - 31 retrospective studies included, authors note significant heterogeneity

<table>
<thead>
<tr>
<th>Factor</th>
<th>RFL</th>
<th>Studies</th>
<th>OR (CI)</th>
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<tr>
<td>FVL</td>
<td>RFL</td>
<td>7</td>
<td>2.01 (1.13-3.58)</td>
</tr>
<tr>
<td>NRFL</td>
<td>12</td>
<td>OR 1.73 (1.18-2.54)</td>
<td></td>
</tr>
<tr>
<td>PTG</td>
<td>RFL</td>
<td>9</td>
<td>2.05 (1.18-3.54)</td>
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<tr>
<td>NRFL</td>
<td>5</td>
<td>OR 2.30 (1.09-4.87)</td>
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<tr>
<td>MTHFR</td>
<td>RFL</td>
<td>8</td>
<td>0.98 (0.55-1.72)</td>
</tr>
</tbody>
</table>

- Similar results in subsequent meta-analyses
More Recent Evidence Refutes Association w/APOs

- Larger single studies with more stringent inclusion criteria and enrollment of appropriate controls have diminished or eradicated the association from smaller earlier studies for thrombophilias and adverse pregnancy outcomes (APOs)
    - Case-control study of 311 women with (≥2) RPL / 599 controls
    - FVL: 4.8% vs 4.2%; OR 1.16 (0.6-2.2)
    - PTG: 3.2% vs 2.5%; OR 1.29 (0.57-2.9)
    - Large Danish cohort of women with RPL (n = 363)
    - Live birth rates no different in 1st pregnancy after referral for FVL/PTG carriers vs non-carriers
    - Clotting analyses in women with ≥3 unexplained losses < 14 wks
    - *No evidence of hypercoagulable state in women with unexplained RPL*

Thrombophilias in Pregnancy: *Prospective* Evaluations

- 2 large prospective multicenter observational cohort studies conducted by NICHD-sponsored MFMU Network
  - 5188 unselected singleton pregnancies; 3.8% PTG (+) /2.7% FVL (+)
- The earlier FVL study was originally conceived as a prospective trial of heparin prophylaxis against VTE for FVL mutation carriers with no other risk factors
  - Since accurate information about VTE risk was found to be lacking in background calculations, study designed to more precisely estimate the true VTE risk among “incidental” mutation carriers
- PTG study was a secondary analysis of the earlier FVL study
  - Only 5 women were compound heterozygotes for FVL/PTG mutations
  - All the VTEs (4/3) in both studies were in nonmutation carriers

*Dizon-Townson D, Obstet Gynecol 2005; Silver RM, Obstet Gynecol 2010*
<table>
<thead>
<tr>
<th></th>
<th>FVL Carriers</th>
<th>FVL Noncarriers</th>
<th>Relative Risk (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy loss</td>
<td>8/134 (6.0)</td>
<td>264/4,751 (5.6)</td>
<td>1.1 (0.5-2.2)</td>
<td>.84</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>5/134 (3.7)</td>
<td>141/4,751 (3.0)</td>
<td>1.3 (0.4-2.8)</td>
<td>.60</td>
</tr>
<tr>
<td>Abruption</td>
<td>0</td>
<td>31/4,751 (0.7)</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>SGA &lt; 5th percentile*</td>
<td>6/124 (4.8)</td>
<td>173/4,628 (3.9)</td>
<td>1.2 (0.5-2.6)</td>
<td>.64</td>
</tr>
<tr>
<td>SGA &lt; 10th percentile*</td>
<td>10/124 (8.1)</td>
<td>403/4,628 (9.1)</td>
<td>0.9 (0.5-1.7)</td>
<td>.69</td>
</tr>
</tbody>
</table>

* Based on live-born singletons.

The relationship of the factor V Leiden mutation and pregnancy outcomes for mother and fetus.

