Novel Treatments for Male Infertility

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San Francisco, CA
The Infertility Problem

Affects 6-8 million couples in US (NSFG 2002, NICHD)
History Physical Exam

Semen Analysis x 2

Normal

Further Female Evaluation

Abnormal

Eliminate Gonadotoxins

Abnormal

Treat Female Factor

Normal

Hormone Evaluation

Not Improved

Improved

Treat Female Factor

Urology Referral

Turek PJ. Nat Clin Pract Urol. 2:1, 2005
Treatment of Male Infertility

Diagnostic Evaluation

Correctable Problem?
Healthy Patient?

Maternal Reproductive Potential >1yr?

Yes

Correct Male Factor (Varicocele, blockage)

No

Genetic Evaluation

IUI
IVF
IVF-ICSI

Surgical Treatments are Very Competitive

Vasovasostomy
Epididymovasostomy

VS

An immaculate misconception
by Carl Djerassi
Produced and directed by William Archer
Yerba Buena Center for the Arts
San Francisco, California
6 October 1998, 8:00 pm.
Decision Analysis: Vasectomy Reversal

Decision Modeling: Sensitivity Analysis

Cost per Pregnancy (US $)

Vasectomy Reversal Patency

Reconstruction

ICSI

78%

How Good are Medical Treatments for Male Infertility?
The things that we know....

It is more than likely that the brain itself is only a sort of great clot of genital fluid held in suspense or reserved.

Ezra Pound
1885-1972
Hormones-When to Order?

1. Sperm density < $10 \times 10^6$ sperm/mL
2. Evidence of impaired sexual function (low libido, impotence)
3. Findings of an endocrinopathy (thyroid)


## Endocrine Evaluation-Findings

(n=1035 men)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Findings</th>
<th>% Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>90.4%</td>
</tr>
<tr>
<td>Germ cell failure</td>
<td>Elev. FSH</td>
<td>7.8%</td>
</tr>
<tr>
<td>Pan testis failure</td>
<td>Elev. FSH, LH</td>
<td>0.9%</td>
</tr>
<tr>
<td>Hyperprolactinemia</td>
<td>Elev. PRL</td>
<td>0.5%</td>
</tr>
<tr>
<td>Leydig tumor</td>
<td>Elev. T, Low LH</td>
<td>0.2%</td>
</tr>
<tr>
<td>IHH</td>
<td>Low LH, T</td>
<td>0.2%</td>
</tr>
<tr>
<td>Androgen resistance</td>
<td>Elev. LH, T</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

Both *testosterone* and *FSH* are required for *quantitatively normal* spermatogenesis.
Thinking therapy? Think 60-70 days

Misell et al. J Urol. 175. 242, 2006
Spermatogenic Wisdom

One must show the greatest respect towards any thing that increases exponentially, no matter how small

G. Hardin, 1968
Hyperprolactinemia and Male Infertility

• Elevated prolactin generally presents with low libido and erectile dysfunction. Isolated spermatogenic failure is rare.

• Elevated prolactin is correlated with low testosterone, but not necessarily lower FSH and LH. Estradiol is generally unchanged. Micic et al. Arch Androl. 1985;15;123

• Prolactin needs to be elevated at least 2x normal to cause infertility. Nishimura et al. Arch Androl. 1999; 43: 207

• The treatment of low sperm counts in infertile men with normal prolactin is unsuccessful (4 controlled studies) Vandekerckhove et al. Cochrane Database Syst Rev. 2000, (2) CD000152
Replacement Therapy and Male Infertility

- **Thyroid Disorders.** Fertility returns when thyroid disease is stabilized.

- **Obesity.** Relationship between BMI and male infertility is real. Danish cohort study: 26,303 planned pregnancies. Adjusting for partner BMI, coital frequency, ages and smoking habits

<table>
<thead>
<tr>
<th>BMI</th>
<th>OR of infertility</th>
<th>(CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25.5</td>
<td>1</td>
<td>1.04-1.38</td>
</tr>
<tr>
<td>25-30</td>
<td>1.2</td>
<td>1.13-1.63</td>
</tr>
<tr>
<td>30-35</td>
<td>1.36</td>
<td>1.13-1.63</td>
</tr>
</tbody>
</table>

- **Opiates** cause hypogonadism. Produces hypogonadotrophic hypogonadism) in 75-100% of chronic opiate users. 

