Male Factors in Infertility & Recurrent Pregnancy Loss

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Conflicts of Interest

• Advisory board: Acerus
Objectives

• Approach to assessing the infertile male.
  • History
  • Physical Exam
  • Investigations

• Discussing male contribution to recurrent pregnancy loss
  • Overview
  • DNA Fragmentation
  • Sperm FISH

Approach to the Infertile Male

• Infertility effects 15% of couples
• Male factors contribute to 50% of cases
• Severe male factors (i.e. Azoospermia) affects 1% of general population, and 15% of couples
Approach to the Infertile Male

- **Fertility History**
  - Primary vs. secondary
  - Previous treatment or evaluation
  - Mechanics of Intercourse

- **Past Medical History**
  - Systemic diseases
  - GU infections
  - Congenital Abnormalities (i.e. Undescended testes)
  - Malignancies

Approach to the Infertile Male

- History
- Physical Exam
- Investigations
- +/- Referral
Approach to the Infertile Male

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- **Past Surgical History**
  - Scrotal & inguinal surgery (i.e. vasectomy or hernia repair)
  - Pelvic & abdominal surgery (i.e. RPLND)
  - Transurethral surgery (i.e. TURP)

- **Medication History**
  - Testosterone
  - Chemotherapies

- **Social History & Exposures**
  - Heat exposures
  - Work exposures
  - Lifestyle

- **Physical Exam**
  - Body habitus
  - Gynecomastia & androgenization
  - Scrotal exam (i.e. testis size, vas deferens, epididymis & varicoceles)

- **Investigations**
  - Semen analysis (x2)
    - Volume
    - **Concentration**
    - Motility
    - Morphology
    - (pH, round cells, viability)
Approach to the Infertile Male

- Azoospermia – No sperm!

- **Non-obstructive Azoospermia**
  - Central or testicular failure
    - Adjuvant hormone therapy?
    - Microsurgical Testicular sperm extraction

- **Obstructive Azoospermia**
  - Find the blockage
    - Fix the blockage
    - Retrieve sperm

![Diagram of Approach to the Infertile Male]

- **FSH >7.6IU/L**
  - **FSH <7.6IU/L**
  - **N Testicular Volume**
    - Long-axis >4.6cm
      - Flat Epididymis
      - Likely NOA.
    - Long-axis <4.6cm
      - Full Epididymis
      - Possible (NOA), or obstruction (OA).

- **Diagnostic testicular biopsy.**
  - If ++ sperm = epididymal SR
  - If No sperm = testicular SR
  - Likely Obstructive (OA).
  - Reconstruction, or epididymal/testicular sperm retrieval.

- **Possible testicular failure (NOA).**
  - Therapeutic biopsy.
  - Testicular Sperm Retrieval
  - Possible testicular failure (NOA), or OA.
  - Diagnostic testicular biopsy.
  - If ++ sperm = epididymal SR
  - If No sperm = testicular SR
  - Therapeutic biopsy.
Azoospermia

FSH > 7.6 IU/L

↓ Testicular Volume

Likely NOA.

Therapeutic biopsy. Testicular sperm retrieval.

NOA

Therapeutic biopsy. Testicular sperm retrieval.

FSH < 7.6 IU/L

↓ Testicular Volume

Long-axis > 4.6 cm

Flat Epididymis

Possible (NOA), or Obstructive (OA)

Diagnosis testicular biopsy.

If ++ sperm = Epididymal SR
If No sperm = Testicular SR

Full Epididymis

Likely Obstructive (OA)

Reconstruction, or epididymal/testicular sperm retrieval.

↓ Testicular Volume

Long-axis < 4.6 cm

Flat Epididymis

Possible testicular failure (NOA).

Therapeutic biopsy.

Full Epididymis

Possible testicular failure (NOA), or OA.

