INDIAN FERTILITY SOCIETY

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INDIAN FERTILITY SOCIETY & SIG APPLIED GENETICS

HOW DO WE APPLY GENETICS IN OB / GYN PRACTICE - BASICS TO THE ADVANCES

Infertility is a disease of the reproductive system characterized by inability to achieve pregnancy after 12 or more months of regular unprotected sexual intercourse. An array of factors, including ovulation defects, Spermatogenic failure, elderly age, obesity and genital infections have been linked with infertility, in addition to few genetic anomalies.

The study of genes associated with infertility in human reproduction has expanded the field of translational genetics in pinpointing the underlying cause of human fertility issues.

Many captivating aspects of the molecular basis of infertility in humans remain inadequately understood, however, application of genetic knowledge in this field is encouraging. The growing literature on the genetics of human infertility disorders deserves attention and a critical concise summary is required. Keeping this in mind we have designed panel of Three Pan India workshops to create awareness about the genetics in infertility.

I am sure you would enjoy the meetings and reading the manual.

Dr M Gouri Devi
President - IFS

According to the World Health Organization, infertility is a disease of the reproductive organs and is defined, as the inability of sexually active couples taking no contraceptives to achieve pregnancy within 1 year.

This is a multifaceted disease and many genetic disorders may lead to infertility. Known genetic causes of infertility include chromosomal aberrations, single gene variants and multifactorial inheritance,

Several genetic syndromes may lead to infertility and there are precise markers that can be used for genetic testing of infertility situations. Presently there is a need to develop diagnostic technologies to ascertain infertility related genes. In the coming future, tailing the common genetic variants, mutations, or polymorphisms may provide clinically relevant therapeutics for infertile individuals. Currently, several genetic associations have been performed to identify genes for infertility in humans.

I am sure you would enjouy this clinical symposium and learn nuances of genetics in reproductive biology. I am grateful to Dr Ratna Puri and her team and team from Thermo Fisher Scientific led by Dr Sailesh in organizing this event.

Prof (Dr) Pankaj Talwar
Secretary General -IFS

Infertility affects nearly 7% couples in the reproductive age. Although the etiology is heterogeneous, nearly 50% of infertility cases are of genetic etiology. These genetic causes are varied ranging from abnormalities in chromosomes, single gene disorders and those with a significant environmental impact. Some typically involve male infertility whereas others impact male and female fertility status. In the current era of major technological advances in reproductive genetics and the availability of all testing in India, it is relevant to update ourselves of the appropriate screening and management of couples in pregnancy.

This workshop was designed to address the above felt needs of genetics as applied to infertility. The topics deal with a range of practical situations in clinical practice. The understanding and appropriate utilization of current, basic as well as high-end tests, can appropriately optimize outcomes in pregnancy. Through this workshop we would like to conceptualize that genetics has moved from "bench to bedside".

In the field of infertility and assisted reproduction, knowledge of the possible genetic etiology of infertility helps to suitably test the fetus for genetic disorders to avoid the birth of an affected child. Antenatal screening for aneuploidy in infertility and ART has specific implications that will be touched upon.

And finally, are we adding on to the burden of genetic disorders with the techniques of ART is a burning question to address and counsel families.

Throughout this workshop we hope to invite your participation to allow for an interactive session as well as learn from each other. I would also like to bring to record and thank the Indian Fertility Society, under the leadership of the President and Secretary, for recognizing the era of genetics in clinical practice and encouraging. They have spearheaded the creation of a " special interest group" in Applied Genetics to allow us to meet and exchange ideas to better patient care.