- **Renal Disease.** The fertility of renal transplant patients is normal.
Case study

34 yo male with 4 year history of primary infertility.

Exam: Tall, light facial hair.
10cc (2 x 1.5 cm) testes bilaterally.

Labs: Test. 80 ng/ml (NL 260-1200)
FSH 2 IU/ml (NL 2-10)
LH 3 IU/ml (NL 3-15)
PRL 8 IU/ml (NL 2-8)

Semen: Low volume; azoospermia: fructose present

Hypogonadotrophic hypogonadism

If acquired, consider Sickle cell anemia, Hemachromatosis
Hypogonadotrophic Hypogonadism

- Olfactory nerve and LHRH secreting cells co-migrate during development.

- Migration failure results in defective LHRH and anosmia—Kallmann Syndrome or hypogonadotrophic hypogonadism
Hypogonadotrophic Hypogonadism

Hypothalamus

Anterior Pituitary

Genes associated with migration failure:

Kallmann syndrome interval gene (*KALIG*)

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Inheritance</th>
<th>Gene</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAL-1</td>
<td>X-linked</td>
<td>KALIG-1</td>
<td>Xp22.3</td>
</tr>
<tr>
<td>KAL-2</td>
<td>Autos. dom.</td>
<td>FGFR-1</td>
<td>8p11-12</td>
</tr>
<tr>
<td>KAL-3</td>
<td>Autos. recess.</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>
Gonadotropin Replacement

- Months: 0, 3, 6, 9, 12, 15, 18
- hCG 2000U: 3x wk
- hMG 75IU: 2x wk
- Sperm Count: 15 x10⁶ sperm/mL

O'Dea et al. Fert Steril 70: 3 (28A), 1998
Bouloux et al. Fert Steril. 77: 270, 2002
What About Other Therapies?
Aromatase Inhibitors
(Testolactone, Arimidex)

Theory: estrogen-testosterone imbalance is a cause of decreased spermatogenesis. Seek T/E ratio of 15/1.

Aromatase converts testosterone to estrogens

Aromatase inhibitors block estrogen production

Rx

Oral testolactone 100-200 mg/daily
Oral Anastrozole (Arimidex) 1mg/daily
Follow semen analyses q 3 months.

Side Effects: well tolerated. ? Lipid and bone effects.
Efficacy: T/E ratios increased from 5-7 range to 12-18. Semen volume, concentration improved.

A Pill for Every Ill?

- A problem in the field of male infertility (read “medicine”)
- Everyone wants a “pill.”
- n=500 consecutive male infertility patients surveyed on alternative and hormonal therapies.
- n=481 (96%) completed survey. 31% used alternative therapies:
  - 63% of those men were using antioxidants
  - 12% were using herbal remedies
  - 17% were using testosterone or antiestrogens
  - 1/4 of latter did not inform care providers

How About SERM’s for Oligospermia?

• First generation drug, clomiphene citrate, used to induce ovulation in anovulatory women. Not FDA approved for men in U.S.

• Clomiphene citrate is a racemic mixture of two geometric isomers, enclomifene (E-clomifene) and zuclomifene (Z-clomifene).

E-clomifene

Z-clomifene
How Does Clomiphene Citrate Work?

Nonsteroidal SERM
Acts as anti-estrogen
Increases GnRH output

Rx
12.5-25 mg/day
Check FSH, T in 4 weeks
Monitor semen q 3 mos

Side Effects: gynecomastia, weight gain, visuals, skin
## Does Clomiphene Citrate Really Work?

