
Genetic Testing Using Novel Technologies in ART
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Section 1
Genetic Testing and Male Infertility
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Learning Objectives

After completing this section, participants should better understand:

- Etiologies and risks of genetic abnormalities of infertile men
- Genetic and molecular causes of male infertility
- Genetic screening of male infertility
- Role of genetic counselling
- Influence of genetic testing on ART outcomes

Genomics Era – Tools for Further Studies

- 10% of genes in the human genome may be related to spermatogenesis and fertility
- 400 candidate genes may be responsible for male infertility
- The genetics of male infertility in the era of genomics: a tool for progress

Gersen SL, Keagle L, eds. *The Principles of Clinical Cytogenetics*. New York, NY: Humana Press; 1999.

Prevalence and Risk of Genetic Abnormality of Infertile Men

- Risk exists for miscarriages and having children with chromosomal, congenital defects
  - Men with azoospermia: 10-15%
  - Men with severe oligozoospermia (<5 million/mL): 5%
  - Men with normal sperm concentration: 1%
  - Sex chromosomal aneuploidy (Klinefelter syndrome 47,XXY): 1.5-7%
  - Structural autosomal abnormalities (inversions, balanced translocations): 2%

Practice Committee of American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. *Fertil Steril.* 2012;98:294-301.; Martin R. Sperm cell—genetic aspects. In: Grudzinskas JG, Yovich JL, Simpson JL, et al, eds. *Cambridge Reviews in Human Reproduction.* Cambridge, England: Cambridge University Press; 1995:104-121.

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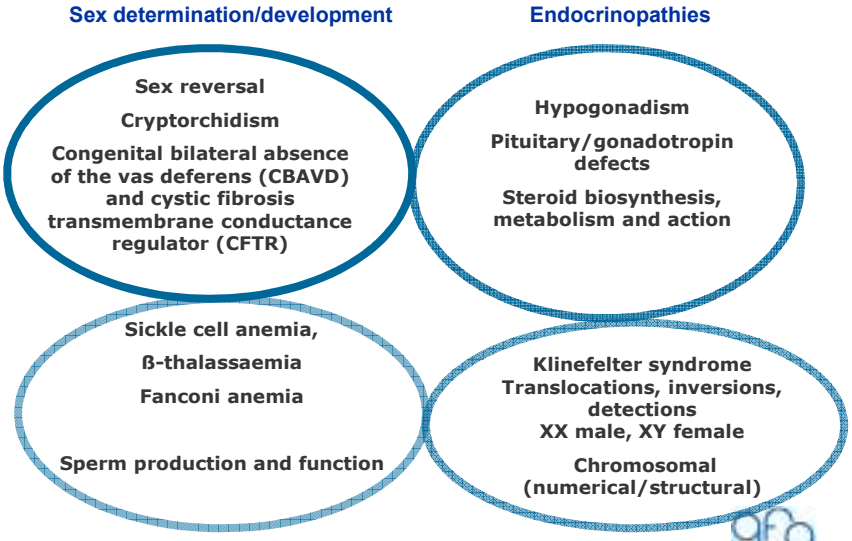
Genetic Etiologies of Male Infertility



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Abnormal karyotypes are less frequent if spermatogenesis is healthier

Sperm concentration	Abnormal karyotype
<20 million/mL	1.76%
>20 million/mL	<1%
>100 million/mL	0.2%

Intra-cytoplasmic sperm injection (ICSI) enables sperm resulting from severely defective spermatogenesis to bypass natural selection processes to initiate pregnancies.

Hirsh AV. The management of infertile men presenting in the assisted conception unit. In: Brinsden PR, ed. *A Textbook of In Vitro Fertilization and Assisted Reproduction*. Boca Raton, FL: CRC Press; 2005:35-60.

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Genetic or Molecular Causes of Male Infertility

- Numerical and structural chromosomal abnormalities
- Monogenetic disorders (cystic fibrosis, androgen receptor defects)
- Mitochondrial DNA mutations
- Multifactorial disorders (mutation in multiple genes often coupled with environmental factors)
- Infertile men with normal karyotype (including germinal mosaics)

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Numerical and Structural Chromosomal Abnormalities

- 47,XXY
- 47,XYY
- Translocations – Robertsonian
- Translocations – reciprocal
- Inversions: paracentric and pericentric
- Y chromosome microdeletion

Martin RH. Cytogenetic determinants of male infertility. *Hum Reprod Update.* 2008;14:379-390.

Klinefelter's Syndrome (47,XXY)

- Frequency of sex chromosome aneuploidy varies from 1.5-7% in sperm from Klinefelter mosaics and 2-45% in non-mosaic 47,XXY karyotype
- Spermatogenesis seems to eliminate extra chromosomes
- Patients are prime candidates for ICSI

Kruse R, Guttenbach M, Schartmann B, et al. Genetic counseling in a patient with XXY/XXXY/XY mosaic Klinefelter's syndrome: estimate of sex chromosome aberrations in sperm before intracytoplasmic sperm injection. *Fertil Steril.* 1998;69:482-485.

Outcome of ART in Klinefelter's Syndrome

- Desirable to eliminate embryos with sex chromosome aneuploidy through preimplantation genetic diagnosis (PGD)
- Recovery of testicular sperm from men with non-mosaic Klinefelter's syndrome (48%) was similar to other cases of non-azoospermia
- 36 healthy children were born after ICSI using sperm from non-mosaic Klinefelter's patients with only one 47,XXY fetus identified

Vernaeve V, Staessen C, Verheyen G, Van Steirteghem A, Devroe P, Tournaye H. Can biological or clinical parameters predict testicular sperm recovery in 47,XXY Klinefelter's syndrome patients? *Hum Reprod*. 2004;19:1135-1139.

47,XYY

- Theoretically, 50% of sperm cells should be abnormal[1]
- Case report: 75 sperm karyotypes from a 47,XYY male resulted in no disomic sperm for sex chromosome[1]
- Increased incidence of sperm aneuploidy for sex chromosomes ranging from 0.3-15%
- Oligozoospermia may indicate more perturbations during meiotic pairing, subsequent loss of germ cells, and production of aneuploid sperm[2]

1. Martin RH. Cytogenetic determinants of male infertility. *Hum Reprod Update*. 2008;14:379-390.
2. Benet J, Martin RH. Sperm chromosome complements in a 47,XYY man. *Hum Genet*. 1988;78:313-315.

Translocations

- Robertsonian translocation carriers have a fusion of the long arms of 2 acrocentric chromosomes
- Risk of meiotic imbalance is primarily determined by characteristics of chromosomes involved and break-point positions
- Sperm karyotype studies of 37 reciprocal translocated heterozygotes have shown that 19-77% of spermatozoa are unbalanced
- Incidence of paternally derived translocation imbalances at prenatal diagnosis is about 12%; therefore, PGD is recommended[1]

1. Boué A, Gallano P. A collaborative study of the segregation of inherited chromosome structural rearrangements in 1356 prenatal diagnoses. *Prenat Diagn.* 1984;4:45-67.

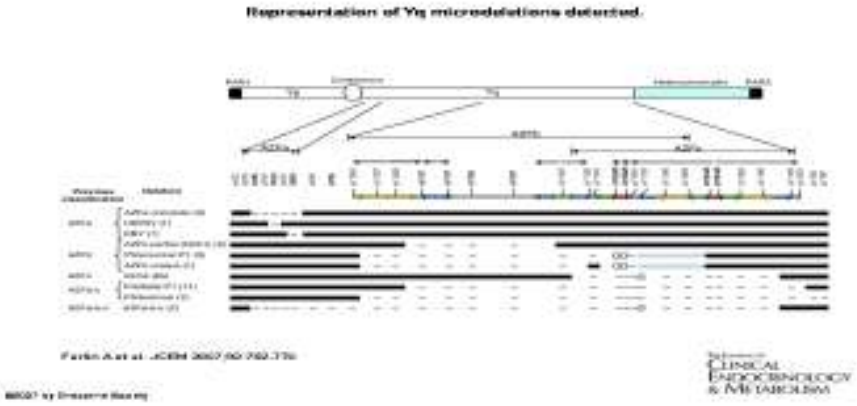
Inversions

- Inversion: 2 chromosome breaks occur in the same chromosome and then heal in an inverted order
- Correct number of genes, but an altered pairing of homologous chromosomes during meiosis
- If a single crossover occurs in the inverted region of the paired chromosomes, the presence of such recombinant chromosomes may result in an offspring with chromosomal duplications and deficiencies
  - Paracentric: same arm (risk of viable recombinants is ~3.8%)
  - Pericentric: both arms, including centromere (risk at prenatal diagnostic is 10-15%)

Pettenati MJ, Rao PN, Phelan MC, et al. Paracentric inversions in humans: a review of 446 paracentric inversions with presentation of 120 new cases. *Am J Med Genet.* 1995;55:171-187.; Anton E, Vidal F, Blanco J. Role of sperm FISH studies in the genetic reproductive advice of structural reorganization carriers. *Hum Reprod.* 2007;22:2088-2092.

Azoospermia Factor (AZF)

- AZF are located in three areas of Yq11: AZFa, AZFb, AZFc



Ferlin A, Arredi B, Speltra E, et al. Molecular and clinical characterization of Y chromosome microdeletions in infertile men: a 10-year experience in Italy. *J Clin Endocrinol Metab*. 2007;92:762-770.; Morris RS, Gleicher N. Genetic abnormalities, male infertility, and ICSI. *Lancet*. 1996;347:1277.

Y Chromosome Microdeletions

- Incidence is 3-18% in men with severe sperm production abnormalities, including azoospermia
- Two-thirds of men with deletions in the azoospermia factor **c** region have sperm in ejaculate. Testicle production is present in azoospermic men. Recommended: testicular sperm extraction (TESE)
- Men with azoospermia factor **b** deletions are unlikely to have sperm in ejaculate or recover sperm with TESE. TESE is not recommended
- 9% of men with azoospermia factor **a** deletion have non-obstructive azoospermia and Sertoli cell-only pattern
  - Partial deletion: germ cells found on testis biopsy
  - Complete deletion: no sperm cells retrieved

Hung AJ, King P, Schlegel PN. Uniform testicular maturation arrest: a unique subset of men with nonobstructive azoospermia. *J Urol*. 2007;178:608-612.

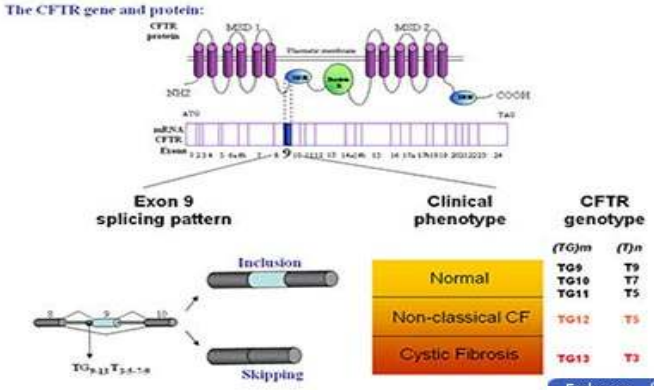
Monogenic Disorder and Multifactorial Disorders

- Monogenic genetic disorders occur as a direct consequence of a single gene being defective
  - Cystic fibrosis carriers (bilateral congenital absence of the vas deferens)
  - Defect in the androgen receptor
- Multifactorial disorders result from mutations in multiple genes, often coupled with environmental factors

Poongothai J, Gopenath TS, Manonayaki S. Genetics of human male infertility. *Singapore Med J*. 2009;50(4):336-347.

