

Infertility, In Vitro Fertilization (IVF) and Genetic Testing

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Outline

- Infertility 101
- Treatment Options
 - ▣ In Vitro Fertilization
 - ▣ Egg Freezing
 - ▣ Egg Donor/Surrogacy
- Genetic Testing
 - ▣ Preconception
 - ▣ Preimplantation
 - ▣ Prenatal
- The Future
 - Three Parent IVF



**Do you have any friends or family members
that have struggled with infertility?**

a. Yes

b. No



Infertility



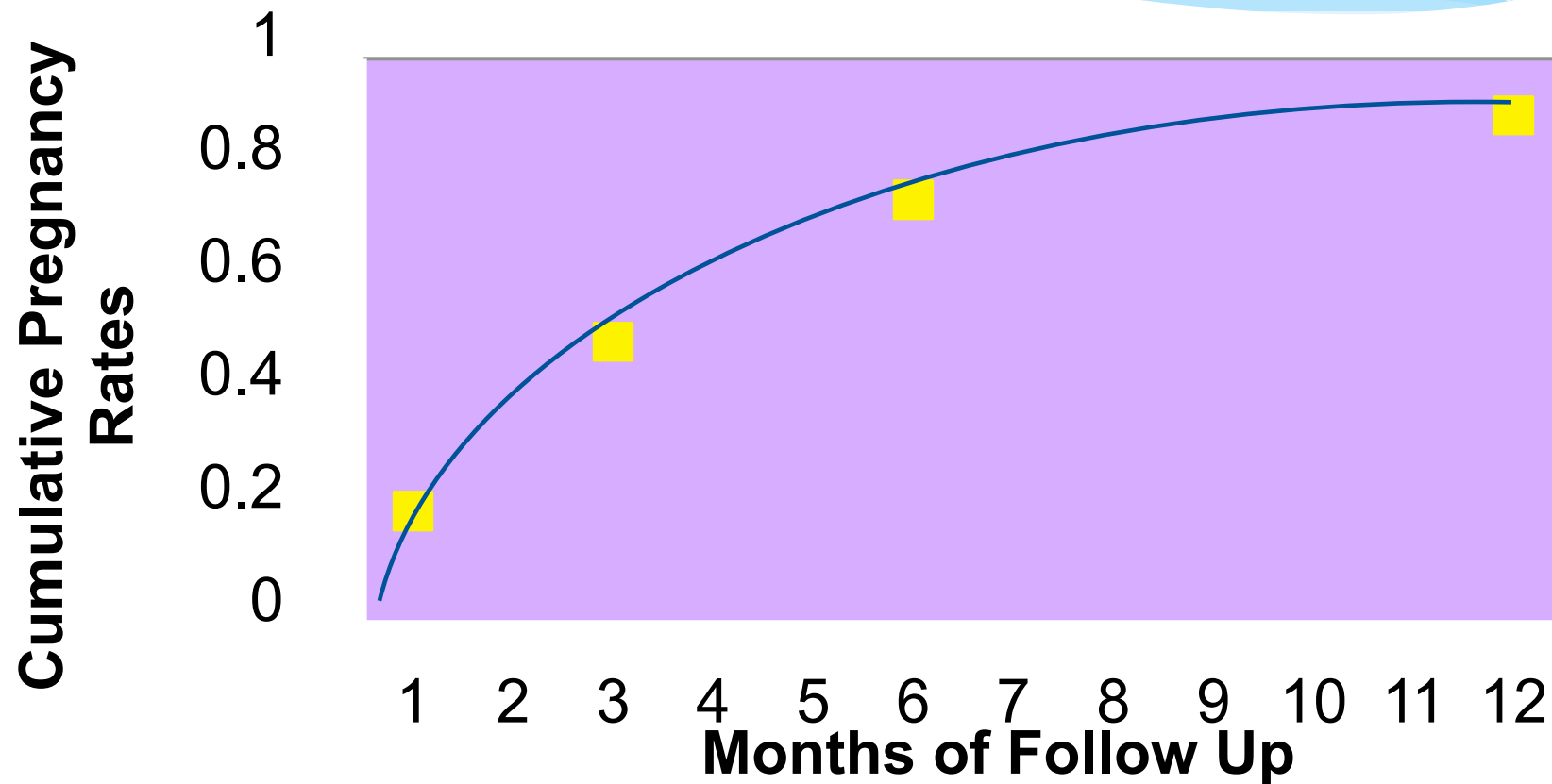
What percentage of the population is subfertile or infertile?

- a. 1-2%**
- b. 10%**
- c. 15%**
- d. 20%**
- e. 25%**

Overview of Infertility

- Definition: 1 year of well-timed, unprotected intercourse without a pregnancy
- 15% of population is infertile (subfertile)

Normal Fertility

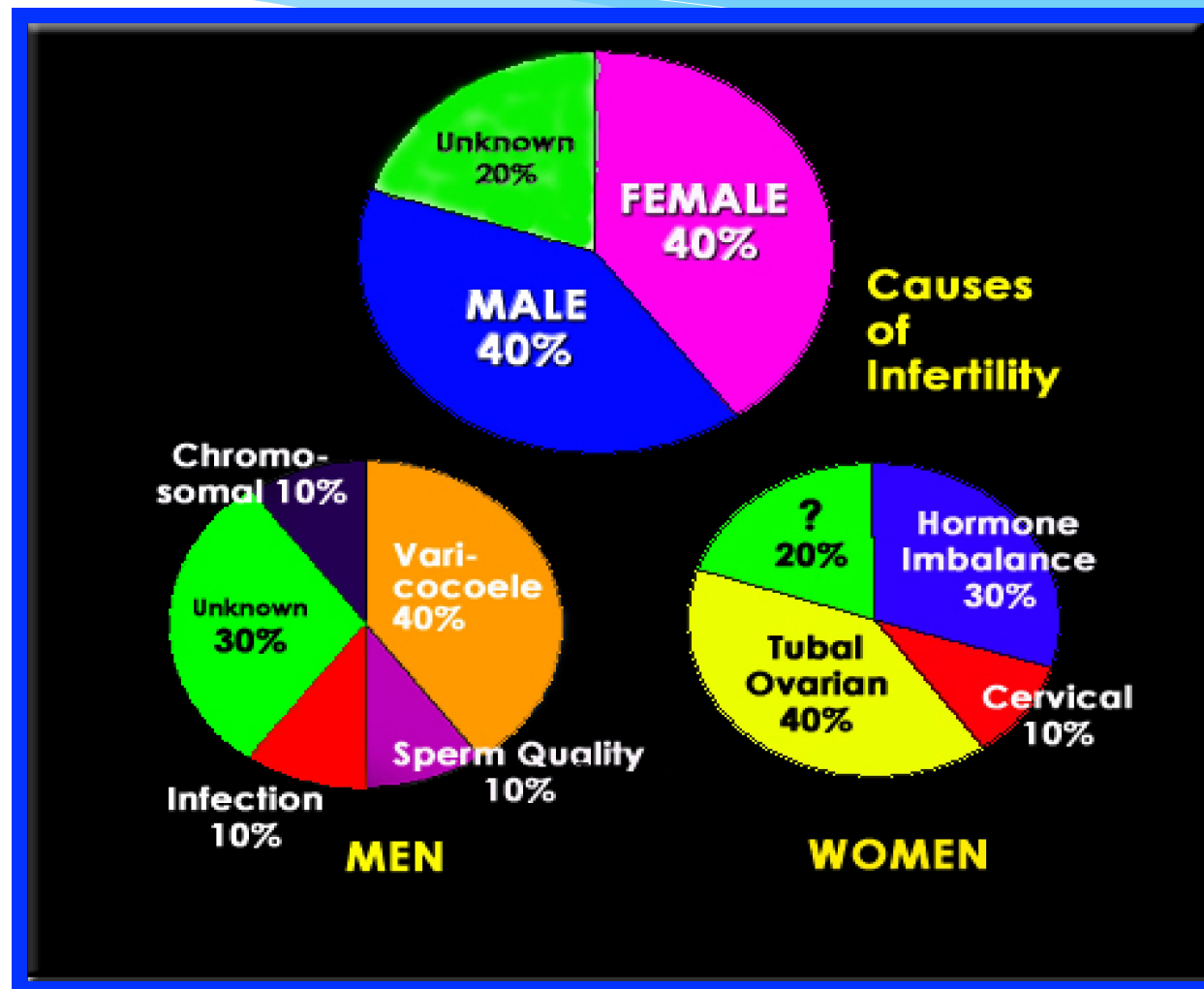




What factor might cause problems with fertility?

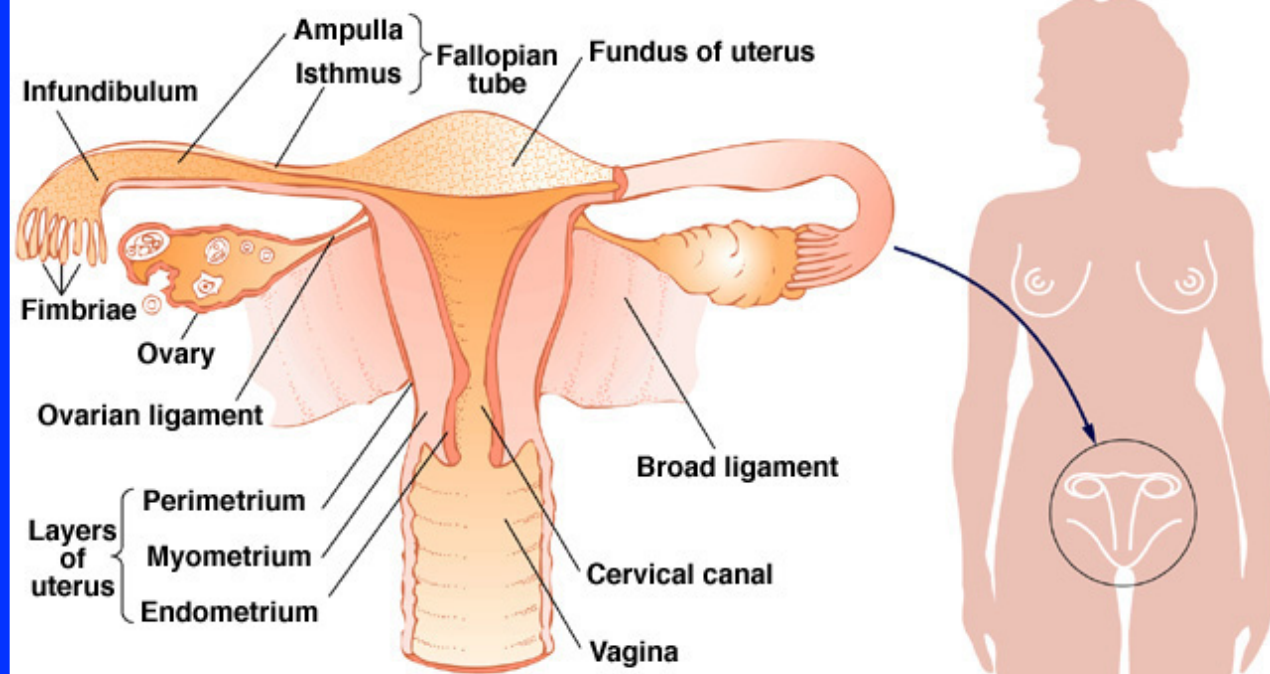
- a. birth control pill use > 10 years**
- b. drinking a glass of wine/beer every day**
- c. eating disorder**
- d. hiking for 45 minutes per day**
- e. history of recurrent yeast infections**

Causes of Infertility



Female Reproductive Organs

Internal Sexual & Reproductive Organs(F)