Dizon-Townson D; Miller C; Sibai B; Speng C; Thom E; Wendel G Jr; Wendelton K; Samuels P; Corvinno MA; Morwood D; Sorokin Y; Meis P; Miodowska M; O’Sullivan MJ; Conway D; Wagner RJ; Gabbe SG. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Obstetrics & Gynecology. 106(3):517-24, 2005 Sep.

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<table>
<thead>
<tr>
<th></th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P</th>
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<tr>
<td>Pregnancy loss</td>
<td>0.98</td>
<td>0.49-1.95</td>
<td>.951</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>1.30</td>
<td>0.56-3.02</td>
<td>.536</td>
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<tr>
<td>SGA 5%</td>
<td>1.39</td>
<td>0.67-2.89</td>
<td>.377</td>
</tr>
<tr>
<td>SGA 10%</td>
<td>1.34</td>
<td>0.80-2.25</td>
<td>.267</td>
</tr>
<tr>
<td>Abruption</td>
<td>2.23</td>
<td>0.52-9.58</td>
<td>.280</td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td>1.18</td>
<td>0.57-2.44</td>
<td>.659</td>
</tr>
<tr>
<td>Preterm delivery</td>
<td>1.39</td>
<td>0.87-2.21</td>
<td>.165</td>
</tr>
<tr>
<td>GA at delivery</td>
<td>--</td>
<td>--</td>
<td>.941</td>
</tr>
</tbody>
</table>

CI, confidence interval; SGA, small for gestational age; GA, gestational age.

Multivariable analysis adjusted for maternal age, race, parity, prior pregnancy loss, prior SGA neonates, and family history of thromboembolism.

Prothrombin gene G20210A mutation and obstetric complications.

Silver RM; Zhao Y; Speng C; Sibai B; Wendel G Jr; Wendelton K; Samuels P; Curtis SN; Sorokin Y; Miodowska M; O’Sullivan MJ; Conway D; Wagner RJ. Obstetrics & Gynecology. 115(1):14-20, 2010 Jan.
Association between Thrombophilias and APOs? (2014)

- Prospective cohort study of unselected pregnant women at 3 tertiary Canadian hospitals (n=7343)
  - Blood drawn in early 2nd trimester and tested for FVL and PTG mutation genotypes after delivery
  - Main outcome measure: composite of pregnancy loss, SGA < 10th %ile BW, preeclampsia, abruption
- 507 women were heterozygous for FVL/PTG (6.9%)
  - Of these women, 11.6% had placentally-mediated pregnancy complication, compared to 11.2% without
  - RR= 1.04 (95% CI: 0.81-1.33)


Testing for Common and “Other” Thrombophilias

- Current available data show no indication for testing for common thrombophilias for a history of recurrent pregnancy loss or other adverse outcomes, and no documented benefit from rx
- No association with MTHFR polymorphism (heterozygous or homozygous) for any adverse pregnancy outcomes: including RPL and VTE
- Less common “thrombophilias” have even less data to support either association with APOs or treatment and should not be tested (ACOG PB, 2011)
  - “Promoter mutation” in PAI-1 gene, anti-protein Z Ab’s, “protein Z deficiency”, anti-annexin ab’s

Does “Treating” Thrombophilias Improve Outcome?

- Recent review by ACMG: Bradley LA, Genet Med 2012 (Apr)
  - While some association may be present, analysis of data is adequate that anticoagulation is ineffective for this indication (except for APS), and had higher risk of harm

  - Randomized multicenter trial, 207 women with thrombophilia and RPL
  - ASA vs Lovenox/placebo vs ASA/Lovenox
  - No differences in outcomes between groups

LMWH vs None for Preventing APOs in Women with Thrombophilia? (TIPPS Trial 2014)

- Open-label randomized trial: pregnant women with thrombophilia at risk for VTE or placentally-mediated adverse outcomes (by hx)
  - Primary outcome (composite): (1) major VTE, (2) severe PECL < 32 weeks, (3) BW < 10\textsuperscript{th} %ile, (4) pregnancy loss

- 289 women were randomized
  - Primary outcome no different in dalteparin vs no rx for both intention-to-treat (17% v 19%) or on-rx (20% v 17%)
  - No difference in major bleeding but ↑ in minor bleeding with dalteparin (20% v 9%, p = 0.01)

- Antepartum prophylactic dalteparin did not reduce risk of APOs in women with thrombophilias

Figure 2: Subgroup analysis forest plot with risk ratio (95% CI) for the primary composite outcome. The primary composite outcome was major VTE or severe/neonatal pre-eclampsia, SGA infant (4th/10th percentile), or pregnancy loss. SGA: small for gestation.

