

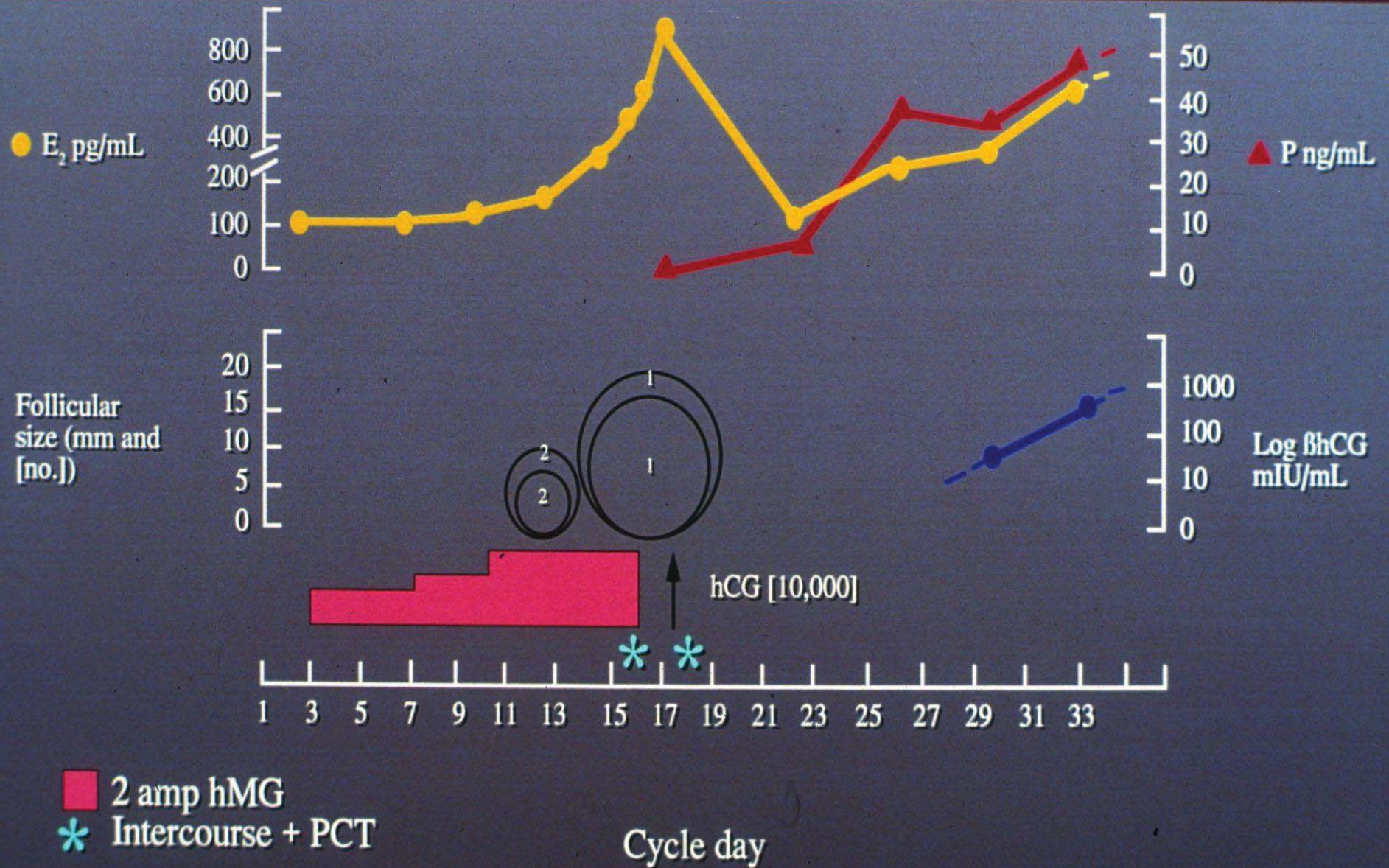
*Assisted Reproductive*  
*Technologies: Present and*  
*Future*