Causes of Female Infertility

- Ovary
- Tubes
- Uterus
- Hormones
- Chromosomes

Causes of Female Infertility – Ovary

- AGE
- Problems with ovulation
- Premature ovarian failure



**Do women continue to produce eggs
throughout their life (*i.e., from puberty until
death*)?**

a. Yes

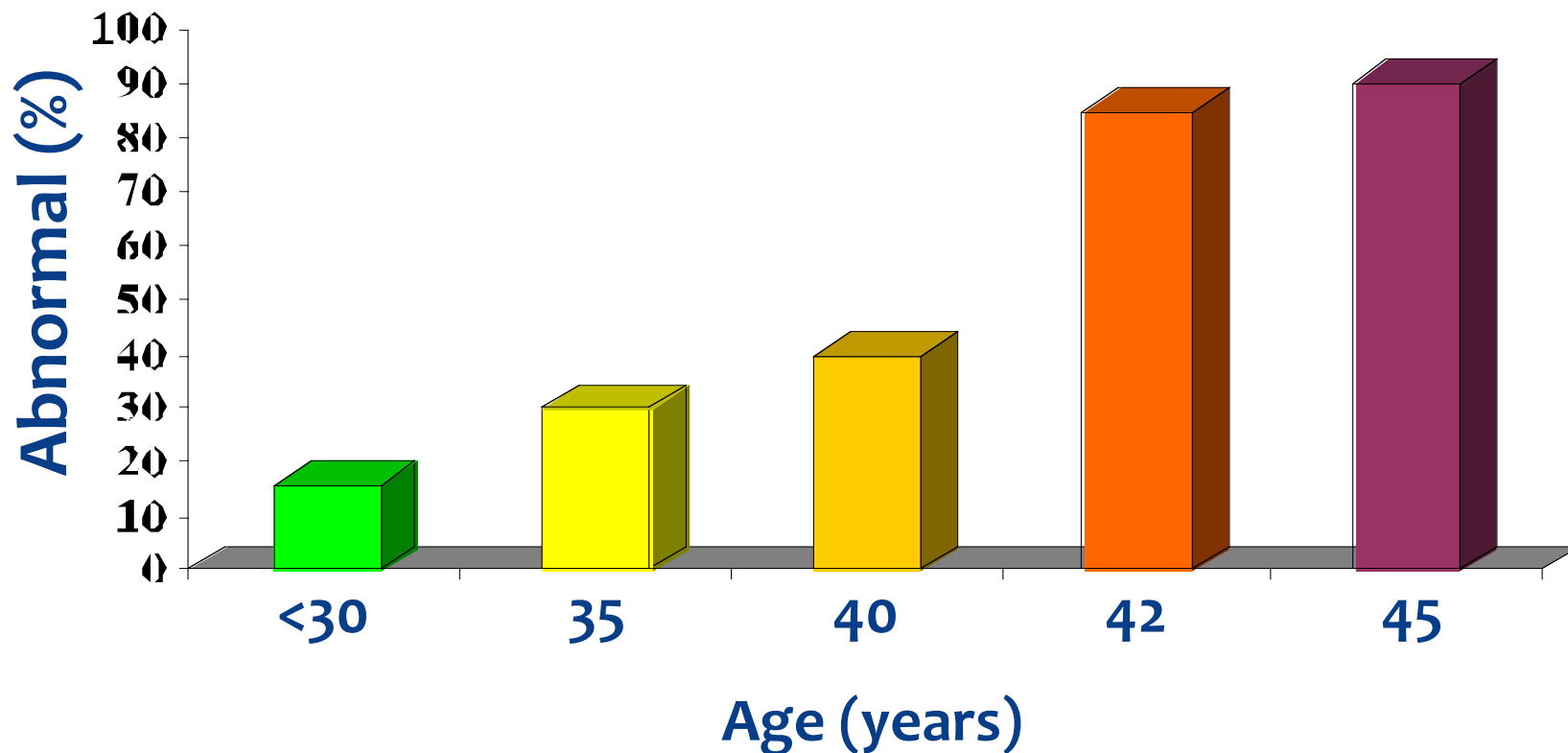
b. No

Ovary Problems - Female Age

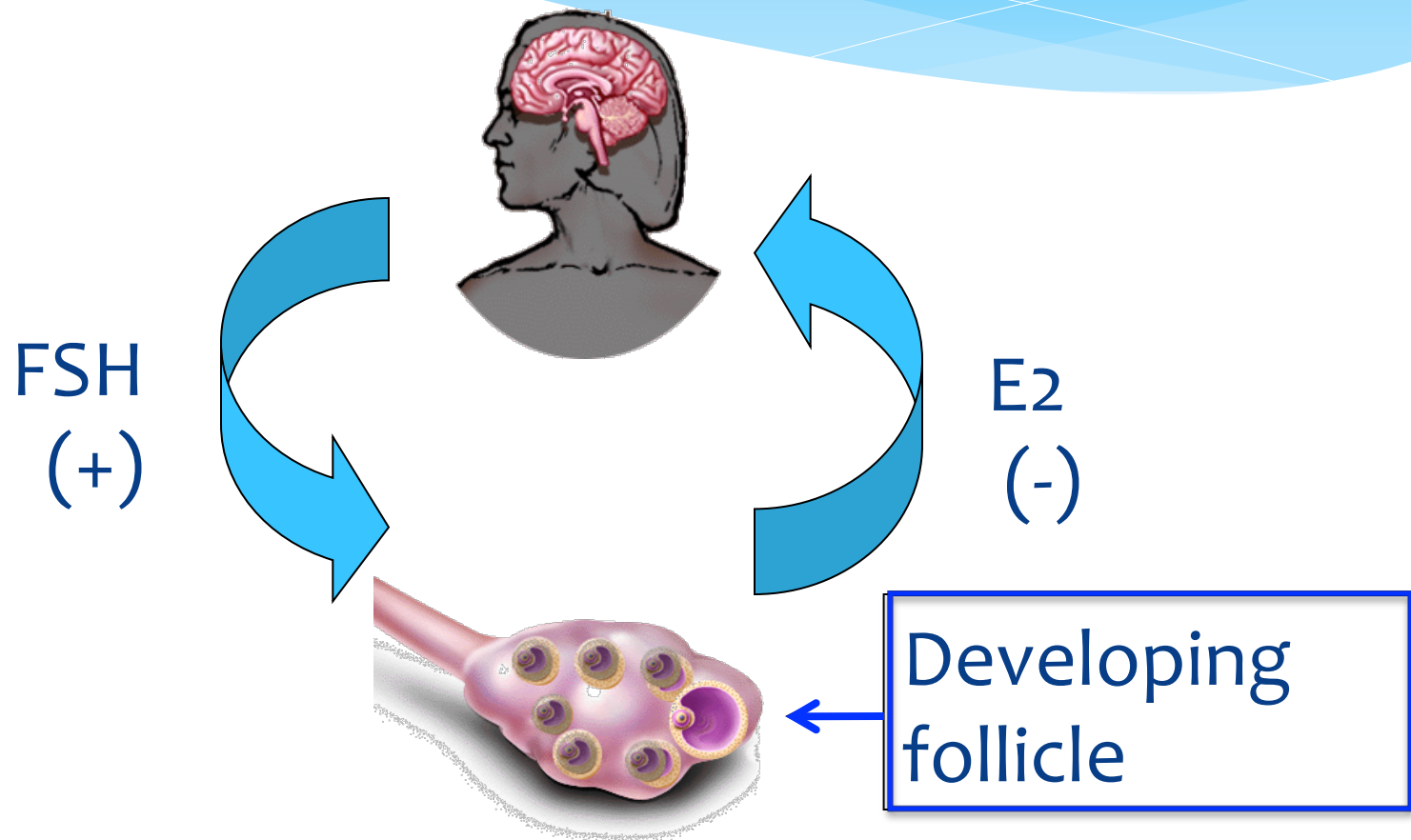
Women are born with their lifetime egg supply

- Significant decline at age 37-38
- Rare pregnancies after age 44
- 100,000 eggs left at time of puberty
- Fertility initially declines at age 27
- Significant decline at age 37-38
- Rare pregnancies after age 44

Prevalence of genetically abnormal oocytes in infertile women



Physiology



FSH=Follicle Stimulating Hormone

E2=Estradiol

Ovary Problems - Causes of Anovulation

- Hormone imbalance
- Obesity
- Anorexia
- Significant stress
- Patients display:
 - ❑ Irregular menstrual cycles
 - ❑ Skipped cycles
 - ❑ Minimal or absent premenstrual symptoms

Ovary Problems – Premature Ovarian Failure

- Menopause prior to age 40
 - ❑ Decreased Estrogen
 - ❑ Increased FSH
- Causes
 - ❑ Autoimmune
 - ❑ Genetic
 - ❑ Idiopathic
- 1-2% pregnancy rate



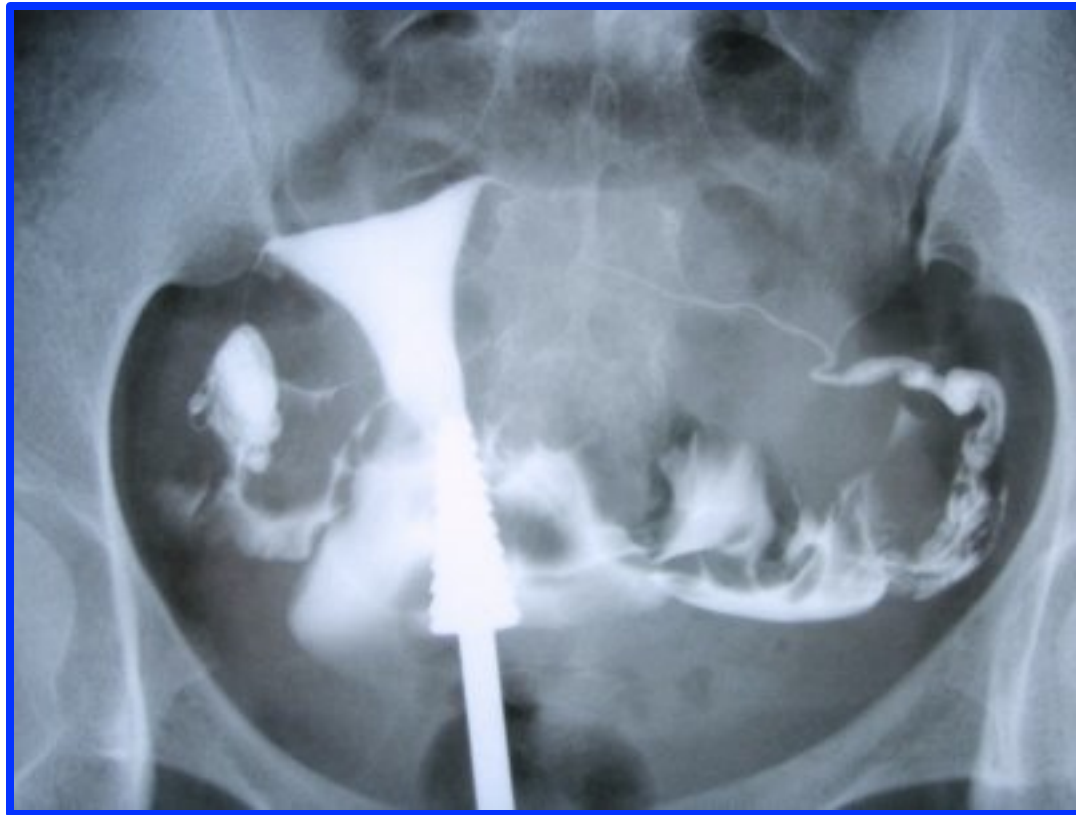
What factor would not cause a woman's fallopian tubes to be blocked?

- a. diabetes**
- b. ruptured appendix**
- c. endometriosis**
- d. chlamydia infection**
- e. tubal ligation**

Causes of Female Infertility – Fallopian Tubes

- Infection (chlamydia)
- Endometriosis
- Tubal ligation (female sterilization)

Open Tubes



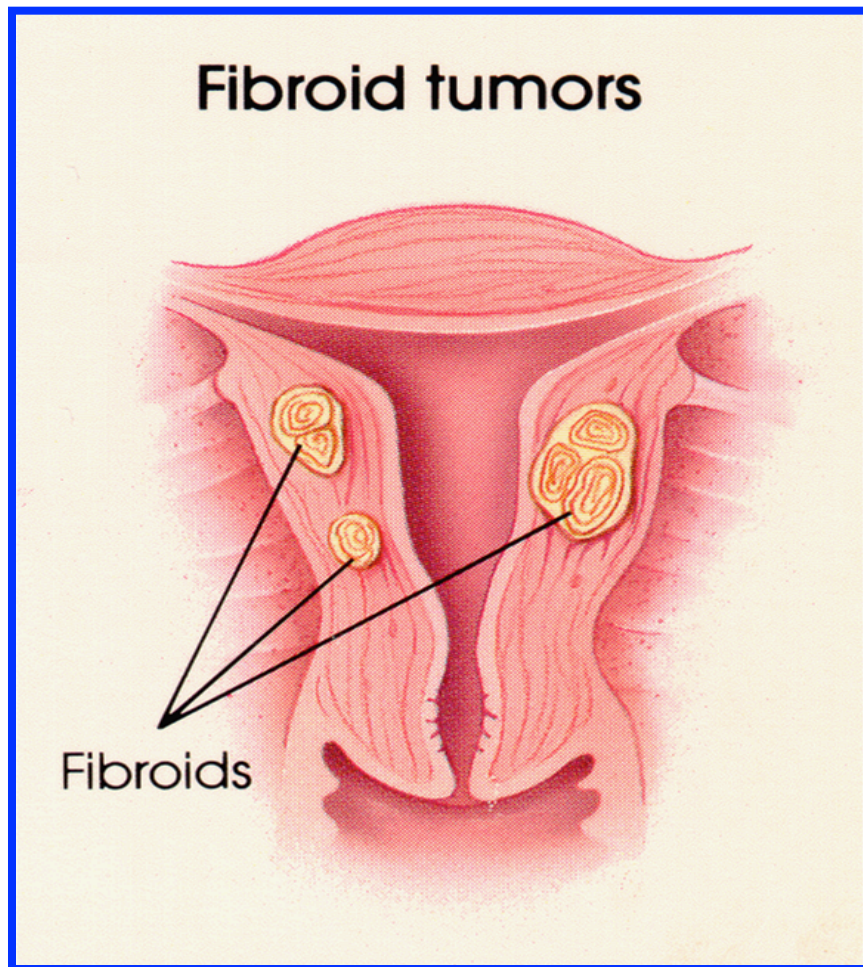
Blocked Tubes



Female Infertility - Uterus

- **Uterus**
 - **Fibroids**
 - **Polyps**
- Mullerian (congenital) defects
 - Absent
 - Bicornuate/Septum