Cystic Fibrosis Gene Mutations

- CFTR mutation rate
  - CBAVD: 50%
  - Obstructive azoospermia: 15%



McCallum T, Milunsky J, Munarriz R, Carson R, Sadeghi-Nejad H, Oates R. Unilateral renal agenesis associated with congenital bilateral absence of the vas deferens: phenotypic findings and genetic considerations. *Hum Reprod*. 2001;16:282-288.

Cystic Fibrosis Mutation Screening in CBAVD

- Strong association exists between male infertility caused by CBAVD and CFTR gene mutations
- Cases of obstructive azoospermia without CBAVD can be associated with CFTR gene mutations

Results of the screening test for the CFTR mutation of the 5T allele

Procedure	Couples screened	One carrier n (%)	Two carriers n (%)
IUI	552	23 (4.0)	1 (0.2)
IVF	604	36 (5.9)	4 (0.7)
ICSI and MESA-TESE	1,350	98 (7.3)	9 (0.7)
Azoospermia[a]	121	23 (19.0)	2 (1.7)

Note: [a] Extrapolated from ICSI and MESA-TESE group
IUI: intrauterine insemination; IVF: in vitro fertilization; MESA: microsurgical epididymal sperm extraction

Riccaboni A, Lalatta F, Caliari I, Bonetti S, Somigliana E, Ragni G. Genetic screening in 2,710 infertile candidate couples for assisted reproductive techniques: results of application of Italian guidelines for the appropriate use of genetic tests. *Fertil Steril.* 2008;89:800-808.

Outcome of Chromosomal Abnormalities in Infertile Men

Abnormality type per concentration category	Chromosomal abnormality per concentration category	Consequences for offspring
Azoospermia (gonosomal - 7, translocation - 1, translocation and invertion - 1)	15.2% (12/79)	NI-6 (M and CA)-2 M-1
0–1 million/mL (gonosomal – 3, translocation – 2, inversion – 4)	3.1% (10/319)	NI-8 (M and CA)-2
1–5 million/mL (gonosomal – 2, inversion – 1)	1.2% (3/251)	NI-2 M-1
5–10 million/mL (translocation–3)	1.4% (3/211)	(M and CA)-2 M-1
10–20 million/mL (gonosomal – 3, translocation – 3)	3.1% (6/191)	NI-3 (M and CA)-3
20 million/mL (translocation – 2, inversion – 2)	2.3% (4/172)	NI-2 (M and CA)-2

NI: Chromosomal abnormality without increased risk for miscarriage or child with congenital anomalies;
M: Chromosomal abnormality with increased risk miscarriage only;
M and CA: Chromosomal abnormality with increased risk miscarriage and child with congenital anomalies

Dul EC, van Echten-Arends J, Groen H, Dijkhuizen T, Land JA, van Ravenswaaij-Arts CM. Chromosomal abnormalities in azoospermic and non-azoospermic infertile men: numbers needed to be screened to prevent adverse pregnancy outcomes. *Hum Reprod.* 2012;27:2850-2856.

Future Novel Technologies

- Microarrays: identification of gene expression profiles of infertile phenotypes
- Genomic analysis: determine differentially transcribed genes
- Proteomic: determine protein expression profiles of fertile and infertile men
- Metabolomic: mass spectroscopy and nuclear magnetic resonance spectroscopy can be used to create metabolite profiles
  - Clinical applications of metabolomics include gamete selection (assessing the best sperm to use for ART) and genomic testing (screening for aneuploidy)

Shima JE, McLean DJ, McCarrey JR, Griswold, MD. The murine testicular transcriptome: characterizing gene expression in the testis during the progression of spermatogenesis. *Biol Reprod.* 2004;71:319-330.

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Epigenetics and Spermatogenesis

- Several genes in testes are regulated through epigenetic mechanisms
- Hypermethylation (MTHFR, PAX8, NTF3, SFN, HRAS, JHM2DA, IGF2, H19, and others) is associated with poor semen parameters
- There is a direct correlation between epigenetic aberrations and spermatogenesis
- Environmental factors impact epigenome and male infertility
- ICSI and round spermatid injection (ROSNI) may increase the incidence of imprinting disorders and adversely affect embryonic development

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The Role of Genetic Counseling

- Identifiable genetic risks
- Information and explanation of genetic defect causes
- Current PGD options to select embryos for transfer
- Avoid passing on genetic abnormality to children
- Transmitting genes causing male infertility is of less concern with the small risk of associated somatic disease, but potential parents need to be aware

Conclusions

- Men with non-obstructive azoospermia or severe oligozoospermia (<5 million/mL) are at increased risk for having a definable genetic abnormality
- Individuals with normal karyotypes may have abnormal cell lines in their testes
- It is necessary to offer karyotype and Y chromosome analysis before ICSI with such sperm
- Genetic counseling should be provided whenever a genetic abnormality is detected
- Genetic information helps improve ICSI outcomes

Learning Objectives

After completing this section, participants should better understand:

- The etiologies and risks of genetic abnormalities of infertile women
- Genetic and molecular causes of female infertility
- Genetic screening for female infertility
- The role of genetic counseling
- Novel technologies for genetic testing of female infertility

Prevalence and Risk of Genetic Abnormality of Infertile Women

- In about 10% of female infertile subjects, genetic abnormalities could be present, including chromosome aberrations and single gene mutations
- The frequency of chromosomal abnormalities in female infertility is about 5%
  - 2.8% have numerical sex chromosome abnormalities
  - 2.2% have structural autosomal abnormalities

Gekas J, Thepot F, Turleau C. Chromosomal factors of infertility in candidate couples for ICSI: an equal risk of constitutional aberrations in women and men. *Hum Reprod*. 2001;16:82-90.

Prevalence and Risk of Genetic Abnormality of Infertile Women

Table 6 Classification of genetic causes of female infertility

Chromosomal aberrations (homogenous or mosaicism)
- Sex chromosomes
  - Turner syndrome and gonadal dysgenesis with short stature (45,X; mosaicisms such as 45,X/46,XX and 45,X/47,XXX; Xq isochromosome; del(Xq); del(Xp); r(X); etc)
  - Gonadal dysgenesis with Y-cell line
    - Mixed dysgenesis (45,X/46,XY)
    - 46,XY gonadal dysgenesis (Swyer syndrome)
    - True hermaphroditism with Y-cell line
  - X-autosomal translocation
  - 47,XXX and mosaicisms
- Autosomes
  - Robertsonian translocations
  - Reciprocal translocations
  - Inversions

Gene mutations
- X-linked
  - Fragile X syndrome (FRAXA)
  - Kallmann syndrome
  - Complete androgen insensitivity syndrome
- Autosomal
  - Complex genetic syndromes in which fertility is a minor manifestation[a]
  - Infertility as major manifestation
    - Genes for β-subunit of FSH and genes for LH and FSH receptors
    - Gene for GnRH receptor
    - BPES (blepharophimosis, ptosis, epicanthus inversus)
    - Denys-Drash syndrome
    - Fresier syndrome

Chromosomal aberrations confined to oocytes
- Advanced age

Foresta C, Ferlin A, Gianaroli L, Dallapiccola B. Guidelines for the appropriate use of genetic tests in infertile couples. *Eur J Hum Genet*. 2002;10:303-312.

Numerical and Structural Chromosomal Abnormalities

- Structural aberrations
  - Translocations
  - Chromosomal inversions
  - Supernumerary and marker chromosomes
- Constitutional aneuploidies
  - Turner syndrome
  - 47,XXX
  - Down syndrome (trisomy 21)
- Aneuploidy in gametes
  - Maternal age effect

Structural Aberrations - Translocations

- Reciprocal or Robertsonian translocations
  - Reduction in fertility
  - Spontaneous abortions and birth defects
  - Meiotic process impediment[1]
  - Production of genetically unbalanced gametes
  - Failure of meiosis and subsequent elimination of germ cells[2]
  - If non-homologous pairing involves X and Y chromosomes during meiosis I, it will interfere with X inactivation, resulting in a lethal gene-dosage effect on the germ cells[1]
  - Interactions of the translocation chromosomes with other parts of the nucleus may produce errors in meiosis and cell death[3]

1. Forejt J. X-Y involvement in male sterility caused by autosome translocations—a hypothesis. In: Fraccaro M, Rubin B, Rubin B, eds. *Genetic Control of Gamete Production and Function*. New York, NY: Academic Press; 1982:261-273.
2. Miklos GLG. Sex-chromosome pairing and male infertility. *Cytogenet Cell Genet*. 1974;13:558-577.
3. Chandley AC, McBeath S, Speed RM, Yorston L, Hargreave TB. Pericentric inversion in human chromosome 1 and the risk for male sterility. *J Med Genet*. 1987;24:325-334.

Structural Aberrations – Inversions and Supernumerary and Marker Chromosomes

- Inversion – formation of a pairing loop
  - Meiotic process impediment[1]
  - Reduction in rate of recombination leads to a breakdown of meiosis[2]
  - If crossing over occurs, unbalanced gametes can be produced[3]
- Supernumerary and marker chromosomes
  - Carriers of marker chromosomes are at risk of infertility due to meiotic arrest and instability[4]

1. Forejt J. X-Y involvement in male sterility caused by autosome translocations—a hypothesis. In: Fraccaro M, Rubin B, Rubin B, eds. *Genetic Control of Gamete Production and Function*. New York, NY: Academic Press; 1982:261-273.
2. Brown GM, Leversha M, Hulten M, Ferguson-Smith MA, Affara NA, Furlong RA. Genetic analysis of meiotic recombination in humans by use of sperm typing: reduced recombination within aheterozygous paracentric inversion of chromosome 9q32-q34.3. *Am J Hum Genet*. 1998;62:1484-1492. 3. Chandley AC. Infertility and chromosome abnormality. In: Clarke JR, ed. *Oxford Reviews in Reproductive Biology*. Vol 6. Oxford, United Kingdom: Oxford University Press; 1987:1-46. 4. Chandley AC, McBeath S, Speed RM, Yorston L, Hargreave TB. Pericentric inversion in human chromosome 1 and the risk for male sterility. *J Med Genet*. 1987;24:325-334.

Constitutional Aneuploidies – Turner Syndrome

- 45,X is the characteristic karyotype in Turner syndrome patients (1/5,000 – 1/10,000), occurring in ~55% of cases
- Frequency of all karyotypes associated with the syndrome[1]

Table 1. The relative frequencies of the karyotypes associated with Turner syndrome

Karyotype	Description	Frequency of Turner cases (%)
45,X	One X chromosome missing	55
46,X,i(Xq) or one of its variants	Isochromosome X most commonly seen in mosaic form together with a 45,X lineage	20
Various structural	For example deletions and ring X chromosomes	10
46,XX/45,X	Mosaic Turner or normal	10
Various Y mosaics	Mosaicism with one cell line having a normal Y or structurally rearranged Y chromosome	5

- Primary amenorrhea occurs in 25% and secondary amenorrhea in 0.5–1% of women with 45,X[2]
- In 10% of patients with a 45,X cell line and up to 50% of women with 45,X/X chromosome mosaicism, pubertal development and menstruation can be present, but short-lived[3]

1. Shah K, Sivapalan G, Gibbons N, Tempest H, Griffin DK. The genetic basis of infertility. *Reproduction*. 2003;126:13-25.
2. Reindollar RH, Novak M, Tho SP, McDonough PG. Adult-onset amenorrhea: a study of 262 patients. *Am J Obstet Gynecol*. 1986;155:531-543. 3. Zhong Q, Layman LC. Genetic considerations in the patient with Turner syndrome--45,X with or without mosaicism. *Fertil Steril*. 2012;98:775-779.