**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**

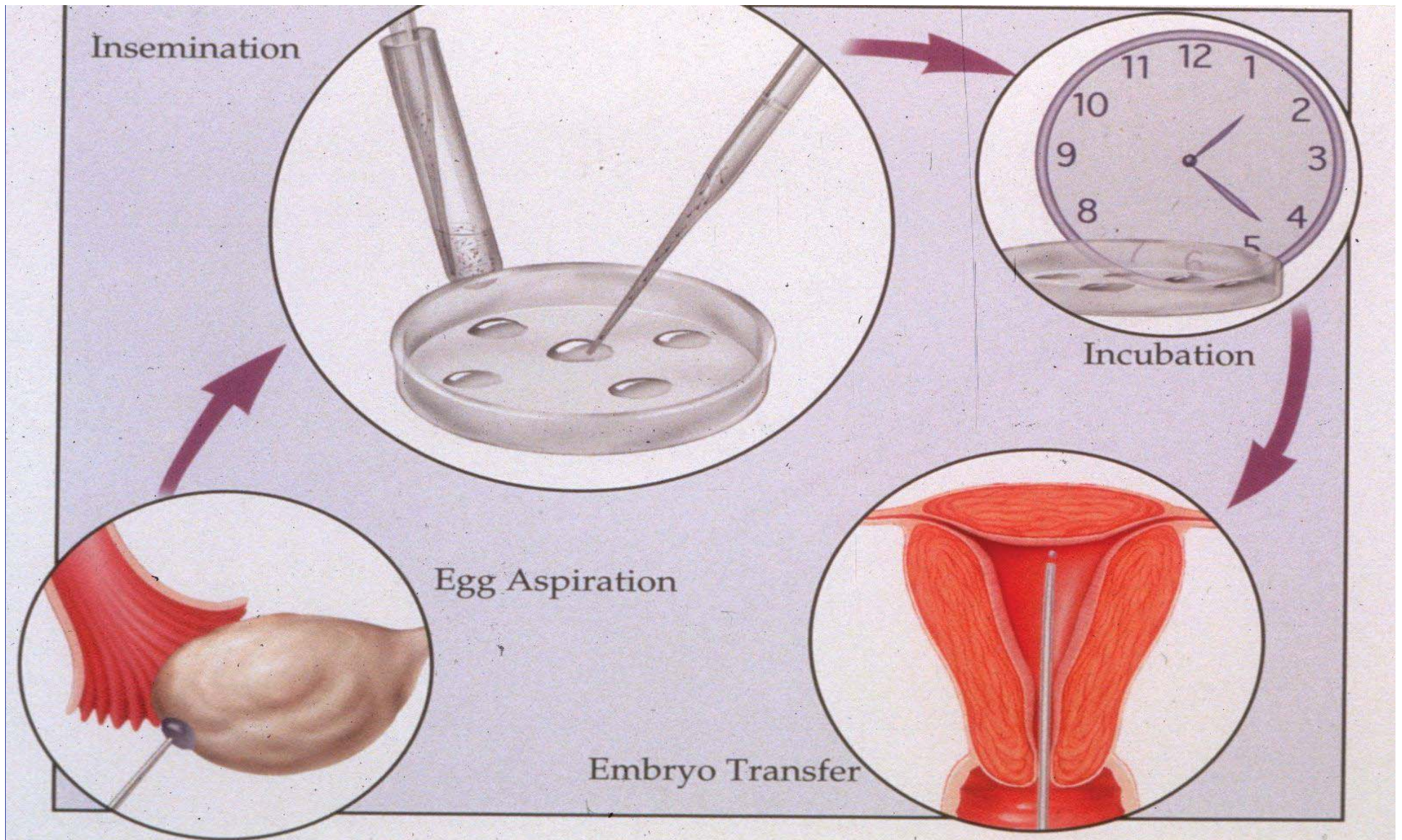
# An hMG-hCG Cycle



Adapted from Navot and Rosenwaks, 1987.

# 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



©TTC

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.

## IVF Embryo Culture and Transfer

# 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 ??

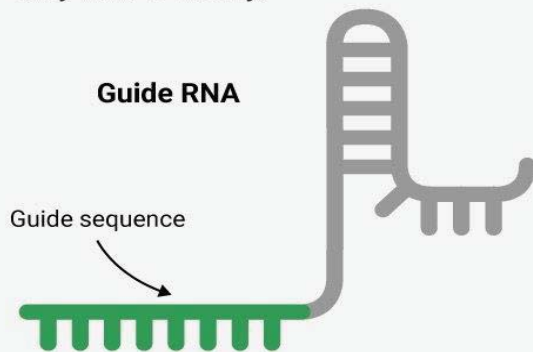
## *How Will CRISPR Technology change PGD ??*

- *Clustered Regularly Interspaced Short Palindromic Repeats*
- **Rapidly remove/add genes using Cas nuclease and synthetic guide RNA**
- **Used to modify mosquitos 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 ??**

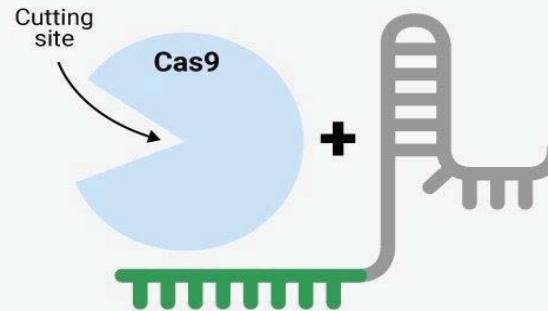
# CRISPR Technology

## EDITING A GENE USING THE CRISPR/CAS9 TECHNIQUE

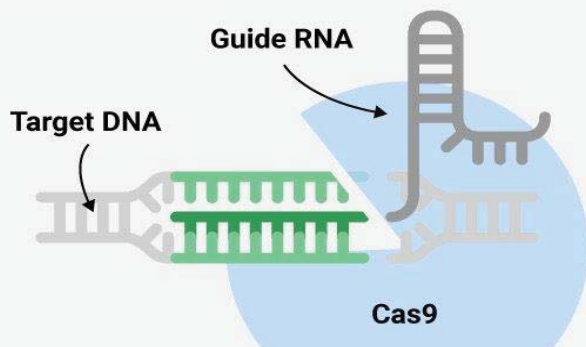
- 1** Scientists create a genetic sequence, called a "guide RNA," that matches the piece of DNA they want to modify.