Female Infertility - Uterus

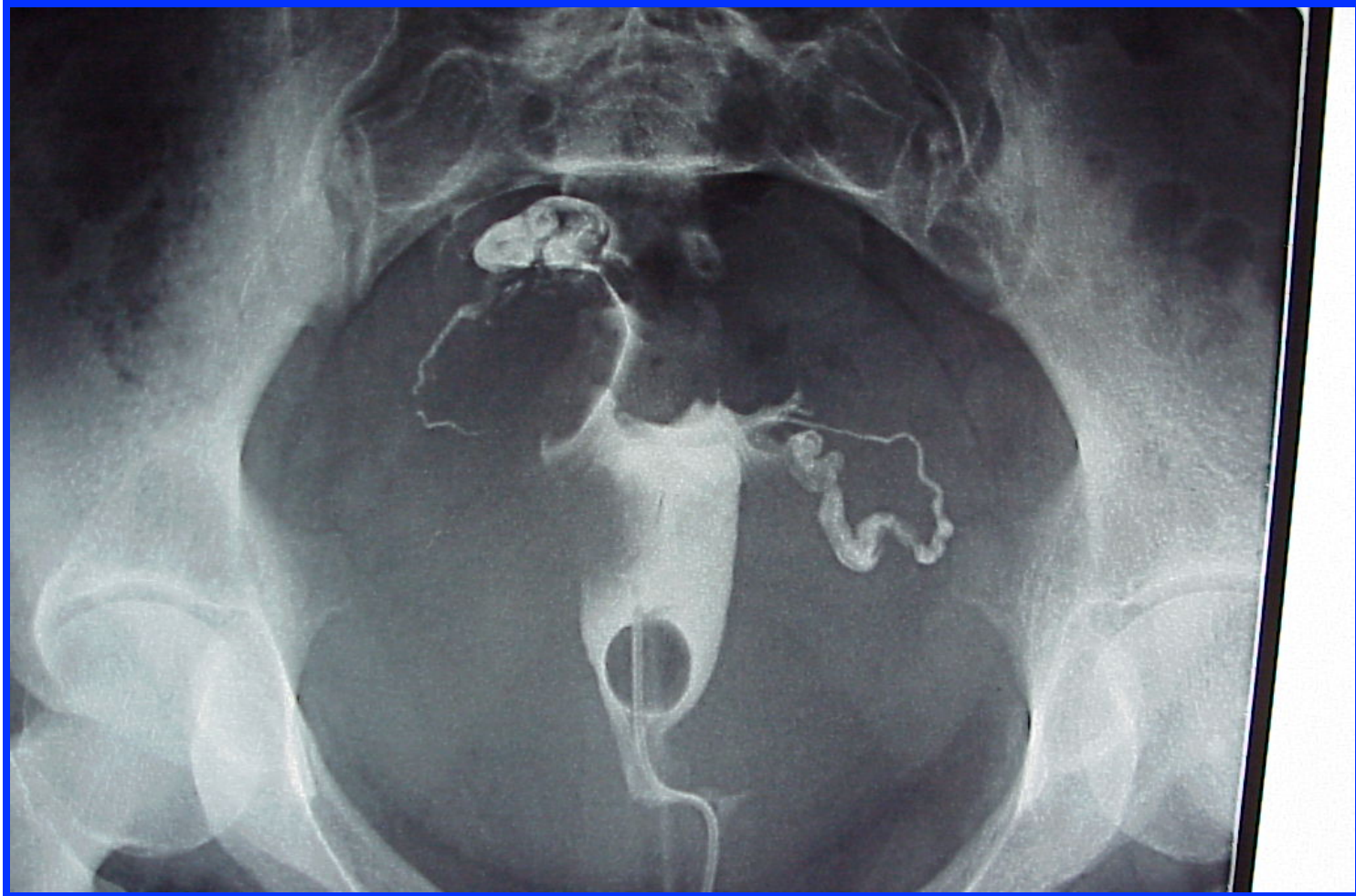


- Uterine muscle tumor
- Benign (>95%)
- 25-30% of women

Normal Shape of Uterus



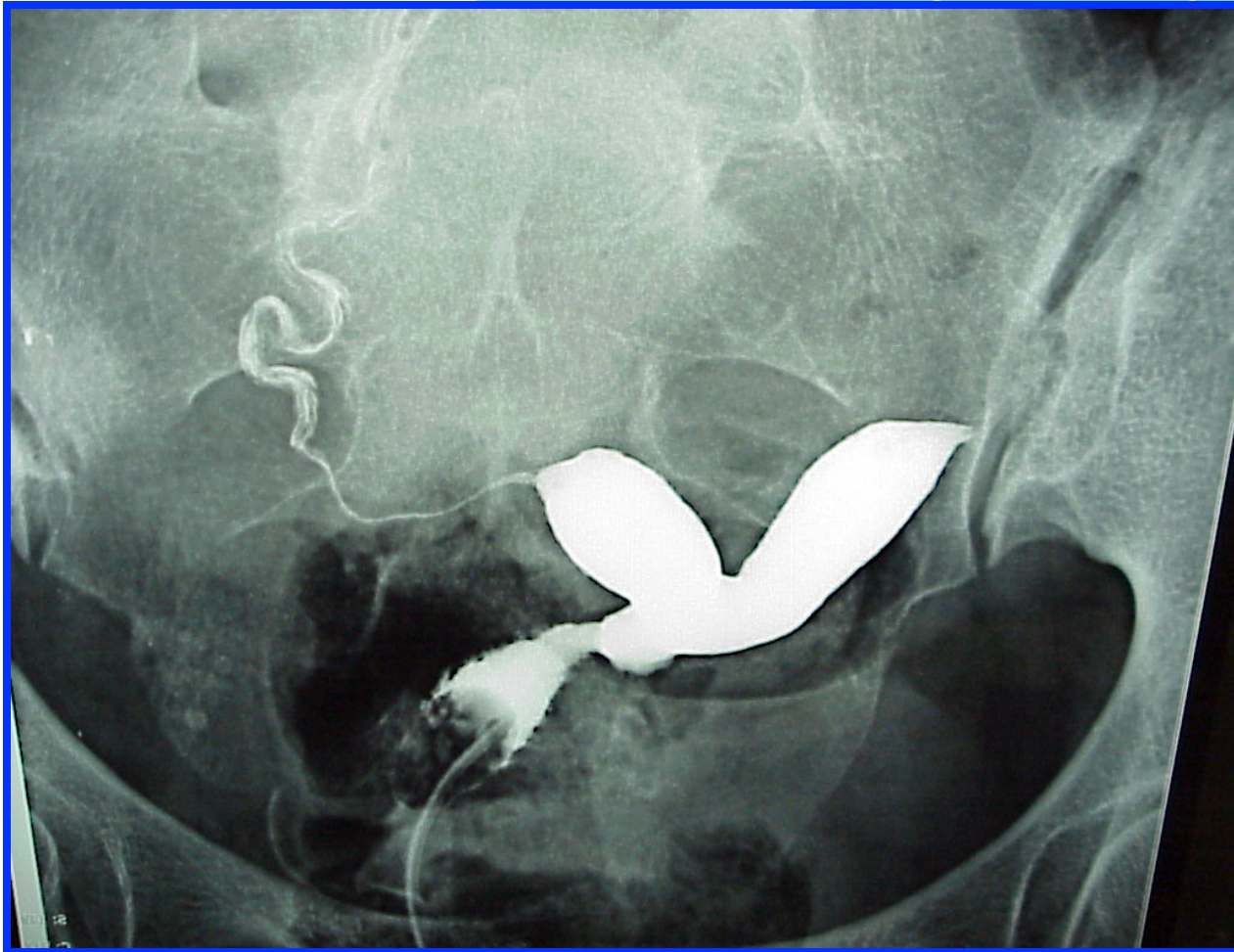
Fibroid Uterus



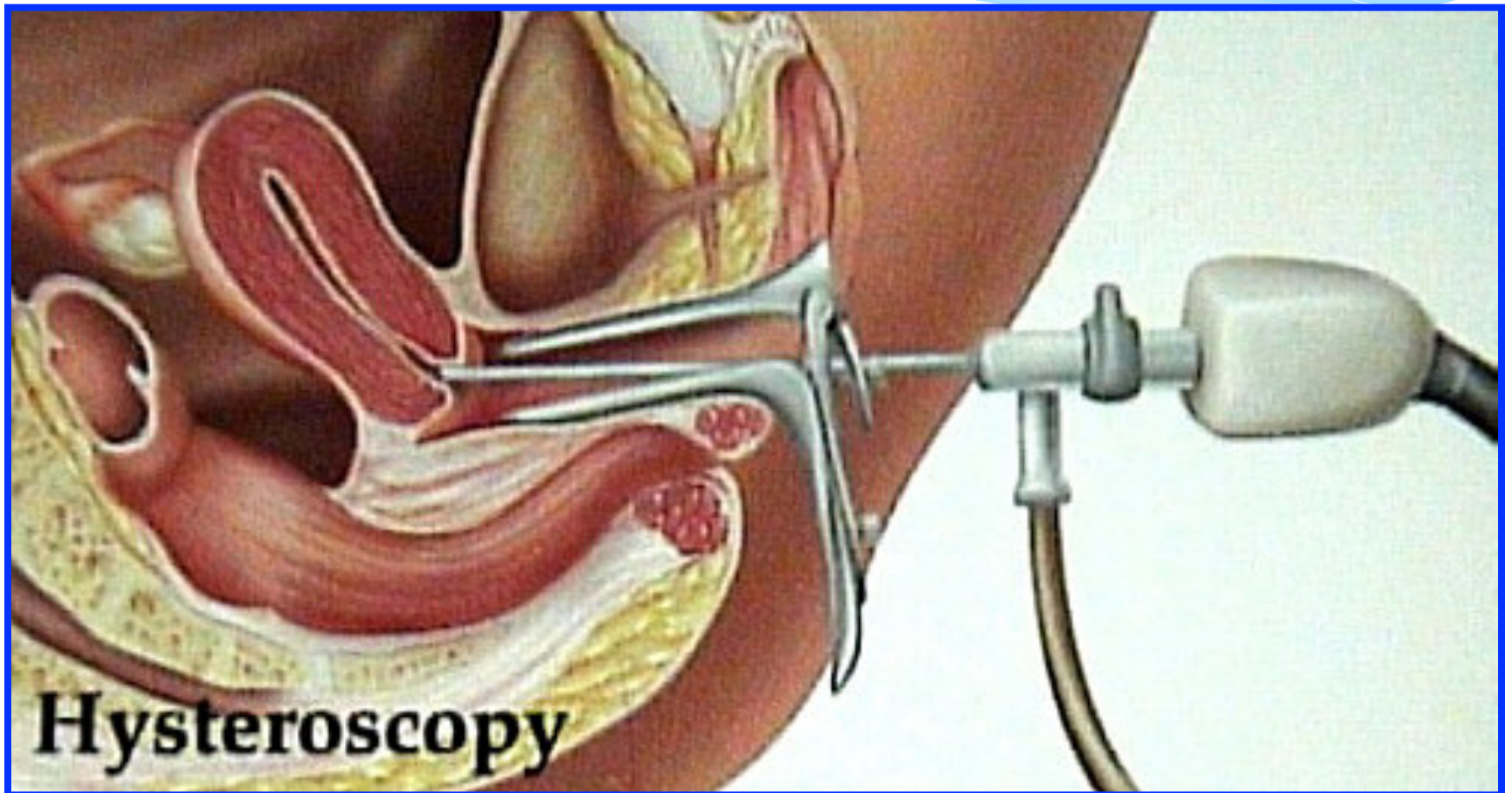
Female Infertility - Uterus

- Uterus
 - Tumors
 - Fibroids
 - Polyps
 - **Mullerian defects (congenital)**
 - **Absent uterus**
 - **Bicornuate/septate**

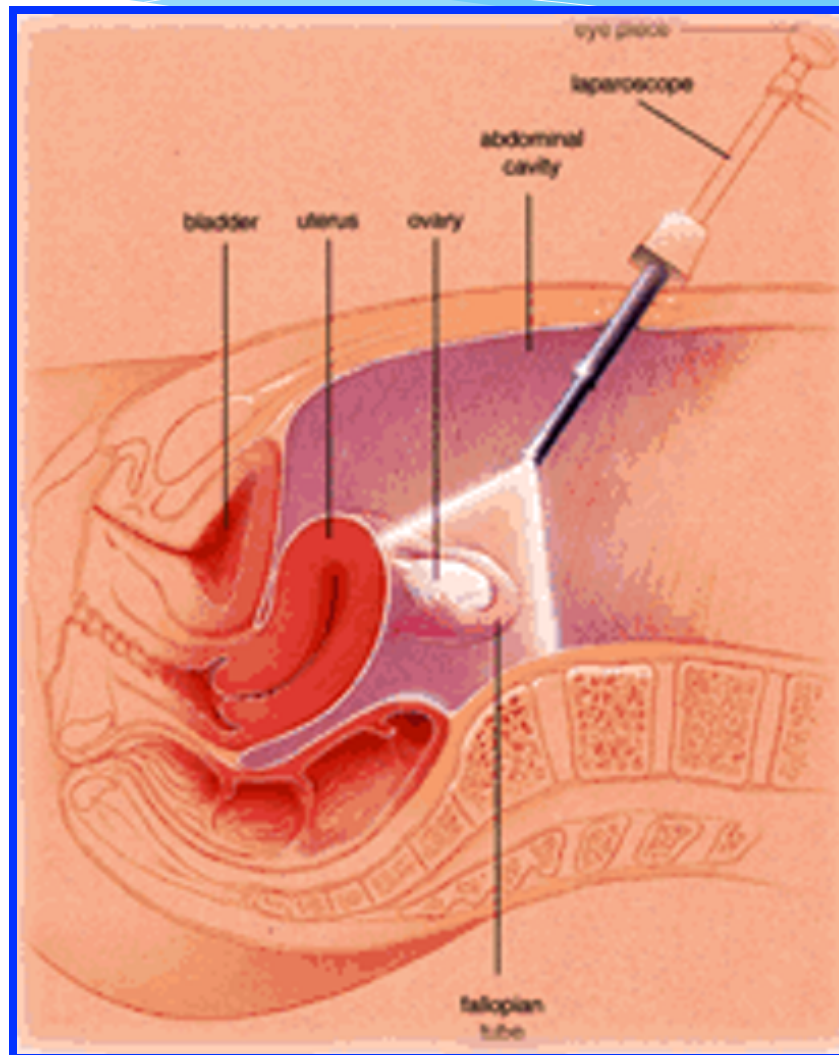
Mullerian Defect



Treatment with Hysteroscopy



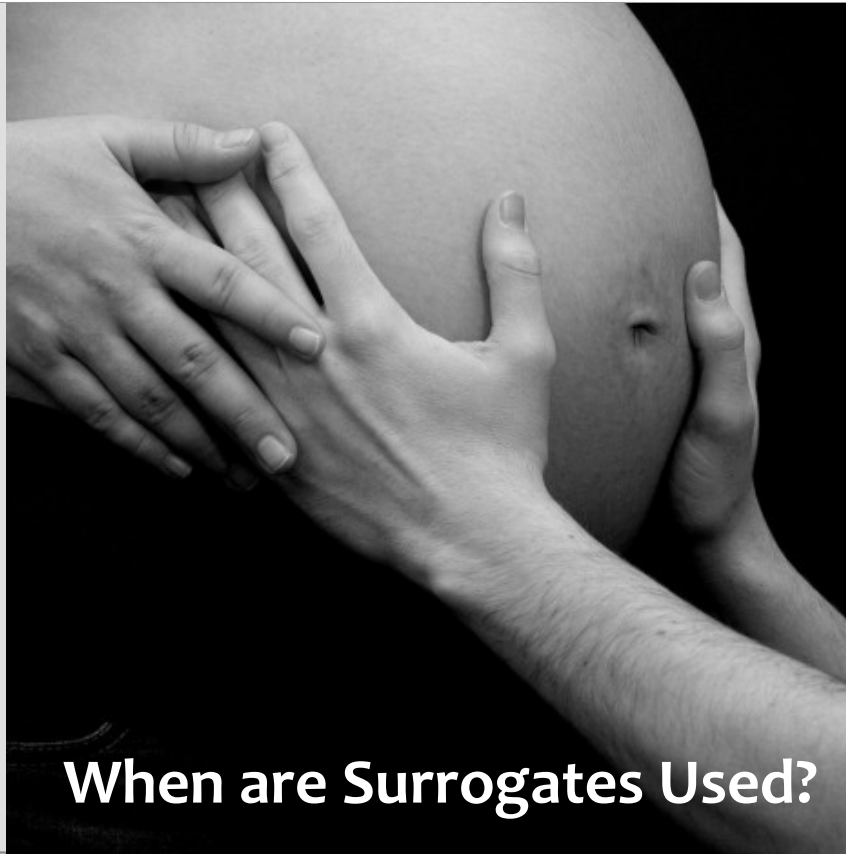
Treatment with Laparoscopy





Surrogate Mothers, Inc.

Alternatives to Infertility since 1984. ©



When are Surrogates Used?

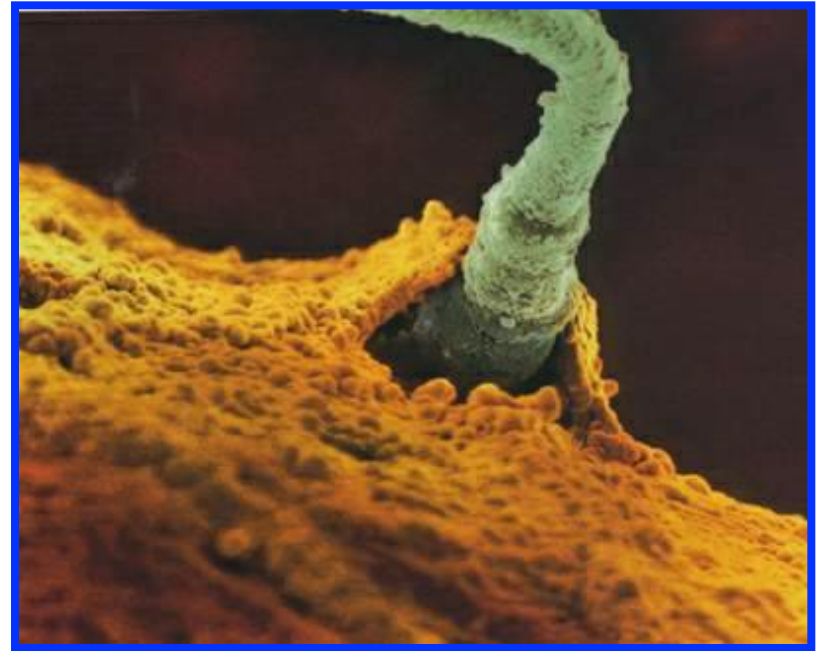
Female Infertility - Hormones

- Endocrine abnormality (hormones)
 - ❑ Thyroid
 - ❑ Prolactin
 - ❑ Polycystic ovary syndrome (PCOS)
 - Estrogen, insulin
 - ❑ Hypothalamic hypogonadism
 - Stress
 - Exercise (ballet dancer)

Other Causes of Female Infertility

- Other Causes
 - Chromosome abnormalities
 - Turner's syndrome (XO)
 - Androgen Insensitivity (XY)
 - Male pseudohermaphrodite
 - Female phenotype
 - Blind vaginal canal
 - Inguinal hernia (50%)

Sperm Are Also Required!!



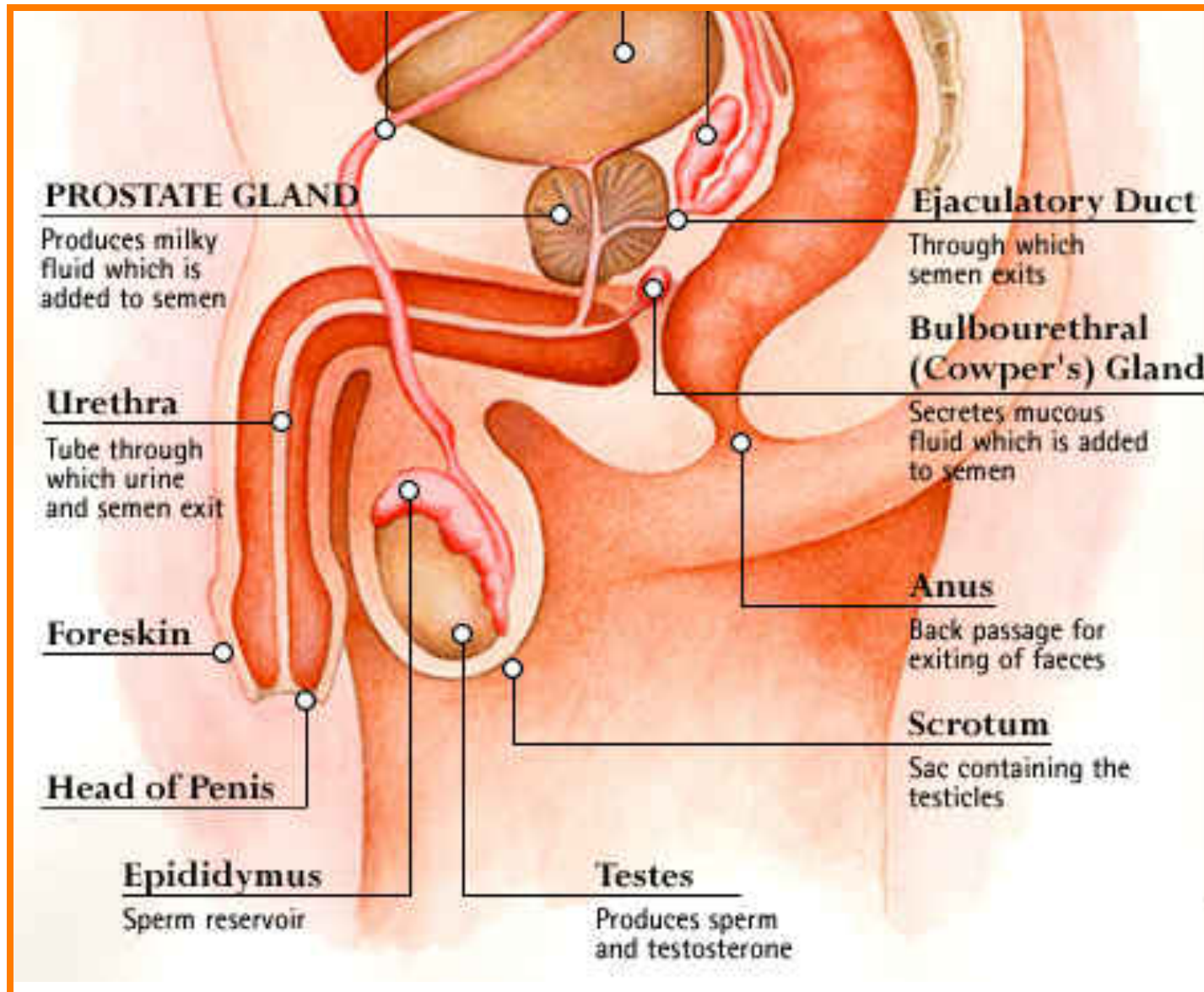


Do men continue to produce sperm throughout their life (from puberty until death)?