### Efficacy Studies for Idiopathic Male Infertility

<table>
<thead>
<tr>
<th>Author</th>
<th>Number Patients</th>
<th>Semen Improvement</th>
<th>Pregnancy Rate</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foss, 1973</td>
<td>114</td>
<td>NR</td>
<td>17% both</td>
<td>Neg.</td>
</tr>
<tr>
<td>Paulson, 1979</td>
<td>40</td>
<td>70% vs 40%</td>
<td>35% vs 17%</td>
<td>Posit.</td>
</tr>
<tr>
<td>Ronnberg, 1980</td>
<td>56</td>
<td>78% vs 21%</td>
<td>10% vs 3%</td>
<td>Posit.</td>
</tr>
<tr>
<td>Abel 1980</td>
<td>187</td>
<td>0% vs 0%</td>
<td>17% vs 17</td>
<td>Neg.</td>
</tr>
<tr>
<td>Wang, 1980</td>
<td>37</td>
<td>NR</td>
<td>36% vs 0%</td>
<td>Posit.</td>
</tr>
<tr>
<td>Micic, 1985</td>
<td>101</td>
<td>32% vs 7%</td>
<td>13% vs 9</td>
<td>?</td>
</tr>
<tr>
<td>Sokol, 1988</td>
<td>46</td>
<td>NR</td>
<td>9% vs 32%</td>
<td>Neg.</td>
</tr>
<tr>
<td>Check, 1988</td>
<td>100</td>
<td>NR</td>
<td>58% vs 16%</td>
<td>Posit.</td>
</tr>
<tr>
<td>WHO, 1992</td>
<td>200</td>
<td>NR</td>
<td>8% vs 12%</td>
<td>Neg.</td>
</tr>
</tbody>
</table>

"Hung jury"
Does Clomiphene Citrate Really Work?

- Cochrane review of anti-estrogens for idiopathic oligospermia.
- Included randomized trials, > 3 months duration.
- n=10 trials with n=738 patients.
- Antiestrogens “had a positive effect on endocrine outcomes.”
- No difference in pregnancy rate: OR 1.26 (.99-1.56).
- Overall pregnancy rate no different: 15.4% treated vs. 12.5% untreated.

Vandekerckhove et al. Cochrane Database Syst Rev. 2000, (2) CD000151
Is There a Rationale Use for Clomiphene Citrate in Male Infertility?

**Potential application:**
Improving anterior pituitary function (LH and FSH) in men with secondary hypogonadotrophic hypogonadism (HH) due to:

- Idiopathic causes
- Prolactinoma resection
- Acromegaly
- Diabetes
Response to Clomiphene Citrate in HH men with Male Infertility

- n=10 men; 2 centers; 5 years. Testosterone <164 ng/dL
- Treated 3 classes of HH with CC 50mg 3x weekly

<table>
<thead>
<tr>
<th>Category</th>
<th># Pts</th>
<th>Semen Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>With anosmia (Kallmann)</td>
<td>4</td>
<td>0/4</td>
</tr>
<tr>
<td>No anosmia (idiopathic, acquired)</td>
<td>4</td>
<td>3/4</td>
</tr>
<tr>
<td>Panhypopituitary patients</td>
<td>2</td>
<td>1/2</td>
</tr>
</tbody>
</table>

- Stated that CC may work for idiopathic, adult onset, HH

How Semen Quality Changes in Hypogonadal Men on Clomiphene Citrate

Carson Lawall MD
Uche Ezeh MD
Blake Tyrell MD
Paul Turek MD

ASRM 2004
Assess changes in hormones, symptoms and semen quality in men taking clomiphene citrate for secondary hypogonadotrophic hypogonadism.
• Prospective analysis of men treated with CC.

• Inclusion criteria:
  - Total testosterone < 250ng/mL.
  - Normal or Low LH level.
  - Clinical symptoms (ED, infertility, libido)

• Given Clomid at 12.5-25mg daily. Hormone response assessed 3 weeks later. Titrated treatment to achieve testosterone levels in the 400-700ng/mL range
• 22 men enrolled. Mean age 40 y.o. (range 21-56)

• Treatment indications:
  - Infertility: 14 patients
  - Infertility/libido: 2 patients
  - ED and libido: 2 patients
  - ED: 1 patient
  - Infertility and ED: 1 patient
  - Decreased libido: 1 patient
  - ED and gynecomastia: 1 patient

• Pathologic conditions:
  - Prolactinoma Rxn: 4 patients
  - Acromegaly: 2 patients
  - Intracranial germinoma: 1 patient
  - Idiopathc: 15 patients
Results

• Chemical response to clomiphene citrate:

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Pre-clomiphene</th>
<th>Post-clomiphene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Testosterone</td>
<td>143 ng/mL</td>
<td>479 ng/mL</td>
</tr>
<tr>
<td>FSH</td>
<td>3.4 mIU/mL</td>
<td>6.9 mIU/mL</td>
</tr>
<tr>
<td>LH</td>
<td>2.0 mIU/mL</td>
<td>5.7 mIU/mL</td>
</tr>
</tbody>
</table>

86% of patients had >50% increase in testosterone.