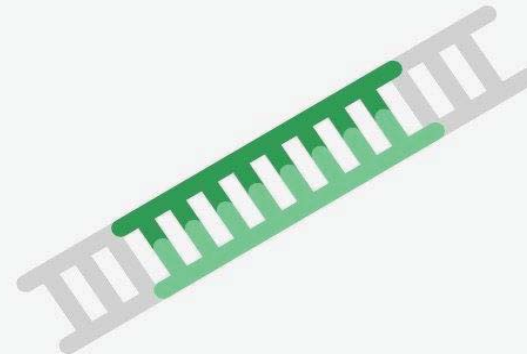
- 2** This sequence is added to a cell along with a protein called Cas9, which **acts like a pair of scissors** that cut DNA.



- 3** 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.



- 4** 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!



# PGD Trophectoderm Embryo Biopsy



## *Comprehensive Chromosomal Screening (CCS)*

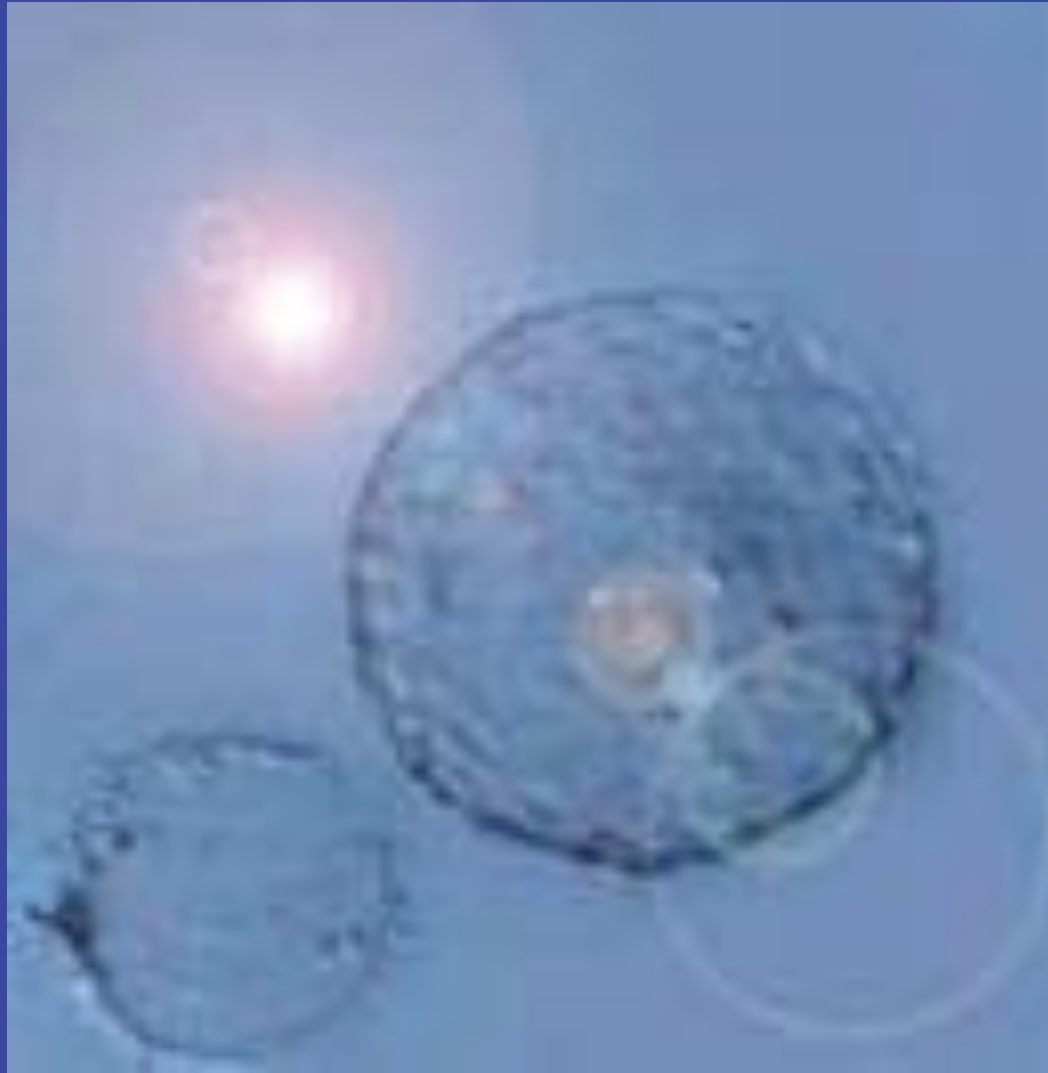
- **Next Generation Sequencing (NGS)**
- **Quantitative PCR (qPCR)**
- **Single Nucleotide Polymorphism (SNP array)**
- **DNA Fingerprinting**
- **Comparative Genomic Hybridization (array CGH)**
- *Whole Genome Sequencing (future)*