Constitutional Aneuploidies – 47,XXX and Trisomy 21

- 47,XXX
  - Incidence is 1/1000 females
  - The extra X chromosome is of maternal origin in 95% of cases and has a strong association with increased maternal age[1]
  - Normal weight, height, and mental function are present
  - Normal pre-pubertal development and fertility are present, but with early onset of menopause (30 years of age)[2]
- Trisomy 21
  - Frequency is 1/700 births
  - Rare possibility to reproduce

1. Hassold T, Abruzzo M, Adkins K, et al. Human aneuploidy: incidence, origin, and etiology. *Environ Mol Mutagen*. 1996;28:167-75.
2. May KM, Jacobs PA, Lee M, et al. The parental origin of the extra X chromosome in 47,XXX females. *Am J Hum Genet*. 1990;46:754-761.

Aneuploidy in Gametes – Maternal Age

- Risks of aneuploid gametes include trisomic offspring and pregnancy loss (25% in first trimester)
- Three hypotheses have been postulated:
  - *Production Line Hypothesis*: Oocytes that enter meiosis first are the first to be ovulated, and those entering last are ovulated last; the latter are more prone to non-disjunction[1]
  - *Local Factors Hypothesis*: Ovarian environment compromised with aging, in terms of oxygen concentration, pH, and hormone concentration; implicated in progressive loss of normal chromosomal disjunction during later meiosis[2-4]
  - *Limited Pool Hypothesis*: Oocytes depletion of the ovary leaves the remnants more prone to non-disjunction[5]

1. Henderson SA, Edwards RG. Chiasma frequency and maternal age in mammals. *Nature*. 1968;218:22-28. 2. Crowley PH, Gulati DK, Hayden TL, Lopez P, Dyer R. A chiasma-hormonal hypothesis relating Down's syndrome and maternal age. *Nature*. 1979;280:417-418. 3. Sugawara S, Mikamo K. Absence of correlation between univalent formation and meiotic nondisjunction in aged female Chinese hamsters. *Cytogenet Cell Genet*. 1983;35:34-40. 4. Eichenlaub-Ritter U, Chandley AC, Gosden RG. The CBA mouse as a model for age-related aneuploidy in man: studies of oocyte maturation, spindle formation and chromosome alignment during meiosis. *Chromosoma*. 1988;96:220-226. 5. Peters H, McNatty KP. *The Ovary*. London, England: Granada Publishing; 1980.

Monogenic and Multigenic Causes of Female Infertility

- Hypogonadotropic hypogonadism
  - Normosmic hypogonadotropic hypogonadism (nHH)
  - Kallmann syndrome (KS)
- Hypergonadotropic hypogonadism
  - Premature Ovarian Failure (POF)
  - Autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED)
  - Blepharophimosis–ptosis–epicanthus syndrome (BPES) type 1
- Eugonadism
  - Spontaneous ovarian hyperstimulation syndrome (sOHSS)
  - Mullerian aplasia
  - Endometriosis
  - Polycystic ovary syndrome (PCOS)
  - Leiomyomata

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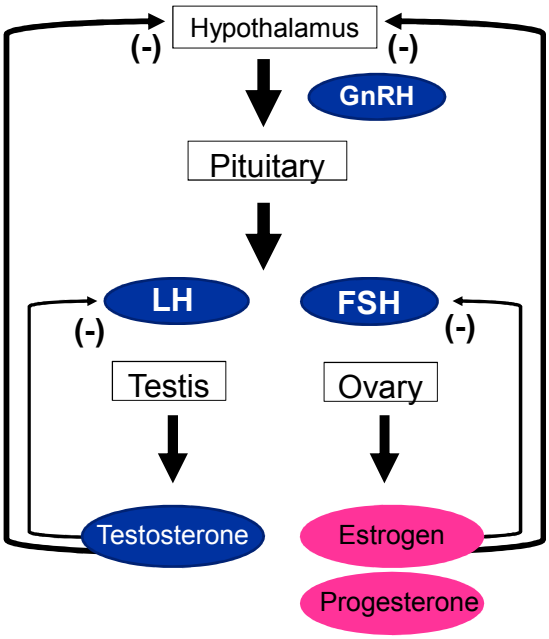
Hypothalamic–Pituitary–Gonadal Axis

Gonadotropin releasing hormone (GnRH) is responsible for sexual development and reproductive function by acting on the hypothalamic–pituitary–gonadal axis



Hypothalamic–Pituitary–Gonadal Axis (con't.)

- GnRH is released in a pulsatile fashion in order to bind to its cell surface receptor on pituitary gonadotropes. This binding induces follicle stimulating hormone (FSH) and luteinizing hormone (LH) synthesis
- FSH and LH (gonadotropins) bind to their G-protein coupled receptors in the gonads. This binding induces steroids and gamete development
- Sex steroids are responsible for the inhibitory negative feedback on the gonadotropin stimulus
- GnIH (gonadotropin inhibitory hormone), inhibins, and antimullerian hormone (AMH) also play important roles in reproductive function[1,2]

1. Bentley GE, Ubuka T, McGuire NL, et al. Gonadotrophin-inhibitory hormone: a multifunctional neuropeptide. *J Neuroendocrinol*. 2009;21:276-281.
2. Plant TM. Hypothalamic control of the pituitary-gonadal axis in higher primates: key advances over the last two decades. *J Neuroendocrinol*. 2008;20:719-726.

Hypogonadotropic Hypogonadism

- Symptoms typical of estrogen deficiency include absence of breast development or hypoestrogenic amenorrhea due to absence of negative feedback to the hypothalamus and pituitary gland
- GnRH deficiency
- Sense of smell
  - If normal: normosmic hypogonadotropic hypogonadism (nHH)
  - If impaired: Kallmann syndrome (KS)
- A small percentage of patients can regain reproductive function with treatment[1]

Pitteloud N, Acierno JS Jr, Meysing AU, Dwyer AA, Hayes FJ, Crowley WF Jr. Reversible Kallmann syndrome, delayed puberty, and isolated anosmia occurring in a single family with a mutation in the fibroblast growth factor receptor 1 gene. *J Clin Endocrinol Metab*. 2005;90:1317-1322.

Hypogonadotropic Hypogonadism - Etiology

- KAL1 gene mutations cause nHH/KS in 35-40% of patients[1-2]
  - Inheritance of KAL1 is X-linked recessive; only males are affected
- GNRHR gene mutations cause nHH in 4% of patients
  - First form of recessive autosomal inheritance of the pathology[3-4]
  - Variable phenotypes from complete absence of puberty to partial pubertal development or constitutional delay[5]
  - GNRHR gene mutations do not solely cause KS; additional autosomal disease causative genes are involved[6]
- CHD7 is the causative gene of CHARGE syndrome,[7] but it can be mutated in nHH/KS patients without this syndrome
- nHH/KS phenotypic features are caused by 24 additional genes
  - Mainly ligand/receptor partners involved in GnRH regulation are impaired
- Mutations in 6 other genes determine combined pituitary hormone deficiency (CPHD)
  - Growth hormone deficiency associated with absence of 1+ pituitary hormones
  - Inheritance can be autosomal recessive or dominant, or X-linked recessive

1. Franco B, Guioli S, Pragliola A, et al. A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. *Nature*. 1991;353:529-536. 2. Legouis R, Hardelin JP, Levilliers J, et al. The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules. *Cell*. 1991;67:423-435. 3. de Roux N, Young J, Misrahi M, et al. A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. *N Engl J Med*. 1997;337:1597-1602. 4. Layman LC, Lee EJ, Peak DB, et al. Delayed puberty and hypogonadism caused by mutations in the follicle-stimulating hormone beta-subunit gene. *N Engl J Med*. 1997;337:607-611. 5. Kim HG, Pedersen-White J, Bhagavath B, Layman LC. Genotype and phenotype of patients with gonadotropin-releasing hormone receptor mutations. *Front Horm Res*. 2010;39:94-110. 6. Bhagavath B, Podolsky RH, Ozata M, et al. Clinical and molecular characterization of a large sample of patients with hypogonadotropic hypogonadism. *Fertil Steril*. 2006;85:706-713. 7. Vissers LE, van Ravenswaaij CM, Admiraal R, et al. Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. *Nat Genet*. 2004;36:955-957.

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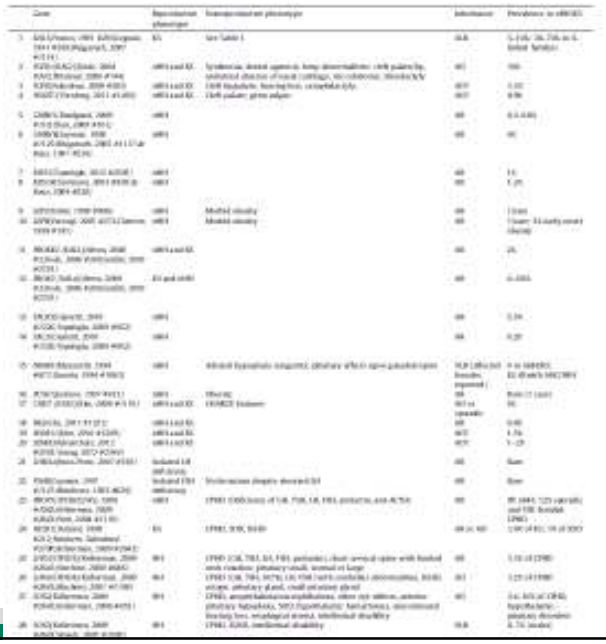
Hypogonadotropic Hypogonadism – Etiology (con't.)



Among the genes identified, only two (KAL1 and NR0B1) are X-linked recessive, while 12 are autosomal recessive and 6 are autosomal dominant

Layman LC. The genetic basis of female reproductive disorders: etiology and clinical testing. *Mol Cell Endocrinol*. 2013;370:138-148.

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Hypogonadotropic Hypogonadism – Clinical Considerations

- Digenic/oligogenic gene mutation identification has complicated counseling of these patients
- A single mutated gene is sufficient to cause the pathology, and a second mutation can exacerbate the phenotype
- Mutation screening in FGFR1 (10%), CHD7 (6%), GNRHR (5%), and TACR3 (6%) is sufficient to cover 16% of KS and 25% of nHH patients, thus simplifying genetic counseling
- FGFR1 and CHD7 are inherited in an autosomal dominant fashion; thus screening for them could be sufficient to diagnose nHH/KS

Hypergonadotropic Hypogonadism - Phenotype

- Symptoms typical of estrogen deficiency: absence of breast development or hypoestrogenic amenorrhea due to lack of negative feedback to the hypothalamus and pituitary gland
- High levels of FSH and LH, indicating that the problem resides in the ovary[1]
- Cardiac anomalies are found in one-half of patients and renal complications in one-third
- Dysgenetic gonads in patients phenotypically female but with a 46,XY karyotype (Swyer syndrome)
  - Swyer syndrome is caused by SRY mutations in 15% of cases[2-3]

1. Layman LC. Genetics of human hypogonadotropic hypogonadism. *Am J Med Genet*. 1999;89:240-248.
2. Jäger RJ, Anvret M, Hall K, Scherer G. A human XY female with a frame shift mutation in the candidate testis-determining gene SRY. *Nature*. 1990;348:452-454.
3. Sim H, Argentaro A, Harley VR. Boys, girls and shuttling of SRY and SOX9. *Trends Endocrinol Metab*. 2008;19:213-222.