• A subset of 11 men with infertility had pre- and post-treatment semen analysis available for comparison
Results

• Semen quality response (mean values) to clomiphene citrate treatment (n=11 men):

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-clomiphene</th>
<th>Post-clomiphene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>2.5 mL</td>
<td>2.6 mL</td>
</tr>
<tr>
<td>Sperm concentration</td>
<td>15.7 mill/mL</td>
<td>30.8 mill/mL</td>
</tr>
<tr>
<td>Motility</td>
<td>15%</td>
<td>24%</td>
</tr>
<tr>
<td>Total Motile Sperm</td>
<td>11 million</td>
<td>33 million</td>
</tr>
</tbody>
</table>

• **Responders**: 7/11 men (64%) had a >50% increase in total motile sperm count. Gains mainly in counts (5.8x). 2/7 men conceived naturally.

• **Non-responders**: 2/4 had bilateral varicoceles and 2/4 had extensive pituitary resection.
Conclusions

• Daily clomiphene citrate may be a rationale treatment for men with secondary hypogonadism.

• Two-thirds of men with known and unknown causes of pituitary dysfunction responded chemically.

• Semen parameters showed dramatic increases and natural pregnancies occurred in treated men.

• Is there a role for clomiphene citrate in male infertility after all?
Case study

• 35 yo man presents with low libido, erectile dysfunction and infertility.
• Diagnosis of Type II diabetes 2 years earlier. Treated with multiple oral agents, including metformin.
• Diagnosed with hypogonadism (T=172) and started on T gel replacement. Libido and erections improved.
• I switched to clomiphene citrate 25mg daily. T=472.
• Conceived 9 months later. Staying on clomiphene for near term.
Injectable FSH for Very Low Sperm Counts

- n=33 men with severely low sperm counts and failed IVF-ICSI.
- Given 3 months of recombinant FSH at 150 IU/mL (n=23) or no treatment (n=10) prior to next IVF-ICSI cycle.
- Could FSH improve ICSI fertilization and pregnancy rates?
- Found a trend toward higher fertilization rates (62% vs 47%).
- Couldn’t really assess differences in pregnancy rates (low #’s).

**HOWEVER, mean ejaculated sperm concentrations increased from 1.3 million/mL, to 3.8 million/mL (normal > 20) in the treated group.** Controls showed no change.

- SO, can we drive the failing, azoospermic testis to make 100 or even 1000 sperm in the ejaculate and avoid costly and invasive sperm retrieval procedures?
Case study (2)

- 38 yo man with infertility, azoospermia and CML.
- FNA mapping show a single site of sperm.
- Underwent 3 months of recombinant FSH therapy
- Retrieved enough sperm for all eggs at IVF-ICSI by testis microdissection.
- Couple now has healthy twins.
Injectable FSH for NonObstructive Azoospermia

**Concept:** Give rFSH to infertile men with testis failure who demonstrate pockets of sperm on FNA mapping.

**Objective:** To improve sperm yield from testicular dissection procedures or to eliminate need for such procedures (by finding sperm in ejaculate) with rFSH therapy.
## Injectable FSH for NonObstructive Azoospermia

<table>
<thead>
<tr>
<th>Treatment</th>
<th>#Pts</th>
<th>Mean Age</th>
<th>% Sperm Success</th>
<th># Ejaculated Sperm</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH -</td>
<td>30</td>
<td>36</td>
<td>77%</td>
<td>0</td>
</tr>
<tr>
<td>FSH +</td>
<td>17</td>
<td>37</td>
<td>85%</td>
<td>3 (18%)</td>
</tr>
</tbody>
</table>
The Medical Management of Male Infertility: Does it Work?

For **specific** treatment of medical conditions with known correctable pathology (Kallmann, hyperprolactinemia, acquired HH): *Absolutely!*

For **empirical** treatment of medical conditions with unclear pathology: *Well, maybe!*
Woody Allen on sex:

That was most fun I’ve ever had without laughing...

Annie Hall (1977)
Men’s Reproductive Health: Erectile Dysfunction

1. Erectile dysfunction and heart disease involve endothelial cell dysfunction.
2. PDE5 inhibitors were developed to treat angina.
3. Erectile dysfunction is a marker of silent cardiac and vascular disease.
4. Erectile dysfunction predicts occurrence of significant cardiovascular events 5-10 years later.
1. Male infertility and subsequent testis cancer
2. Male infertility and subsequent prostate cancer
Male Infertility and Germ Cell Cancer
An Epidemiologic Study:
Do Infertile Men Have Higher Rates of Testis Cancer?