Marc A. Rodger, William M. Hague, John Kingdom, Susan R. Kahn, Alan Karovitch, Mathew Sermer, Anne Marie Cleme...

Antepartum dalteparin versus no antepartum dalteparin for the prevention of pregnancy complications in pregnant women with thrombophilia (TIPPS): a multinational open-label randomised trial


http://dx.doi.org/10.1016/S0140-6736(14)60793-5
Case #3

- 31 year-old G1 P0101
- 1st encounter was at 30+ weeks for in-house consult
  - ↑ BPs 2 weeks before admission
  - Delivered for HELLP syndrome; also IUGR, AEDF
- Labs in-house
  - (-) ACA and B2GP; “borderline” LAC
- Labs 12 weeks after discharge from hospital
  - All labs repeated and all negative

**Antiphospholipid Syndrome**

Antiphospholipid syndrome (APS) is an autoimmune disorder defined by the presence of characteristic clinical features 
and/or antibodies to phospholipids. Antiphospholipid antibodies (aPLs) are a diverse group of antibodies that may be directed against or cross-react with various phospholipids bound to proteins, including cardiolipin, phosphatidylserine, and protease-activated receptors (PARs). APS is characterized by a triad of clinical features: recurrent miscarriages, fetal loss, and/or thrombosis. The diagnosis of APS requires evidence of clinical manifestations along with the presence of one or more of the following antibodies: anti-cardiolipin (aCL) or anti-beta-2-glycoprotein I (aB2GPI) antibodies. APS can also be classified as primary or secondary based on the absence or presence, respectively, of a detectable underlying disease (e.g., systemic lupus erythematosus, HIV infection). The management of APS involves a multidisciplinary approach that includes medication, lifestyle modifications, and close monitoring of clinical outcomes. The therapeutic goal is to prevent adverse pregnancy outcomes and recurrent thrombotic events. Antiphospholipid antibodies can be detected by in vitro methods, such as ELISA or Western blot techniques. The presence of these antibodies is often associated with antiphospholipid syndrome, which may require specific anticoagulant therapy to prevent further complications.
Antiphospholipid Syndrome (APS)

- Autoimmune disorder defined by presence of characteristic clinical features (including RPL) AND specified levels of circulating APL antibodies.
  - Despite clinical significance and prevalence of APS, controversy has surrounded indications for and types of tests for diagnosis.

- Primary clinically relevant antigenic determinant for APL antibodies is β-glycoprotein I.
  - Ubiquitous, multifunctional plasma protein with a regulatory role in coagulation, fibrinolysis, and other physiologic systems.

Testing for APS

  - Only the 3 APL antibodies endorsed (LAC, ACA, anti-β2GP) can and should be used to establish a diagnosis.

- Some laboratories offer testing, often in a broad panel of tests, for other APL and autoimmune antibodies.

- Results from such additional assays do little to improve the accuracy of diagnosing APS and testing for them is not recommended.

