Infertility, In Vitro Fertilization (IVF) and Genetic Testing

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HRC
"Bringing Dreams to Life Since 1988"

HUNTINGTON Reproductive Center
MEDICAL GROUP
REPRODUCTIVE ENDOCRINOLOGY & INFERTILITY

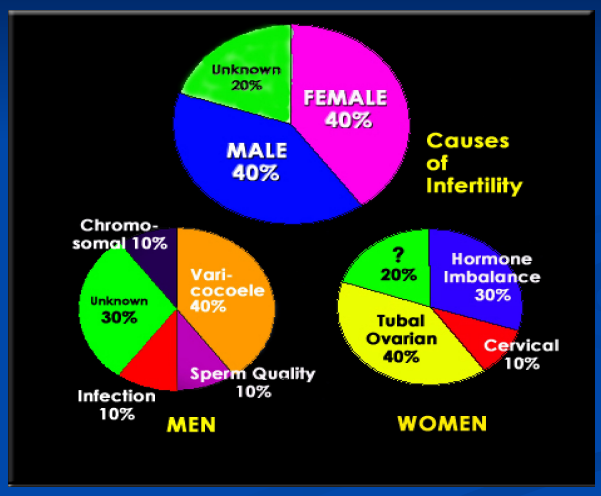
History of Infertility

- First written document dealing with infertility may be found in the Kahoun papyrus (oldest Egyptian medical text), dated to 2200-1950 BC
- The early attribution of infertility to women persisted for centuries and was often considered divine punishment
- The only solution to infertility was prayer – to Egyptian Isis, Phoenician Astarte, Greek Aphrodite, or Roman Venus
- The role of the human male in reproduction was not understood in past civilizations and is still veiled in mystery for many of the primitive tribes

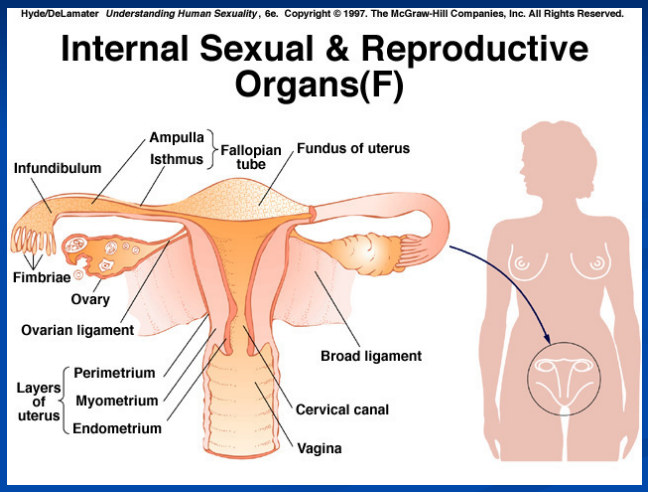
Overview of Infertility

- Definition: 1 year of well-timed, unprotected intercourse without a pregnancy
- 10-15% of population is infertile
- 15-20 % of couples have unexplained infertility (work-up is negative)

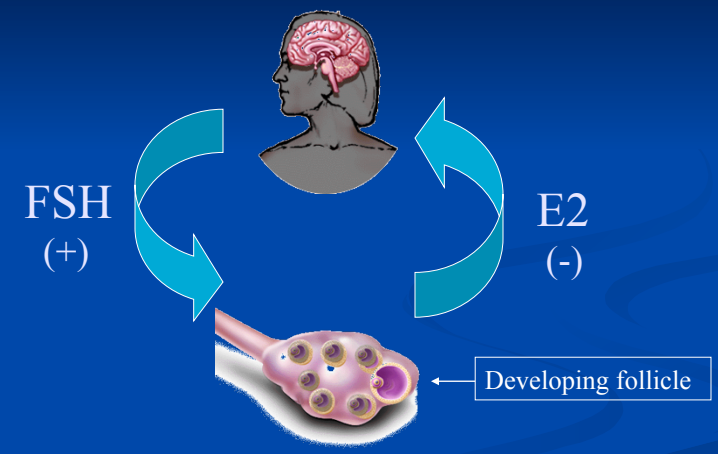
Causes of Infertility



Female Reproductive Organs



Physiology



FSH=Follicle Stimulating Hormone

E2=Estradiol

Ovary - Female Age

- Women are born with their lifetime egg supply
  - 4 million at 20 weeks gestation
  - 400,000 at birth
- 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

Causes of Female Infertility

- Ovary
- Tubes
- Uterus
- Cervix
- Hormones
- Chromosomes

Causes of Female Infertility - Ovary

- Age
- Problems with ovulation
- Premature ovarian failure

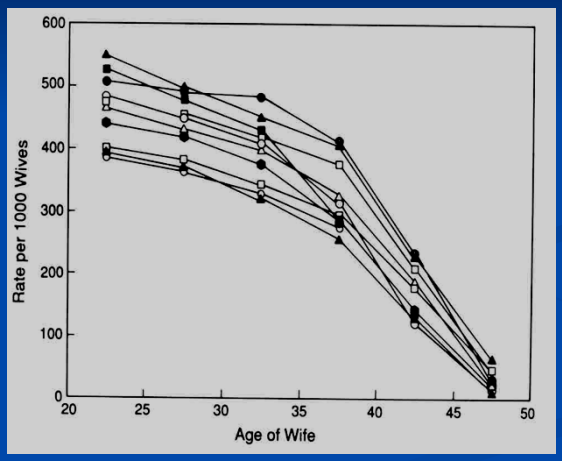
Percentage of Married Women Who are Infertile

