Assisted Reproductive Technologies: Present and Future

Paul Kaplan, M.D.
The Assisted Reproductive Technologies (ART)

- In Vitro Fertilization (IVF)
- Intracytoplasmic Sperm Injection (IVF/ICSI)
- Donor Oocyte IVF
- Frozen Embryo Thaw and Transfer
- Cryopreservation/In Vitro Maturation of Oocytes
In Vitro Fertilization (IVF)

- Daily S/C or IM FSH/hMG injection
- Follicular monitoring with serum estradiol and transvaginal ultrasound
- HCG given to trigger ovulation (LH surge)
- Transvaginal oocyte retrieval and insemination
- Embryo culture and transcervical embryo transfer
- Embryo cryopreservation for future F.E.T.
- Pregnancy rate of 40-50 % per cycle
In IVF, eggs are harvested from the woman’s ovary and fertilized in the laboratory with sperm. The embryos are then transferred into the uterus.
Intracytoplasmic Sperm Injection (ICSI)

- Standard IVF Stimulation and oocyte retrieval
- Injection of a single sperm into each oocyte
- Embryo culture and transcervical embryo transfer
- Currently used in almost 50% of IVF cycles for treatment of male factor and unexplained causes
- Pregnancy rate of 40-50% per cycle
Intracytoplasmic Sperm Injection (ICSI)
Future Directions in ART

- The "-omics" revolution in non-invasive screening
- Preimplantation genetic diagnosis (PGD)
  - with gene therapy?
  - Nuclear and/or cytoplasmic oocyte transfer
  - Embryonic Stem Cell Line Development
  - Gamete Stem Cell Development
Future Directions in ART (Con’t)

- Embryo Cloning - Reproductive/Therapeutic
- Adult Cell Gamete Cloning - sperm/oocyte
- Adult Somatic Cell Cloning
The “-omics” Revolution in Infertility

- **Genomics**: The branch of molecular biology concerned with the structure, function, evolution, and mapping of genomes.

- **Proteomics**: The set of proteins expressed by the genetic material of an organism under a given set of environmental conditions.
The “-omics” Revolution in Infertility

- **Metabolomics**: The systematic study of the unique chemical fingerprints that specific cellular processes leave behind.

- **Embryomics**: The identification, characterization and study of the diverse cell types which arise during embryogenesis.
Preimplantation Genetic Diagnosis (PGD)

- **Goal:** Identify Genetically Abnormal Embryos
- **IVF/ICSI + Embryo Culture**
- **Trophectoderm Biopsy of Blastocyst (~day 5)**
- **CCS (comprehensive chromosomal screening)**
- **Transfer of Normal Blastocysts/Frozen Embryos**
- **Next Step ??**
How Will CRISPR Technology change PGD??

- *Clustered Regularly Interspaced Short Palindromic Repeats*
- Rapidly remove/add genes using Cas nuclease and synthetic guide RNA
- Used to modify mosquitoes so that they cannot transmit malaria
- Major potential for embryo gene defect correction
- Ongoing human research in China, UK, and US on nonviable embryos
- Huge ethical issues ??
## CRISPR Technology

### Editing a Gene Using the CRISPR/Cas9 Technique

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong></td>
<td>Scientists create a genetic sequence, called a &quot;guide RNA,&quot; that matches the piece of DNA they want to modify.</td>
</tr>
<tr>
<td><strong>2</strong></td>
<td>This sequence is added to a cell along with a protein called Cas9, which acts like a pair of scissors that cut DNA.</td>
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<tr>
<td><strong>3</strong></td>
<td>The guide RNA homes in on the target DNA sequence, and Cas9 cuts it out. Once their job is complete, the guide RNA and Cas9 leave the scene.</td>
</tr>
<tr>
<td><strong>4</strong></td>
<td>Now, another piece of DNA is swapped into the place of the old DNA, and enzymes repair the cuts. Voilà, you've edited the DNA!</td>
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PGD Trophectoderm Embryo Biopsy
Comprehensive Chromosomal Screening (CCS)