Hypergonadotropic Hypogonadism – Etiology

	Gene	Reproductive phenotype	Nonreproductive phenotype	Inheritance	Prevalence in POF
	SRY (Jäger, 1990 #433; Sinc, 2009 #1385)	PA and POF	Swyer syndrome in 46,XY males	Sporadic, Y linked	10% in 46,XY
	POF1	Xq26–q28		?	?
	POF2	X13.3–q21.1		?	?
	DIAPH2 (Bione, 1998 #43)	Gene within POF2		Disruption in one X-autosome translocation	1 Case; no point mutations in the gene
1	FMR1 (Conway, 2000 #1346; Uzielli, 1999 #947)	POF (gene within POF1)		XLD	1–5% Sporadic; 12–15% familial
2	FOXL2 (Crisponi, 2000 #2055; Loiseau, 2009 #1681)	POF3	Blepharophimosis–ptosis–epicanthus syndrome	AD	Rare without BPES
3	BMP15 (Di Pasquale, 2004 #238; Rossetti, 2009 #1880)	POF4		XLD	2%
4	NOBOX (Qin, 2007 #1894; Qin, 2009 #2067)	POF5		AD, sporadic	0.1%
5	FIGLA (Zhao, 2008 #1885)	POF6		AD, sporadic	2%
6	NR5A1 (Lourenço, 2009 #1888)	POF7	Adrenal failure	AR	8% (2/25)
7	? (Jääskeläinen, 2009 #2268; Jiang, 1998 #2269)	POF		AR	Rare outside of Finland
8	AIRE (Consortium, 1997 #103; Nagamine, 1997 #682)	POF	APECED	AR	Rare
9	GALT (Kaufman, 1979 #448)	POF	Galactosemia	AR	Rare
10	EIF2B2 (Fogli, 2004 #298; Fogli, 2003 #297)	POF	Ovarioleukodystrophy	AR	Rare unless white matter abnormalities of brain
11	EIF2B4 (Fogli, 2004 #298; Fogli, 2003 #297)	POF	Ovarioleukodystrophy	AR	Rare unless white matter abnormalities of brain
12	EIF2B5 (Fogli, 2004 #298; Fogli, 2003 #297)	POF	Ovarioleukodystrophy	AR	Rare unless white matter abnormalities of brain
13	CYP19A1 (Simsir, 2001 #1850)	POF	Adrenal failure	AR	Rare
14	CYP19A1 (Bo, 2002 #2302)	POF	Sexual ambiguity	AR	Rare

Layman LC. The genetic basis of female reproductive disorders: etiology and clinical testing. *Mol Cell Endocrinol*. 2013;370:138-148.

Hypergonadotropic Hypogonadism – Premature Ovarian Failure (POF)

- Patients (46,XX karyotype) show premature ovarian insufficiency
- 14 genes known to be causative, accounting for 15% of patients
- FMR1 gene is most commonly involved and most largely known, and causes Fragile X syndrome (FRAXA)
  - FRAXA is an X-linked dominant disorder; affected males show variable mental retardation, facial dysmorphism, and macroorchidism
  - FRM1 normally presents with 5-50 CGG trinucleotide repeats in an untranslated region; expansion of this trinucleotide from 50 to up to 200 repeats causes premutation. Premutated alleles in women predisposes to further expansion during meiosis
  - > 200 repeats in males are causative of FRAXA due to full inactivating mutation; the mechanism entails methylation of the FMR1 promoter
  - 16% of women carriers of the premutated allele will develop POF[1]
  - POF patients have a 3-4% risk of being carriers of the premutated allele if they are the only affected individual in the family. This incidence increases to 12-15% if another female is affected by POF in the pedigree[2]

1. Allingham-Hawkins DJ, Babul-Hirji R, Chitayat D, et al. Fragile X premutation is a significant risk factor for premature ovarian failure: the International Collaborative POFin Fragile X study--preliminary data. *Am J Med Genet*. 1999;83:322-325.
2. Conway GS, Payne NN, Webb J, Murray A, Jacobs PA. Fragile X premutation screening in women with premature ovarian failure. *Hum Reprod*. 1998;13:1184-1187.

Hypergonadotropic Hypogonadism – Other Diseases

- Autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED)
  - Systemic autoimmune disorder
- Blepharophimosis–ptosis–epicanthus syndrome (BPES), type 1
  - Rare autosomal dominant disorder caused by mutations in FOXL1 gene

Eugonadism - Spontaneous Ovarian Hyperstimulation Syndrome (sOHSS), Mullerian Aplasia, and Endometriosis

- Spontaneous ovarian hyperstimulation syndrome (sOHSS)
  - Caused by FSHR activating mutation[1,2] in which receptor is constitutively active
  - Autosomal dominant inheritance
- Mullerian aplasia
  - Absence of the uterus and vagina
  - Affects 1/5000 women and 10% of women with primary amenorrhea[3]
  - Unknown etiology
- Endometriosis
  - Inflammatory disorder resulting in pelvic pain and infertility
  - 5-10% of women between puberty and menopause affected; 7-fold increase if familial
  - Multifactorial and polygenic disease with unknown etiology; genome-wide association studies (GWAS) are ongoing

1. Smits G, Olatunbosun O, Delbaere A, Pierson R, Vassart G, Costagliola S. Ovarian hyperstimulation syndrome due to a mutation in the follicle-stimulating hormone receptor. *N Engl J Med*. 2003;349:760-766. 2. Vasseur C, Rodien P, Beau I, et al. A chorionic gonadotropin-sensitive mutation in the follicle-stimulating hormone receptor as a cause of familial gestational spontaneous ovarian hyperstimulation syndrome. *N Engl J Med*. 2003;349:753-759. 3. Reindollar RH, Byrd JR, McDonough PG. Delayed sexual development: a study of 252 patients. *Am J Obstet Gynecol*. 1981;140:371-380.

Eugonadism - Polycystic Ovary Syndrome (PCOS) and Leiomyomata

- Polycystic ovary syndrome (PCOS)
  - Defined as hyperandrogenic anovulation with or without polycystic appearing ovaries[1]
    - Hyperandrogenemia causes hirsutism
    - Higher levels of free estrogens result in increased risk of endometrial cancer
    - Hyperinsulinemia increases risk of type 2 diabetes
  - Most common cause of anovulation due to infrequent LH surges, affecting 5-8% of women
  - Unknown etiology; GWAS are ongoing
- Leiomyomata
  - Fibroids (benign smooth muscle tumors of the uterus) of clonal or somatic origin can cause bleeding/hysterectomy
  - More than 1/3 of women suffer from leiomyomata
  - Etiology still not well defined

1. Azziz R, Carmina E, Dewailly D, et al. Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. *J Clin Endocrinol Metab*. 2006;91:4237-4245.

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Role of the Clinician in Counseling of These Patients

- Hypogonadotropic hypogonadism
  - FGFR1 and CHD7 should be tested for mutations by sequencing the DNA of all coding exons and splice junctions
  - TACR3 and GNRHR tests could also be included to diagnose up to 25% of nHH cases
- Hypergonadotropic hypogonadism
  - Karyotype to identify Turner syndrome
  - 46,XX patients with POF should be offered FMR1 testing by polymerase chain reaction (PCR) and Southern blot for triplet repeat expansion analysis
- GWAS are ongoing and will provide information about additional causative genes

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Role of the Clinician in Counseling of These Patients (con't.)

- Eugonadal disorders
  - sOHSS: FSHR gene DNA sequencing for protein-coding exons and splice junctions
  - Mullerian aplasia: WNT4 DNA sequencing
  - Endometriosis, fibroids, or PCOS: No reliable tests are currently available
- Structural or numerical chromosomal abnormalities
  - Preimplantation genetic screening (PGS) using comprehensive chromosome screening (CCS) analysis platforms should be proposed to women considering ART, especially women of advanced maternal age or translocation carriers

Future Novel Technologies

- Novel technologies to be used in the future include:
  - Targeted deep resequencing aimed at simultaneous screening of all genes involved in hypergonadotropic and hypogonadotropic hypogonadism
  - Whole exome sequencing
  - Whole genome sequencing
- The costs of these technique are progressively decreasing
- Logistical and ethical problems will emerge from the interpretation of the data these technologies provide, especially in terms of genetic counseling

Conclusions

- Genetic causes of female infertility vary from structural and numerical chromosomal imbalances to monogenic and multigenic conditions, mainly impairing the hypothalamic–pituitary–gonadal axis
- Comprehensive counseling exploiting currently available diagnostic tools is needed in order to inform the patient about prognostic perspectives
- PGD/PGS ensure encouraging outcomes especially when the cause of infertility is advanced maternal age
- New technology, such as molecular screening techniques, will bring new insight into the etiology of female infertility by increasing the throughput and decreasing the cost of analysis

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Learning Objectives

After completing this section, participants should better understand:

- Definitions of preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS)
- Indications for the use of PGD and PGS
- Technical aspects of PGD and PGS
- Advantages and disadvantages of different biopsy methods
- Controversies and perspectives in preimplantation genetic testing (PGT)

Definition of PGT

- **Preimplantation genetic testing** describes procedures involving the removal of one or more nuclei from oocytes (polar bodies) or embryos (blastomeres or trophectoderm cells) to test for mutations in gene sequence or aneuploidy before transfer
- **Preimplantation genetic testing** includes **PGD** and **PGS**

Definition of PGD and PGS

- **Preimplantation genetic diagnosis (PGD)** is used when one or both parents carry a gene mutation or a chromosomal rearrangement and testing is performed to determine whether that specific mutation or an unbalanced chromosomal complement has been transmitted to the oocyte or embryo
- **Preimplantation genetic screening (PGS)** is used when the parents are known or presumed to be chromosomally normal and their embryos are screened for aneuploidy

Practice Committee of the Society for Assisted Reproductive Technology; Practice Committee of the American Society for Reproductive Medicine. Preimplantation genetic testing: a Practice Committee opinion. *Fertil Steril*. 2007;88:1497-1504.

Indications for PGD – High Risk

- Single gene disorders:
  - Carriers of autosomal dominant disorders (risk – 50%), such as myotonic dystrophy (DMPK)
  - Carriers of autosomal recessive disorders (risk – 25%), such as spinal muscular atrophy (SMA1)
  - Female carriers of X-linked disorders Duchenne/Becker muscular dystrophy (DMD)
  - Carriers of mutations such as BRCA-1 mutation
- Known chromosomal abnormalities (deletions, translocations, inversions)
- When human leukocyte antigen (HLA) matching is performed

Goossens V, Harton G, Moutou C, Traeger-Synodinos J, Van Rij M, Harper JC. ESHRE PGD Consortium data collection IX: cycles from January to December 2006 with pregnancy follow-up to October 2007. *Hum Reprod*. 2009;24:1786-1810.

Indications for PGS – Low Risk

- Advanced maternal age
- History of recurrent early pregnancy loss
- Repeated implantation failure
- Severe male factor infertility
- Sex selection or family balancing
- Other indications

Goossens V, Harton G, Moutou C, Traeger-Synodinos J, Van Rij M, Harper JC. ESHRE PGD Consortium data collection IX: cycles from January to December 2006 with pregnancy follow-up to October 2007. *Hum Reprod*. 2009;24:1786-1810.

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Differences Between PGD/PGS

- Primary aim
  - PGD: identify embryos unaffected uniquely and specifically by a inheritable disease
  - PGS: identify euploid embryos for successful pregnancy
- Fertility
  - PGD: often fertile
  - PGS: infertile or subfertile
- Preliminary work-up
  - PGD: needed in order to determine needed assays
  - PGS: not needed

Methods of Genetic Analysis

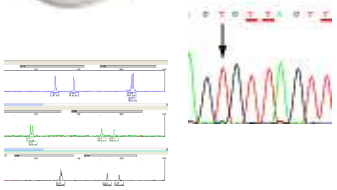
- Fluorescence *in situ* hybridization analysis (FISH-analysis)
- Array comparative genomic hybridization
- Single nucleotide polymorphism (SNP) array
- PCR amplification and sequencing
- Real-time quantitative PCR
- Next-generation sequencing

Bodurtha J, Strauss JF 3rd. Genomics and perinatal care. *N Engl J Med*. 2012;366:64-73.