Dr Ratna Dua Puri
Convenor

- **Professor and Chairperson, Institute of Medical Genetics and Genomics, Sir**
- **Ganga Ram Hospital, New Delhi**
- **M.D. Pediatrics (AFMC); D.M. Medical Genetics (SGPGI, Lucknow)**
- **Past President, Society of Fetal Medicine**
- **Founding Member, Society of Indian Academy of Medical Genetics**
- **Dharam Vira Award of Excellence** in recognition of meritorious service rendered to Sir Ganga Ram Hospital - 2010
- Young Investigators Award, Tokyo, Japan 2006
- Member of the Department of Health Research – ICMR, Task Force
- Ongoing Research Projects
  - A Study of Whole Exome Sequencing in Anomalous Euploid Foetuses
  - **Multicentric** Collaborative Study of the Clinical , Biochemical and Molecular Characterization of Lysosomal Storage Disordrs in India
  - **The Outcome in Fetuses with Increased Nuchal Translucency in the First Trimester**
  - **Establishing Registry for rare and potentially treatable Genetic Disorders**
  - Establishing Center for Education and Training in Genetic Medicine
- Publications – 85

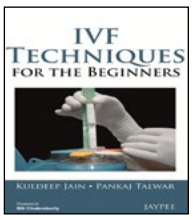
Dr M Gouri Devi
M.D

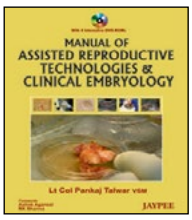
- Director, Gouri Hospitals Ltd.
- Director, Ridge IVF Group.(Runs a chain of IVF centres)
- President, Indian fertility society
- Ex-Secretary General, Indian Fertility Society
- Executive, AOGD governing council
- Member, Executive Board, NARCHI, DGES, FPSI
- Ex Vice President, NARCHI
- Chairperson, Advocacy &Ethics Committee, IFS.
- State Quality Assurance Committee (SQAC)Govt of NCT of Delhi.
- Member: MTP advisory committee, Govt Of NCT of Delhi
- Member Advisory committee on ethical practices in the field of obstetrics, Govt of NCT, Delhi
- Recipient of Kanak Goel Award 1995-1996 from IMA.
- Chairman's Appreciation Award by IMA AMS – 2002
- Dr. APJ Abdul Kalam Excellence Award – 2017
- Economic Times Award one of the Most Inspiring Gynecologists of India

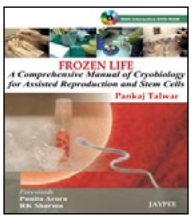
She is a keen academician, has organized many conferences, has been a speaker in many national and international conferences. Has many publications to her credit

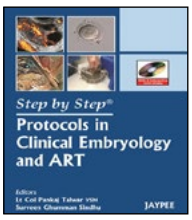
Col Pankaj Talwar, VSM
Professor and HOD
ART Centre, Army Hospital, New Delhi

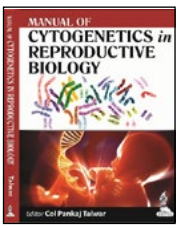
- Sec IFS.
- Secretary Fertility preservation society of India.
- Editorial board of multiple Infertility journals.
- Member Advisory committee ICMR
- Member Infertility committee FOGSI
- Editor Nexus / Artext – E bulletin of IFS
- Awarded Vishisht seva medal by the President of India for working in field of infertility
- Associate Editor FSR
- Set up four centres for Armed forces .
- Experience of 10,000 and ET cycles .
- Member International society of fertility preservation.
- Trained Human Embryonic Stem Cell Derivation – Israel
- Trained in ovarian cortex freezing (fertility preservation) - Paris
- Trained in PGD – Germany, Spain
- Trained in QA/QC-Spain
- Edited 6 books