4. ACOG PB #111, 2011.
Antiphospholipid (aPL) antibody syndrome: Diagnosis

Clinical criteria
1. Vascular thrombosis: One or more episodes of arterial, venous, or small vessel thrombosis in any tissue or organ
2. Morbidity in pregnancy
   - One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation
   - One or more premature births of a morphologically normal neonate prior to the 34th week of gestation secondary to eclampsia or severe pre-eclampsia or recognized features of placental insufficiency
   - Three or more unexplained consecutive spontaneous miscarriages before the 10th week of gestation

Laboratory criteria (Must be present on 2 or more occasions at least 12 weeks apart)
1. Lupus anticoagulant present
2. Anticardiolipin antibody OR Anti-β2-glycoprotein: medium or high titer of IgG or IgM isotype (>40 µg of IgG or IgM phospholipid or >99th percentile)

Specific Impact of Lupus Anticoagulant

- Positive test for LAC is a stronger risk factor for thrombosis and adverse pregnancy outcomes after 12 weeks than (+) results for ACA or B2GP

- Case-control study of risk factors for stroke among women in general population < age 50
  - 17% of stroke patients vs 0.7% of controls (+) for LAC (OR 43.1)
  - Risk increased by use of OCs (OR 201.0) or smoking (OR 87.0)

References:
1. Giannakopoulos MB, NEJM 2013
Physiologic Rationale for Obstetric APS Therapy

- **Aspirin**
  - APL antibodies against β2GPI activate platelets → synthesis of thromboxane A2
  - ASA shown to ↓ placental thromboxane production in vitro and in women with RPL \( \text{(Peaceman AM, AJOG 1993, Obst Gynecol 1995)} \)

- **Heparin**
  - Targets inflammatory reaction from interaction of APL and cellular binding
  - Impacts complement activation pathways (more than anticoagulant effect \( \text{(Girardi G, Nature Med 2004)} \))
  - In vitro: attenuates APL-mediated trophoblast apoptosis

- **Initial treatment studies (1980s)**
  - ASA/heparin alone, small series, poorly-defined criteria and controls: largest study (1988), 42 pts, open-label ASA, 88% v 10% live vs hx

APS Treatment Trials

- **Majority opinion: ASA + Heparin**
    - 90 women with RPL (median # losses = 4) and (+) APL
    - All started ASA with (+) HCG, random allocation to UFH (5000 U BID) when +FHM on scan. Endpoint live ≥ 34 wks (or Sab)
    - Live birth rate ↑ with ASA/heparin: 71% v 42% (OR 3.4; 1.4-8.1)
  - Kutteh WH. AJOG 1996
    - Similar protocol and rates: 80% v 44%
  - Subsequent meta-analyses confirmed superiority of ASA + heparin over ASA alone \( \text{(Ziakis PD, Obst Gyene 2010; Empson M, Cochrane 2005)} \)

- **Best type of heparin: UFH vs LMWH?**
  - Majority of studies suggest LMWH equivalent to \( \text{(Noble et al, Fertil Steril 2005, Laskin CA, J Rheum 2009)} \) or superior to UFH \( \text{(Stephenson, J ObGyn Can 2004)} \) for treatment of APS
What Doesn’t Work for APS

- The range of immunomodulatory agents used to treat RPL in conjunction with (or without) APL have shown no benefit at best, and inferior outcomes at worst, when used with or instead of ASA and heparin

- Prednisone – poorer outcomes
  - Prednisone + ASA vs placebo (RCT, n = 66)
    - No differences in live birth rates (Laskin CA, NEJM 1997)
    - ↑ rates of prematurity (62% vs 12%, p < 0.001), HTN (13% vs 5%) and diabetes (15% vs 5%) in treatment group
  - In Cochrane analysis, prednisone and ASA also associated with higher rates of PTD (PPROM) and GDM compared to: (1) placebo, (2) ASA alone, and (3) ASA/heparin (Empson et al. Cochrane 2005)

Empiric Therapy for RPL without APS? (Hint: No)

- SPIN Study (Scotland)
  - 294 women with ≥ 2 consecutive unexplained losses (> 10 and < 24 weeks); APL & thrombophilia (-)
  - Randomized to LMWH/ASA vs surveillance
  - Pregnancy loss rates equivalent: 22% vs 20%, OR 0.91 (0.52-1.59) (Clark P, et al. Blood 2010)