From 3 national U.S. surveys

Age (years)	Infertile
20-24	7.0
25-29	8.9
30-34	14.6
35-39	21.9
40-44	28.7

Menken et al, Science 1989;23:1389

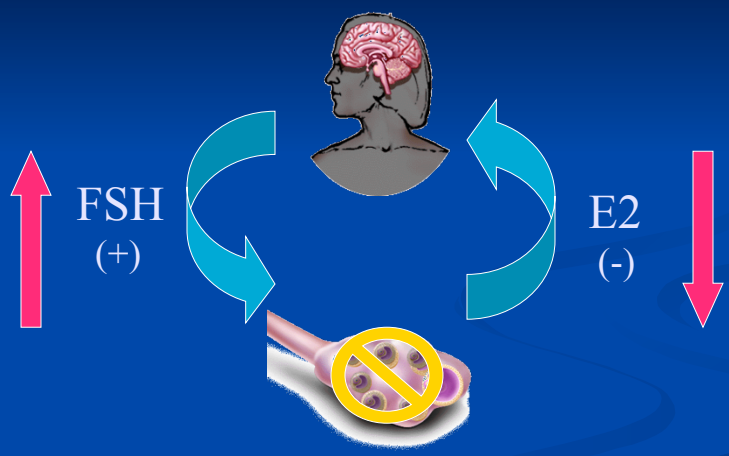
Fertility and age: natural populations



Marital fertility rates in natural populations (no contraception) as a function of age of wife

Science 1986;23:1389

Effects of Aging on the Ovary



FSH=Follicle Stimulating Hormone

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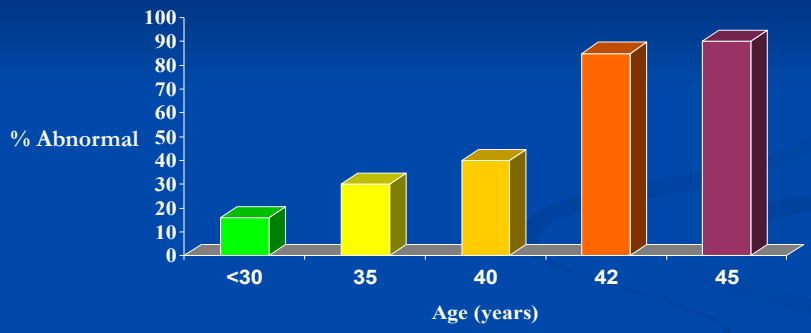
Why does fertility decline with increasing maternal age?

- Decline in the *number* of eggs
  - Every month there is loss of a group of eggs
- Decline in the *quality* of eggs
  - As the egg ages, errors in the dividing embryo increase
  - These errors may result in aneuploidy (an incorrect number of chromosomes)

