Recurrent Pregnancy Loss

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Learning Objectives
- Identify possible causes of early pregnancy loss
- Outline basic evaluation for recurrent pregnancy loss (RPL)
- Review current treatment approaches for these patients

Definition
- Classical: 3 or more consecutive pregnancy losses before 20 weeks gestation
- Expanded: 2 or more consecutive losses
  - Risk of further loss similar for 2 versus 3 consecutive losses
  - Initiation of evaluation appropriate after 2 losses based on patient age and desire

Recurrent Loss Epidemiology
- 5% of couples attempting pregnancy have 2 or more consecutive losses
- 1% have 3 or more consecutive losses
- Most clinicians consider RPL even if losses are not consecutive

Miscarriage Epidemiology
- 34% pregnancy loss in prospective cohort of healthy women
  - 22% unrecognized - detected by assay only
  - 12% clinically recognized
- Obstetrical history predictive
  - prior success: 4.6% chance of loss
  - prior loss: 19.24% chance of loss

Miscarriage or Recurrent Pregnancy Loss?
- A single SAB, unless a successful pregnancy intervenes, increases the risk for the next pregnancy
- Distinction between “sporadic” and “recurrent” loss blurred
- Effect of maternal age: SAB risk approaches 50% by age 40 for both aneuploid and euploid losses

Wilcox NEJM 1988;319:189-194
### Miscarriage Recurrence Risk

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<tr>
<th>Outcome</th>
<th>Prior Losses</th>
<th>Recurrence Risk %</th>
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Warburton D, Fraser FC: Am J Human Genet 16:1, 1964

### PCOS & Pregnancy Loss

- **Pregnancy loss ↑ with PCOS**
  - Franks S, Ann Int Med 93
  - Jacobs HS BRJOBGYN 93

- **GnRH-a ↓ miscarriage in PCOS women**

- **RSA patients with ↑ LH, DHEAS or T more likely to miscarry**

### Metformin Reduces Pregnancy Loss in PCOS

- Retrospective study of PCOS women who became pregnant
  - Group 1: metformin during pregnancy (n=101)
  - Group 2: control (n=31)
- Early loss rate 12.9% vs 41.9% (p=0.001)
- Prior SPAB: 15.7% vs 58.3% (p=0.005)


### Etiology

#### Uterine Malformations

- 10-15% recurrent 1st trimester losses have congenital anomaly
- Variations of the double uterus the most common
- Septate loss rates 25-90% - usually amenable to resection
- Bicornuate loss rates 40% - uncertain benefit of surgery


#### Uterine Fibroids

- Unclear relationship between uterine leiomyomata and RPL
  - Large submucosal fibroids distort the cavity or occupy a large subendometrial area
  - ? Mechanism(s) - mechanical constriction or inadequate placentation resulting from poorly vascularized endometrium
Acquired Uterine Defects

**Etiology**

- Infection
  - No infectious agent has been proven to cause recurrent pregnancy loss
  - Colonization with *Ureaplasma urealyticum* leading to empiric antibiotics
  - Certain infections have been associated with spontaneous loss
    - *Toxoplasma gondii*, rubella, HSV, CMV, measles, coxsackie

**Genetic Factors**

- Trisomy (50%)
  - 9/16 all lethal
  - #21 Down Syndrome usually due to meiotic non-disjunction
- Monosomy X (20%)
- Triploidy (15%)
- Tetraploidy (5%)
- Mosaicism (2%)

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**Etiology**

- Genetic Factors

<table>
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<th>% Chromosomal Abnormal by Gestational Age</th>
<th>% abnormal</th>
<th>Gestational age</th>
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<td>-1</td>
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**Etiology**

- Genetic Factors

- Parental abnormalities in 3-5% of couples with recurrent loss
- Balanced translocation most common
  - Reciprocal (60%) or Robertsonian (40%)
  - 25-50% risk of pregnancy loss
  - May eventually produce normal offspring