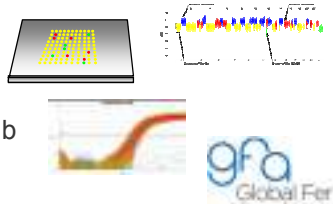
Applications and Methods for PGT

9-chromosome FISH:
For known chromosomal abnormality, gender selection (X-linked mutation)

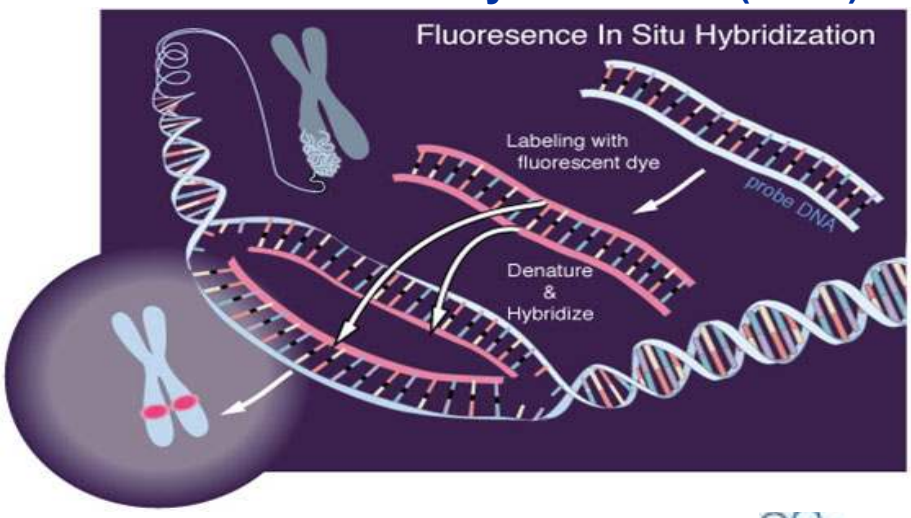
PCR and sequencing:
For autosomal single gene mutation, X-linked single gene mutation, HLA matching



24-chromosome screening platforms (aCGH, aSNP, qPCR):
Different applications according to the resolution of the platform. Mainly, chromosome copy number variations and chromosomes imbalances greater than 2 Mb are detectable

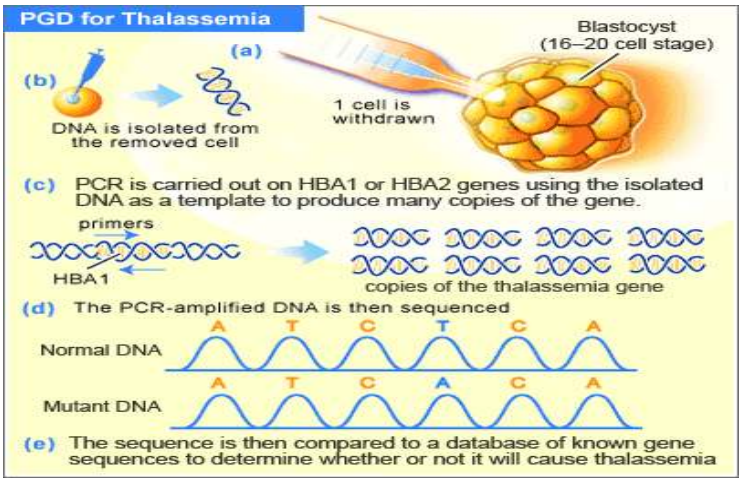


Fluoresence in Situ Hybridization (FISH)



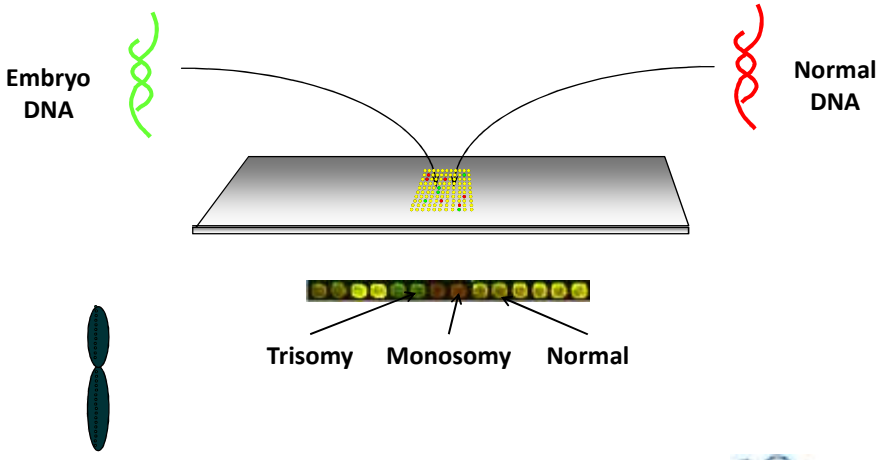
Bishop R. Applications of fluorescence *in situ* hybridization (FISH) in detecting genetic aberrations of medical significance. *Bioscience Horizons.* 2010;3:85-95.

PCR Amplification and Sequencing



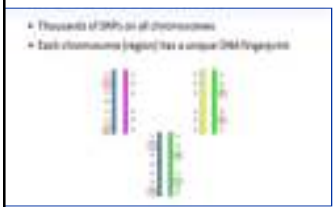
Sermon K, De Rijcke M, Lissens W, et al. Preimplantation genetic diagnosis for Huntington's disease with exclusion testing. *Eur J Hum Genet.* 2002;10:591-598.; Thornhill AR, Snow K. Molecular diagnostics in preimplantation genetic diagnosis. *J Mol Diagn.* 2002;4:11-29.; Rechitsky S, Kuliev A, Tur-Kaspa I, Morris R, Verlinsky Y. Preimplantation genetic diagnosis with HLA matching. *Reprod Biomed Online.* 2004;9:210-221.; Fiorentino F, Kokkali G, Biricik A, et al. Polymerase chain reaction-based detection of chromosomal imbalances on embryos: the evolution of preimplantation genetic diagnosis for chromosomal translocations. *Fertil Steril.* 2010;94:2001-2011.

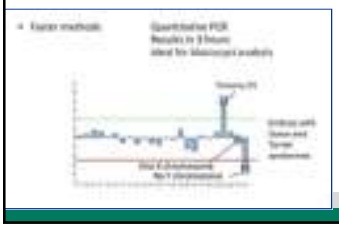
Array Comparative Genome Hybridization (aCGH)



Harton GL, Munné S, Surrey M, et al. Diminished effect of maternal age on implantation after preimplantation genetic diagnosis with array comparative genomic hybridization. *Fertil Steril*. 2013;100:1695-1703.

24-chromosome Screening Platforms





Comparison of methods for preimplantation CCS

Reported characteristics	CCS method		
	aCGH	SNP array	qPCR
Validation on cell lines samples	no	yes	yes
Accuracy	NR	94 – 99%	97-99%
Consistency between PB and oocyte	94%	NR	NR
Minimum turn-around time	12h	24 h	**4 h**
Blastocyst biopsy and fresh embryo transfer	Partial	NO	YES
Lab work-load	HIGH	HIGH	**LOW**
Number of probes	2-32 K	263-370 K	NR
Reported minimum detectable imbalance	2.5 Mb	1.7 Mb	NR
Direct monogenic disease screening	-	+	+
Contamination screening	-	+	+
Origin of aneuploidy screening	-	+[c]	NR

Adapted from Treff NR, Scott RT Jr. Methods for comprehensive chromosome screening of oocytes and embryos: capabilities, limitations, and evidence of validity. *J Assist Reprod Genet*. 2012;29:381-390.

Next Generation Sequencing

Evaluation of targeted next-generation sequencing–based preimplantation genetic diagnosis of monogenic disease

Nathan R. Treff, Ph.D.,[a,b,c] Anastasia Fedick, B.S.,[a,b] Xin Tao, M.S.,[a] Batsal Devkota, Ph.D.,[a] Deanne Taylor, Ph.D.,[a,c] and Richard T. Scott Jr., M.D.[a,c]

[a] Reproductive Medicine Associates of New Jersey, Morristown, New Jersey; [b] Molecular Genetics, Microbiology and Immunology, and [c] Obstetrics, Gynecology, and Reproductive Sciences, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, New Brunswick, New Jersey

- 100% consistent with TaqMan Allelic Discrimination assay
- 100% consistent with reference lab genotypes
- 100% reliable
- Reasonable cost
- Turn-around time of 24 hours from biopsy to results
- Can detect chromosomal aneuploidies, translocations, and single-gene diseases using a single trophectoderm biopsy

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Challenges of Single-cell PCR

- Quality of embryo
- Limited amount of DNA
  - Requires nested PCR or a high number of amplification cycles (>45)
  - Robust and high-fidelity polymerase
  - Hot-start PCR
- Allele dropout (ADO)
- Failed amplification
  - Use of linked polymorphic markers
- Meiotic recombination (cross-over)
  - Markers flanking the gene of interest

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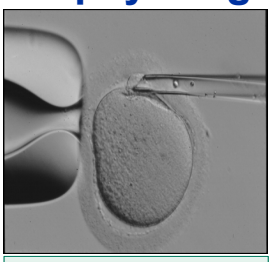
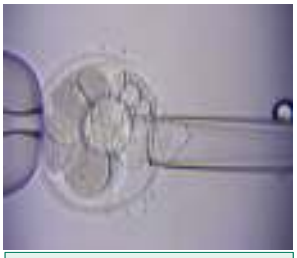
Biopsy Stage for PGD/PGS



Polar body biopsy[1,2]

- Paternal and post-zygotic errors not detected
- Need 2nd PB biopsy
- High rate of false positives
- Impacts embryo development
- Expensive, time-consuming



Blastomere biopsy[3-5]

- Extensive experience worldwide
- Small reduction in embryo viability
- High impact of mosaicism
- Single cell analysis issue

Trophectoderm biopsy[4,6,7]

- More robust genetic analysis
- High clinical predictive value
- No impact of biopsy
- Low impact of mosaicism
- Reduced number of embryos/cycles
- Most cost effective

1. Capalbo A, Bono S, Spizzichino L, et al. Sequential comprehensive chromosome analysis on polar bodies, blastomeres and trophoblast: insights into female meiotic errors and chromosomal segregation in the preimplantation window of embryo development. *Hum Reprod*. 2013;28:509-518. 2. Levin I, Almog B, Shwartz T. Effects of laser polar-body biopsy on embryo quality. *Fertil Steril*. 2012;97:1085-1088. 3. Mertzanidou A, Wilton L, Cheng J, et al. Microarray analysis reveals abnormal chromosomal complements in over 70% of 14 normally developing human embryos. *Hum Reprod*. 2013;28:256-264. 4. Scott RT Jr, Upham KM, Forman EJ, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. *Fertil Steril*. 2013;100:697-703. 5. Treff NR, Scott RT Jr. Methods for comprehensive chromosome screening of oocytes and embryos: capabilities, limitations, and evidence of validity. *J Assist Reprod Genet*. 2012;29:381-390. 6. Capalbo A, Wright G, Elliott T, Ubaldi FM, Rienzi L, Nagy ZP. FISH reanalysis of inner cell mass and trophectoderm samples of previously array-CGH screened blastocysts shows high accuracy of diagnosis and no major diagnostic impact of mosaicism at the blastocyst stage. *Hum Reprod*. 2013;28:2298-2307. 7. Scott RT Jr, Upham KM, Forman EJ, Zhao T, Treff NR. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. *Fertil Steril*. 2013;100:624-630.