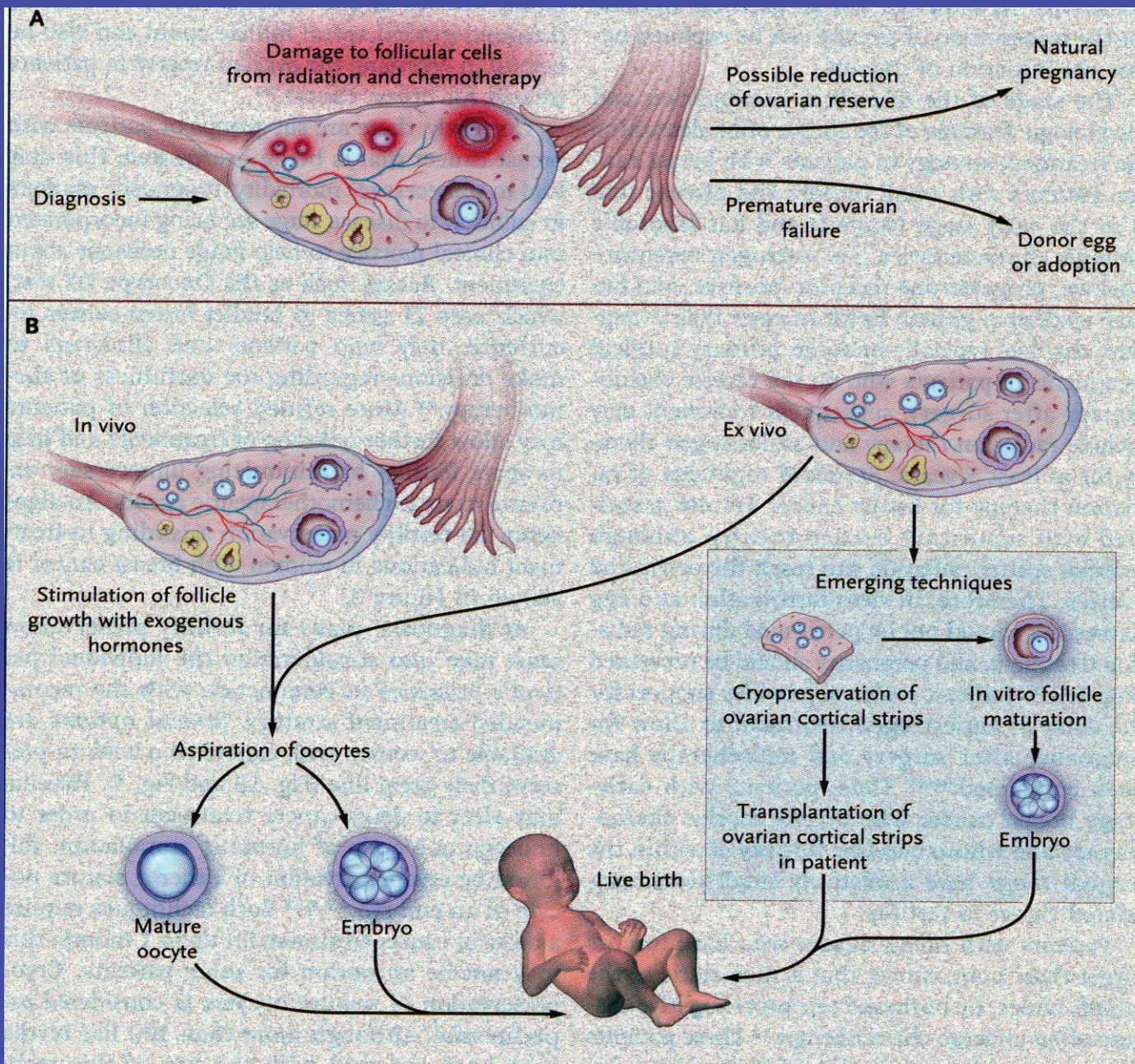
# Oocyte Cryopreservation

- 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

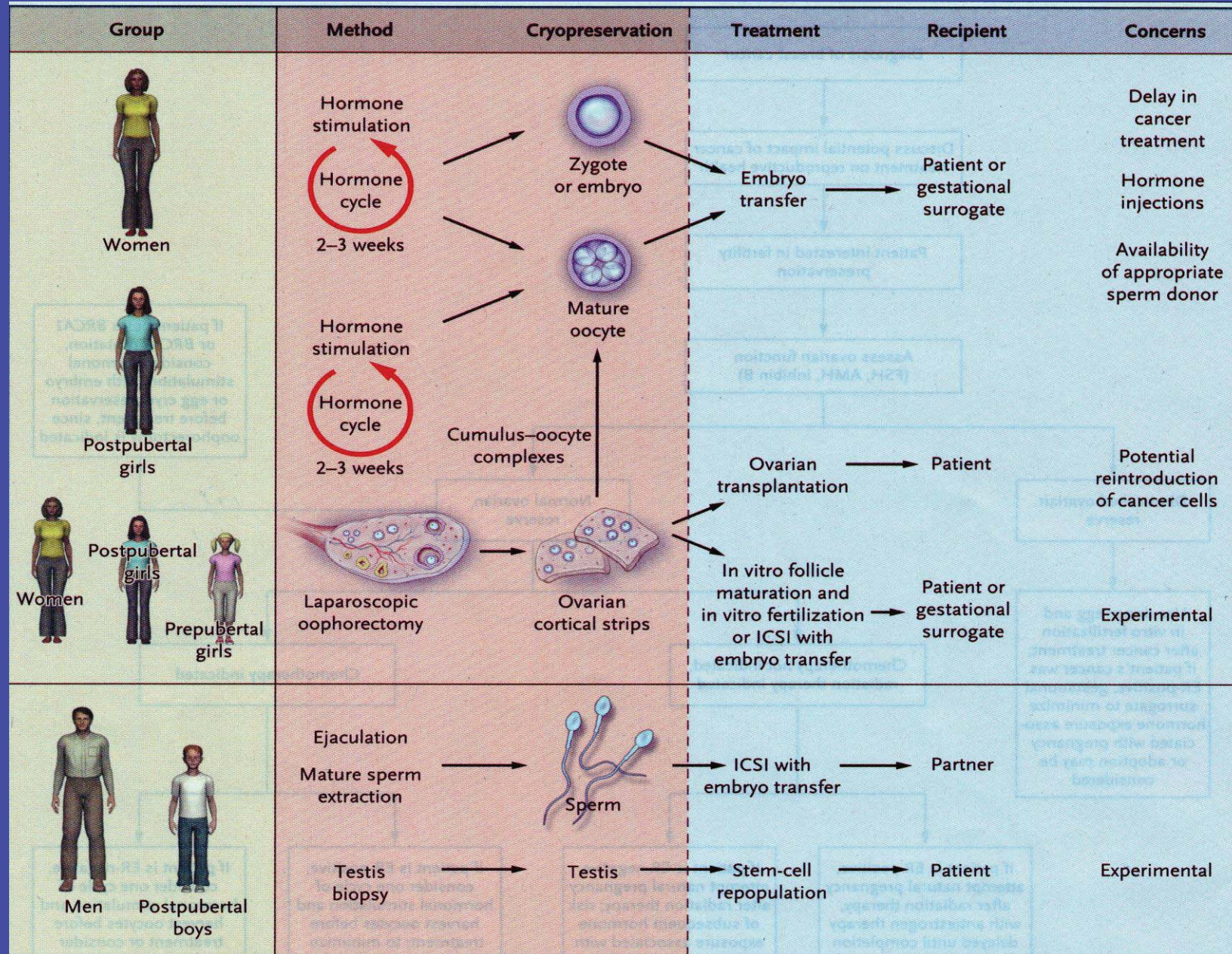