- Next Generation Sequencing (NGS)
- Quantitative PCR (qPCR)
- Single Nucleotide Polymorphism (SNP array)
- DNA Fingerprinting
- Comparative Genomic Hybridization (array CGH)
- Whole Genome Sequencing (future)
Oocyte Cryopreservation

- Preservation of Oocytes Prior to Fertilization
- TV Retrieval of Stimulated Oocytes
  - Future: Unstimulated Oocytes with IVM
- Desiccation and Cryopreservation
- Delayed Thaw and IVF/ICSI Embryo Culture
- Transfer of Healthy Embryos
Oocyte Desiccation for Cryopreservation
Fertility Preservation

A. Damage to follicular cells from radiation and chemotherapy
   - Possible reduction of ovarian reserve
   - Premature ovarian failure
   - Natural pregnancy
   - Donor egg or adoption
   - Diagnosis

B. Stimulation of follicle growth with exogenous hormones
   - In vivo
   - Ex vivo
   - Aspiration of oocytes
   - Mature oocyte
   - Embryo
   - Transplantation of ovarian cortical strips in patient
   - Emerging techniques
   - Cryopreservation of ovarian cortical strips
   - In vitro follicle maturation
   - Embryo
   - Live birth
### Fertility Preservation

<table>
<thead>
<tr>
<th>Group</th>
<th>Method</th>
<th>Cryopreservation</th>
<th>Treatment</th>
<th>Recipient</th>
<th>Concerns</th>
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<tbody>
<tr>
<td>Women</td>
<td>Hormone stimulation</td>
<td>Zygote or embryo</td>
<td>Embryo transfer</td>
<td>Patient or gestational surrogate</td>
<td>Delay in cancer treatment</td>
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<tr>
<td></td>
<td>Hormone cycle 2–3 weeks</td>
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<td>Hormone injections</td>
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<tr>
<td>Postpubertal girls</td>
<td>Hormone stimulation</td>
<td>Mature oocyte</td>
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<td></td>
<td>Availability of appropriate sperm donor</td>
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<tr>
<td></td>
<td>Hormone cycle 2–3 weeks</td>
<td>Cumulus–oocyte complexes</td>
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<tr>
<td>Women</td>
<td>Laparoscopic oophorectomy</td>
<td>Ovarian cortical strips</td>
<td>Ovarian transplantation</td>
<td>Patient</td>
<td>Potential reintroduction of cancer cells</td>
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<td>Postpubertal girls</td>
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<td>In vitro follicle maturation and</td>
<td>Patient or gestational surrogate</td>
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<td></td>
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<td>in vitro fertilization or ICSI with embryo transfer</td>
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<tr>
<td>Prepubertal girls</td>
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<tr>
<td>Men</td>
<td>Ejaculation</td>
<td>Sperm</td>
<td>ICSI with embryo transfer</td>
<td>Partner</td>
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<td>Postpubertal boys</td>
<td>Mature sperm extraction</td>
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<td>Testis biopsy</td>
<td>Testis</td>
<td>Stem-cell repopulation</td>
<td>Patient</td>
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<td>Experimental</td>
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IVF Treatment of Genetic Mitochondrial Disease

- “Mitochondrial DNA Replacement Therapy”
- Performed by Donor Egg Nuclear Transfer
- 1:200 incidence of pathogenic mtDNA mutation
- Recently approved by British Parliament
- Currently in active IRB-approved study at OHSU
Stem Cell Gamete Production

A. Goal is cost-effective, ethically-acceptable source of sperm and oocytes
   - Reduction of risks with donor gametes
   - Alternative to somatic cell cloning

B. Potential treatment for cancer patients, age-related infertility, and severe male factor

C. Reduction of multiple gestation by SET
Mouse Embryonic Stem Cell Spermatid
Mouse Blastocysts Fertilized by Embryonic Stem Cell Spermatids
Milestones of infertility medicine

1967
Clomid comes on the market

1969
Pergonal and human chorionic gonadotropin marketed

1978
Louise Brown, first “test-tube baby,” born

1981
First IVF baby in America

1984
GIFT technique developed by Ricardo Asch of San Antonio
First “donor baby” (eggs and sperm) born to surrogate mother in Australia