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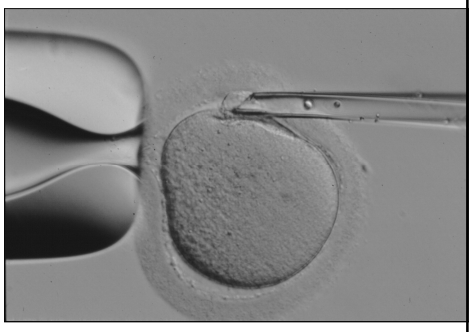
Polar Body Approach to PGD – Chromosomal and Single Gene Disorders (PB1 and PB2)

- Comparable prevalence of meiosis I and II errors
  - 1/3 are isolated events not detected by PB1 testing
- Limited diagnostic value of blastomere analysis
- >1/3 of meiotic errors are complex, indicating overall disturbance in female meiosis[1]
- Accurate embryo genotype assessment requires combined oocyte and embryo testing, particularly for chromosomal disorders
- Detrimental impact of biopsy on embryo development[2]
- High false positive and false negative diagnosis rate[3]
- Expensive and time-consuming



1. Kuliev A, Rechitsky S, Verlinsky O. *Atlas of Preimplantation Genetic Diagnosis*. 3rd ed. Boca Raton, FL: Taylor & Francis; 2014. 2. Levin I, Almog B, Shwartz T. Effects of laser polar-body biopsy on embryo quality. *Fertil Steril*. 2012;97:1085-1088. 3. Capalbo A, Bono S, Spizzichino L, et al. Sequential comprehensive chromosome analysis on polar bodies, blastomeres and trophoblast: insights into female meiotic errors and chromosomal segregation in the preimplantation window of embryo development. *Hum Reprod*. 2013;28:509-518.

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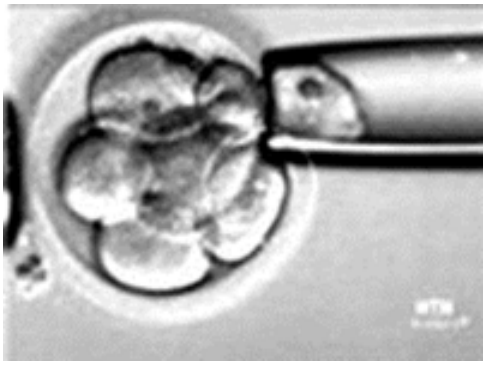
Blastomere Biopsy

- Advantages
  - Diagnosis of hereditary parental abnormality
  - Possible sex determination
  - Sufficient time for diagnosis
  - Highest worldwide experience
- Disadvantages
  - Highest level of chromosome mosaicism at this stage
  - Limits in performing interphase FISH and molecular-genetic diagnosis (1 or 2 cells)
  - Single cell analysis

Embryo day 3



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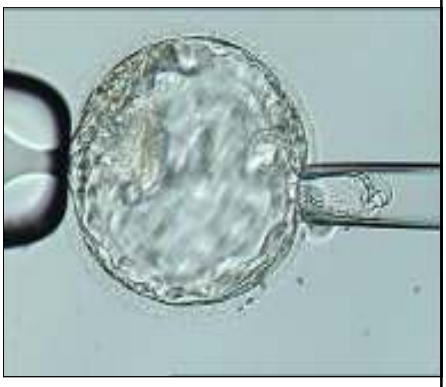
Blastocyst Biopsy

- Advantages
  - More DNA, so more robust diagnosis
  - Blastocysts have less mosaicism
  - Low error = low miscarriage rate (4%)
  - No damage to the embryos
  - Facilitates single embryo transfer
  - Least time-consuming and most cost-effective
- Disadvantages
  - aCGH and aSNP analysis turnaround times not compatible with fresh embryo transfer



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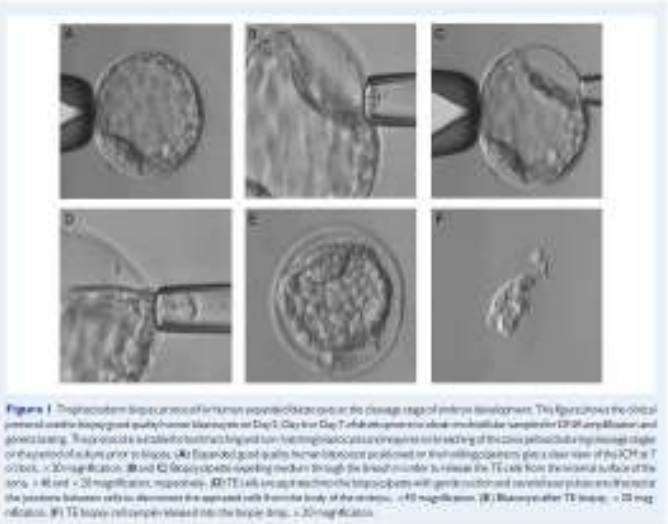
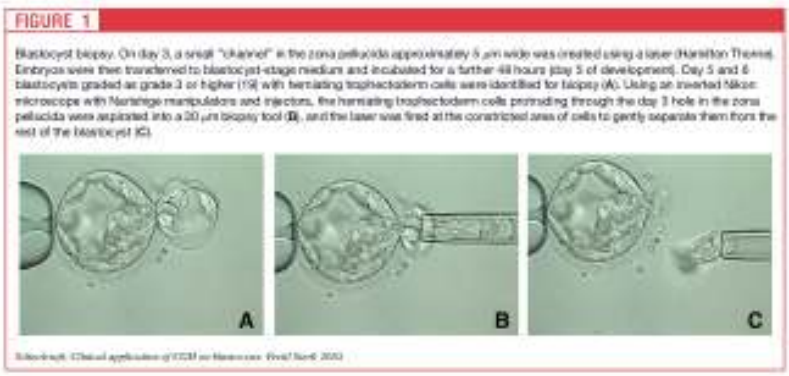
Methods of Blastocyst Biopsy



Trophectoderm biopsy without zona breaching at the cleavage stage

Capalbo A, Rienzi L, Cimadomo D, et al. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts [published online ahead of print March 19, 2014]. *Hum Reprod.*

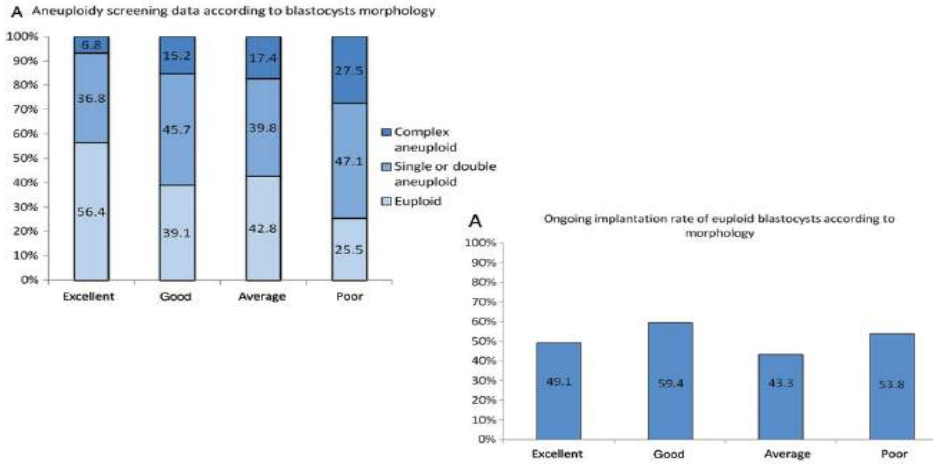
Methods of Blastocyst Biopsy (con't.)



Trophectoderm biopsy with zona breaching at the cleavage stage

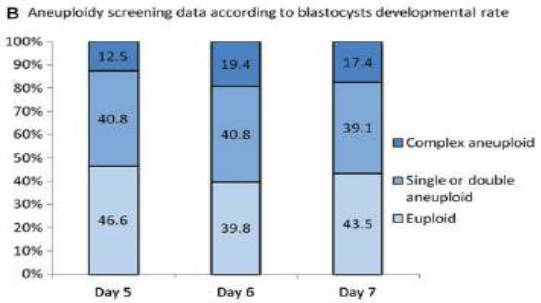
Schoolcraft WB, Fragouli E, Stevens J, Munne S, Katz-Jaffe MG, Wells D. Clinical application of comprehensive chromosomal screening at the blastocyst stage. *Fertil Steril.* 2010;94:1700-1706.

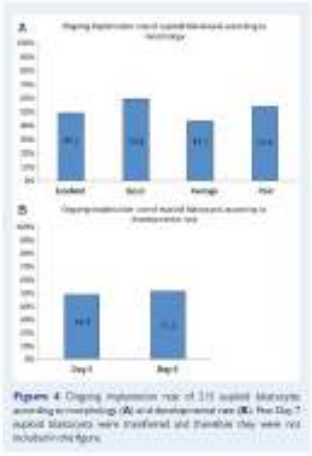
Conventional Parameters of Blastocyst Evaluation are not Predictive of Euploidy



Capalbo A, Rienzi L, Cimadomo D, et al. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts [published online ahead of print March 19, 2014]. *Hum Reprod.*

Conventional Parameters of Blastocyst Evaluation are not Predictive of Euploidy (con't.)





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Prognosis Depending on Age and Cohort Size

# Day 5 embryos	% patients with normal embryos (% normal embryos)				
	Egg donors	< 35 years old	35 – 39 years old	40 - 42 years old	> 42 years old
1-3	99% 69%	95% 68%	79% 49%	61% 34%	37% 17%
4-6	100% 77%	100% 73%	97% 52%	81% 31%	67% 13%
7-10	100% 62%	100% 58%	100% 45%	97% 27%	95% 22%
> 10	100% 67%	100% 59%	100% 51%	100% 41%	100% 17%

Calculated using 875 cycles, 4600 embryos
Euploidy decreased with age (P <.01) but NOT cohort size

Ata B, Kaplan B, Danzer H, et al. Array CGH analysis shows that aneuploidy is not related to the number of embryos generated. *Reprod Biomed Online*. 2012;24:614-620.

Implantation After PGS

RCT	Patient group	Fresh or freezing	Genetic method	IR after PGS for 24 chrom. vs control
Yang et al. 2012	<35	Day 5 biopsy, day 6 fresh transfer	aCGH	40% increase
Schoolcraft et al. 2011	>35 (av. 39)	Day 5 biopsy, freezing, fresh transfer	aSNP	32% increase
Forman et al.2013	>35	Day 5 biopsy, day 6 fresh transfer	qPCR	32% increase
Scott et al. 2013	20-42 (av. 32)	Day 5 biopsy, day 6 fresh transfer	qPCR	28% increase

All randomised controlled trials (RCTs) show at least 30% increase in implantation rate (IR) after PGS for 24 chromosome analysis in comparison to no PGS

Yang Z, Liu J, Collins GS, et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. *Mol Cytogenet*. 2012;5:24.; Schoolcraft WB, Treff NR, Stevens JM, Ferry K, Katz-Jaffe M, Scott RT Jr. Live birth outcome with trophectoderm biopsy, blastocyst vitrification, and single-nucleotide polymorphism microarray-based comprehensive chromosome screening in infertile patients. *Fertil Steril*. 2011;96:638-640.; Forman EJ, Hong KH, Ferry KM, et al. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. *Fertil Steril*. 2013;100:100-107.; Scott RT Jr, Upham KM, Forman EJ, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. *Fertil Steril*. 2013;100:697-703.