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**Etiology**

- Genetic Factors

- Homologous Robertsonian translocation
  - 1/2500 couples
  - Precludes successful reproduction
  - Heterozygous may lead to partial monosomy or trisomy; "milder" phenotypical expression

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**Etiology**

**Genetic Factors**

- Speculation about single gene mutations
  - Blastocyst formation
  - Implantation
  - Morphogenesis of vital organs
- DNA Activitation
  - Epigentics
  - Methylation

**Skewed X inactivation**

- Preferential inactivation (>90%) of one of the X alleles
- May be lethal to a male offspring
- May result in X-autosome translocations
- Trisomy mosaicism in the germline

**Advanced Maternal Age**

- Impact on risk for pregnancy loss cannot be over-emphasized
- Increased rates of maternally-derived trisomies
- Oocytes recruited later in life more likely to be abnormal or experience meiotic error
- Non-chromosomal factors

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**Outcome of Pregnancies from Patients Before and After PGD for Chromosomal Translocation**

- Mean age 32.5 ± 3.9 years

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**Fertility and Miscarriage Rates as a Function of Maternal Age**


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**Decline in the Number of Oocytes from Birth to Menopause**

Etiology  
Thrombophilia

- Pregnancy is a hypercoagulable state
- Women with heritable or acquired thrombophilic disorders have significantly increased risks of pregnancy loss

Kutteh Semin Reprod Med 2006;24(1):54-65

Etiology  
Thrombophilia

- Antiphospholipid Antibodies (APA)
- Lupus Anticoagulant
- Heterozygous Factor V Leiden (G1691A)
- Factor II-prothrombin mutation (G20210A)
- PAI-1 Deficiency
- Antithrombin III Deficiency
- Protein S & C Deficiency
- Elevated Factor VIII
- Hyperhomocysteinemia (MTHFR C677T and A1298C)

Etiology  
Thrombophilia

- Venous

Most common acquired:
- Anti-phospholipid antibodies (APAs)
- Activated Protein C resistance
- Hyperhomocysteinemia (MTHFR C677T and A1298C)
- Other possible abnormalities
  - Anti-thrombin deficiency
  - Protein C or S deficiency
  - Elevated Factor VIII

Etiology  
Thrombophilia

- Arterial

Hyperhomocysteinemia
- APAs
- Lupus anticoagulant

Etiology  
Thrombophilia

- Factor V Leiden
  - Autosomal dominant
  - Acquired activated protein C resistance in pregnancy, OCP use and in presence of APAs
  - Heterozygotes: 7X increase lifetime risk thrombosis; 15X increase during pregnancy or OCP use
  - Homozygotes: 50-100X increase lifetime risk thrombosis
**Etiology**

**Thrombophilia**

- **Prothrombin G20210A Mutation**
  - Higher plasma prothrombin concentrations, augmented thrombin generation
  - Heterozygotes: 2-3% Whites
  - Conflicting prevalence studies among RPL
  - Recent critical review suggests an association

- **Hyperhomocysteinemia polymorphisms**
  - **C677T thermolabile MTHFR**
    - Heterozygous: 10-20% Whites
      - Normal or slightly elevated homocysteine
      - Increased homocysteine when combined with B vitamin deficiencies
    - Homozygous: 10% Whites
      - Significantly increased homocysteine
  - A1298C often occurs with thermolabile C677T
    - 33% frequency in Dutch population
    - Combined heterozygosity results in hyperhomocysteinemia and decreased plasma folate levels

- **Hyperhomocysteinemia**

  - Significant association between hyperhomocysteinemia and RPL
    - Mechanism: interference in embryonic development through defective chorionic villous vascularization
  - Known association with later pregnancy-related complications

- **Anti-thrombin Deficiency**
  - Physiologic inhibitor of coagulation
    - Type I: quantitative; decreased antigen and function; caused by gene deletions, nucleotide changes
    - Type II: qualitative; normal antigen levels, decreased function; caused by point mutations with single amino acid changes leading to a dysfunctional protein