Prevalence of genetically abnormal oocytes in infertile women



Ovary - Causes of Anovulation

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

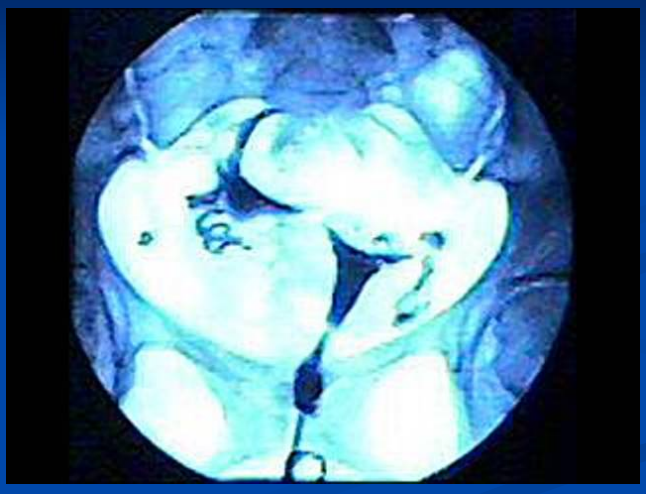
Ovary – Premature Ovarian Failure

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

Causes of Female Infertility – Fallopian Tubes

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

Open Tubes

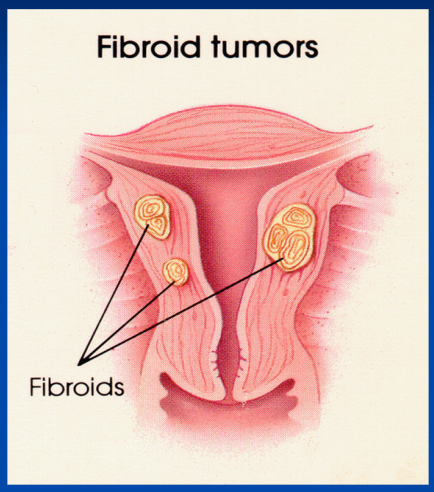


Blocked Tubes

Female Infertility - Uterus

- Uterus
  - Fibroids
  - Polyps

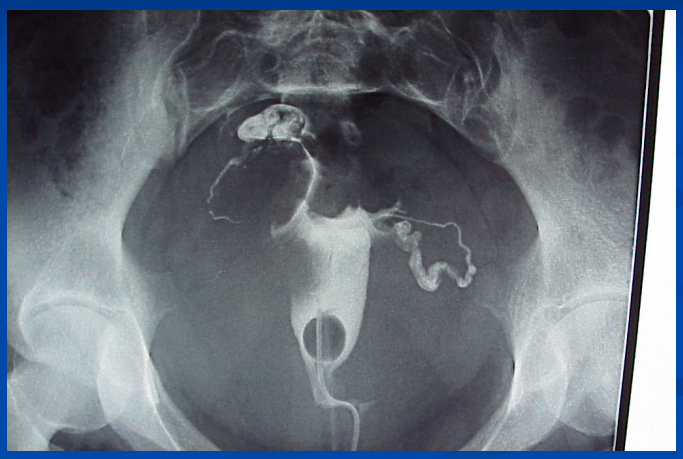
Female Infertility



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

Normal Shape of Uterus

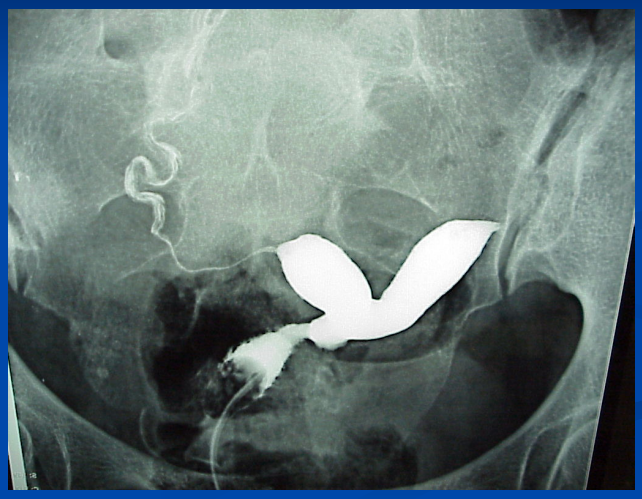
Fibroid Uterus



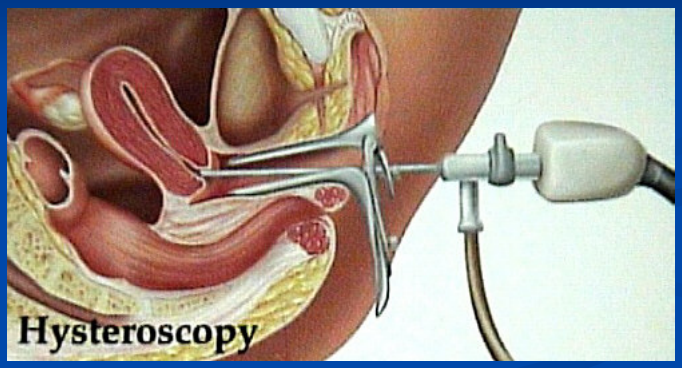
Female Infertility - Uterus

- Mullerian defects (congenital)
  - Absent uterus
  - Bicornuate/septate

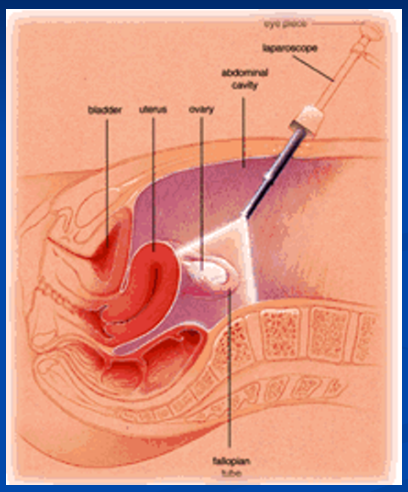
Mullerian Defect



Treatment with Hysteroscopy



Treatment with Laparoscopy



Female Infertility - Cervix

- Cervix
  - Post-surgical
    - Stenosis
    - Mucus changes

Female Infertility - Hormones

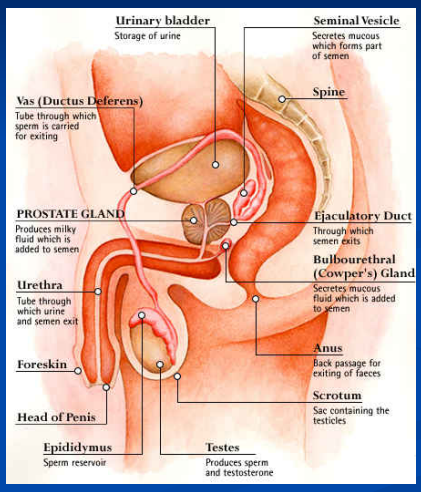
- Endocrine abnormality (hormones)
  - Thyroid
  - Prolactin
  - Polycystic ovary syndrome (PCOS)
    - Estrogen, insulin
  - Hypothalamic hypogonadism
    - Stress
    - Exercise (athlete)

Other Causes of Female Infertility

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

Sperm Are Also Required!!

Male Reproductive Organs

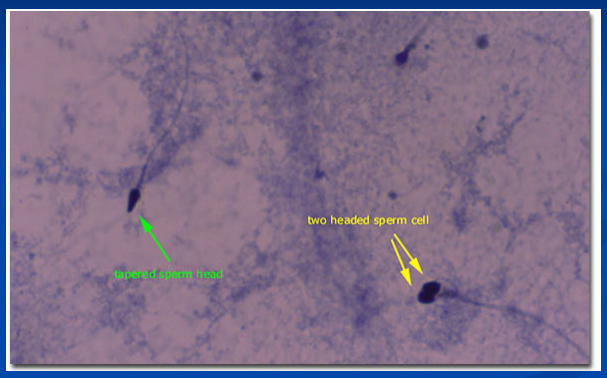


Causes of Male Infertility

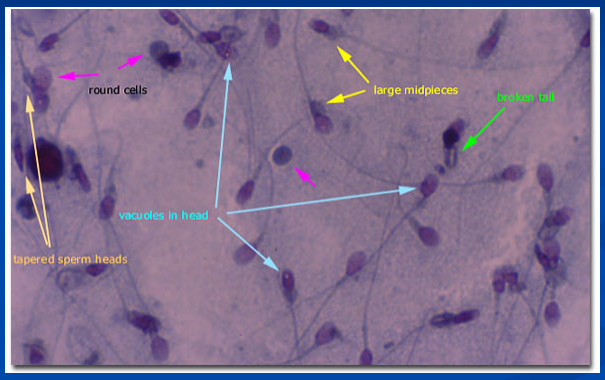
- Abnormality in sperm production
- Abnormality in sperm function
- Obstruction in the ductal system