Clinical Evidence of Blastocyst Stage PGS: RCT

Table 3 Comparison of laboratory findings and clinical outcome among IVF patients undergoing SET with embryo assessment by aCGH + morphology (Group A) and blastocyst morphology alone (Group B)

	A	B	p
Fresh blastocyst transfer *according to morphology assessment:*	55 (100)	48 (100)	
Grade 5/6	31 (56.4)	28 (58.3)	
Grade 4	21 (38.2)	19 (39.6)	0.677[a]
Grade 3	3 (5.4)	1 (2.1)	
Clinical pregnancy	39 (70.9)	22 (45.8)	0.017[a]
Ongoing pregnancy (≥20wks GA)	38 (69.1)	20 (41.7)	0.009[a]
Missed abortion	1 (2.6)	2 (9.1)	0.597[b]

Notes: All data reported as *n* (%). SET = single embryo transfer; aCGH = array comparative genomic hybridization; GA = gestational age [a] by Chi-squared test [b] by Fisher's exact test.

- Females age <35 years
- aCGH
- Blastocyst stage biopsy on day 5 with fresh embryo transfer on day 6

Yang Z, Liu J, Collins GS, et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. *Mol Cytogenet*. 2012;5:24.

Clinical Evidence of Blastocyst Stage PGS: RCT (con't.)

TABLE 1

A. Patient (n = 127) clinical and cycle (n = 130) information.

Maternal age (y)	**37.8 (range 30–42)**
Day 3 FSH	7.39 ± 2.2
Antimullerian hormone	2.98 ± 2.6
Antral follicle count	17.3 ± 8.1
No. of oocytes retrieved	19.1 ± 8.3
No. of oocytes fertilized by ICSI	12.8 ± 5.5
Sperm motility	52.3%
Sperm concentration	86.9 million/mL
Good blastocyst development (grade ≥3BB)	38%
No. of blastocysts biopsied and vitrified	5.9 ± 3.5

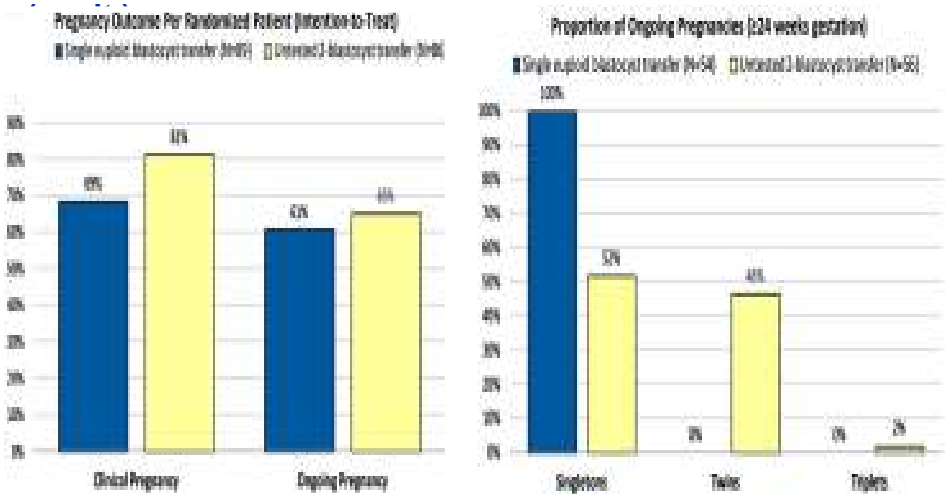
B. Patient comprehensive chromosome screening (CCS) and clinical outcome.

CCS results (n = 125 cycles)	
No result	4.5%
All aneuploid cycle	20%
Euploid blastocysts	47.4% (356/751)
Outcome results (n = 100 fresh frozen embryo transfers)	
Blastocyst survival after warming	96.8% (179/185)
Mean no. of euploid blastocysts transfered	1.78
Biochemical pregnancy	87% (87/100)
Clinical pregnancy (fetal heart tone)	73% (73/100)
Missed abortion	2.7% (2/73)
Implantation rate (fetal heart tone)	64.6% (115/178)
Euploid babies born	113 – 71% live birth rate per transfer – 55.9% live birth rate per oocyte retrieval

- Females age >35 years
- aSNP
- Blastocyst stage biopsy and freezing and frozen embryo transfer

Schoolcraft WB, Treff NR, Stevens JM, Ferry K, Katz-Jaffe M, Scott RT Jr. Live birth outcome with trophectoderm biopsy, blastocyst vitrification, and single-nucleotide polymorphism microarray-based comprehensive chromosome screening in infertile patients. *Fertil Steril*. 2011;96:638-640.

Clinical Evidence of Blastocyst Stage PGS: RCT



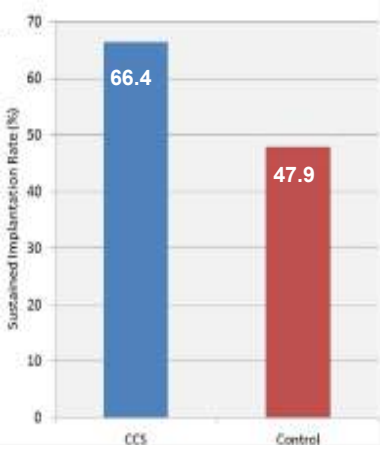
- Females age >35 years
- qPCR
- Blastocyst stage biopsy and day 6 fresh embryo transfer

Forman EJ, Hong KH, Ferry KM, et al. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. *Fertil Steril.* 2013;100:100-107.

Clinical Evidence of Blastocyst Stage PGS: RCT (con't.)

The demographics of the comprehensive chromosomal screening (CCS) and control groups were equivalent as were basic parameters of follicular stimulation during their treatment cycles.

Characteristic	CCS study group	Nonintervention (control group)
Patients (n)	72	83
Age (y)	32.2 ± 0.5	32.4 ± 0.5
Undergoing oocyte donation (n)	3	5
Mature follicles on day of hCG (≥14 mm)	11.4 ± 1.1	12.4 ± 1.8
Peak E_2 level on day of hCG (pg/mL)	2,432 ± 88	2,648 ± 92
Number coasted, n (%)	0 (0)	0 (0)
Proportion of cases using ICSI (%)	100	100
Clinical diagnoses, n (%)		
Male factor	38 (52.8)	40 (48.2)
Tubal factor	9 (12.5)	11 (13.3)
PCOS	8 (11.1)	10 (12.0)
Endometriosis	6 (8.3)	7 (8.4)
Idiopathic	11 (15.3)	15 (18.1)



- Female age 20-42 years (average 32)
- qPCR
- Blastocyst stage biopsy and day 6 fresh embryo transfer

Scott RT Jr, Upham KM, Forman EJ, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. *Fertil Steril.* 2013;100:697-703.

Future PGD/PGS Strategy

- Improved diagnostic strategies for determining chromosome number
- Extend to microdeletions and microduplications
- Assessment may target genes essential for embryonic development
- Combination of single gene and aneuploidy screening
- Viability assessment (reduced time, accurate amplification, readily available, cost-effective)
- Combine chromosomal screening with novel genetic testing applications such as epigenetics and transcriptomics, from the same biopsy

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Conclusions

- PGD is a complex and extensive process
- PGD is presently applied to a wide range of indications, including those of genetic or non-genetic nature, and is also combined with 24-chromosome aneuploidy testing
- Indications for PGD expand beyond diagnostic purposes and include treatment of siblings requiring HLA-compatible stem cell transplantation

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Conclusions (con't.)

- PGS offers
  - High-efficiency elective single embryo transfer
  - Increased pregnancy rate per cycle started
  - Faster time to pregnancy
  - Avoidance of unnecessary embryo transfers
  - Avoidance of cryopreservation of non-viable embryos
  - Prognostic information (recurrent IVF failure patients)

References

- Allingham-Hawkins DJ, Babul-Hirji R, Chitayat D, et al. Fragile X premutation is a significant risk factor for premature ovarian failure: the International Collaborative POFin Fragile X study--preliminary data. *Am J Med Genet*. 1999;83:322-325.
- Anton E, Vidal F, Blanco J. Role of sperm FISH studies in the genetic reproductive advice of structural reorganization carriers. *Hum Reprod.* 2007;22:2088-2092.
- Ata B, Kaplan B, Danzer H, et al. Array CGH analysis shows that aneuploidy is not related to the number of embryos generated. *Reprod Biomed Online*. 2012;24:614-620.
- Azziz R, Carmina E, Dewailly D, et al. Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. *J Clin Endocrinol Metab*. 2006;91:4237-4245.
- Benet J, Martin RH. Sperm chromosome complements in a 47,XYY man. *Hum Genet.* 1988;78:313-315.

References

- Bentley GE, Ubuka T, McGuire NL, et al. Gonadotrophin-inhibitory hormone: a multifunctional neuropeptide. *J Neuroendocrinol*. 2009;21:276-281.
- Bhagavath B, Podolsky RH, Ozata M, et al. Clinical and molecular characterization of a large sample of patients with hypogonadotropic hypogonadism. *Fertil Steril*. 2006;85:706-713.
- Bishop R. Applications of fluorescence *in situ* hybridization (FISH) in detecting genetic aberrations of medical significance. *Bioscience Horizons.* 2010;3:85-95.
- Bodurtha J, Strauss JF 3rd. Genomics and perinatal care. *N Engl J Med*. 2012;366:64-73.
- Brown GM, Leversha M, Hulten M, Ferguson-Smith MA, Affara NA, Furlong RA. Genetic analysis of meiotic recombination in humans by use of sperm typing: reduced recombination within aheterozygous paracentric inversion of chromosome 9q32-q34.3. *Am J Hum Genet*. 1998;62:1484-1492.

References

- Boué A, Gallano P. A collaborative study of the segregation of inherited chromosome structural rearrangements in 1356 prenatal diagnoses. *Prenat Diagn.* 1984;4:45-67.
- Capalbo A, Bono S, Spizzichino L, et al. Sequential comprehensive chromosome analysis on polar bodies, blastomeres and trophoblast: insights into female meiotic errors and chromosomal segregation in the preimplantation window of embryo development. *Hum Reprod*. 2013;28:509-518.
- Capalbo A, Rienzi L, Cimadomo D, et al. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts [published online ahead of print March 19, 2014]. *Hum Reprod.*

References

- Capalbo A, Wright G, Elliott T, Ubaldi FM, Rienzi L, Nagy ZP. FISH reanalysis of inner cell mass and trophectoderm samples of previously array-CGH screened blastocysts shows high accuracy of diagnosis and no major diagnostic impact of mosaicism at the blastocyst stage. *Hum Reprod*. 2013;28:2298-2307.
- Chandley AC. Infertility and chromosome abnormality. In: Clarke JR, ed. *Oxford Reviews in Reproductive Biology*. Vol 6. Oxford, United Kingdom: Oxford University Press; 1987:1-46.
- Chandley AC, McBeath S, Speed RM, Yorston L, Hargreave TB. Pericentric inversion in human chromosome 1 and the risk for male sterility. *J Med Genet*. 1987;24:325-334.
- Conway GS, Payne NN, Webb J, Murray A, Jacobs PA. Fragile X premutation screening in women with premature ovarian failure. *Hum Reprod*. 1998;13:1184-1187.
- Crowley PH, Gulati DK, Hayden TL, Lopez P, Dyer R. A chiasma-hormonal hypothesis relating Down's syndrome and maternal age. *Nature*. 1979;280:417-418.