a. Yes

b. No

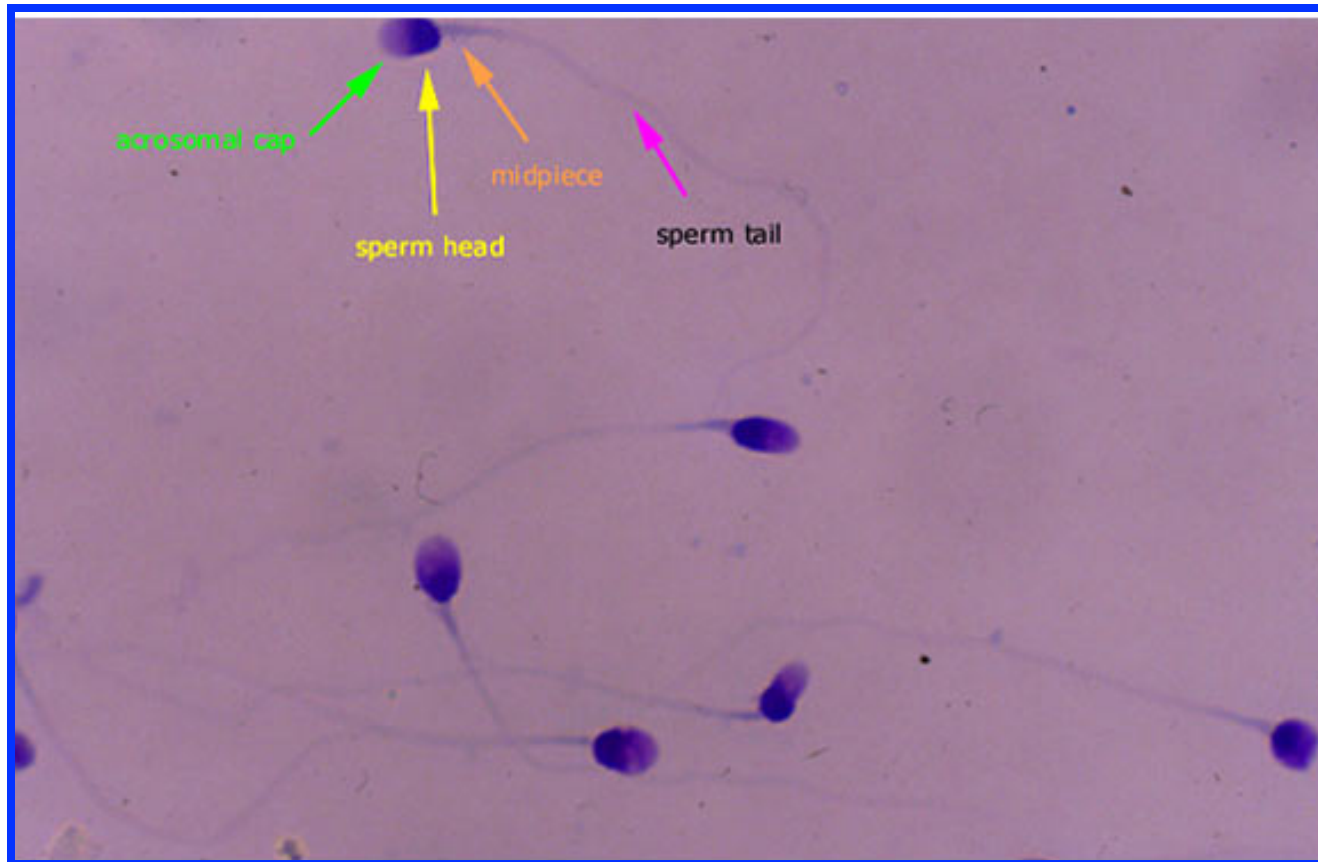
Male Reproductive Organs



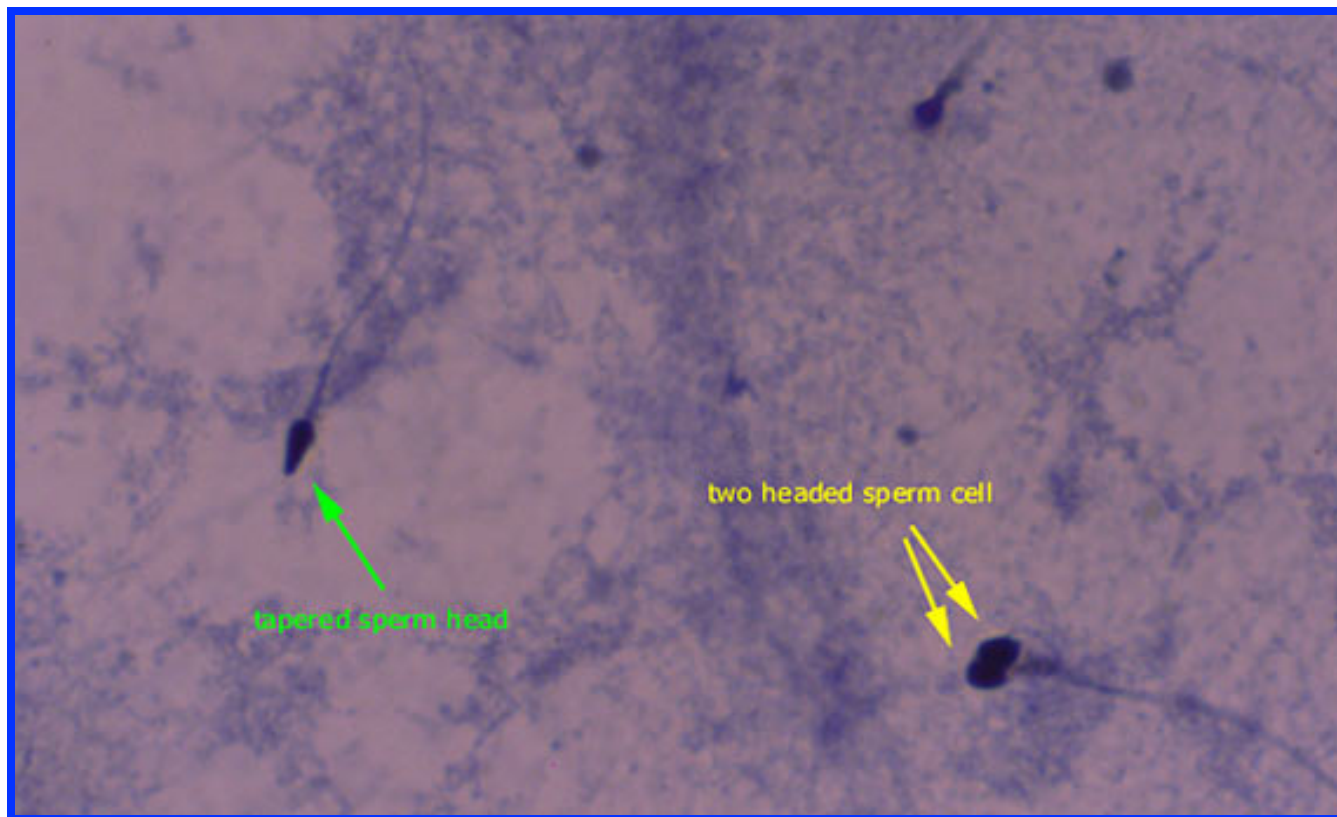
Sperm: Semen Analysis

- Volume: ≥ 2 mL
- Concentration: $\geq 15,000,000$ per mL
- Motility: $\geq 36\%$
- Normal morphology:
 - ▣ Kruger strict criteria: $\geq 14\%$ normal
 - ▣ Best predictor of fertilizing ability

Normal Sperm Morphology



Abnormal Morphology



Sperm

- **How many are needed for fertilization?**
- Natural conception
 - ❑ 20,000,000
- Intra-uterine insemination
 - ❑ 1,000,000
- In-vitro fertilization (IVF)
 - ❑ 10,000
- Intra-cytoplasmic sperm injection (ICSI)
 - ❑ 1

Causes of Male Infertility

- Abnormality in sperm production
- Abnormality in sperm function
- Obstruction in the ductal system

Abnormalities of Sperm Production

- **Genetic**
 - ❑ Y chromosome microdeletions
- Damage to testes – anatomical
 - ❑ Cryptorchidism
 - ❑ Varicocele
- Infection
 - ❑ Mumps orchitis
- Gonadotoxins

Obstructions in Ductal System

- Vasectomy
- Congenital bilateral absence of the vas deferens

Male Infertility - Lifestyle

- Tobacco
- Marijuana
- Alcohol
- Cocaine
- Steroids
- Heat
- Exercise

Infertility: Initial Evaluation

- Egg supply
- Sperm supply
- Fallopian tubes
- Uterus

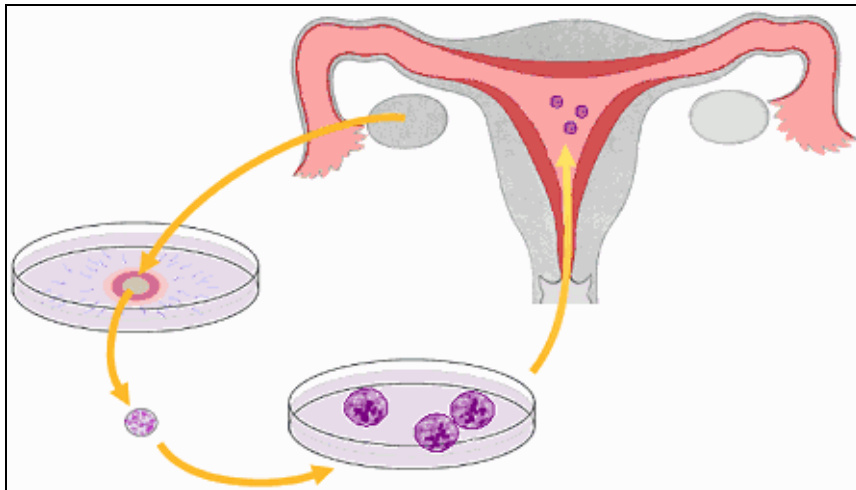
Unexplained Infertility

- Work-up is negative
- 15-20 % of couples

Infertility Treatments

- Improve Timing of Intercourse
- Intrauterine insemination (IUI)
- In Vitro Fertilization (IVF)
 - ❑ Standard IVF
 - ❑ IVF with egg donor
 - ❑ IVF with surrogate

In Vitro Fertilization



In Vitro Fertilization - History

- 1978 – First “test tube” baby was born in England
- 1981 – IVF in U.S.
- Started with GIFT and ZIFT
- 2016 - >98% IVF with transfer of embryo to uterus

IVF Statistics-2013 (Latest Report)

- **497 U.S. clinics offer IVF** (467 reporting data to CDC)
- **190,773 cycles of ART treatment**
- **54,323 live-birth deliveries**
- **67,996 infants born**

Who Needs IVF?

- Failed other treatments
- Tubal damage
- Significant male factor
- Absent uterus
- **Carriers of genetic diseases**
- Gender selection
- Fertility preservation/Cancer patients
- Lesbian-Gay Couples

IVF and Ethics

- Life vs. destruction of life (embryos)
- Gender Selection
- Genetic testing of embryos
 (“designer” babies)
- Reproductive “laws”



Who should decide if a couple/person can have infertility treatment with IVF?


- a. state governments**
- b. national fertility agencies (ASRM)**
- c. reproductive specialists**
- d. psychologists/psychiatrists**
- e. the couple/person**



**Should a 52 year old postmenopausal woman
be able to use her daughter's eggs to have a
child with her new 28 year old husband?**

a. Yes

b. No

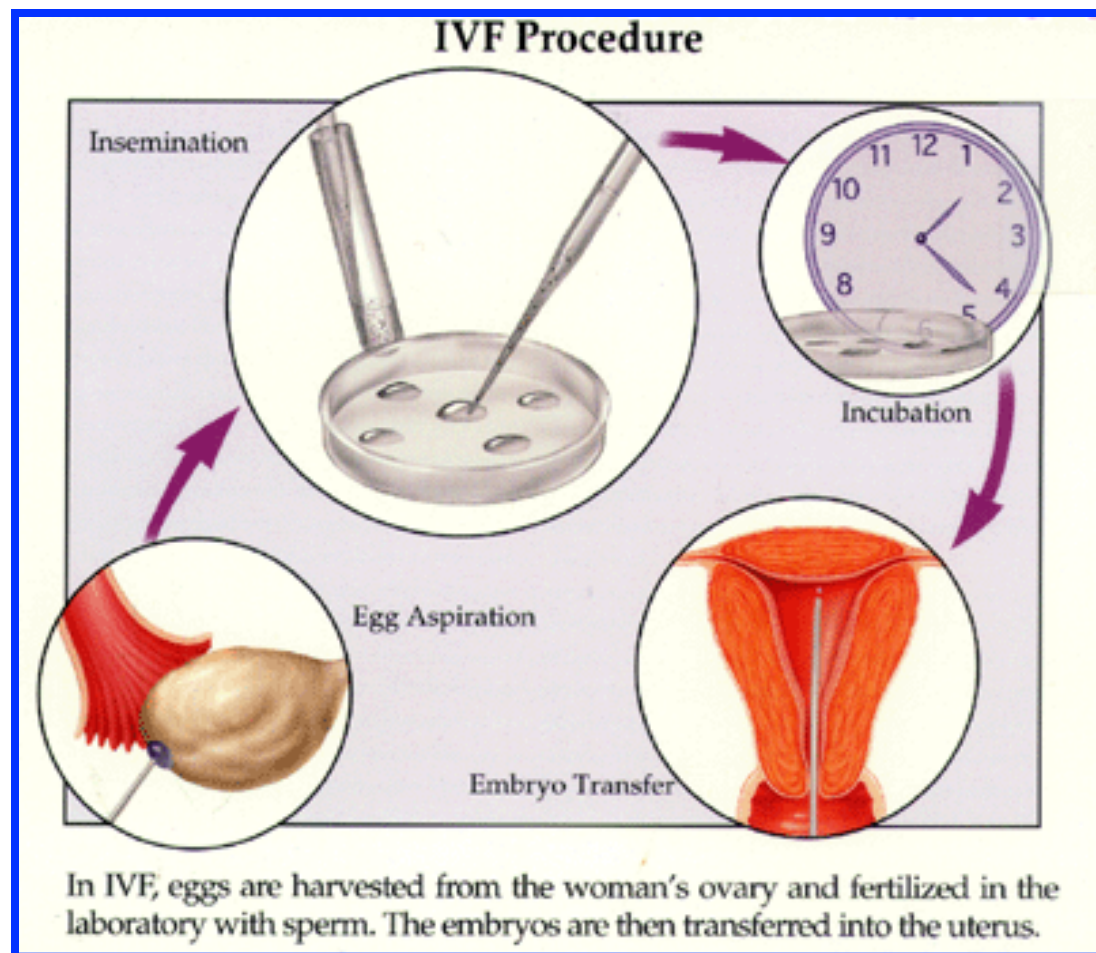


**Should a woman be able to have sperm
extracted from her husband's newly dead
body so that she can have "their child"?**

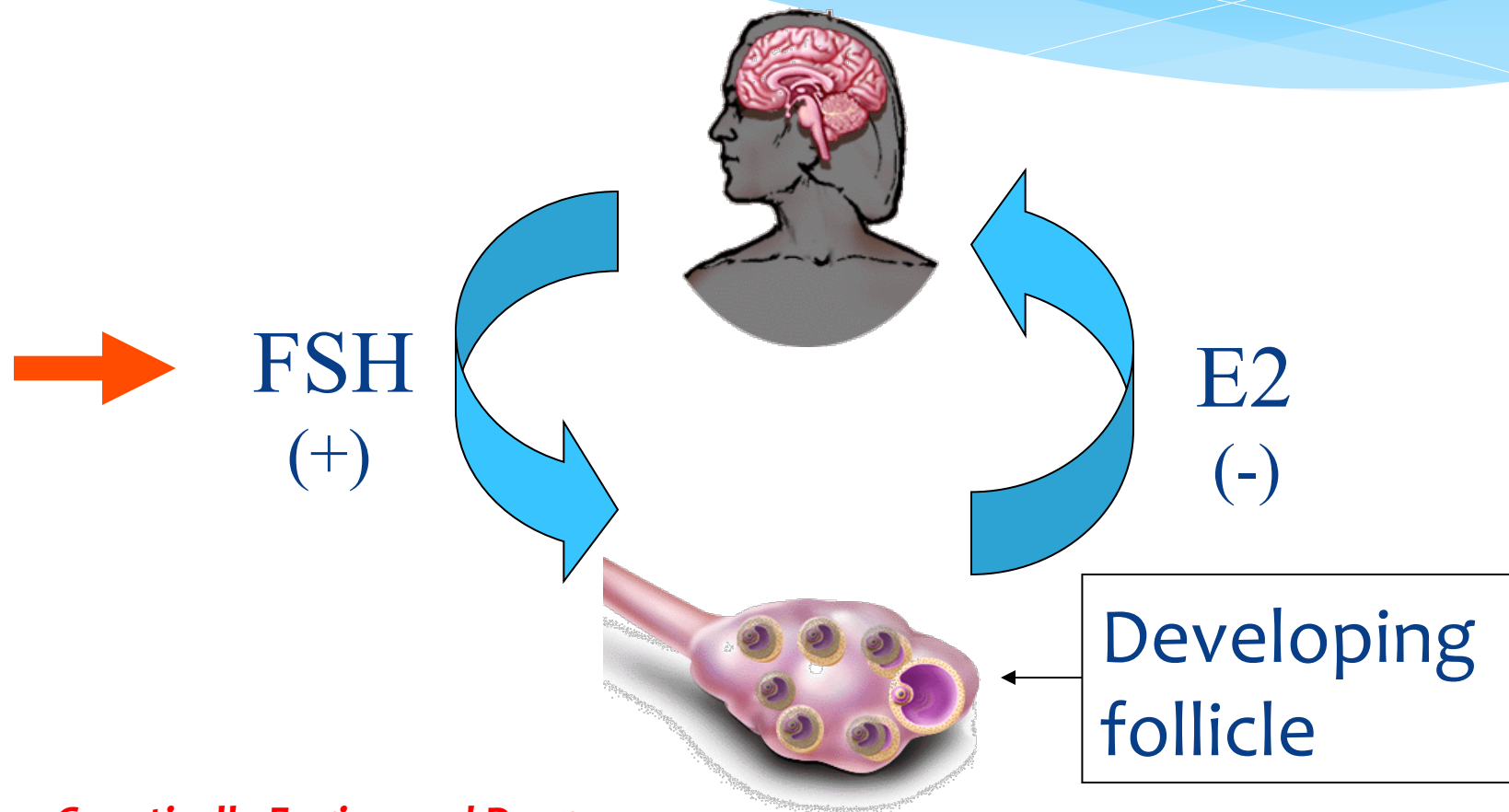
a. Yes

b. No

IVF – Step by Step



How Does Recombinant FSH Work To Increase Egg Number?