# Oocyte Desiccation for Cryopreservation



# Fertility Preservation



# Fertility Preservation



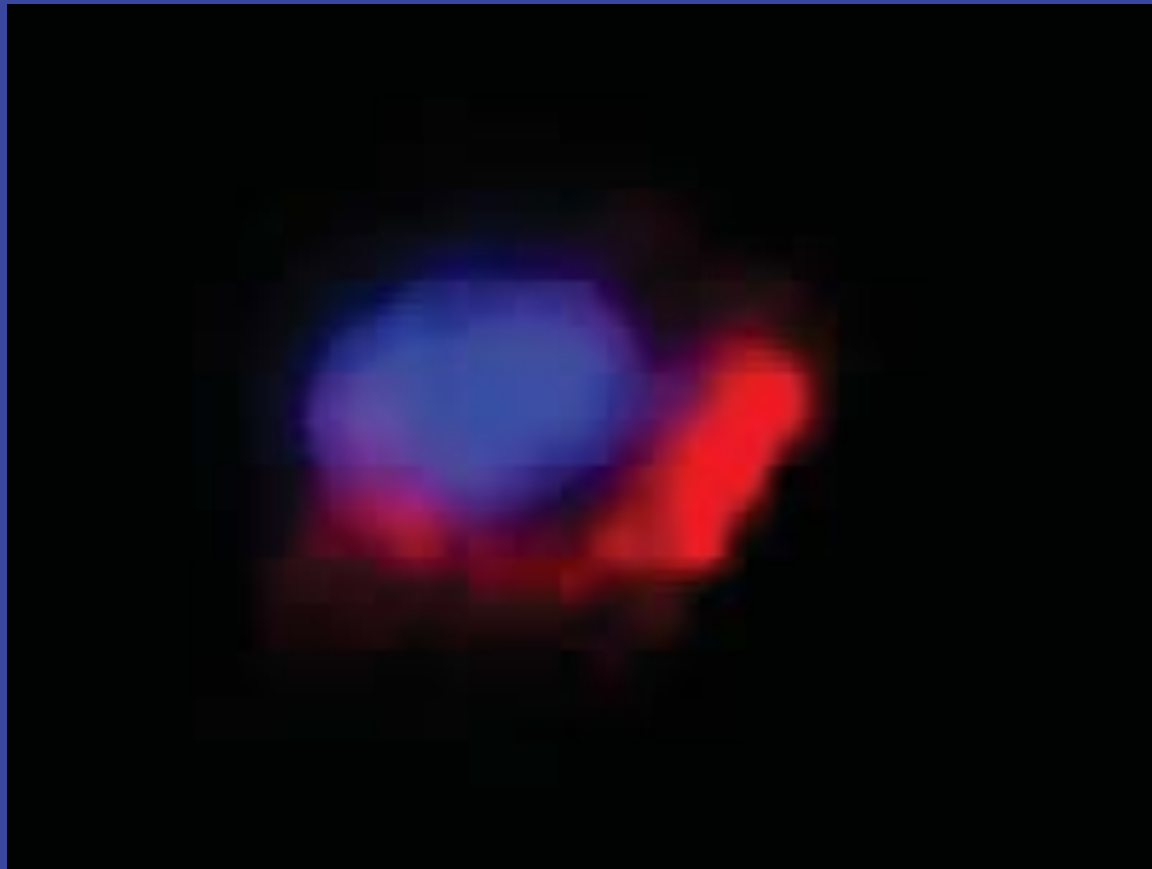
# IVF Treatment of Genetic Mitochondrial Disease