- **Anti-thrombin Deficiency**
  - Autosomal dominant
  - Prevalence Type I heterozygous carriers: 1/2000 – 1/5000
  - Prevalence Type II heterozygous carriers: 3/1000
  - Most thrombogenic of inherited thrombophilia: 20-50% lifetime risk
  - Associated increased risk stillbirth and fetal loss
Etiology  
**Thrombophilia**

- **Protein C Deficiency**
  - Down-regulates coagulation cascade; deficiencies lead to unregulated fibrin formation
  - Autosomal dominant: > 160 mutations
  - Type I: quantitative
  - Type II: decreased function
  - Associated with 2nd trimester losses

- **Protein S Deficiency**
  - Principal cofactor of activated Protein C; mimics C deficiency; questionable association with pregnancy loss
  - Autosomal dominant: > 160 mutations; prevalence 0.15-0.8% general population; acquired forms in multiple disease states
  - Type I: quantitative
  - Type II: decreased function
  - Type III: low free protein, normal antigen, reduced activity

Etiology  
**Luteal Phase Defect**

- Luteal phase defect is a controversial cause of RPL
  - Studies proving LVD as a cause of RPL lacking
  - No convincing studies showing LVD treatment improves pregnancy outcome
  - 80% of women with low midluteal progesterone proceed to term
  - 20% of fertile women have abnormal endometrial biopsies
  - P4 drops after meals & standing

Etiology  
**Endocrine Factors**

- Poorly controlled diabetes
- Overt hyperthyroidism
- Overt hypothyroidism
- No evidence that asymptomatic systemic endocrinologic or metabolic disorders are a cause of RPL

Etiology  
**Autoimmune Factors**

- Certain autoimmune diseases are associated with pregnancy loss
  - Systemic lupus erythematosus
    - 1st trimester loss: 10% risk
    - 2nd and 3rd trimester loss: 6%
  - Anti-phospholipid syndrome
    - 2nd trimester loss: 38%

- Anti-phospholipid antibodies (aPL)
  - autoantibodies recognizing various combinations of phospholipids, phospholipid-binding proteins; or both
- Anti-phospholipid syndrome (APS) - clinical association between aPL and syndrome of hypercoagulability
Etiology  Autoimmune Factors

- APS diagnostic criteria:
  - Clinical features
    - Vascular thrombosis or
    - Loss of fetus at or after 10 weeks or
    - Preterm delivery at or before 34 weeks or
    - 3 or more consecutive SAB before 10 weeks

- Laboratory features
  - Anti-cardiolipin (aCL) antibodies: IgG or IgM at moderate or high levels on 2 or more occasions at least 6 weeks apart
  - Lupus anticoagulant (LA) antibodies: detected on 2 or more occasions at least 6 weeks apart

Etiology  Autoimmune Factors

- Other anti-phospholipid antibodies
  - Anti-phosphatidylserine: nearly always associated with APS, highly correlated to cardiolipin binding
  - Other antibodies have less correlation
    - No consistency among reported studies
    - No independence from aCL

- Other auto-antibodies NOT associated with RPL
  - Anti-nuclear antibodies may be more common among women with RPL but their presence or absence do not predict subsequent pregnancy outcome

- Anti-thyroglobulin and anti-thyroid peroxidase are markers of increased risk for pregnancy loss if identified early in pregnancy

- Some small studies suggest a slight association in RPL; other larger studies do not

- Subsequent pregnancy outcomes not affected

Etiology  
Alloimmune Factors

- Immune response to non-self components of pregnancy
  - Cytotoxic antibodies
  - Absence of maternal blocking antibodies
  - Inappropriate sharing of HLA
  - Disturbances in natural killer cell function and distribution

Etiology  
Alloimmune Factors

- Cytotoxic antibodies
  - Maternal response to paternal antigens
  - Present in normal pregnancies
  - More common in fertile couples than those with RPL
  - No bearing on subsequent pregnancy outcome

Etiology  
Alloimmune Factors

- Blocking antibodies
  - Theory: maternal anti-fetal antibodies block maternal cell-mediated response; if absent, then fetal rejection occurs