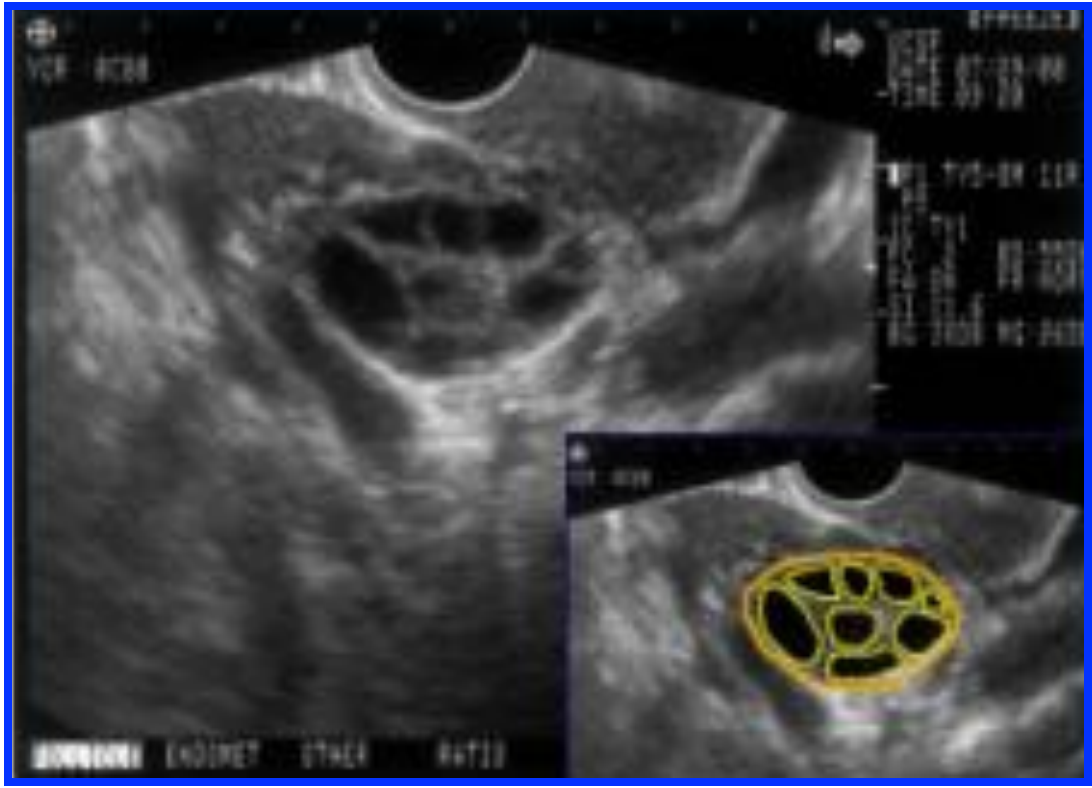


Genetically Engineered Drug

FSH=Follicle Stimulating Hormone

E2=Estradiol

Ovarian Hyperstimulation



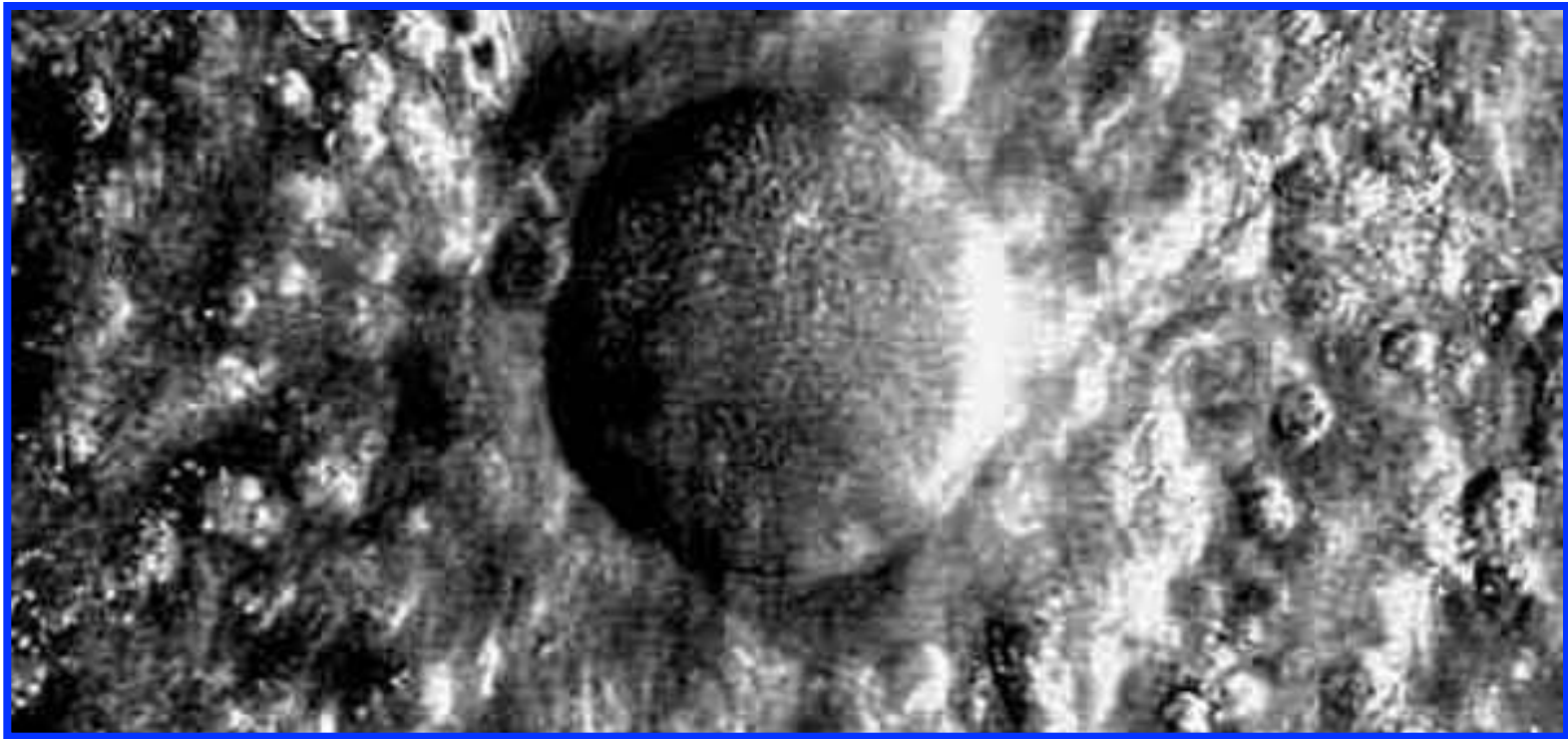
Egg Retrieval



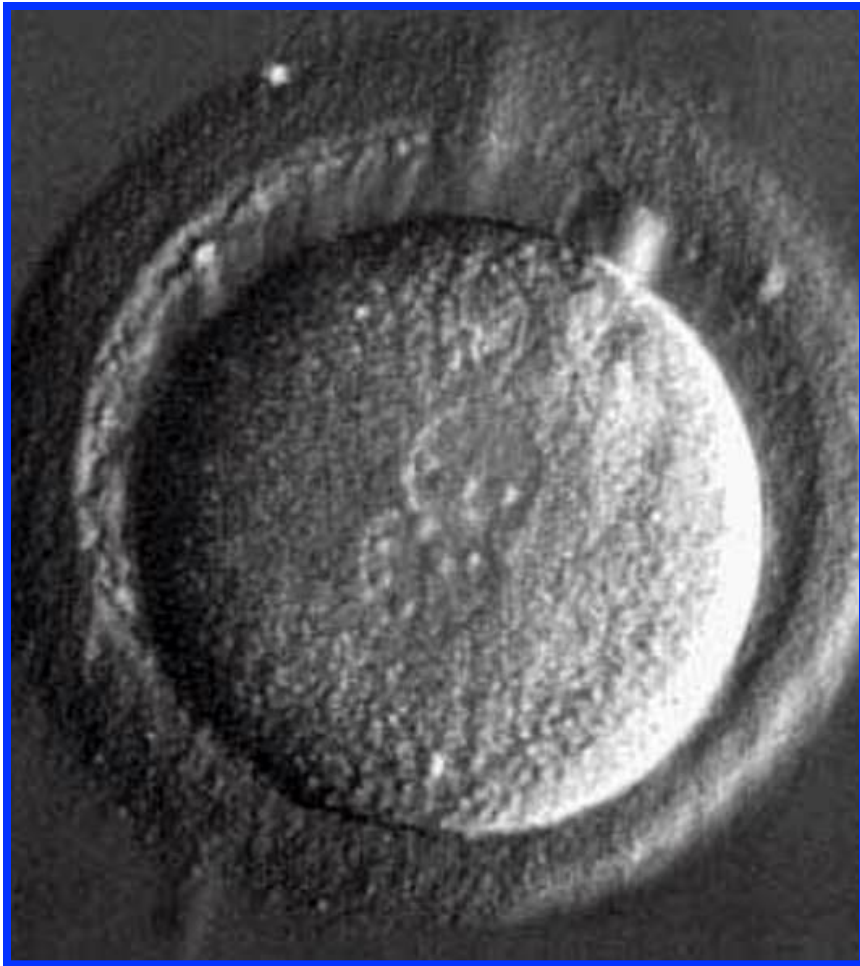
Good Egg



Bad Egg

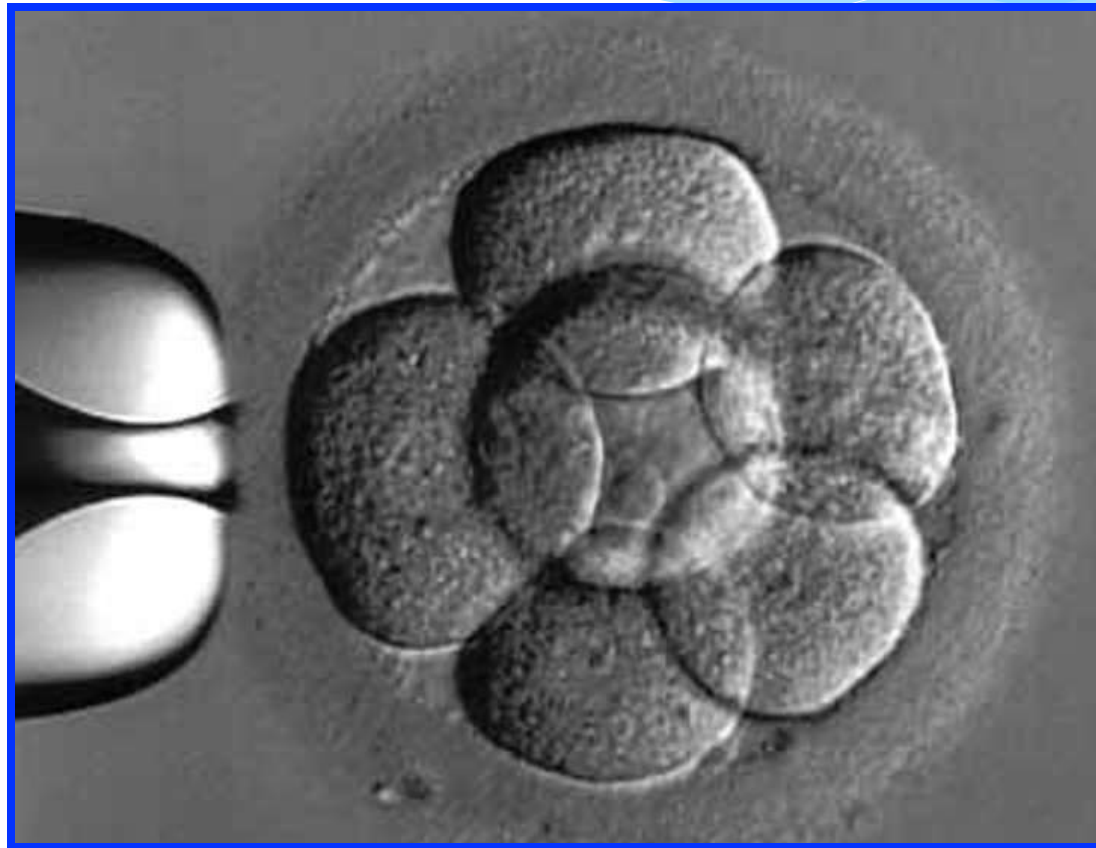


Fertilization



- 2 Pronuclei (2PN)
- 1 day after egg retrieval

Day 3 Embryo



Day 3 Embryo



Blastocyst – Day 5

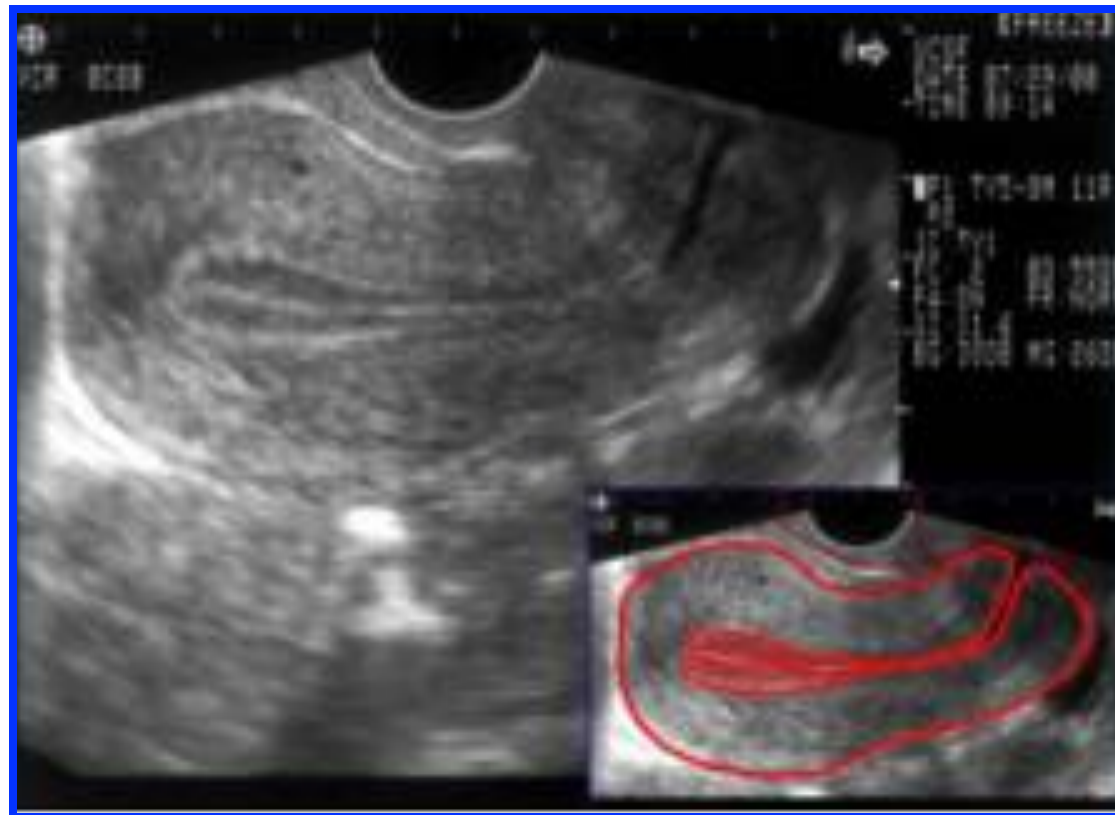


Source of Stem Cells – PGS biopsy stage

Hatching Blastocyst



Embryo Transfer



Special IVF Procedures

- Assisted hatching
- Intracytoplasmic sperm injection (ICSI)
- Preimplantation genetic diagnosis (PGD)
- Freezing
- Egg donation
- Surrogacy