- **“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

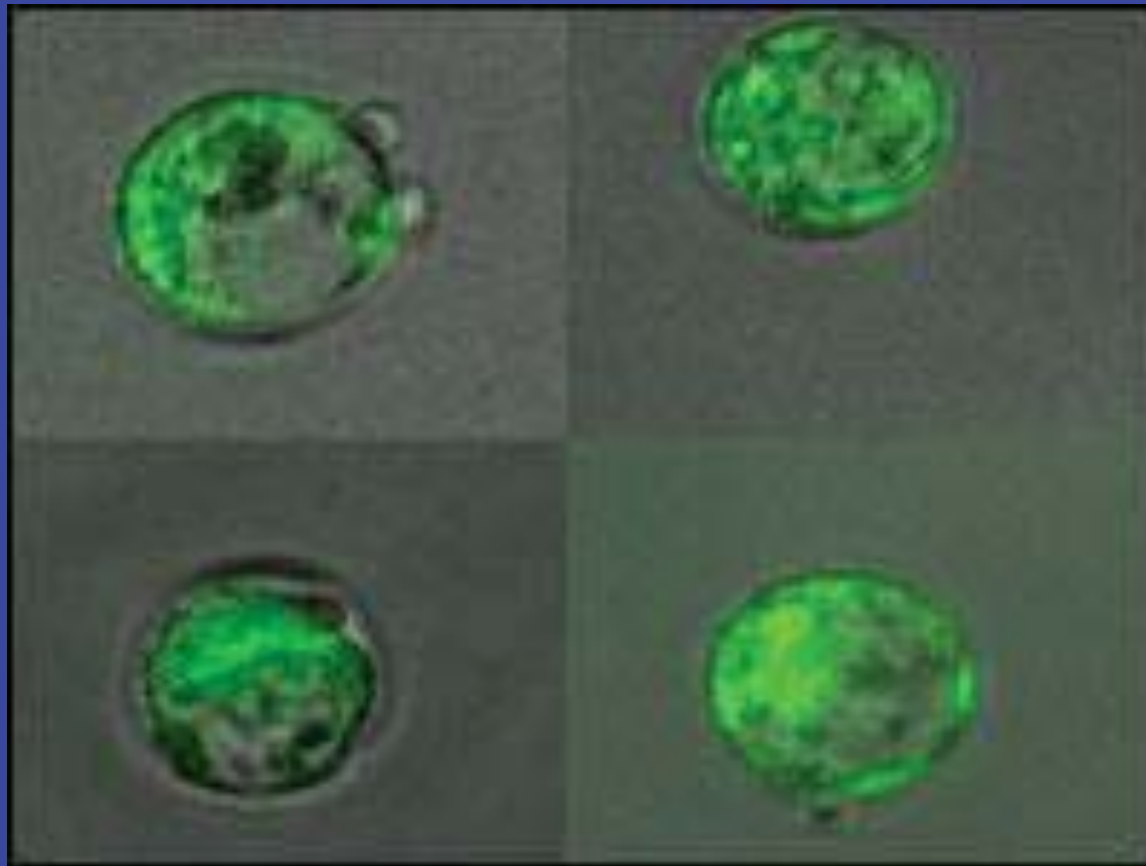
- A. Goal is cost-effective, ethically-acceptable source of sperm and oocytes**
  - Reduction of risks with donor gametes
  - Alternative to somatic cell cloning
  
- B. Potential treatment for cancer patients, age-related infertility, and severe male factor**
  
- C. Reduction of multiple gestation by SET**

# Mouse Embryonic Stem Cell Spermatid



NPR Dec. 2004

# Mouse Blastocysts Fertilized by Embryonic Stem Cell Spermatids



NPR Dec. 2004



# Milestones of infertility medicine



**1969**

Pergonal and human chorionic gonadotropin marketed

**1981**

First IVF baby in America

**1967**

Clomid comes on the market

**1978**

Louise Brown, first "test-tube baby," born

**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

**1990**

Mark Sauer reports pregnancies in postmenopausal women

**1987**

ZIFT technique introduced

Lupron comes on the market

**1986**

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

**1991**

First preimplantation genetic screening (for cystic fibrosis)

**1993**

Supreme Court decides frozen embryos cannot be implanted against the father's will

**1992**

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

# 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 ?????



© AFP/Getty Images

**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**

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# Polycystic Ovary Disease: A Common Endocrine Disorder in Women

Paul Kaplan, M.D.