- Dutch Trial (NEJM 2010)
  - 364 women with ≥ 2 unexplained losses < 20 weeks
  - Randomized to: (1) ASA, (2) ASA/LMWH, (3) placebo
  - No treatment arm improved live-birth rates compared to placebo (Kaandorp SP, et al. NEJM 2010)

- **Randomized, placebo-controlled trial**
  - Recurrent: ≥ 2 consecutive miscarriages < 15 wks, conception with same partner, no live births after Sabs
  - Normal karyotypes both partners and (-) APL, FVL/PTG, Pro S/C
  - Enoxaparin 40 mg/day vs placebo injection daily: mean inclusion @ 8 weeks; 72% of women had at least 3 Sabs
- **258 women enrolled and randomized**
  - Live-birth rates for enoxaparin v placebo were 66.6% v 72.9% (risk difference -6%; 95% CI -17 to 5.1; p = 0.34)
  - No differences in secondary outcomes for pregnancies going beyond 20 weeks (PECL, SGA, IUFD, PTD)

*Pasquier E, et al. Blood 2015; 125: 2200-5*
Prior Losses and Preconception ASA?

- Commonly used with IVF despite meta-analyses showing no impact on pregnancy or livebirth rates. *Sristoladis CS, Cochrane 2011; Groevenfeld E, Hum Reprod Update 2011*
- Recent NICHD-sponsored RPCT in women with 1-2 prior losses
- ASA (or placebo) given daily until completion of 6 cycles or 36 weeks gestation
- Slow enrollment led to “expansion” of criteria
  - 1 or 2 losses (from 1); loss > 20 weeks (from <20); losses > 1 yr before enrollment; up to 2 liveborn
- Primary outcome was livebirth
  - No differences for ASA in either overall or the sub-strata groups
  - No difference in pregnancy loss rates in any group

*Schisterman EF, Siver RM, et al. Lancet. Apr 2 2014*
IVIG and RPL
- Proposed on basis of direct antibody effects and role for immunomodulation
- Majority of use data from uncontrolled and/or retrospective open-label series
- One large multicenter RPCT ([Stephenson MD, Human Reprod 2010])
  - 47 women with ≥ 3 unexplained losses, IVIG vs saline q14-21 days preconception then q4 wks until 20 wks
  - Live birth rates equivalent: 70% v 63%, OR 1.4 (0.4-4.6)
  - For pregnancies with (+) FHM @ 6 wks: 94% both arms
  - 6 RCTs included, OR live birth for IVIG 0.9 (0.55-1.54)

Natural Killer Cells and RPL?
- Some studies suggest differences in peripheral blood NK cell levels in women with RPL
  - Based on uterine/endometrial NK cell data with RPL ([Quenby S, et al, Hum Reprod 2002])
- However, both phenotypic and functional differences exist between uterine and peripheral NK cells
  - Data demonstrate that peripheral blood NK testing gives no useful info on uterine NK cells ([Moffett A, et al. BMJ 2004])
NK Cells: Allegations of Harm

- Despite the scientific evidence, soon after NK cells were described, the notion evolved that “killing” the embryo by uNK was responsible for miscarriages and IVF failure
  - Any woman with “high” levels was offered a range of therapies to suppress NK cells from attacking embryo or having other ill-defined effects on immune system (Chao et al, Am J Repro Immun 1995)
  - The emotive claims outlined by centers offering such therapies lack any supportive evidence
    - “If the women’s immune system for any reason identifies the embryo as foreign…she will begin to fight it” (Ndukwe G 2015, zitawest.com)
    - “…some women’s cells are so aggressive they attack the pregnancy, thinking the fetus is a foreign body” (Shehata H 2014, bbc.co.uk/news)
    - “…there are couples who produce embryos that are misinterpreted by the immune system as cancer cells…These embryos are repudiated …until the uterus behaves like a ‘den of lions’ and each pregnancy attempt fails…” (Allan Beer Center 2015, repro-med.net)

Natural Killer Cells and RPL? (2)