Normal Sperm Morphology

Abnormal 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

Abnormalities of Sperm Production

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

Abnormalities of Sperm Function

- Antisperm antibodies
- Genital tract inflammation
  - Prostatitis
- Varicocele
- Failure of acrosome reaction
- Problems with sperm binding/penetration

Obstructions in Ductal System

- Vasectomy
- Congenital bilateral absence of the vas deferens
- Epididymis/ejaculatory ducts
  - Congenital or acquired

Male Infertility - Lifestyle

- Tobacco
- Marijuana
- Alcohol
- Cocaine
- Steroids (can be permanent)
- Heat
- Exercise

Infertility: Initial Evaluation

- Eggs
  - Ovulation (Progesterone)
  - Egg quality (FSH, Estradiol)
- Sperm
  - Presence
  - Quality
- Gamete transport/Implantation
  - Hysterosalpingogram

Sperm: Semen Analysis

- Volume: ≥ 2 mL
- Concentration: ≥ 20,000,000 per mL
- Motility: ≥ 50%
- Normal morphology: ≥ 40% normal
  - Krueger strict criteria: ≥ 14% normal
  - Best predictor of fertilizing ability

Infertility Treatments

- Improve Timing of Intercourse
- Intrauterine insemination (IUI)
  - Clomiphene citrate (Clomid) + IUI
  - FSH + IUI
- In Vitro Fertilization (IVF)
  - "Standard" IVF
  - Egg donation + IVF
  - Egg Freezing + IVF

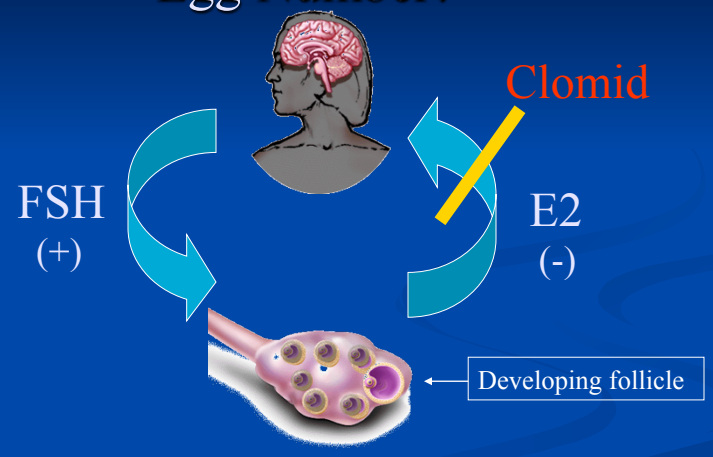
Infertility Treatment Options

- IUI, FSH or FSH + IUI
- Patients with unexplained infertility

Treatment	Cycles	Pregnancy	Pregnancy per cycle
IUI	30	1	2.7%
FSH	49	3	6.1%
FSH+IUI	34	9	26.4%

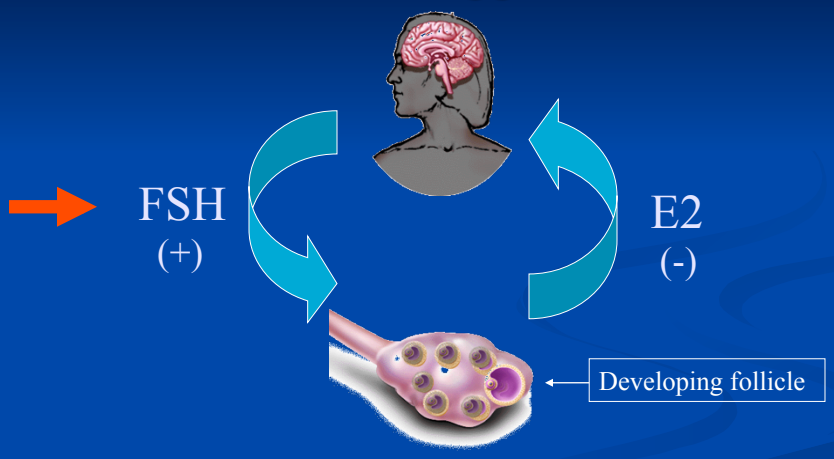
Serhall et al, Fertil Steril 1988;49:602

How Does Clomid Work To Increase Egg Number?



FSH=Follicle Stimulating Hormone E2=Estradiol

How Does Recombinant FSH Work To Increase Egg Number?



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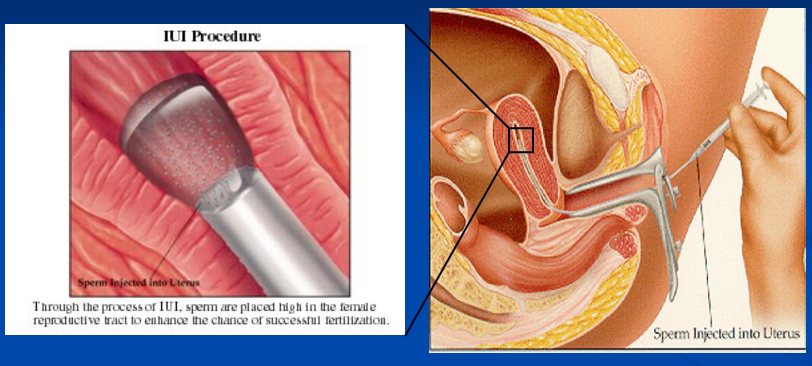
Intrauterine Insemination (IUI)



Goal is to Maximize the Chance of Fertilization

- **Increase Number of Eggs**
- **Position Sperm Closer to Eggs**

In Vitro Fertilization

Historical Perspective

- 1978 Louise Joy Brown, first IVF baby
- 1981 Elizabeth Carr, first IVF baby in USA
- 1983 First birth after egg donation
- 1985 First birth from cryopreserved embryo
- 1985 Transvaginal ultrasound for follicle monitoring
- 1990 First report of births after PGD
- 1990 First report of egg donation to older mothers
- 1992 First human birth after ICSI

Who Needs IVF?