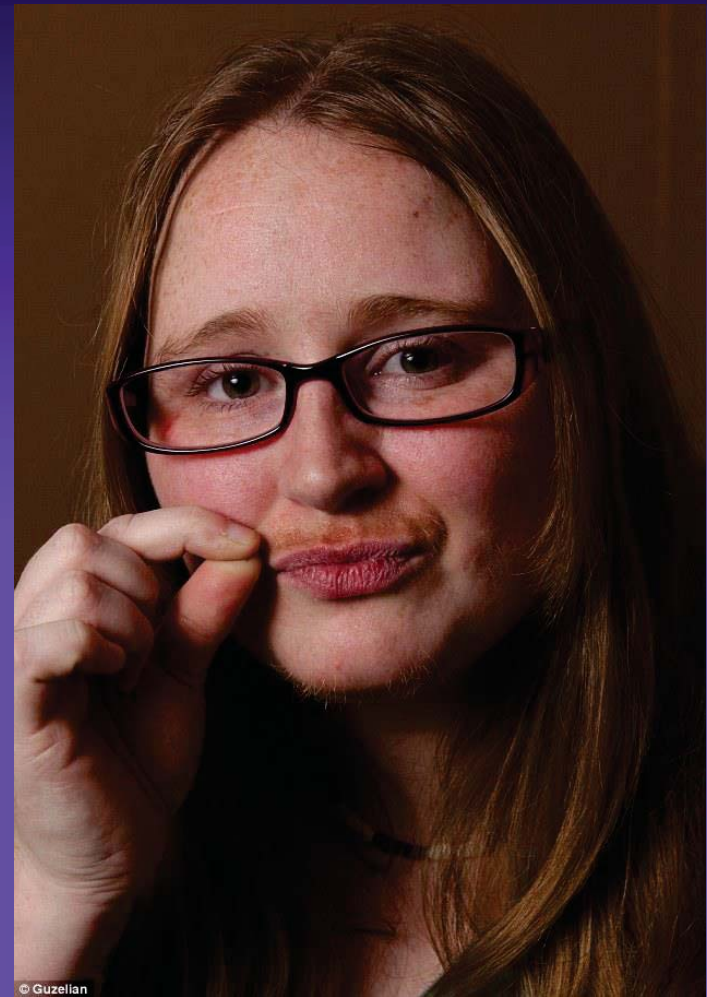
Clinical Professor of Reproductive Endocrinology - OHSU

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

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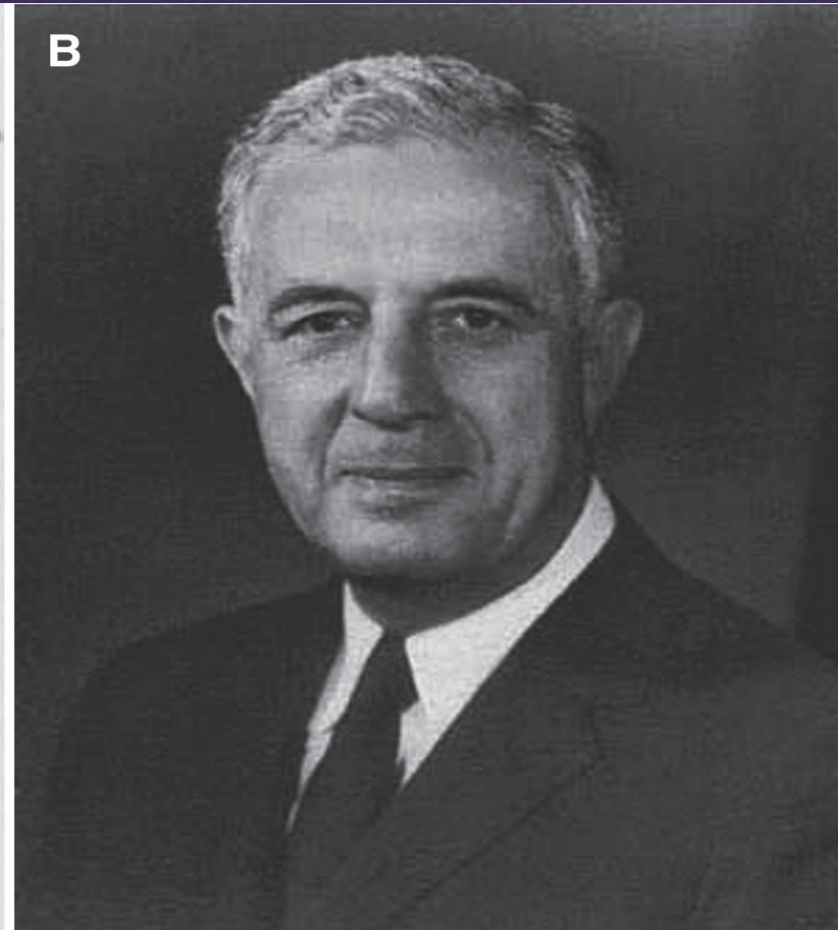
- ❑ 1935 “Stein-Leventhal Syndrome”
- ❑ Observed association of amenorrhea and polycystic ovaries (at surgery)
- ❑ Currently 30,000 published articles on PCOS
- ❑ Now recognized as the leading cause of infertility

# Irving Stein, M.D.





# Michael Leventhal, M.D.



# PCOS: A NEW PARADIGM

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“ 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?

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- ❑ Irregular Menstual 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)

Rotterdam ESHRE/ASRM Group. Hum Reprod 2004;19:41-47.

Chang RJ. Am J Obstet Gynecol 2004;191:713-17.