Diagnostic testicular biopsy.
Choosing Advanced Investigations

- **Hormone Profile**
  - AM Testosterone, Estradiol, LH, FSH, Prolactin
  - Order when: sperm concentration is <10 million

- **Genetic Testing**
  - CFTR Gene
  - Order when: absent vas deferens
  - Karyotype & Y-Chromosome Microdeletion
  - Order when: sperm concentration <5 million

- **DNA Fragmentation**
  - Multiple assays available
  - Unexplained infertility, recurrent pregnancy loss

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  - DNA Fragmentation
  - Sperm FISH
  - Parental Karyotype
Recurrent Pregnancy Loss

- Pregnancy loss occurs in ~0.8-25% of clinical pregnancies.
- Recurrent pregnancy loss (RPL): 2 or more failed clinical pregnancies.
  - Incidence: 5% of couples experience 2 failed clinical pregnancies
  - Incidence: 1% of couples experience >2 failed clinical pregnancies

1 Franssen MT, Korevaar JC, Leschot NJ, Bosuyt PM, Gerssen-Schoorl KB, Wouters CH, Hansson KB, Hochstenbach R, Madan K et al. Selective chromosome analysis in couples with two or more miscarriages: case-control study. BMJ 2005;331: 137-141.
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  • Incidence: 1% of couples experience >2 failed clinical pregnancies
• Developmental or genetic abnormalities present in 86-91% of analyzed pregnancy tissues when embryo is present.\(^1\)
• Chromosomal abnormalities identified in pregnancy tissues (45% of single sporadic miscarriages; 39% in subsequent miscarriages).\(^1\)

\(^1\) Franssen MT, Kooistra JC, Laschot NJ, Bossuyt PM, Knekt AG, Gerssen-Schoorl KB, Wouters CH, Hanssen KB, Hochstenbach R, Madan K et al. Selective chromosome analysis in couples with two or more miscarriages: case-control study. BMJ 2005;331: 137-141.

Recurrent Pregnancy Loss

• **Female Factors are critical**

• **Female Age**
  • Women <35 years of age \(\rightarrow\) miscarriage rate of 9-12%
  • Women >40 years of age \(\rightarrow\) miscarriage rate of 50%

• **Etiology**
  • Unexplained (50-75%)
  • Antiphospholipid Syndrome (8-42%)
  • Anatomic (1.8-37.6%)
  • Cytogenetic (2-5%)
  • Hormone or metabolic
  • Alloimmune
  • Lifestyle – environmental factors
  • Male factors

\(^2\) Ibrahim & Johnstone. 2018. The male contribution to recurrent pregnancy loss.
Achieving Successful Pregnancies in RPL Couples?

• Despite majority of cases lacking an identifiable etiology, achieving a future pregnancy may occur in 50-60% of couples.
• Cumulative live birth rates with natural conception & medical management 55-74%.
• Live birth rates 31-35% per cycle with IVF/PGD.

Do Sperm Play A Critical Role in RPL?
Sperm Impact Fertilization & Embryogenesis

Evidence for a Strong Paternal Effect on Human Preimplantation Embryo Development and Blastocyst Formation

Laurent Janni¹ and Yves J. Menezo²
¹Unité de IVF, Pélissériques, Charnont-Ferrand, and ²I.R.H./Fondation Mérieux, Bron, France

Assisted reproductive technology

Delayed fertilization and poor embryonic development associated with impaired semen quality

Raphael Ron-El M.D. *, Hanna Nachum, Arie Herman M.D., Abraham Golan M.D., Eliahu Caspi M.D., Yigal Soffer M.D.

Sperm integrity is critical for normal mitotic division and early embryonic development*

Maureen Moomjy, Liliana T. Colombo, Lucinda L. Veeck, Zev Rosenwaks, Gianpiero D. Palermo

Sperm Impact Implantation & Fetal Development

Some semen abnormalities may cause infertility by impairing implantation rather than fertilization

J. H. Check, D. Katsoff, M. L. Check

The role of genomic imprinting in implantation *

Edward E. Wallach M.D. (Associate Editor), Ran Goshen M.D. †‡§, Zion Ben-Rafael M.D. ‡, Bernard Gonik M.D. †, Orit Lustig M.Sc. †, Vasilios Tannos M.D. †, Nathan de-Groot Ph.D. †, Abraham A. Hochberg Ph.D. †

The Role of Imprinted Genes in Fetal Growth

Monica Miozzo Giuseppe Simoni

• Sperm derived from infertile men result in:
  • Reduced fertilization.
  • Impaired blastocyst formation.
  • Poor embryonic morphology.
  • Abnormal sperm centrosome results in embryonic chromosomal mosaicism and lack of bipolar spindle.