1985
Maryland passes legislation requiring insurance coverage for IVF
First ultrasound-guided, nonsurgical IVF

1986
Richard Marrs delivers first U.S. baby developed from a frozen embryo

1987
ZIFT technique introduced
Lupron comes on the market

1990
Mark Sauer reports pregnancies in postmenopausal women

1991
First preimplantation genetic screening (for cystic fibrosis)

1992
Fertility Clinic Success Rate and Certification Act calls for uniform definition of success; to take effect October 1994

1993
Supreme Court decides frozen embryos cannot be implanted against the father’s will
In Vitro Fertilization (IVF) - 2017

- SART Data: 68,782 IVF babies born in 2014 in U.S.
- IVF babies now constitute almost 2% of U.S. births
- Estimated 500,000 IVF babies born in 2015 in world
- IVF births now almost 4% of births in Europe
- Estimated >7,000,000 IVF births by Jan. 2015

- Who Knew ?????
The world's first IVF baby Louise Brown (2nd right) posing with her son Cameron, her mother Lesley Brown and IVF pioneer Professor Robert Edwards in 2008
Polycystic Ovary Disease: A Common Endocrine Disorder in Women

Paul Kaplan, M.D.
Clinical Professor of Reproductive Endocrinology - OHSU
Courtesy Senior Research Associate, Human Physiology
University of Oregon
Case Presentation – Jenn A.

- 23 Y. O. G0 P0 menarche age 13
- BMI 29. Hx of “weight problems”.
- Menses Q 60 -180 days. Start BCPS age 15.
- Moderate acne & hirsutism age 14-15
- Family Hx T2 diabetes and infertility
Polycystic Ovary Syndrome

- A Common Female Endocrine Dysfunction
  - Affects 1 of every ~12 women in U.S.
- Key Features:
  - Oligo/Amenorrhea
  - Abnormal Androgen Production & Metabolism
- Probable Genetic Etiology
  - Conveys evolutionary “metabolic efficiency”
  - Autosomal dominant/variable penetration
PCOS - History

- 1935 “Stein-Leventhal Syndrome”
- Observed association of amenorrhea and polycystic ovaries (at surgery)
- Currently 30,000 published articles on PCOS
- Now recognized as the leading cause of infertility
Irving Stein, M.D.
PCOS: A NEW PARADIGM

“PCOS is a metabolic disorder affecting multiple body systems that requires comprehensive and long-term evaluation and management.”

John Nestler, M.D.  Fertility & Sterility  November, 1998
PCOS: Evolutionary Benefits

- Metabolic "Thriftiness"
  - Maximal caloric conservation
  - Longevity in animal studies
  - Stress-induced ovulation (↓ LH  P/F )
  - Rate of oocyte atresia (↑ Insulin levels)
How Do Women with PCOS Present?

- Irregular Menstrual Periods
- Hirsutism
- Facial Acne
- Overweight
- Infertility
- Acanthosis Nigricans (café au lait spots)
Acanthosis Nigricans
PCOS: Diagnosis

N.I.H. Definition (2 of 2)
- Oligo/Anovulation
  - Cycles > 35 days apart or < 7 per year
- Abnormal Androgen Production & Metabolism
  - Clinical (Hirsutism/Acne) or Lab (T, A, DHEA-S)

ESHRE/ASRM Rotterdam 2003 (2 of 3)
- Oligo/Anovulation
- Androgen Excess
- Polycystic Ovaries (12 or > follicles/ovary on U/S)

PCOS

YOUNG

REPRODUCTIVE AND HYPERANDROGENIC DYSFUNCTION

LATER YEARS

CARDIOMETABOLIC DISORDERS
PCOS: CVD Classification

- **Classic (75%)**
  - RD/NIH Criteria + Overweight (BMI > 25)
  - 40% Risk of IGT or T2DM by age 40 (5X controls)
  - Dyslipidemia in 70% of Classic PCOS (IR effect)

- **Ovulatory (Lean) (12.5%)**
  - Medium risk profile

- **Nonhyperandrogenic (12.5%)**
  - Lowest Risk

*Assessment of Cardiovascular Risk in PCOS. JSEM May 2010;95:5.*
PCOS: Clinical Consequences