References

- de Roux N, Young J, Misrahi M, et al. A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. *N Engl J Med*. 1997;337:1597-1602.
- Dul EC, van Echten-Arends J, Groen H, Dijkhuizen T, Land JA, van Ravenswaaij-Arts CM. Chromosomal abnormalities in azoospermic and non-azoospermic infertile men: numbers needed to be screened to prevent adverse pregnancy outcomes. *Hum Reprod*. 2012;27:2850-2856.
- Eichenlaub-Ritter U, Chandley AC, Gosden RG. The CBA mouse as a model for age-related aneuploidy in man: studies of oocyte maturation, spindle formation and chromosome alignment during meiosis. *Chromosoma*. 1988;96:220-226.
- Ferlin A, Arredi B, Speltra E, et al. Molecular and clinical characterization of Y chromosome microdeletions in infertile men: a 10-year experience in Italy. *J Clin Endocrinol Metab*. 2007;92:762-770.

References

- Fiorentino F, Kokkali G, Biricik A, et al. Polymerase chain reaction-based detection of chromosomal imbalances on embryos: the evolution of preimplantation genetic diagnosis for chromosomal translocations. *Fertil Steril.* 2010;94:2001-2011.
- Forejt J. X-Y involvement in male sterility caused by autosome translocations—a hypothesis. In: Fraccaro M, Rubin B, Rubin B, eds. *Genetic Control of Gamete Production and Function*. New York, NY: Academic Press; 1982:261-273.
- Foresta C, Ferlin A, Gianaroli L, Dallapiccola B. Guidelines for the appropriate use of genetic tests in infertile couples. *Eur J Hum Genet*. 2002;10:303-312.
- Forman EJ, Hong KH, Ferry KM, et al. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. *Fertil Steril*. 2013;100:100-107.

References

- Franco B, Guioli S, Pragliola A, et al. A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. *Nature*. 1991;353:529-536.
- Gekas J, Thepot F, Turleau C. Chromosomal factors of infertility in candidate couples for ICSI: an equal risk of constitutional aberrations in women and men. *Hum Reprod*. 2001;16:82-90.
- Gersen SL, Keagle L, eds. *The Principles of Clinical Cytogenetics*. New York, NY: Humana Press; 1999.
- Goossens V, Harton G, Moutou C, Traeger-Synodinos J, Van Rij M, Harper JC. ESHRE PGD Consortium data collection IX: cycles from January to December 2006 with pregnancy follow-up to October 2007. *Hum Reprod*. 2009;24:1786-1810.
- Harton GL, Munné S, Surrey M, et al. Diminished effect of maternal age on implantation after preimplantation genetic diagnosis with array comparative genomic hybridization. *Fertil Steril*. 2013;100:1695-1703.

References

- Hassold T, Abruzzo M, Adkins K, et al. Human aneuploidy: incidence, origin, and etiology. *Environ Mol Mutagen*. 1996;28:167-75.
- Henderson SA, Edwards RG. Chiasma frequency and maternal age in mammals. *Nature*. 1968;218:22-28.
- Hirsh AV. The management of infertile men presenting in the assisted conception unit. In: Brinsden PR, ed. *A Textbook of In Vitro Fertilization and Assisted Reproduction*. Boca Raton, FL: CRC Press; 2005:35-60.
- Hung AJ, King P, Schlegel PN. Uniform testicular maturation arrest: a unique subset of men with nonobstructive azoospermia. *J Urol*. 2007;178:608-612.
- Jäger RJ, Anvret M, Hall K, Scherer G. A human XY female with a frame shift mutation in the candidate testis-determining gene SRY. *Nature*. 1990;348:452-454.

References

- Kim HG, Pedersen-White J, Bhagavath B, Layman LC. Genotype and phenotype of patients with gonadotropin-releasing hormone receptor mutations. *Front Horm Res*. 2010;39:94-110.
- Kruse R, Guttenbach M, Schartmann B, et al. Genetic counseling in a patient with XXY/XXXY/XY mosaic Klinefelter's syndrome: estimate of sex chromosome aberrations in sperm before intracytoplasmic sperm injection. *Fertil Steril.* 1998;69:482-485.
- Kuliev A, Rechitsky S, Verlinsky O. *Atlas of Preimplantation Genetic Diagnosis*. 3rd ed. Boca Raton, FL: Taylor & Francis; 2014.
- Layman LC. Genetics of human hypogonadotropic hypogonadism. *Am J Med Genet*. 1999;89:240-248.
- Layman LC. The genetic basis of female reproductive disorders: etiology and clinical testing. *Mol Cell Endocrinol*. 2013;370:138-148.

References

- Layman LC, Lee EJ, Peak DB, et al. Delayed puberty and hypogonadism caused by mutations in the follicle-stimulating hormone beta-subunit gene. *N Engl J Med*. 1997;337:607-611.
- Legouis R, Hardelin JP, Levilliers J, et al. The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules. *Cell*. 1991;67:423-435.
- Levin I, Almog B, Shwartz T. Effects of laser polar-body biopsy on embryo quality. *Fertil Steril*. 2012;97:1085-1088.
- Martin RH. Cytogenetic determinants of male infertility. *Hum Reprod Update.* 2008;14:379-390.
- Martin R. Sperm cell—genetic aspects. In: Grudzinskas JG, Yovich JL, Simpson JL, et al, eds. *Cambridge Reviews in Human Reproduction.* Cambridge, England: Cambridge University Press; 1995:104-121.
- May KM, Jacobs PA, Lee M, et al. The parental origin of the extra X chromosome in 47,XXX females. *Am J Hum Genet*. 1990;46:754-761.

References

- McCallum T, Milunsky J, Munarriz R, Carson R, Sadeghi-Nejad H, Oates R. Unilateral renal agenesis associated with congenital bilateral absence of the vas deferens: phenotypic findings and genetic considerations. *Hum Reprod*. 2001;16:282-288.
- Mertzanidou A, Wilton L, Cheng J, et al. Microarray analysis reveals abnormal chromosomal complements in over 70% of 14 normally developing human embryos. *Hum Reprod*. 2013;28:256-264.
- Miklos GLG. Sex-chromosome pairing and male infertility. *Cytogenet Cell Genet*. 1974;13:558-577.
- Morris RS, Gleicher N. Genetic abnormalities, male infertility, and ICSI. *Lancet*. 1996;347:1277.
- Pettenati MJ, Rao PN, Phelan MC, et al. Paracentric inversions in humans: a review of 446 paracentric inversions with presentation of 120 new cases. *Am J Med Genet.* 1995;55:171-187.

References

- Peters H, McNatty KP. *The Ovary*. London, England: Granada Publishing; 1980.
- Pitteloud N, Acierno JS Jr, Meysing AU, Dwyer AA, Hayes FJ, Crowley WF Jr. Reversible Kallmann syndrome, delayed puberty, and isolated anosmia occurring in a single family with a mutation in the fibroblast growth factor receptor 1 gene. *J Clin Endocrinol Metab*. 2005;90:1317-1322.
- Plant TM. Hypothalamic control of the pituitary-gonadal axis in higher primates: key advances over the last two decades. *J Neuroendocrinol*. 2008;20:719-726.
- Poongothai J, Gopenath TS, Manonayaki S. Genetics of human male infertility. *Singapore Med J*. 2009;50(4):336-347.
- Practice Committee of American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. *Fertil Steril*. 2012;98:294-301.

References

- Practice Committee of the Society for Assisted Reproductive Technology; Practice Committee of the American Society for Reproductive Medicine. Preimplantation genetic testing: a Practice Committee opinion. *Fertil Steril*. 2007;88:1497-1504.
- Reindollar RH, Byrd JR, McDonough PG. Delayed sexual development: a study of 252 patients. *Am J Obstet Gynecol*. 1981;140:371-380.
- Reindollar RH, Novak M, Tho SP, McDonough PG. Adult-onset amenorrhea: a study of 262 patients. *Am J Obstet Gynecol*. 1986;155:531-543.
- Rechitsky S, Kuliev A, Tur-Kaspa I, Morris R, Verlinsky Y. Preimplantation genetic diagnosis with HLA matching. *Reprod Biomed Online*. 2004;9:210-221.

References

- Riccaboni A, Lalatta F, Caliari I, Bonetti S, Somigliana E, Ragni G. Genetic screening in 2,710 infertile candidate couples for assisted reproductive techniques: results of application of Italian guidelines for the appropriate use of genetic tests. *Fertil Steril*. 2008;89:800-808.
- Schoolcraft WB, Fragouli E, Stevens J, Munne S, Katz-Jaffe MG, Wells D. Clinical application of comprehensive chromosomal screening at the blastocyst stage. *Fertil Steril*. 2010;94:1700-1706.
- Schoolcraft WB, Treff NR, Stevens JM, Ferry K, Katz-Jaffe M, Scott RT Jr. Live birth outcome with trophectoderm biopsy, blastocyst vitrification, and single-nucleotide polymorphism microarray-based comprehensive chromosome screening in infertile patients. *Fertil Steril*. 2011;96:638-640.

References

- Scott RT Jr, Upham KM, Forman EJ, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. *Fertil Steril*. 2013;100:697-703.
- Scott RT Jr, Upham KM, Forman EJ, Zhao T, Treff NR. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. *Fertil Steril*. 2013;100:624-630.
- Sermon K, De Rijcke M, Lissens W, et al. Preimplantation genetic diagnosis for Huntington's disease with exclusion testing. *Eur J Hum Genet*. 2002;10:591-598.
- Shah K, Sivapalan G, Gibbons N, Tempest H, Griffin DK. The genetic basis of infertility. *Reproduction*. 2003;126:13-25.

References

- Shima JE, McLean DJ, McCarrey JR, Griswold, MD. The murine testicular transcriptome: characterizing gene expression in the testis during the progression of spermatogenesis. *Biol Reprod.* 2004;71:319-330.
- Sim H, Argentaro A, Harley VR. Boys, girls and shuttling of SRY and SOX9. *Trends Endocrinol Metab*. 2008;19:213-222.
- Smits G, Olatunbosun O, Delbaere A, Pierson R, Vassart G, Costagliola S. Ovarian hyperstimulation syndrome due to a mutation in the follicle-stimulating hormone receptor. *N Engl J Med*. 2003;349:760-766.
- Sugawara S, Mikamo K. Absence of correlation between univalent formation and meiotic nondisjunction in aged female Chinese hamsters. *Cytogenet Cell Genet*. 1983;35:34-40.
- Thornhill AR, Snow K. Molecular diagnostics in preimplantation genetic diagnosis. *J Mol Diagn*. 2002;4:11-29.

References

- Treff NR, Scott RT Jr. Methods for comprehensive chromosome screening of oocytes and embryos: capabilities, limitations, and evidence of validity. *J Assist Reprod Genet*. 2012;29:381-390.
- Vasseur C, Rodien P, Beau I, et al. A chorionic gonadotropin-sensitive mutation in the follicle-stimulating hormone receptor as a cause of familial gestational spontaneous ovarian hyperstimulation syndrome. *N Engl J Med*. 2003;349:753-759.
- Vernaeve V, Staessen C, Verheyen G, Van Steirteghem A, Devroe P, Tournaye H. Can biological or clinical parameters predict testicular sperm recovery in 47,XXY Klinefelter's syndrome patients? *Hum Reprod*. 2004;19:1135-1139.
- Vissers LE, van Ravenswaaij CM, Admiraal R, et al. Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. *Nat Genet*. 2004;36:955-957.

References

- Yang Z, Liu J, Collins GS, et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. *Mol Cytogenet*. 2012;5:24.
- Zhong Q, Layman LC. Genetic considerations in the patient with Turner syndrome--45,X with or without mosaicism. *Fertil Steril*. 2012;98:775-779.