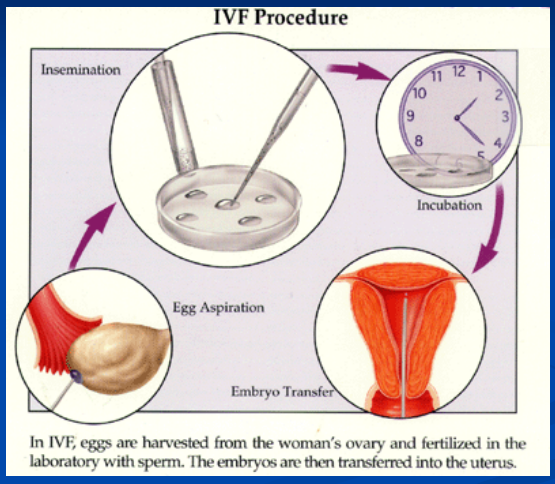
- Failed other treatments
- Tubal damage
- Significant male factor
- Absent uterus
- Carriers of genetic diseases
- Family Balancing
- Cancer patients
- Non-traditional Lifestyles

IVF Statistics - 2005

- 422 U.S. programs offer IVF
- 134,260 cycles of ART treatment
- 9,649 donor oocyte cycles
- 38,910 deliveries (birth of 52,041 neonates)

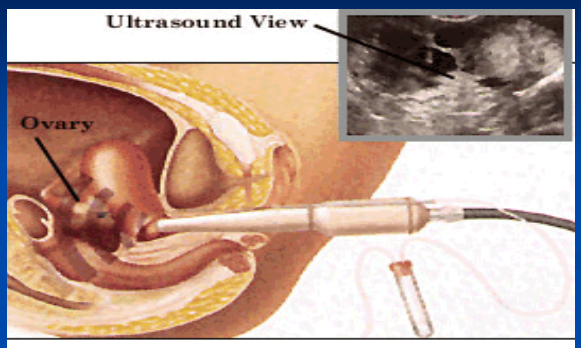
CDC 2005 National Report

In Vitro Fertilization (IVF)



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.

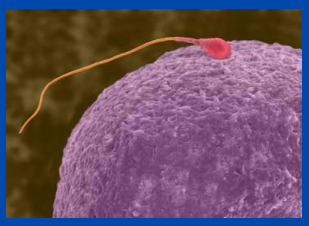
Egg Retrieval

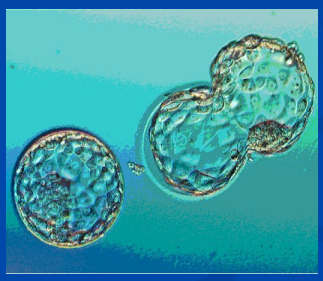


Using ultrasound to view the ovary, the physician inserts the needle that runs alongside the ultrasound probe through the wall of the vagina into the ovary and removes the egg for use in IVF or GIFT.

Egg Retrieval

Fertilization

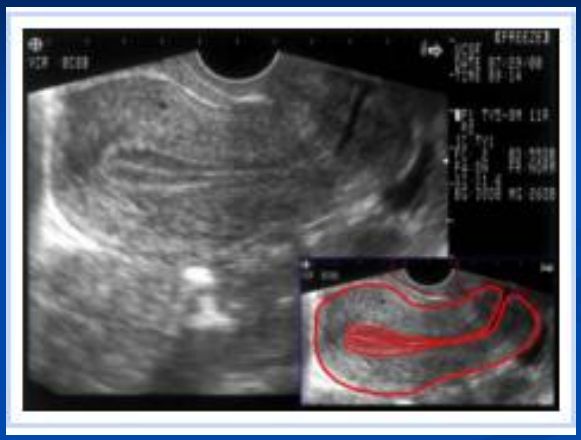




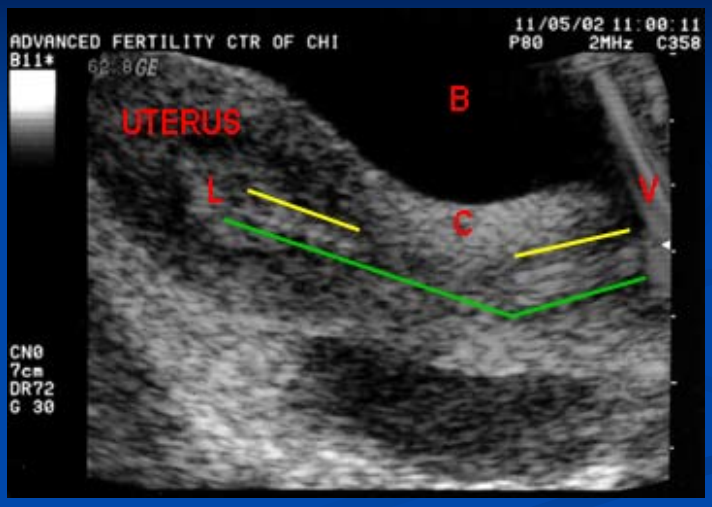
Fertilization

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

Embryo Transfer



Embryo Transfer



How Many Embryos are Transferred?