Assisted Hatching



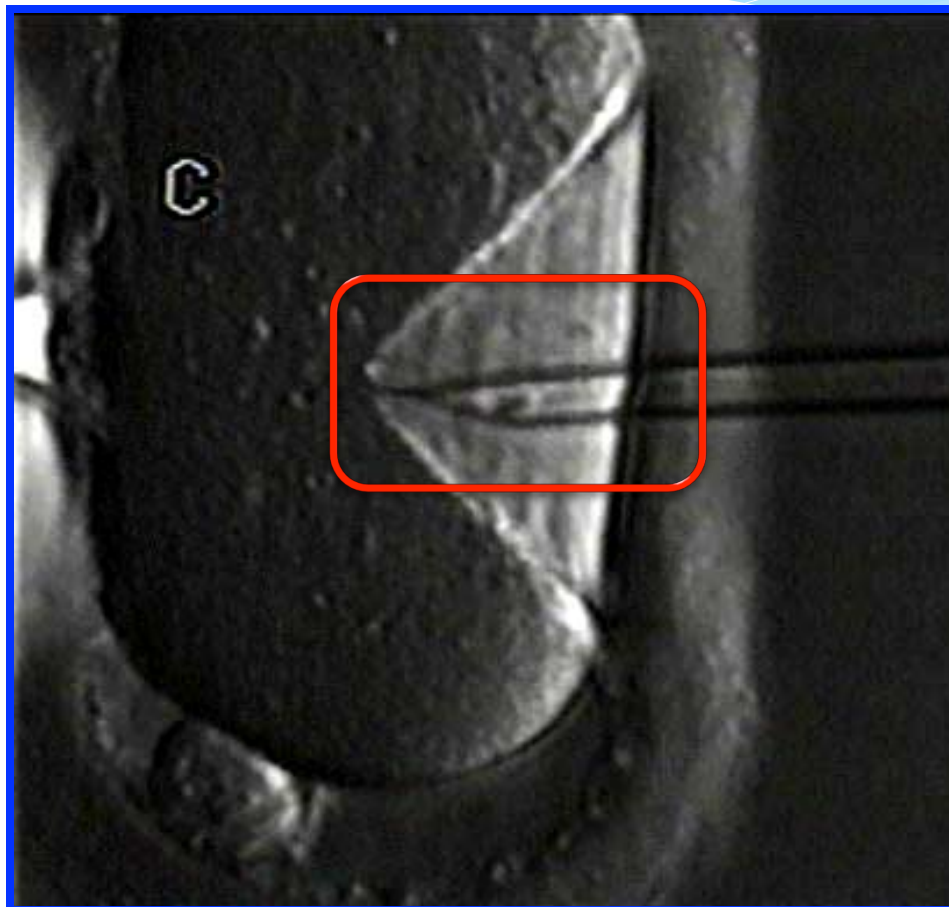
Empty Zona



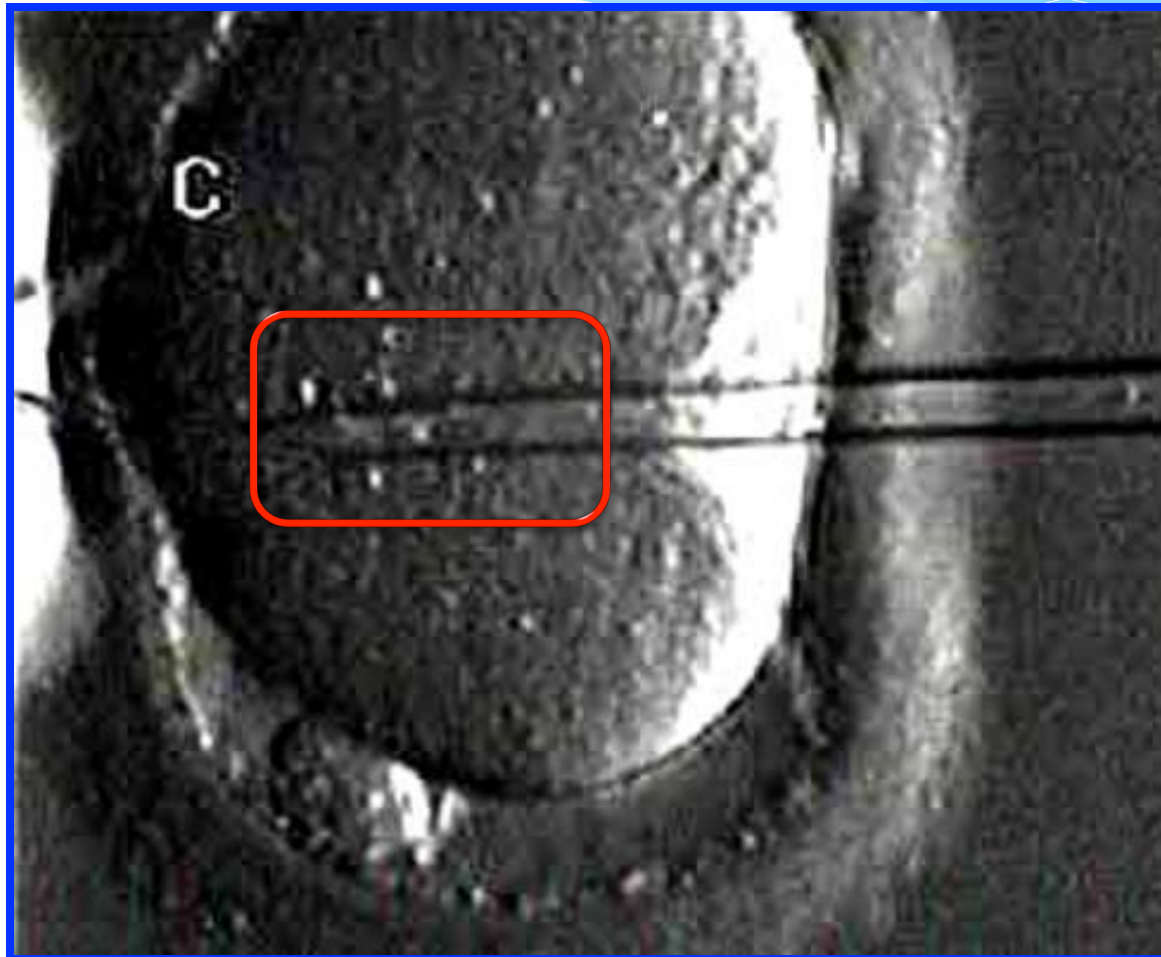
ICSI



ICSI



ICSI





What are the risks of IVF?

- a. bleeding requiring blood transfusion**
- b. pelvic infection**
- c. increased risk of congenital anomalies**
- d. multiple pregnancies**
- e. all of the above**



Who should decide how many embryos are transferred?

- a. The Doctor**
- b. The Couple/Person**
- c. American Society of Reproductive Medicine**
- d. Government**
- e. Religious Leaders**

How Many Embryos are Transferred?

- **Related to age and embryo quality**
 - ❑ **<35 = 1-2**
 - ❑ **35-37 = 2**
 - ❑ **38-40 = 2-3**
 - ❑ **>40 = 3**
 - ❑ **For patients with 2 or more failed IVF cycles, or a poor prognosis, can add more based on clinical judgment**

What Happens to the Other Embryos?

- **Freeze Embryos**
- **Donate For Research/Stem Cells**
- **Embryo Adoption**
- **Discard**



What Would You Do With Your Embryos?

- a. Freeze and Store Them**
- b. Donate For Research (e.g., Stem Cells)**
- c. Donate To Others For Adoption**
- d. Discard**

IVF Success Rates - 2013

- **U.S. Fertility Centers From SART/CDC**
- **Female age**
 - ❑ **<35 – 55% (47%)**
 - ❑ **35-37 – 47% (39%)**
 - ❑ **38-40 – 38% (28%)**
 - ❑ **41-42 – 27% (16%)**
 - ❑ **43-44 – 16% (8%)**
 - ❑ **> 44 – 7% (3%)**

IVF Statistics Live Births - 2013

- **73.4% singletons**
- **25.7% twins**
- **0.9% triplets or more**

Singleton Pregnancy



Twin Pregnancy



Triplet Pregnancy



IVF and Multiple Pregnancy

- **Maternal complications**
- **Fetal complications**
- **Cost**
- **“Selective reduction”**
- **Single embryo transfer vs. success rates**
- **Divorce**

Cost of IVF

- IVF cycle + medications = \$10,000-20,000
- Assisted hatching = \$500
- ICSI = \$1,500
- Freezing = \$700
- Storage = \$600/year
- PGS = Minimum \$3,500
- Egg Donor = Minimum \$15,000
- Surrogate = Minimum \$40,000

Not Covered By Insurance In Most States!

Egg Donation



Give The Gift Of Life...
And Make A Difference

EXCEPTIONAL DONORS

Exceptional Donors, Inc. was founded to help couples struggling with the pain of infertility in the Portland, Oregon area and throughout the United States. Egg donation is a wonderful way for families to grow. And we can help. Allow us to assist you as you begin your journey toward becoming a parent.

We are always searching for exceptional donors. If you are between 19 and 32 years of age, healthy, intelligent, athletic, artistic, musically talented, attractive, or possess other exceptional qualities, you could be a candidate for our program.

We offer \$5,000 compensation for first-time donors, and more for subsequent donations.

*Call 866.296.1015 or visit www.exceptionaldonors.com
to learn more about becoming an exceptional egg donor.*



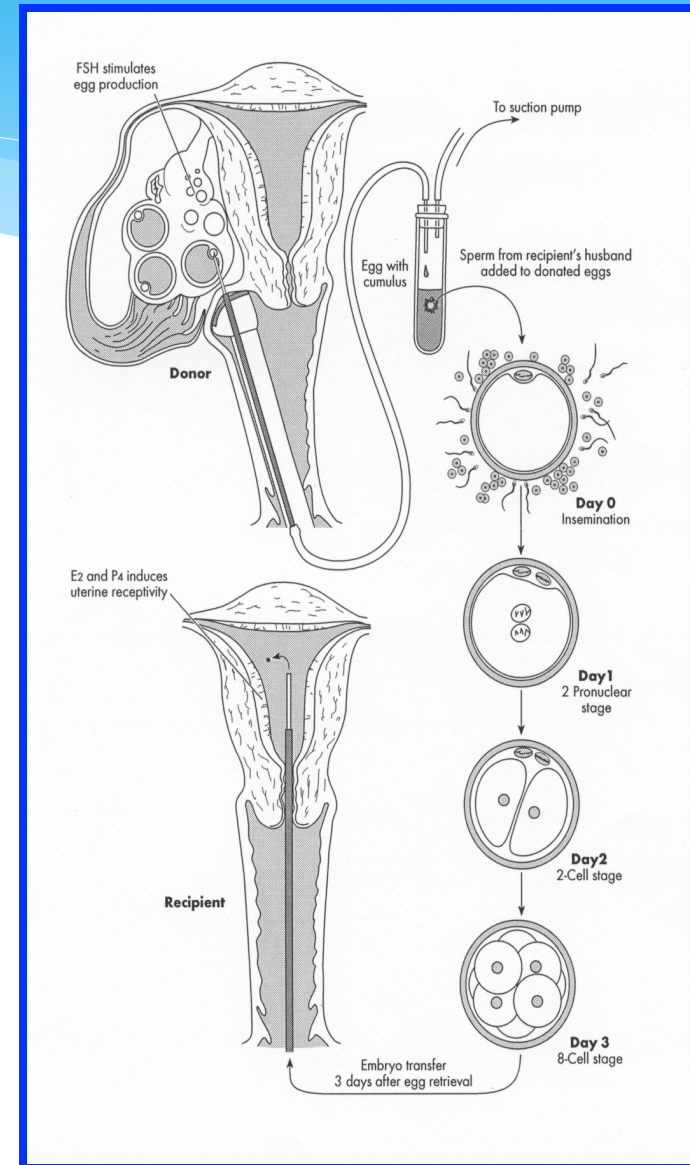
Would you consider being an egg or sperm donor?

a. Yes

b. No

Egg donation

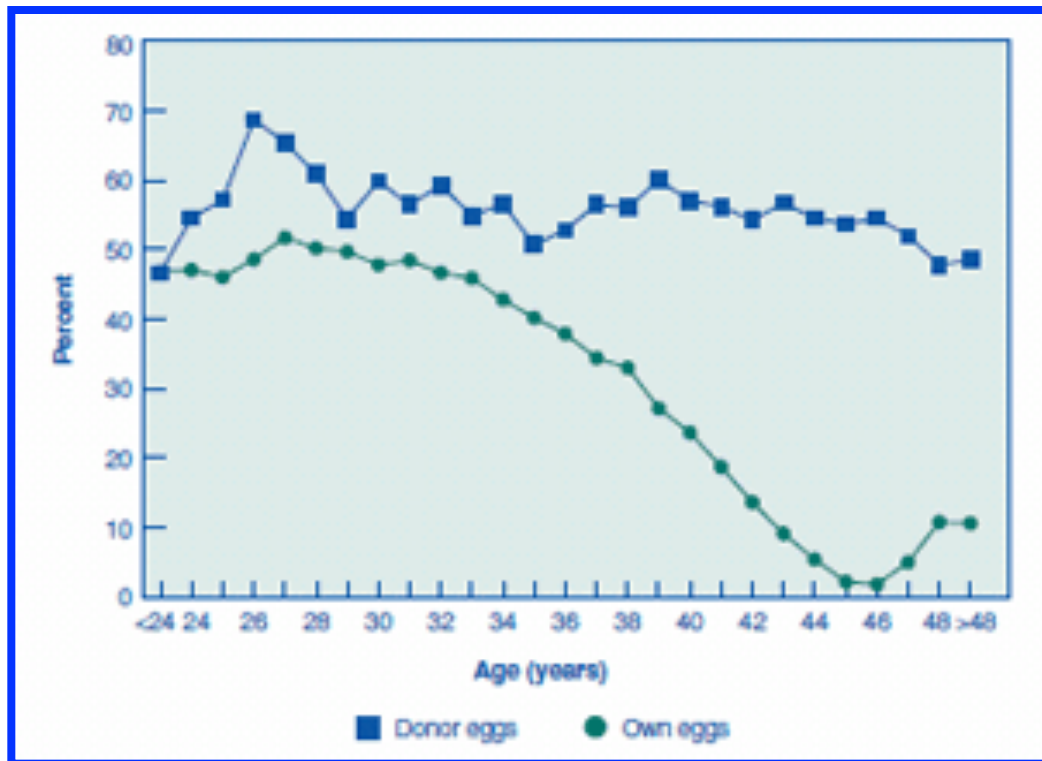
- IVF for two
- Donor
 - ❑ Standard controlled ovarian hyperstimulation
 - ❑ Egg retrieval
- Recipient
 - ❑ Embryo transfer



Who are candidates to be an egg donor ?

- 21-30 years old (older if a friend or relative)
- FSH <10
- Good personal health and family history
- Normal psych, drug, genetic screening
- Preferably prior egg donation experience
 - ❑ How many eggs were produced?
 - ❑ Did pregnancy result?