Etiology  
Alloimmune Factors

- Blocking antibodies
  - Not present in normal pregnancies, yet are often present in RPL
  - Detected by the non-specific mixed lymphocyte response assay

Etiology  
Alloimmune Factors

- Animal model: B-cell deficient (agammaglobulinemic) mice have normal pregnancy outcomes
  - Human agammaglobulinemics have successful pregnancies
  - Presence or absence not predictive of subsequent outcome

Etiology  
Alloimmune Factors

- Parental HLA sharing
  - Theory: if parents are antigenically similar, mother is less likely to develop blocking antibodies
  - Studies contradictory; some show increased sharing in HLA-B and HLA-DR loci
  - Most show no associations

Porter Semin Reprod Med 2000;18(4):393-400
Etiology  Alloimmune Factors

- Natural killer cells
  - Theory: CD56+ NK-like cells secrete a transforming growth factor-β-like substance crucial to the maintenance of pregnancy
  - Present in endometria and early gestational decidua of women with RPL

- Murine models show activation of NK cells increases the rate of abortion, depletion of NK cells has opposite effect
- Human studies show no association of testing and successful pregnancy
- No correlation between blood testing and endometrial NK activity
  - Hormonally dependent

Etiology  T helper (Th1) immunodystrophism

- Theory: aberrant or inappropriate Th1 stimulation may result in overproduction of cytokines that have deleterious effect on conceptus
- Dichotomous Th1 versus Th2 cytokine profile associated with human pregnancy loss and success

- No significant difference in semen parameters among men whose partners have RPL compared to WHO standards and men fathering successful pregnancies
- No difference in incidence of anti-sperm antibodies
- Aside from cytogenetic abnormalities, male factor contribution to RPL unknown

DNA Fragmentation may result in early embryo loss
  - Hum Reprod. 2006 Nov;21(11):2876-81; Check JH: Arch Androl. 2005 Mar-Apr;51(2):121-4

Etiology  Male Factor

- RPL males have higher incidence of sperm aneuploidy:
  - Oligoasthenoteratosperma 35-74%
  - Fertile donor sperm 4-7%

Etiology  Environmental Factors

- Confirmed association
  - Ionizing irradiation
  - Organic solvents
  - Alcohol
  - Mercury
  - Lead

- Suspected association
  - Caffeine (> 300 mg/day)
  - Hyperthermia/fever
  - Cigarette smoking

- Unknown association
  - Pesticides
**Etiology**  
- Diagnostic x-rays  
- Air travel  
- Microwave ovens  
- Diagnostic ultrasounds  
- Electromagnetic fields  
- Video display terminals  
- Aspartame

**Environmental Factors**  
- Chocolate  
- Drinking water  
- BGH  
- Phytoestrogens  
- Phthalates  
- Herbicides  
- Hair dyes  
- Nail polish  
- Saccharin  

**Idiopathic**  
- More than 50% of couples with RPL have no explanation despite extensive evaluation(s)  
- Informative and sympathetic counseling appears to play an important role  
  - 70% live birth rates reported in couples with unexplained RPL who undertake an untreated subsequent pregnancy

**Evaluation**  
**History**  
- Pattern and trimester of pregnancy losses and whether a live embryo or fetus was present  
- Exposure to environmental, toxins or drugs  
- Known gynecological or obstetrical infections  
- Features associated with APS

**Lab Tests**  
- Saline Sonogram or hysteroscopy  
- Hysterosalpingogram  
- Luteal phase endometrial biopsy; repeat in next cycle if abnormal  
- Placental CGH analysis  
- Parental karyotypes  
- Lupus anticoagulant, thrombophilia testing  
- Anticardiolipin & Antiserine antibodies IgG and IgM  
- HgbATC or 2hr IGTT  
- TSH, TPO, Prolactin

Evaluation

Tests
- Antiphosphatidylserine antibody IgG and IgM
- Platelet count
- Thrombophilia mutations and functional assays
- Thyroid stimulating hormone