• Abnormal hypo-osmotic swelling test associated with decreased implantation.
  • Paternally imprinted genes are associated with placental proliferation and invasiveness.
  • Paternally imprinted genes are associated with fetal growth.
Clinical Associations to RPL

- **Increased RPL associated with advanced paternal age**
  - Miscarriage rate of 32.4% in fathers >45yrs\(^1\)
  - Miscarriage rate of 13.7% in fathers who were <30yrs\(^1\)
  - Miscarriage rate increases with age\(^2\)
    - OR = 1.2 (age 30-34), OR = 1.5 (age 35-39), OR = 1.3 (age 40-64)

- **Increased RPL among men that smoke\(^3\)**
  - OR 1.81 (95% CI 1.00-3.29)

- Increased risk of RPL identified with smoking, drinking, occupational exposures and environmental factors (OR 11.965; 95% CI 1.49-95.62).\(^4\)

\(^1\)Belloc 2008, Reprod Biomed Online; \(^2\)De la Rochebrochard 2002, Human Repro; \(^3\)Venners, 2004 Am J Epidim; \(^4\)Ruixue et al. 2013

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Sperm DNA Fragmentation & RPL

- Mounting evidence that sperm DNA fragmentation may contribute to idiopathic recurrent pregnancy loss.
- Multiple sperm DNA Fragmentation Assays available
  - Sperm Chromatin Structure Assay (SCSA)
  - Sperm Chromatin Dispersion (SCD)
  - TUNEL
  - COMET
Sperm DNA Fragmentation & RPL

- **Meta-analysis** (Robinson et al. 2012)
  - 16 cohort studies (14 prospective), n=2969 couples.
  - 15/16 studies evaluated in IVF/ICSI population.
  - Increased miscarriage rates in men with higher DNA fragmentation (RR 2.16, 95% CI 1.54 – 3.03).
  - Subgroup analysis:
    - TUNEL assay had the strongest association with miscarriage rate (RR 3.94, 95% CI 2.45-6.32)

- **Meta-analysis** (Zhao et al. 2014)
  - 16 cohort studies (n = 3106 couples)
  - Increased risk of miscarriage OR 2.28 (95% CI 1.55-3.35)

- **Meta-analysis** (Tan, Taskin, Albert, Bedaiwy (UBC) 2019)
  - 12 prospective, 2 retrospective case control studies, n=530 men with recurrent pregnancy loss, n=639 fertile controls.
  - DNA fragmentation 11.98% higher in recurrent pregnancy loss

- **Meta-analysis** (McQueen et al. 2019)
  - 13 studies, case-control, n=579 males of partners with recurrent pregnancy loss and n=434 controls
  - DNA fragmentation elevated among men from recurrent pregnancy loss 11.91% (95% CI 4.97-18.86)
• Compared 22 couples with RPL & 20 fertile men
• **RPL men had:**
  • Decreased progressive motility (30.2% vs 51.5%)
  • Increased abnormal morphology (74.8% vs 54.2%)
  • Increased DNA fragmentation (17.1% vs 10.2%)
  • Increased aneuploid sperm (10.6% vs. 1.5%)

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**How can sperm DNA fragmentation lead to miscarriage?**

• Sperm DNA fragmentation relies on oocyte DNA repair mechanisms as sperm do not carry the machinery for DNA repair.
  • Sperm DNA fragmentation increases in men >40 years of age
  • Exacerbated by presumption that oocytes of women >35 years of age have impaired ability to repair damaged DNA.
How can sperm DNA fragmentation lead to miscarriage?

- Sperm DNA fragmentation relies on oocyte DNA repair mechanisms as sperm do not carry the machinery for DNA repair.
  - Sperm DNA fragmentation increases in men >40 years of age
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- Disrupted downstream transcription, gene expression, and errors in developing embryo or placenta.
- Other abnormalities associated with same etiology that lead to DNA fragmentation.
- Associated Epigenetic abnormalities?