- Recent retrospective cohort study (Kitano K, Fert Steril, 12/13)
  - For 552 pts with unexplained RPL, no rx, pNK studied
    - Subsequent miscarriage rate was 22.5%
    - LB rate was 81% with 2 prior Sab, 71% with 3, 65% with 4
  - In multivariable logistic regression, elevated pNK activity not an independent risk factor for subsequent miscarriage
    - Plasma NK activity did show weak inverse correlation with age

- Testing of “NK cell profiles” should not be performed routinely in evaluation of miscarriage in general and RPL in particular 1-4

### Summary of agents used for immunomodulation in assisted reproductive technology

**Moffett & Shreeve, UK, 2015**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cost</th>
<th>Common clinical uses</th>
<th>Some known side effects or adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid Emulsion e.g. Intralipid®</td>
<td>Approx. £300 per infusion in clinic setting</td>
<td>Parenteral nutrition, administered with propofol, cardio-protection in bupivacaine toxicity (Picard, 2006)</td>
<td>Hepatomegaly, jaundice, cholestasis, splenomegaly, thrombocytopenia, leukopenia and fat overload syndrome (&lt;1% occurrence in clinical trials) (FDA, 2007)</td>
</tr>
<tr>
<td>Intravenous Immunoglobulin (IVIG)</td>
<td>Approx. £1500 per infusion (may vary depending on dose) in clinic setting</td>
<td>Primary and secondary antibody deficiency states, haematological disorders, neurological conditions, other uses, e.g. solid organ transplantation (DoH, 2011)</td>
<td>Aseptic meningitis, renal failure, thromboembolism, haemolytic reactions, anaphylactic reactions, lung disease, enteritis, dermatologic disorders and infectious diseases (Stehrn, 2013)</td>
</tr>
<tr>
<td>Granulocyte-Colony Stimulating Factor (G-CSF)</td>
<td>Net price 600 mcg/ml £52.70, 0.5-ml prefilled syringe (BNF, 2015)</td>
<td>Neutropenia (various clinical types), severe or recurrent infections in advanced human immunodeficiency virus infection (BNF, 2015)</td>
<td>Mucositis, splenic enlargement, hepatomegaly, transient hypotension, epistaxis, urinary abnormalities, osteoporosis, exacerbation of rheumatoid arthritis, anaemia, pseudogout (BNF, 2015)</td>
</tr>
</tbody>
</table>

BNF, British National Formulary; DoH, Department of Health; FDA, Food and Drug Administration.

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### NK Cells and “Therapy”: Why is This Still an Issue?

- Recent Cochrane review came out again against “alternate” therapies: “no significant beneficial effect over placebo in improving live birth rates (Wong *et al*, 2014)
  - Paternal leukocyte immunization was banned by FDA in 2002
- Private “miscarriage clinics” and “reproductive immunologists” can use off-label treatments at whim driven by commercial gain since, unlike IVF, specific licensure is not needed
- Even the most informed patients are willing to “try anything” to achieve a live birth
- Still, “it is no longer acceptable for medical practitioners to continue to administer and profit from potentially unsafe and unproven treatments, based on belief and not scientific rationale” *(Moffett & Shreeve, Human Reprod 2015)*
Summary (1)

- Recurrent pregnancy loss (RPL) is a specific and clinically-focused diagnosis: at least 2 embryonic losses at > 9-10 weeks
- Common hereditary thrombophilias are not contributors to RPL (or other adverse pregnancy outcomes) and should not be tested for in this context
- MTHFR mutation testing is not indicated for any obstetric or non-obstetric risk histories

Summary (2)

- Antiphospholipid syndrome (APS) is a real diagnosis based on clinical and lab criteria, and warrants treatment during pregnancy
- ASA/heparin regimens are the only evidence-validated treatment for APS
- Prednisone, IVIG, and other unproven immune modulators have not been shown to have benefit for RPL in well-conducted trials and may be detrimental
- NK cell testing is not validated for screening in RPL, outside of research protocols