# PCOS

**YOUNG**

**LATER YEARS**

**REPRODUCTIVE  
AND  
HYPERANDROGENIC  
DYSFUNCTION**

**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



# 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

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- ❑ 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

RIGHT - 21 X 16

LEFT - 12 X 11

07/04/93

09:07:00

FROZEN

FQ 75 75

SC 06 06

FC FM FM

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EE E2 E2

DR 45 45

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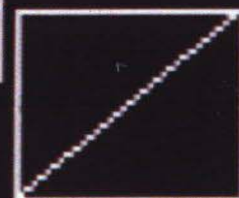
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"String of Pearls" in PCOS



1H



\*WOMEN'S CARE FERTILITY CEN

# Transvaginal Ultrasound of the Ovaries

# 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

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- ☐ 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

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- ❑ 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 May2010;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

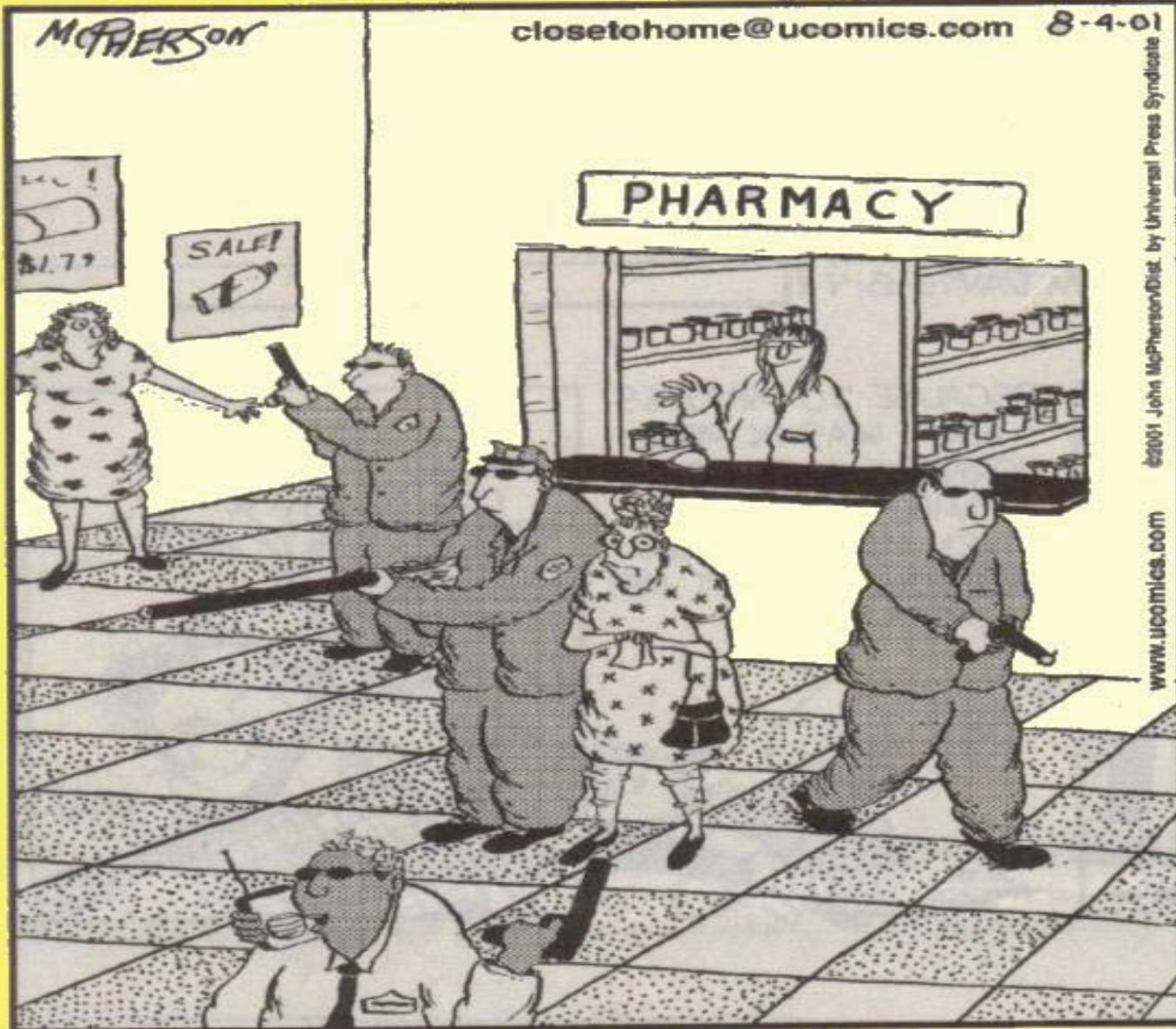
- ❑ Obese (BMI>30) PCOS patients
  - ❑ Adults
  - ❑ Adolescents (who can be compliant)
- ❑ Insulin resistant patients
  - ❑ Fasting Insulin > 12-20 or G/I ratio < 4.5
- ❑ Young patients (age <30) desiring fertility
- ❑ Patients with impaired glucose tolerance (IGT) or Type 2 D.M.

# PCOS : Management Summary

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- ❑ Tailor treatment to life stage
- ❑ Induce regular menses
- ❑ Identify & treat endocrinopathies
- ❑ Identify & treat insulin resistance
- ❑ Ongoing regular medical follow-up

# CLOSE TO HOME



**With prescription drug prices rising exponentially, many drugstores now provide armed escorts to assure that customers reach their cars safely.**