Sperm DNA Fragmentation

**Management**
- Reversible causes. I.e. smoking, prolonged abstinence.
- Varicocelectomy
- Testicular sperm
- ?Antioxidants

Effects of Varicocele Repair

- **Improved DNA Integrity**
  - Meta-analysis 2012
  - Pooled 7 prospective & retrospective studies
  - Among 240 men, those with varicoceles had greater sperm DNA damage compared to 176 fertile controls.
  - Varicocele repair reduced sperm DNA fragmentation compared to pre-op levels in 177/240 men by -3.4% (95% CI, -4.1 to -2.7, p<0.0001), as measured by SCSA, TUNEL, or Comet assay.


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Effects of Varicocele Repair on Spontaneous First Trimester Miscarriage
A Randomized Clinical Trial

Mandana Mansour Ghaniali, Seyyed Alaeddin Asgari, Nassrin Dadrass, Aliakbar Allahkhah, Elham Iran-Pour, Mohammad Reza Safarinejad

- **Improved Miscarriage & Pregnancy Rate & SA**
  - Randomized Trial
  - Mansour 2012
  - 136 couples with recurrent miscarriage.
  - Men randomized to varicocelectomy vs observation.
  - Men were normozoospermic at study onset.
  - Pregnancy rate was greater in VR: 44.1% vs 15.9%, p=0.003
  - Miscarriage rate was lower in VR: 19.1% vs 44.1%, p=0.001
  - Improved sperm concentration, progressive motility, and morphology in the VR group vs observational group.
Reproductive outcomes of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with high levels of DNA fragmentation in semen: systematic review and meta-analysis

Sandro C. Esteves, M.D., Ph.D., Matheus Roque, M.D., Cara K. Bradley, Ph.D., and Nicolás Garrido, Ph.D.

ANDROFERT, Center for Male Reproduction, São Paulo, Brazil; ORIGEN, Center for Reproductive Medicine, Rio de Janeiro, Brazil; Geneva, Sydney, New South Wales, Australia; and IVI Foundation, Valencia, Spain

Testicular Sperm in High DNA Fragmentation

- **Meta-analysis** (Esteves et al. 2017)
  - 5 studies – 143 patients with ejaculated & testis derived sperm.
  - 4 studies – 3840 cycles ICSI.
  - Testis sperm reduced SDF -24.58% (95% CI -32.53 to -16.64%).
  - No difference in fertilization rate: OR 0.81 (95% CI 0.58-1.15).
  - Testis-ICSI **increased CPR**: OR 2.42 (95% CI 1.57-3.73).
  - Testis-ICSI **increased LBR**: OR 2.58 (95% CI 1.54-4.35).
  - Testis-ICSI **lower miscarriage rates**: OR 0.28 (95% CI 0.11-0.68).
  - **Caution**: Retrospective case series, different SDF assays and thresholds used.
Sperm FISH

- Sperm from men with RPL have\(^1\)
  - 2.7 times increased rate of sex chromosome aneuploidy
  - 3.3 times greater rate of chromosome 13 or 21 aneuploidy
  - 6 times greater rate of chromosome 18 aneuploidy
- Increased incidence of disomy 16 in men with RPL (60%).
- Utility: Considering donor sperm if grossly abnormal result?

\(^1\)Ramasamy, Scovell, Kovac, Cook, Lamb, Lipshultz. 2015. Fluorescence in situ hybridization detects increased sperm aneuploidy in men with recurrent pregnancy loss. F&S.

Parental Karyotyping

- Retrospective cohort (Carp et al. 2004)
- 916 patients (men and women) with recurrent miscarriages
- 43/458 (9.4%) men had karyotype abnormalities
  - Paternal balanced translocation 21/458 (4.6%)
  - Paternal mosaic for numeric aberration 3/458 (0.6%)
  - Paternal inversion 19/458 (4.1%)
- No differences in live birth rates among parents with chromosomal rearrangements
Conclusions

• Approach to Male infertility relies on a strong history, physical exam and semen analysis.

• Mounting evidence suggests male factors contribute to idiopathic recurrent pregnancy loss.
Conclusions

• Approach to Male infertility relies on a strong history, physical exam and semen analysis.

• Mounting evidence suggests male factors contribute to idiopathic recurrent pregnancy loss.

• DNA fragmentation assays are a next step in assessing male partners with recurrent pregnancy loss.

Thank you

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