Tests NOT useful
- Other anti-phospholipid antibodies
- ANA
- Maternal anti-paternal leukocyte antibodies
- Mixed lymphocyte maternal-paternal cell cultures
- HLA genotyping
- Mouse embryotoxicity assays
- Immunophenotype panels (CD56, CD16)

Hill ASRM 2002 Course 6 p.58-59

Treatment Thrombophilia

For heritable or acquired thrombophilia: lovenox or heparin anticoagulation
For bonafide APS, multiple studies support use of lovenox or heparin and aspirin

Treatment APS

- Aspirin 81 mg po/day
- Subcutaneous heparin 10K-20K units/day divided doses
- Alternative: Lovenox 40-80mg SQ
- Calcium supplementation

Treatment Thrombophilia

For elevated homocysteinemia without thrombosis history
- Supplementation with Vitamin B6, B12 and folic acid
- Metafolin PNV: Neevo, Prenate DHA
- Anticoagulation for history of thrombosis or failure to normalize homocysteine levels
Empiric Treatment

- Use of aspirin alone attractive because of ease of use and relative safety profile, barring contraindication to low-dose aspirin use
- Supporting data lacking

Treatment - Immunotherapy

- "Blocking antibody" hypothesis
- Intravenous immunoglobulin
  - Studies and meta-analyses show no benefit
  - Extremely expensive $7-14,000
  - Side effects: headache, hypotension, nausea
  - Potential anaphylaxis in IgA deficient patients
  - Potential for prion disease transmission due to large pool of donors

Treatment - Immunotherapy

- Progesterone called "nature's immunosuppressant" due to inhibition of immune cells at maternal-fetal interface
- No verification yet through RCT
- Safe and inexpensive
- Dose: 100 mg BID vaginal suppositories, beginning 3 days after ovulation

Supportive Treatment

- 60-90% chance of pregnancy success with supportive care and...
  - Timed intercourse for genetic and idiopathic RPL
  - Surgery for selected anatomic factors
  - Ovulation induction for LPD or irregular menstrual cycles
  - Luteal phase hCG with irregular cycles.

Supportive Treatment

- 60-90% chance of pregnancy success with supportive care and...
  - Immunosuppressive P₄ for presumed alloimmune factors
  - Thyroid replacement for hypothyroidism
    - Keep TSH < 3.5
  - Appropriate anticoagulation for APS/thrombophilias
Management: Genetic Losses

- Consider Microarray CGH or SNP analysis
  - IVF
  - Day 3 biopsy vs Day 5
    - Risk of biopsy vs blast cryo
    - High implantation rates
  - Create probe for single gene defect or aneuploidy
  - Embryo biopsy allows detection of entire genome

Embryo Evaluation “omics”

- GENomics
  - FISH – day 3
  - Array CGH – day 5
  - SNPs
- TRANSciPTOmics
  - Gene transcription
- PROTEomics
  - Proteins
  - Secretomics
- METABOLOmics
  - Metabolites
  - Amino Acids

Biopsy and Preimplantation Genetic Diagnosis of a 3-Day-Old (Eight-Cell) Embryos

Embryos and Blastocysts during Assisted Reproduction (x20)
Analysis by Comparative Genomic Hybridization of a Blastomere Obtained by Biopsy of a Six-to-Eight-Cell Embryo

Management: Genetic Losses
- Drawbacks of CGH/SNPs Microarray
  - Expense
  - Possibility of no transfer
  - 10-25% mosaicism and potential for misidentification
  - No large scale studies supporting benefit for recurrent pregnancy loss

Blastocyst Apposition and Adhesion

Blastocyst Implantation

Maintenance of Early Pregnancy

Summary
- Early pregnancy loss is a frustrating entity for both patients and providers
- Possibility of successful pregnancy outcome high, depending on maternal age and number of prior losses
- Understanding the potential underlying mechanisms of loss along with empathetic supportive care decreases emotional stress and facilitates cost-effective evaluation and therapy
Questions?