51,318 infertile males
• 15 California centers
• 1965 to 1998

California Cancer Registry (CCR)
• 10 SEER Regions 1973 to 2003
  – Testis cancer
  – Prostate cancer
  – Colon cancer
  – Melanoma

Walsh et al. Arch Int Med, Feb 2009
Age-Aggregated Standardized Incidence Ratios (SIR) and 95% Confidence Intervals for Testicular Cancer in Men with and without Male Factor Infertility

<table>
<thead>
<tr>
<th>Fertility Status</th>
<th>No. of Men</th>
<th>SIR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Men</td>
<td>42,283</td>
<td>1.7 (1.2, 2.2)*</td>
</tr>
<tr>
<td>Male Factor Infertility</td>
<td>7,494</td>
<td>3.7 (2.1, 6.1)*</td>
</tr>
<tr>
<td>No Male Factor Infertility</td>
<td>25,159</td>
<td>1.4 (0.9, 2.1)</td>
</tr>
</tbody>
</table>

*P<0.05

Walsh et al. Arch Int Med, Feb 2009

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. Men</th>
<th>Observed cases</th>
<th>Expected cases</th>
<th>SIR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colorectal Cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire cohort</td>
<td>42,283</td>
<td>97</td>
<td>120</td>
<td><strong>0.81 (0.65, 0.98)</strong>*</td>
</tr>
<tr>
<td>With male factor</td>
<td>7,494</td>
<td>25</td>
<td>21</td>
<td>1.21 (0.78, 1.79)</td>
</tr>
<tr>
<td>Without male factor</td>
<td>25,159</td>
<td>41</td>
<td>58</td>
<td><strong>0.71 (0.51, 0.96)</strong>*</td>
</tr>
<tr>
<td><strong>Melanoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire cohort</td>
<td>42,283</td>
<td>161</td>
<td>113</td>
<td><strong>1.42 (1.21, 1.66)</strong>*</td>
</tr>
<tr>
<td>With male factor</td>
<td>7,494</td>
<td>32</td>
<td>18</td>
<td><strong>1.74 (1.19, 2.46)</strong>*</td>
</tr>
<tr>
<td>Without male factor</td>
<td>25,159</td>
<td>80</td>
<td>58</td>
<td><strong>1.38 (1.10, 1.72)</strong>*</td>
</tr>
<tr>
<td><strong>Prostate Cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire cohort</td>
<td>42,283</td>
<td>286</td>
<td>146</td>
<td><strong>1.96 (1.74, 2.20)</strong>*</td>
</tr>
<tr>
<td>With male factor</td>
<td>7,494</td>
<td>77</td>
<td>27</td>
<td><strong>2.90 (2.29, 3.63)</strong>*</td>
</tr>
<tr>
<td>Without male factor</td>
<td>25,159</td>
<td>108</td>
<td>63</td>
<td><strong>1.72 (1.41, 2.08)</strong>*</td>
</tr>
</tbody>
</table>

*P<0.05

Walsh et al. Submitted
Infertile Men have Evidence of Faulty DNA Repair and Abnormal Recombination


Centromeres-blue; SCP-red; MLH1-yellow

The Ultimate Treatment for Male Infertility? Embryonic Stem cells from Adult Testis?

Blastocyst → Primordial germ cell (PGC) → Neonatal testis → Gonocytes → Adult testis

inner cell mass → Embryonic stem cell

Multipotent germline stem cell → Differentiation

Spermatogonial stem cell → Spermatogenesis

Chimeras

Ectoderm Mesoderm Endoderm Teratoma
Can We Create Male *Embryonic* Stem Cells?

Embryonic stem cells
Primordial germ cells
Spermatogonia
CIS

Developing a Human Spermatogonial Stem Cell Line

Mechanical disintegration, collagenase digestion

Wash, plate and incubate @ 34°C
Generation of Human Spermatogonial Cell Lines

Adult Testis Cells

Colonies @ 2 weeks

Transfer to hES media

Creating:

- Patient specific
- Genetically unmodified
- Non-embryo derived

stem cells for men.

Kossack et al.  
Stem Cells 27: 138, 2009
Summary

• A paradigm shift is occurring regarding the causes of male infertility and its disease associations.

• Novel treatments are being pursued and incredibly novel (stem cell) treatments loom in the future.