- Endometrial Cancer (3x risk, up to 1/5)
- Spontaneous Abortion (? ↑ LH Effect)
- Gestational and Type 2 Diabetes (5-7x)
- Cardiovascular Disease (↑ LDL ↓ HDL)
- Hypertension
- Breast Cancer (3-4x risk in limited data)
- Ovarian Cancer
Evaluation of PCOS

- BMI, Waist Circumference, BP
- Baseline FSH, LH, TSH, Prolactin
- Testosterone, DHEA-S
- 17-OH Progesterone (Follicular a.m.)
- Fasting Glucose + Insulin/GTT
- Fasting Lipids & Chemistry Panel
- Transvaginal Ultrasound of Ovaries
Transvaginal Ultrasound of the Ovaries

“String of Pearls” in PCOS
PCOS: Insulin Resistance

- Demonstrated in 60 - 80% of PCOS
  - 95% in Obese PCOS (BMI > 30)

Metabolic Effects:
- Decreased Hepatic SHBG Production (↑ Free T)
- Increased Ovarian Thecal Androgen Production
- Increased Triglycerides and Adverse Lipid Profile
- Obesity/Metabolic Syndrome
- Hypertension
- High Risk of T2 DM (25%)
Metabolic Syndrome: Diagnosis

- Three or more of the following:
  - Hypertension (130/85 or higher or on meds)
  - Elevated Triglycerides (>150 mg/dL or on meds)
  - Reduced HDL (Less than 50 mg/dL for women)
  - Waist circumference > 35 inches for women
  - Fasting Glucose >100 mg/dL or on meds
Metabolic Syndrome: What We Know

- Occurs in 1/6 (16%) of the general population and 60% of obese men and women.
- 10% of people with NGT, 40% with IGT, 85% with T2 DM.
- Prevalence 24% higher in women (40% by age 60) and increases with age.
- Conveys a high risk of T2 DM and cardiovascular disease.
- Significant increased risk with PCOS
Metabolic Syndrome: Treatment

- Lifestyle: Diet, Exercise, Weight Loss
- Correction of Problems: HTN, DM, Lipids
- Regular monitoring/follow-up
- Low-dose aspirin
PCOS: Cardiovascular Disease

- Dyslipidemia
- Hypertension
- Impaired Glucose Tolerance/Type2DM
- Metabolic Syndrome
- Frequent Positive FH CVD before age 55
- Carotid-IMT (10-15% over controls)
- Carotid Artery Calcification
- Multivessel CVD (32% vs. 25%)

Assessment of Cardiovascular Risk in PCOS. JSEM May 2010;95:5.
PCOS: Treatment Options

- Anovulation: Cyclic Progestins, BCPs
  - Prevent D.U.B., Endometrial CA
- Acne/Hirsutism: BCPs, Spironolactone
- Contraception: Low-Androgenic BCPS
- Fertility: Clomiphene, Aromatase Inhibitors, FSH/hMG
- Weight Loss: Low Calorie ADA Diet
- Role of Insulin-Sensitizing Medications
PCOS: Positive Effects of Insulin Sensitizing Agents

- ↑ SHBG (↑ Androgen Binding)
- ↓ Testosterone and Androstenedione
- ↓ Triglycerides and LDL
- Regulation of Menstrual Cycles (30%)
- Weight Loss (Slow)
- Increased Sensitivity to Ovulation Meds
- ? Decreased Risk of Miscarriage
PCOS: Who to Treat with Metformin in 2015

- Obese (BMI>30) PCOS patients
  - Adults
  - Adolescents (who can be compliant)
- Insulin resistant patients
  - Fasting Insulin > 12-20 or G/I ratio < 4.5
- Young patients (age <30) desiring fertility
- Patients with impaired glucose tolerance (IGT) or Type 2 D.M.
Tailor treatment to life stage
Induce regular menses
Identify & treat endocrinopathies
Identify & treat insulin resistance
Ongoing regular medical follow-up
With prescription drug prices rising exponentially, many drugstores now provide armed escorts to assure that customers reach their cars safely.