Current status of ART in the USA



Live birth rates
per transfer
for fresh embryos
from own and
donor eggs,
by age of recipient

Egg Donation - Ethical Issues

- Egg Donor
 - ❑ Known or anonymous
 - ❑ How many times to donate?
- Recipient
 - ❑ How old is too old?

Pregnancy in the Sixth Decade of Life

- USC experience: 1991-2000
 - ▣ 77 recipients of egg donation
 - ▣ Mean age 52.8 + 2.9 years
- Of the 77 women, 42 (54.5%) had live births
- 45 deliveries in 42 women

Pregnancy in the Sixth Decade of Life: Obstetric Complications

- Pre-eclampsia
 - ▣ 35%
- Background Incidence
 - ▣ 3-10%
- Gestational Diabetes
 - ▣ 20%
- Background Incidence
 - ▣ 5%

How old is too old?

- What is the “physiological limit”?
 - ❑ Marked increase in pre-eclampsia
 - ❑ Increase in diabetes



How old is too old to carry a pregnancy?

- a. 44 (rare natural pregnancies occur later)
- b. 51 (average age of menopause in U.S.)
- c. 65 (social security/retirement)
- d. No limit

Genetic Testing



- Preconception
- Preimplantation
- Prenatal
- Postnatal



Preconception Counseling

- Offered to all women
 - ❑ Prenatal vitamins – 400 micrograms folic acid/day, 200 mg DHA
 - ❑ Rubella and Varicella immunity
 - ❑ Thyroid function tests
 - ❑ Blood Type/Rh status
 - ❑ HIV
 - ❑ Hepatitis B and C screening
 - ❑ **Genetic screening for over 100 mutations**

Recessive Genetic Carrier Screening

- Offered to all individuals of reproductive age
- Certain diseases have high carrier rates
 - Cystic Fibrosis 1/24
 - SMA 1/40
- If one partner is positive, the other partner is tested
- If both are carriers -> 25% chance of having an affected offspring
- Treatment: IVF with PGD

Preconception Counseling

- Offered to certain ethnic groups
 - ❑ Mediterranean – thalassemia
 - ❑ African-American – sickle-cell anemia
 - ❑ Caucasian/Latino – cystic fibrosis, SMA
 - ❑ Ashkenazi Jews – 7 autosomal recessive disorders
 - Gaucher disease (1/13), Tay-Sachs (1/30), Familial dysautonomia (1/30), Canavan disease (1/40), Fanconi anemia (1/89), Niemann-Pick disease (1/90), Bloom syndrome (1/100)

The Universal Genetic Test



January 29, 2010 New York Times

Firm Brings Gene Tests to Masses

By [ANDREW POLLACK](#)

REDWOOD CITY, Calif. — The new movie “Extraordinary Measures” is based on the true story of a father who starts a company to develop a treatment for the rare genetic disease threatening to kill two of his children before they turn 10.

Now, a Silicon Valley start-up is making the bold claim that it can help eradicate that disease and more than 100 others by alerting parents-to-be who have the carrier genes.

The company, Counsyl, is selling a test that it says can tell couples whether they are at risk of having children with a range of inherited diseases, including [cystic fibrosis](#), Tay-Sachs, [spinal muscular atrophy](#), [sickle cell disease](#) and Pompe disease (the one afflicting the children in the movie).

Once informed, Counsyl says, couples can take steps like using in vitro fertilization with genetic testing of the embryos, to avoid bearing children who would have the diseases, many of which are incurable and fatal in childhood.

Some genetic testing of prospective parents is done now, but only for a few diseases like cystic fibrosis and Tay-Sachs, and only for certain ethnic groups. Each test can cost hundreds or even thousands of dollars.

Counsyl’s test, which analyzes DNA from saliva samples, costs \$349 for an individual or \$698 for a couple. Similar tests from others are on the way, experts say. The trend shows that new technology could make possible widespread screening for the risk of passing on rare diseases, something that was simply not practical before.

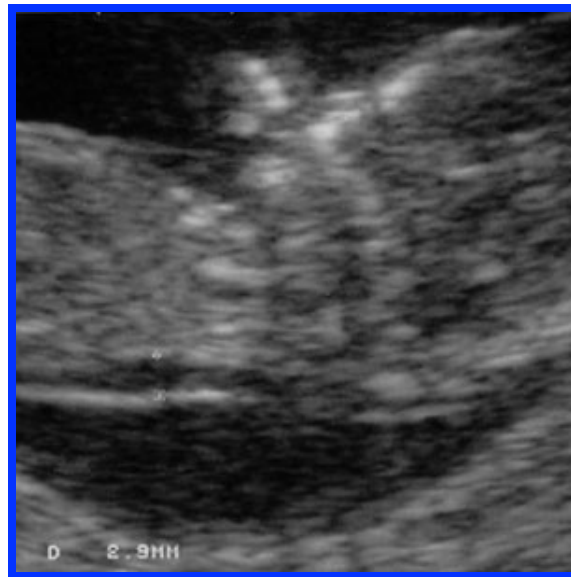
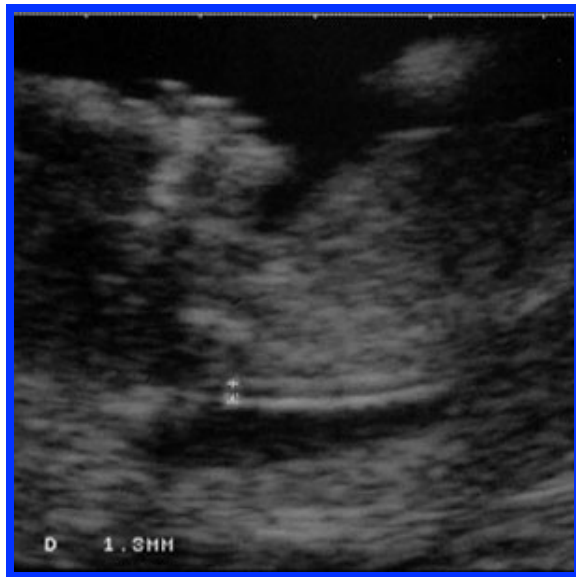
Prenatal Testing

- Ultrasounds
- Serum screens
- Chorionic villus sampling (CVS)
- Amniocentesis

Prenatal Tests - Ultrasound

- Nuchal translucency screening
 - ❑ Performed between 10-13 weeks gestation
 - ❑ Screen for Down Syndrome

Nuchal Translucency



Prenatal Tests - Ultrasound

- Second trimester ultrasound
 - ❑ Detailed exam
 - ❑ Down Syndrome, other trisomies
 - ❑ Cardiac, renal, spinal, limb, brain deformities
 - ❑ Cleft lip/palate

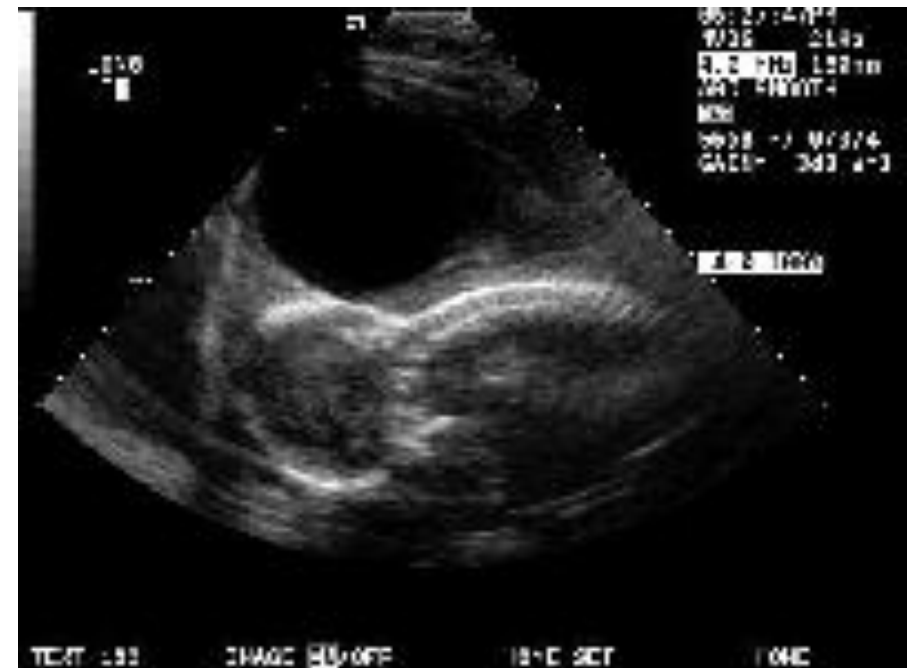
Ultrasound Abnormalities



Echogenic bowels



Ultrasound Abnormalities



First Trimester Chromosome Screening

- Non-invasive prenatal testing (NIPT)
- Detection of fetal cells in maternal blood
- 10 weeks
- Chromosomes 21, 18, 13
- “PLUS”: 22, 16, 22q, 15q, 11q, 8q, 5p, 4p
- Will ultimately replace invasive tests

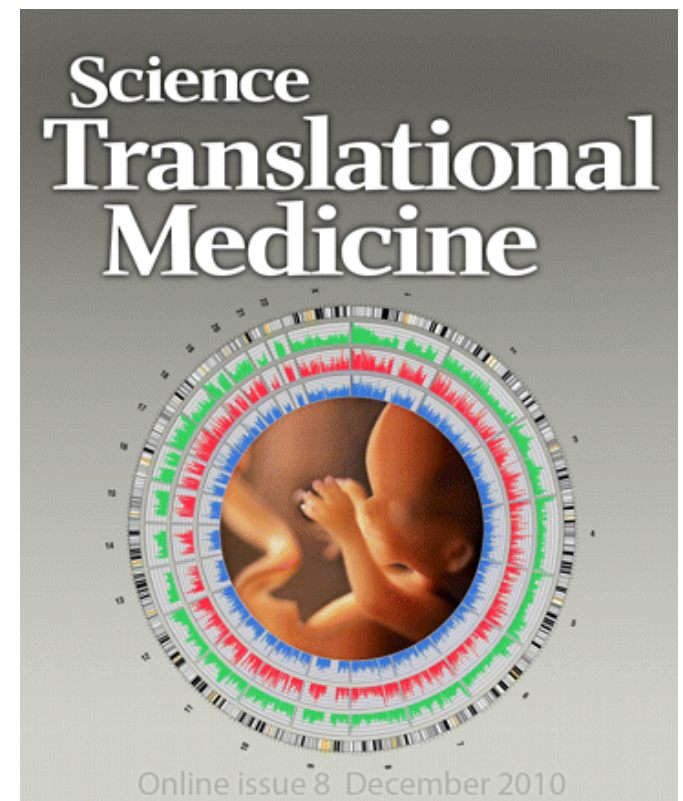
Maternal Plasma DNA Sequencing Reveals the Genome-Wide Genetic and Mutational Profile of the Fetus

Science Translational Medicine, December 8, 2010 (61,1-12)

Sequencing DNA From the Blood of a Pregnant Woman Allows the Complete Genome Of the Fetus to Be Decoded!

~10% of DNA in Maternal Plasma is From the Fetus

A New Era in DNA Testing!!



Prenatal Tests - Serum

- 15-20 weeks gestation
- Quad Screen
 - ❑ Tests for AFP, hCG, uE3 and inhibin A
 - ❑ Neural tube defects, Down syndrome, Trisomy 18, Abdominal wall defects
 - ❑ Readjusts age-related risks

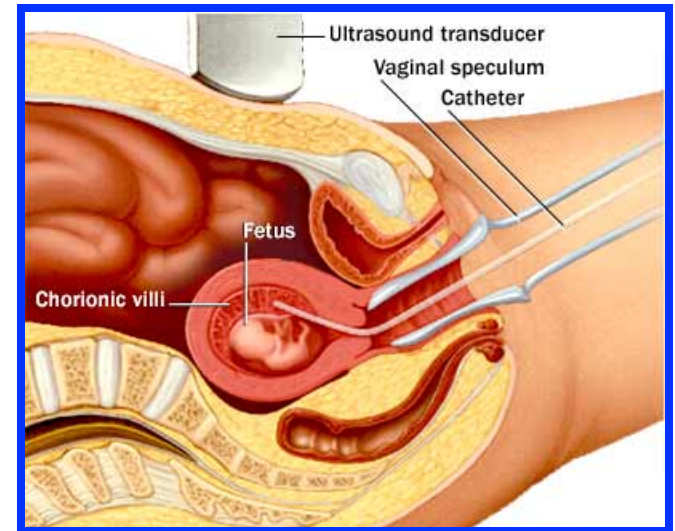
Prenatal Tests

- **Who is offered further testing?**
 - ❑ Advanced maternal age
 - ❑ Previous child or pregnancy with birth defect
 - ❑ Suggestive screening test results
 - ❑ Family history
 - ❑ Positive parental genetic screening

Prenatal Tests – CVS

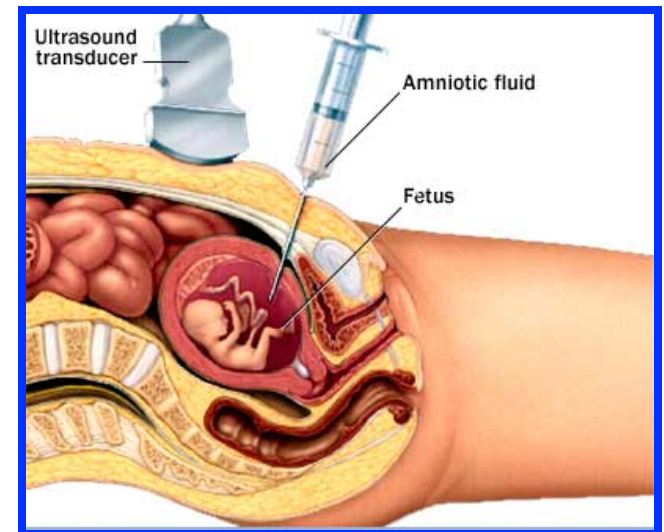
- **Chorionic Villus Sampling**

- ❑ 11-13 weeks gestation
- ❑ Catheter/needle biopsy of placental cells
- ❑ Performed through cervix or abdomen
- ❑ Can test for chromosome and gene defects
- ❑ 1-2% miscarriage rate
- ❑ Digit/limb deficiencies (10 weeks)



Prenatal Tests - Amniocentesis

- Performed at 15-18 weeks gestation
- 10 cc amniotic fluid
- Living cells from fetus in amniotic fluid
- Cells grown in lab for 1-2 weeks
- Results in 3 weeks
- Chromosome and gene defects



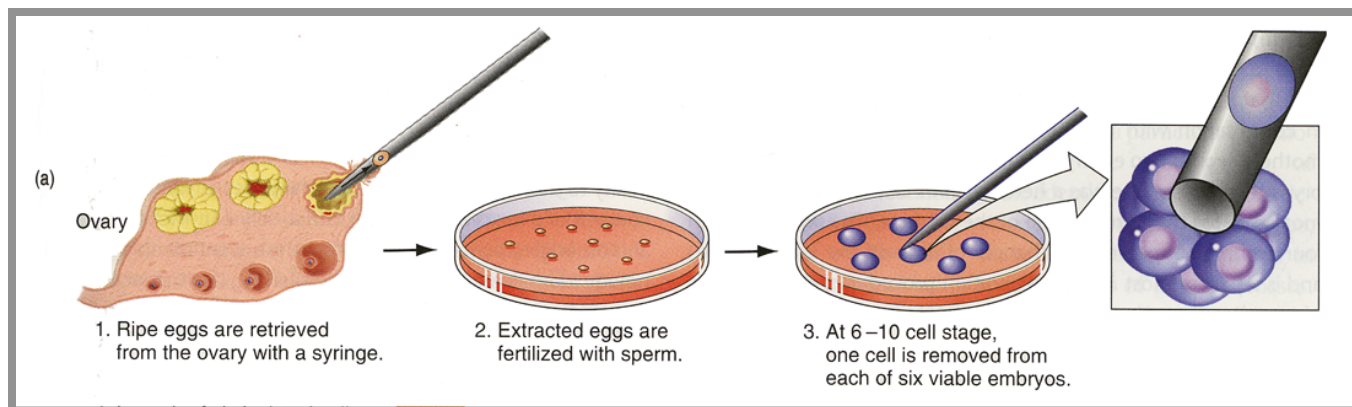
Postnatal Testing

- Most done during first day of life
- Heel stick
- California (mandatory)
 - ❑ Galactosemia
 - ❑ Hypothyroidism (congenital)
 - ❑ Phenylketonuria (PKU)
 - ❑ Sickle Cell Disease (SCD) and Hemoglobinopathies
 - ❑ + 70 Others as of January, 2012