- Related to age and embryo quality
  - <35 = 2
  - 35-37 = 2-3
  - 38-40 = 3-4
  - >40 = up to 5
  - For patients with 2 or more failed IVF cycles, or a poor prognosis, can add more based on clinical judgement

What Happens to the Other Embryos?

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

Special IVF Procedures

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

Assisted Hatching

Intracytoplasmic Sperm Injection (ICSI)

IVF Success Rates - 2005

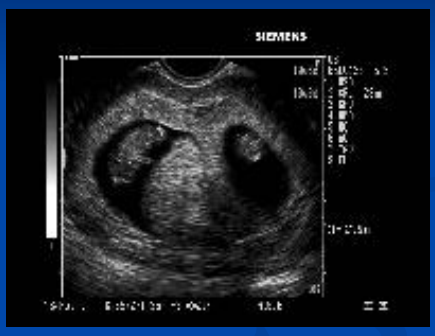
- U.S. Fertility Centers (SART/CDC)
- Female age
  - <35 – 37% (43%)
  - 35-37 – 30% (36%)
  - 38-40 – 20% (27%)
  - >40 – 11% (18%)

IVF Statistics - 2005

- 65.1% singletons
- 32.9% twins
- 4.4% triplets or more

Singleton Pregnancy

Twin Pregnancy

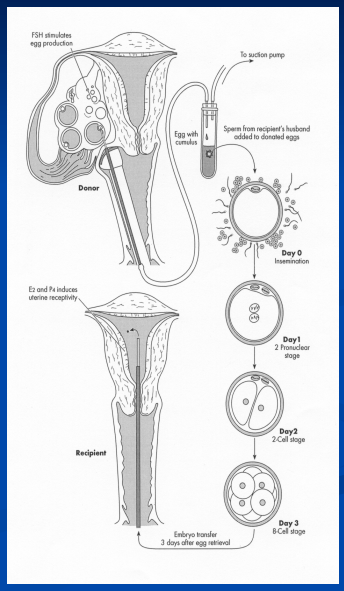


Triplet Pregnancy

Egg Donation

Egg donation

- IVF for two
  - Known/anonymous donor
- <35 years old
- Donor
  - Standard controlled ovarian hyperstimulation
  - Egg retrieval
- Recipient
  - Embryo transfer



Who are candidates to be an egg donor ?

- 21-35 years old (older if a friend or relative)
- FSH <10
- Negative donor
- Good health and genetic history
- Preferably prior egg donation experience
  - How many eggs were produced?
  - Did pregnancy result?

Who are candidates for egg donation ?

- Premature ovarian failure
- Ovarian insufficiency (e.g. FSH>15)
- Physiologic menopause
- Maternal age over 43
- History of poor egg/embryo quality or multiple IVF failures

Egg Donation

- October 23, 1999
- Selling Fashion Models' Eggs Online Raises Ethics Issues
- By CAREY GOLDBERG

- CAMBRIDGE, Mass. -- To the horror and disgust of mainstream infertility groups, a longtime fashion photographer has begun offering up models as egg donors to the highest bidders, auctioning their ova via the Internet to would-be parents willing to pay up to $150,000 in hopes of having a beautiful child.

Egg Donation

Grade A: The Market for a Yale Woman's Eggs

When a Yale undergraduate explored becoming an egg donor for a wealthy couple willing to pay top dollar to the right candidate, she didn't realize how unsettling the process of candidacy would prove to be

by Jessica Cohen

Gestational Surrogacy: Indications

- Absent uterus; congenital or iatrogenic
- Abnormal uterus
- Medical contraindication to pregnancy
- Recurrent pregnancy wastage
- Repeated IVF failures with good embryos

Gestational Carriers

- Friends or relatives
- Agencies
- Age less important
- Previous delivery and parenting
- Benign obstetrical history
- Major psychological evaluation
- Screening similar to recipients

Initial screening-Egg and Sperm Donors (Known and Anonymous)

- Medical History (especially past history of donation in the case of egg donors so protocol and outcome can be reviewed)
- Physical and Clinical Assessment
- Genetic Testing (as required based on Ethnic background and Family and Medical History)
- Serologic (lab) Testing, STD tests, Drug Screening
- Sexual partners of donors are screened for infectious diseases when donors are being evaluated for eligibility
- Screening results for donors are good for 6 months only

Anonymous Egg Donation

Psychocologic Evaluation

Personality Profile administered and evaluated by psychologist (PhD) or licensed social worker trained to work with donors with regards to donor's ability to "meet the demands of the egg donor role". Report sent to MD's office.

Legal Contract

Sent to MD's office stating that the Egg Donation Agreement has been "executed by all parties".

Known Egg/Sperm Donors

- Screenings set up through a specific agency
- Links known donors with psychologists and lawyers trained to perform above assessments and execute necessary legal contracts
- Psych and legal assessment and clearance are especially important between known donors and recipients in which family relations or friendships may cloud the boundaries and guidelines necessary for a successful donor relationship and process

How old is too old?

- Danger to mother
- Decreased life expectancy of parents
- Quality of parenting
- Is 55 a "physiological limit"?

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

Paulson, Tourgeman, Boostanfar et al, JAMA 2002:228;2320.

Pregnancy in the Sixth Decade of Life: Obstetric Complications

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

Pregnancy in the 6th decade of life: Conclusion

- There does not appear to be any definitive medical reason for excluding these women from attempting pregnancy on the basis of age alone

Genetic Testing

- Preconception
- Preimplantation
- Prenatal
- Postnatal

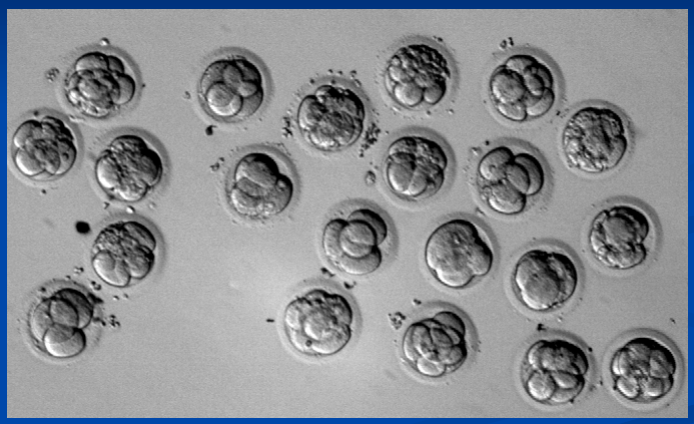
Preimplantation Genetic Diagnosis (PGD)

- Can test embryos for genetic abnormalities prior to implantation
- Has been successfully used in diagnosing and preventing inherited genetic diseases like Cystic Fibrosis, Tay Sach's, Thalassemia, Sickle Cell Anemia and may be potentially used to screen for cancer mutations.
- Uses single cell (blastomere) at 8-cell stage

PGD

- First clinical application described by Handyside, Winston, and Hughes in 1990
- By 2003, estimated >1000 PGD-defined live births (ESHRE Task Force, 2003)

Which Embryo is Disease-Free?

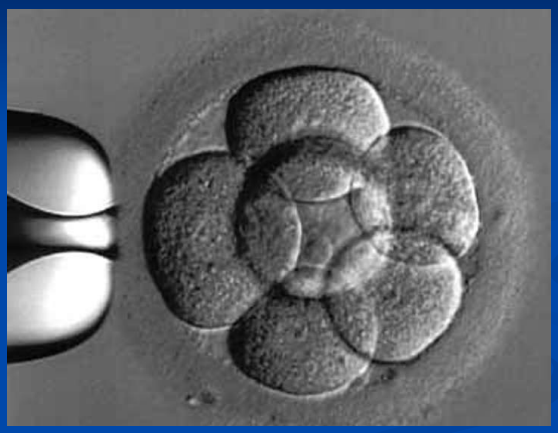


PGD – Clinical Indications

- Single gene defects
- Balanced translocations
- Advanced maternal age (aneuploidy)
- Repetitive IVF failure
- Recurrent pregnancy loss
- Embryo selection

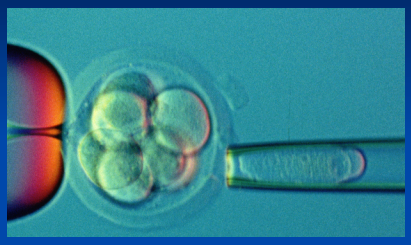
- Achondroplasia
- ADPKD1
- ADPKD2
- Adrenoleukodystroph
- Age-related aneuploidies
- Alpha-thalassemia
- Alpha-1-antitrypsin
- Alport disease
- Amyloid precursor protein (APP) mutation
- ARPKD
- Becker muscular dystrophy
- Beta-thalassemia
- Charcot Marie Tooth disease
- Chromosomal translocations
- Congenital adrenal hyperplasia
- Cystic fibrosis
- Down syndrome
- Duchenne muscular dystophy
- 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 epiphysial dysplasia
- Myotonic dystophy
- 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

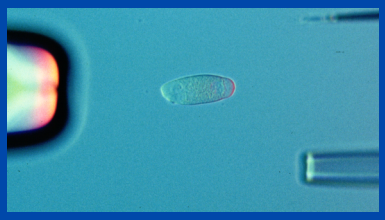
Day 3 Embryo



Pre-Implantation Genetic Testing Stage

PGD – Timing of Biopsy





- Biopsy of a single cell can be performed from an 8-cell embryo after 3 days of culture in the laboratory

PGD

- Fluorescence in situ hybridization (FISH)
  - Aneuploidy/translocations and determining gender (5-10 chromosomes)
- Polymerase chain reaction (PCR)
  - Specific single gene disorders

PGD

- The embryos would continue to grow for 2 more days in the laboratory, awaiting genetic analysis.
- The unaffected embryos are then transferred to the uterus at the blastocyst stage on day 5 of embryo culture and subsequently a child would be born unaffected from the screened genetic disease.