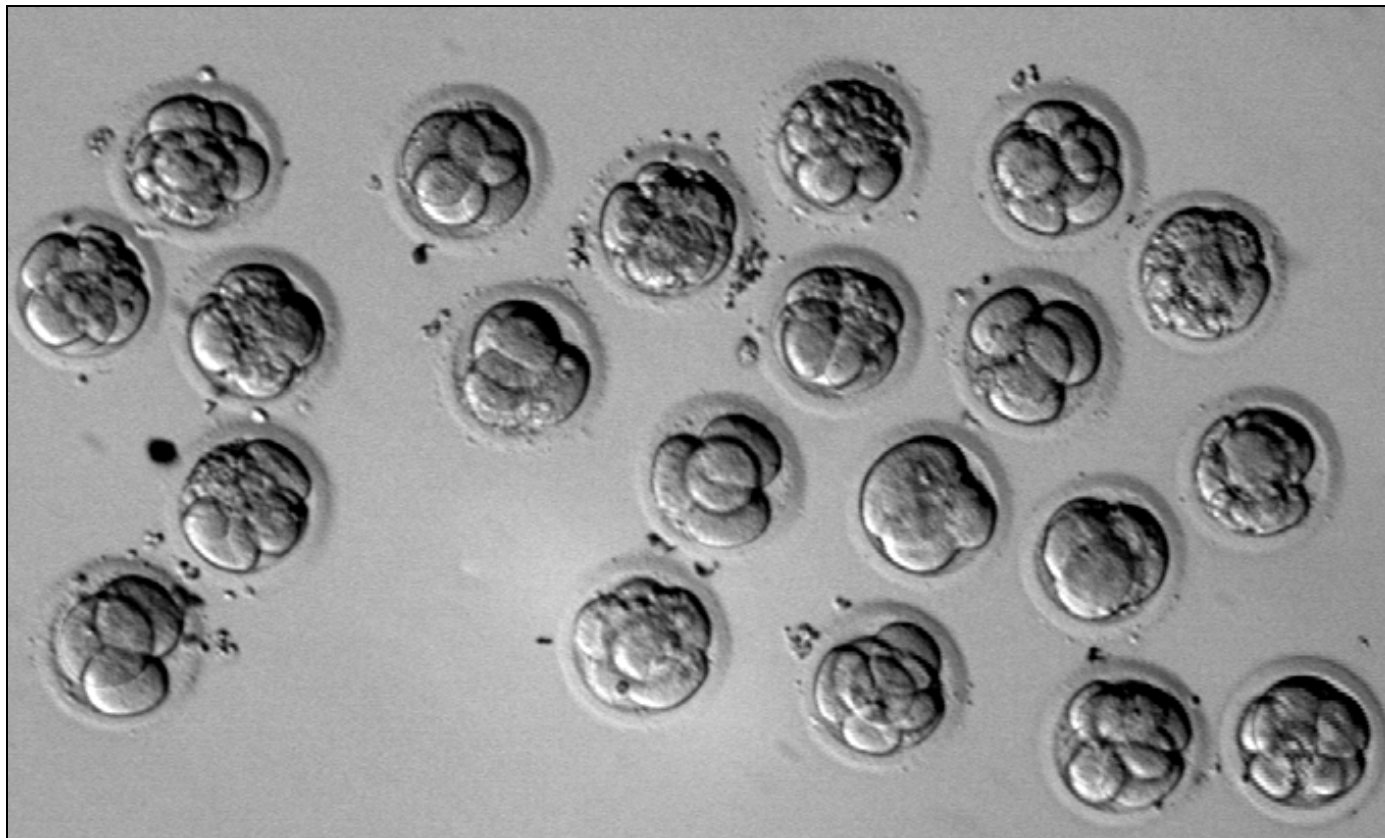
Preimplantation Genetic Diagnosis (PGD)

Preimplantation Genetic Screening (PGS)

- Can test embryos for genetic abnormalities prior to implantation
- First used in 1990 to choose female infant in a family with X-linked disease



Which Embryo is Disease-Free?



PGS – Clinical Indications

- Single gene defects (PGD – diagnosis)
- Balanced translocations
- Advanced maternal age (aneuploidy)
- Repetitive IVF failure
- Recurrent pregnancy loss
- Gender selection

PGS

- **Aneuploidy screening**
 - Next Generation (Next-Gen) Sequencing
 - Micro-array technology comparing DNA in embryo sample to known normal control using thousands of genetic markers

PGD

- **Single Gene Disorders - Methodology**
 - Genetic report documenting specific gene mutation
 - Blood tests from key family members
 - Design molecular DNA test in 4 weeks
 - Uses gene chip microarray analysis

Genetic Disorders Assayed by PGS & Gene Chips

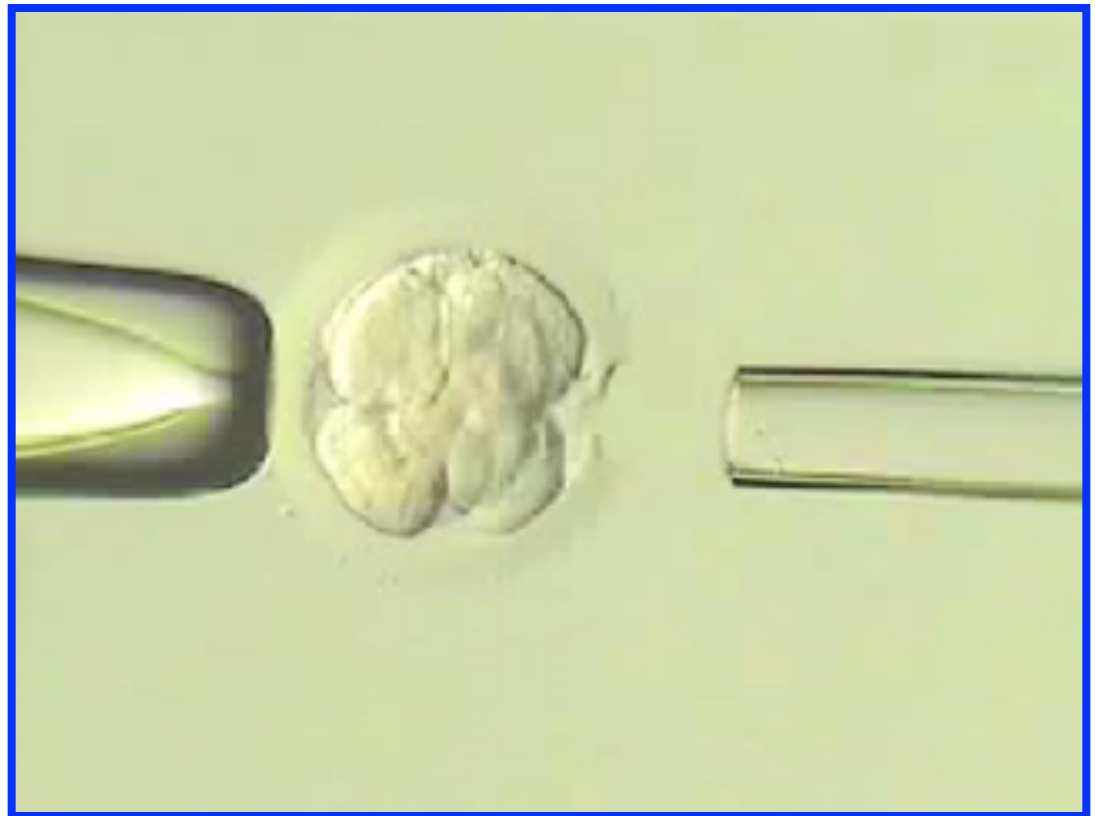
- Achondroplasia
- ADPKD1
- ADPKD2
- Adrenoleukodystroph
- Age-related aneuploidies
- Alpha-thalassemia
- Alpha-1-antitrypsin
- Alport disease
- Amyloid precursor protein (APP) mutation
- ARPKD
- Becker muscular dystrophy
- Beta-thalassemia
- Charcot Marie Tooth disease
- Chromosomal translocations
- Congenital adrenal hyperplasia
- Cystic fibrosis
- Down syndrome
- Duchenne muscular dystrophy
- Dystonia
- Epidermolysis bullosa
- Familial dysautonomia
- Fanconi anemia
- FAP
- Fragile X syndrome
- Gaucher disease
- Hemophilia A and B
- HLA genotyping
- HSNF5 mutation
- Huntington disease
- Hypophosphatasia
- Incontinentia pigmenti
- Kell disease
- Klinefelter syndrome
- LCHAD
- Lesch Nyhan syndrome
- Marfan syndrome
- Multiple epiphyseal dysplasia
- Myotonic dystrophy
- Myotubular myopathy
- NF1 and NF2
- Norrie disease
- Osteogenesis imperfecta
- OTC deficiency
- P53 mutations
- PKU
- Retinitis pigmentosa
- SCA6
- Sickle cell anemia
- Sonic hedgehog mutations
- Spinal muscular atrophy (SMA)
- Tay-Sachs disease
- Tuberous sclerosis
- Turner syndrome
- Von Hippel Lindau
- X-linked hydrocephaly
- X-linked hyper IgM syndrome

PGD for Single Gene Disorders - Advantages

- Safer than elective termination
- More psychologically acceptable for couples
- Provides couples with another option
 - ❑ Adoption
 - ❑ Sterilization
 - ❑ Donor gametes

PGD

- *Pre-implantation genetic diagnosis (PGD)* has been successfully used in diagnosing and preventing inherited genetic diseases like Cystic Fibrosis, Tay Sachs, Thalassemia, Sickle Cell Anemia and may be potentially used to screen for cancer mutations.



PGD

- After a cycle of in-vitro fertilization, biopsy of multiple cells can be performed from a day 5 embryo



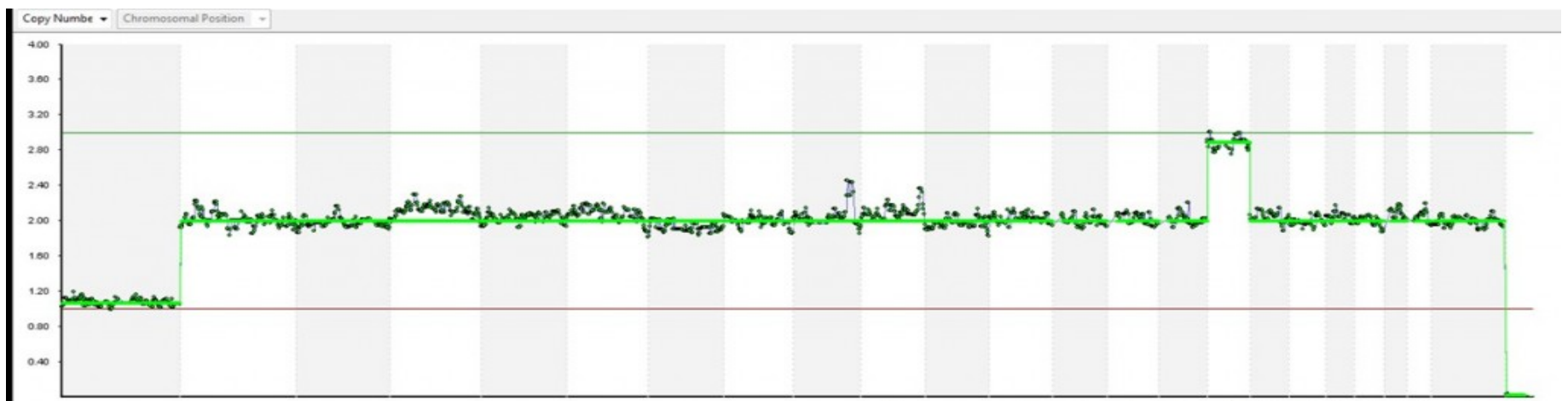
PGD

- Day 5 Blastocyst stage
- Trophectoderm biopsy



PGD and PGS

- The genetic material is then amplified and undergoes next-Generation sequencing (whole genome sequencing) or specific single gene detection with probe and microarray



PGD

- The embryos are frozen in the laboratory, awaiting genetic analysis, and confirmation of which embryos are unaffected with the mutation or aneuploidy.



PGD

- The unaffected embryo(s) are then transferred to the uterus in the following cycle. Subsequently, a child would be born unaffected from the screened genetic disease.



Summary: Who Would Benefit From PGS/PGD?

Couples with a history of --

- Abnormal numbers of chromosomes
- Single gene disorders
- Balanced translocations
- Sex-linked diseases

Couples who --

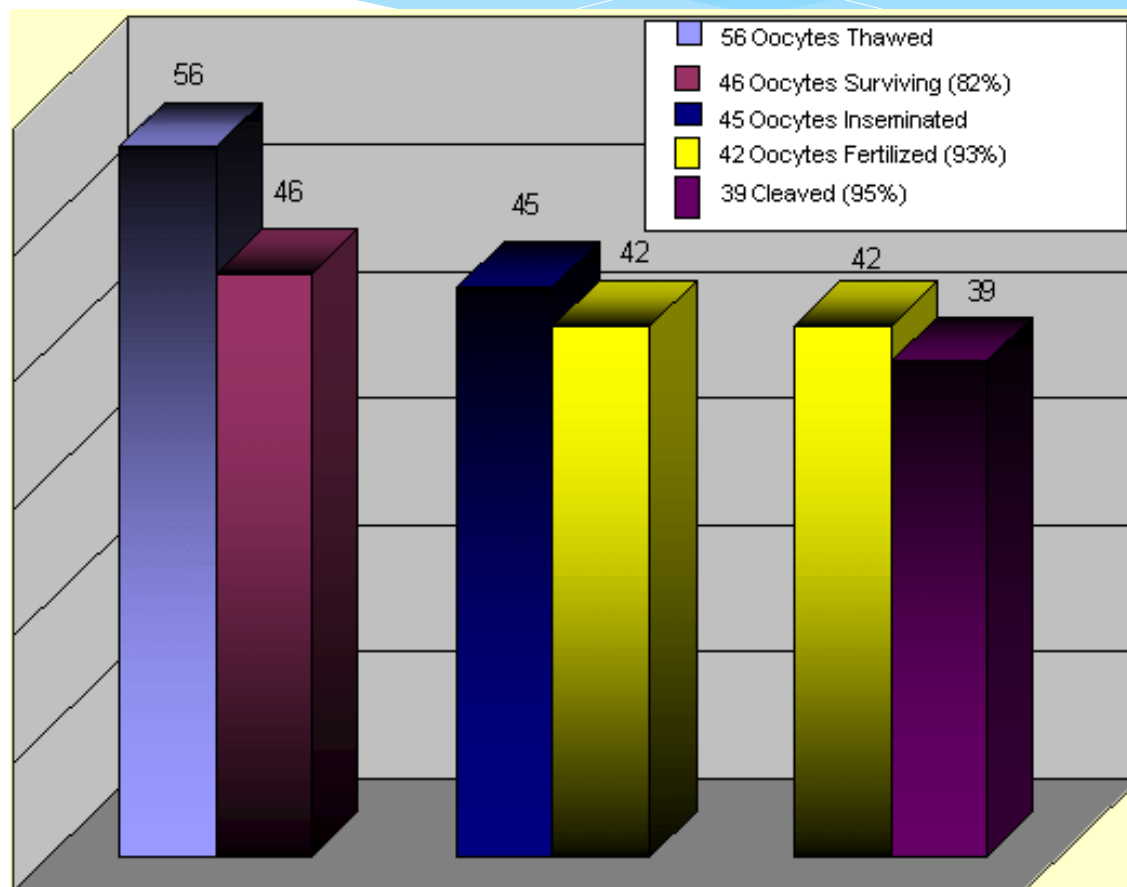
- Desire an offspring of a certain gender



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“Three Parent” IVF

- Gene Replacement for Prevention of Inherited Mitochondrial Disease
- Mitochondrial disease
 - Due to mutations in mitochondrial DNA (mtDNA)
 - mtDNA is maternally inherited
 - Affects tissues with high metabolic demand – heart, brain, Central nervous system, muscles, liver, kidney
 - One in 4,000 children in U.S. are affected

“Three Parent” IVF

- Approved for use in the United Kingdom in February, 2015
- Technique
 - Take the nucleus of one egg (parent) and insert it into the cytoplasm of another egg (donor) which has had its nucleus removed, but still contains mitochondrial DNA
 - Fertilize the hybrid egg with a sperm (parent)

The Future

- Mitochondrial replacement Therapy (MRT)
 - Three parent IVF
- “Designer babies”
- Making gametes from stem cells
- Gene Therapy – correcting genetic disorders

Thank you