PGD Chromosome Panels

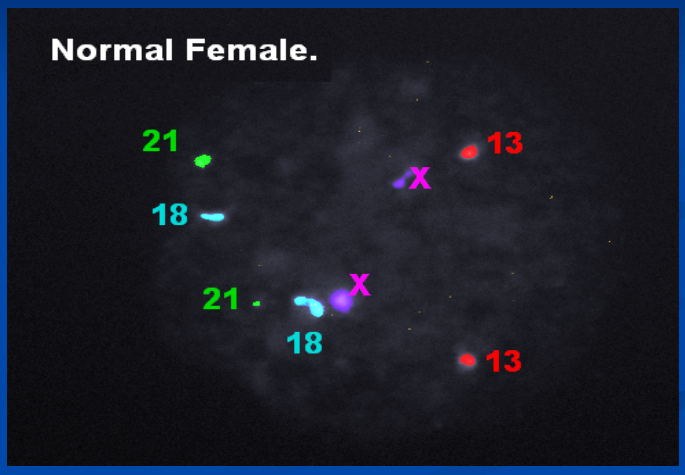
Five Chromosome PGD

- 13, 18, 21, X, Y

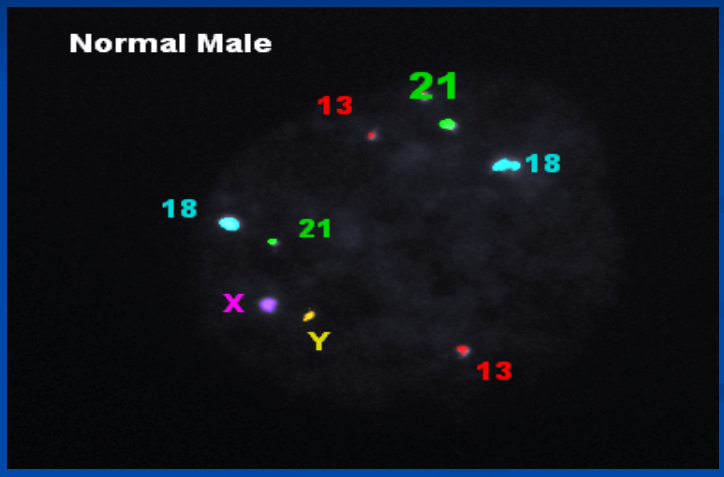
Ten Chromosome PGD

- 8, 9, 13, 15, 16, 18, 21, 22, X, Y

Five Chromosome PGD



Five Chromosome PGD



Ten Chromosome PGD

Prenatal vs. Preimplantation Diagnosis

	PND	PGD
Cells	>100,000	1
Time	2 weeks	6-10 hrs
Accuracy	99%	99%
Cost	Covered	~$5,000

PGD

Gender Selection:

A Big Controversy!

Future considerations

- Oocyte cryopreservation
  - "Pausing the biological clock"
- Cytoplasmic transfer
  - Donation of enucleated oocytes
- Reproduction without gametes
  - Use of nuclear material from somatic cells
  - Donated or synthetic cytoplasm
  - Reconstituted oocytes

WEST COAST FERTILITY CENTERS

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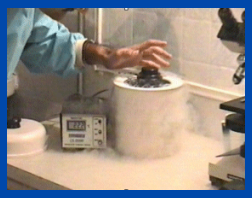
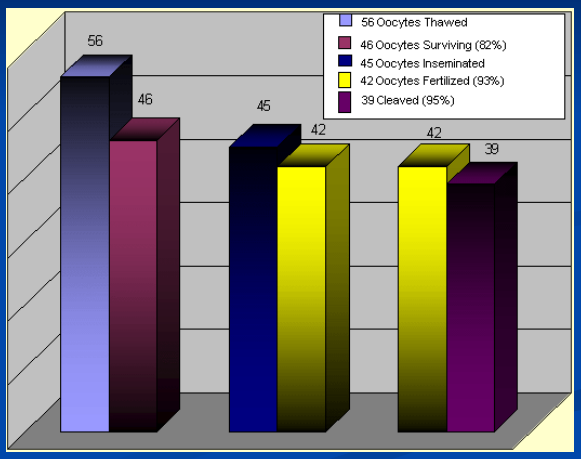
Egg Banking Now Available to Preserve Your Fertility

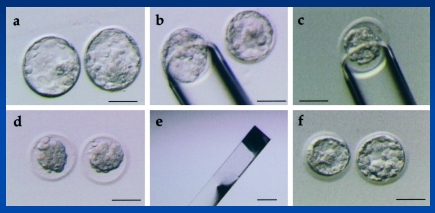


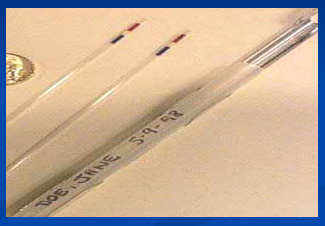


Clinical Applications of Egg Freezing

- Oocyte cryopreservation could be a clinical tool for:
  - Women at risk of losing ovarian function
  - Women desiring fertility preservation (e.g. delayed maternity)
  - Eliminating ethical concerns of embryo cryopreservation
  - Solving the dilemma of abandoned frozen embryos in the IVF laboratory

Oocyte Cryopreservation





- **Slow-freeze Technique**
- **Vitrification (Rapid Freeze) Technique**

Oocyte Freezing: Where do we stand?

- First pregnancy after oocyte cryopreservation was reported by Chen in 1986
- Less than 200 pregnancies have been achieved worldwide
- Survival rate of 80% and fertilization rates of 83%, however low pregnancy rates
- Although pregnancy rates might be improving, rates appear to be significantly less than those seen with standard IVF

Fertility Preservation Options

	Age	Average Cost	Time Requirement	Success Rate
Embryo Freezing	After Puberty	$7,800; $350/year storage fees	2-4 weeks	~40% per 3 embryos transferred under 35; lower in older women
Egg (Oocyte) Freezing	After Puberty	$8,000; $350/year storage fees	2-4 weeks	Experimental; ~3% per egg frozen
Ovarian Tissue Freezing	Before and After Puberty	$12,000; $350/ year storage fees	Outpatient Surgical Procedure	Experimental; no live births to date
Ovarian Transposition	Before or After Puberty	Unknown	Outpatient Surgical Procedure	~50% for ovarian function, pregnancy rates unknown
GnRH Analog Treatment	After Puberty	$500 per dose	1 dose per month in conjunction with chemotherapy	Experimental; study results vary: some show no benefit, others show success
Donor Eggs	Varies, usually 18-25+	$14,000-$20,000	2-4 weeks per cycle	ART with Egg Donation, 40-50%
Surrogacy	Varies, usually 18-25+	$10,000 - $100,000	Varies	Similar to IVF, 20-30%
Adoption	Varies, usually 18-25+	$2,500 - $35,000	Varies Greatly	Not Applicable

Thank you