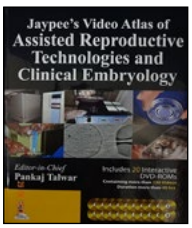


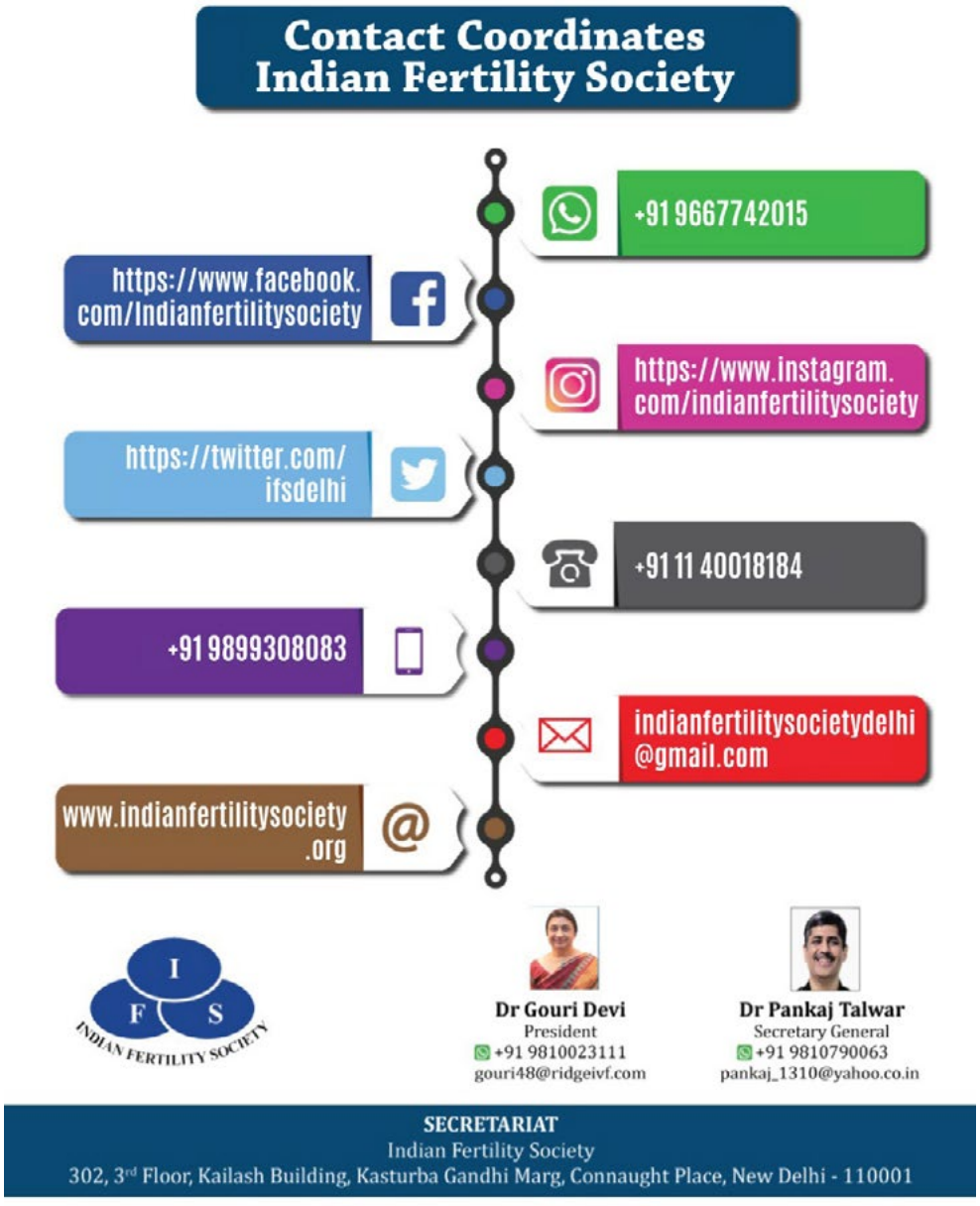








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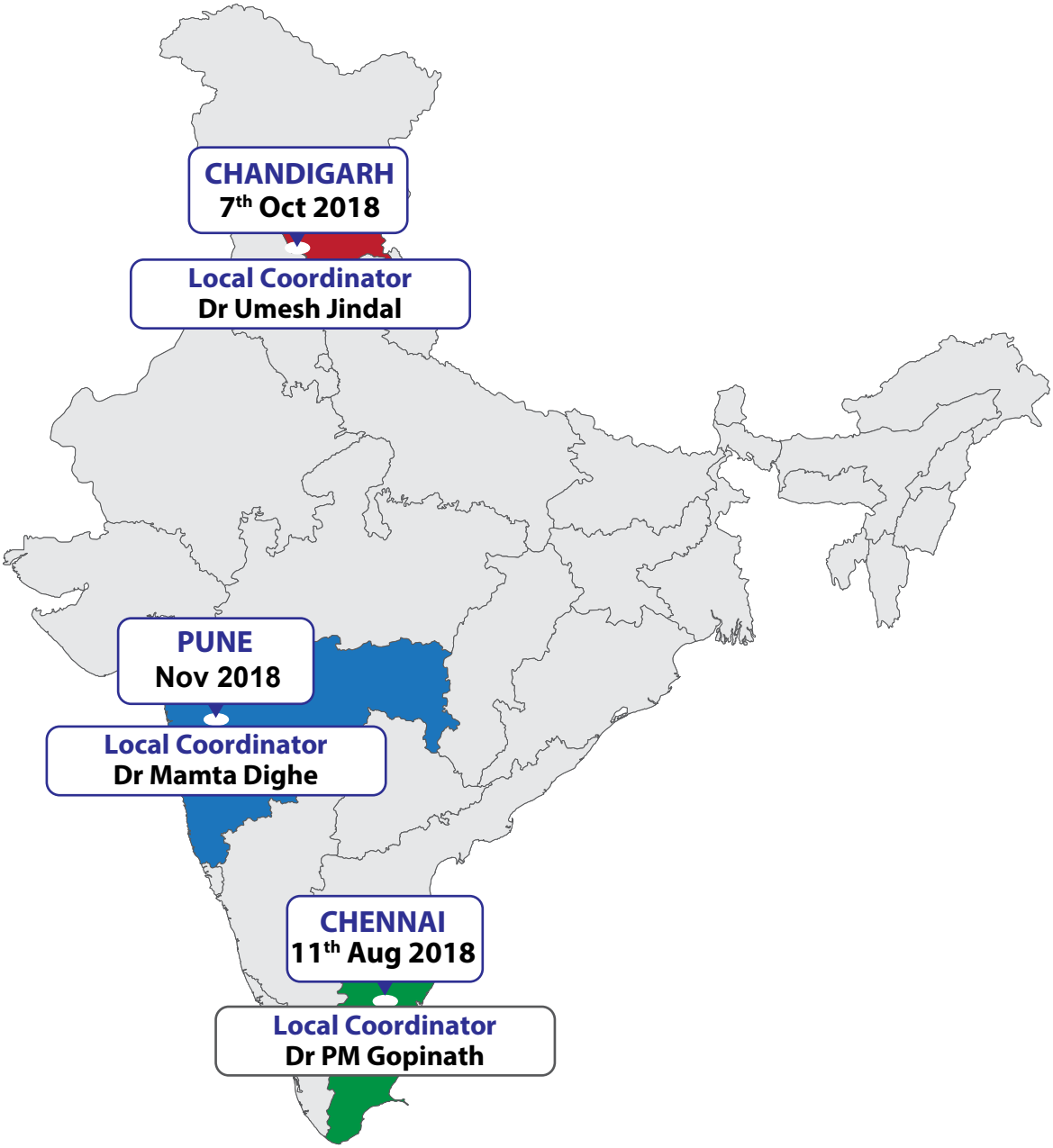
Venue and Dates



List of contributers

Topic	Contributed by
Genetics in the clinic – the time has come	Dr. Ratna Dua Puri
Genetic evaluation in infertility	Dr. Manisha Vajpeyee Dr. Geeta Goswami
Aneuploidy Screening: The how, what and when	Dr. Ratna Dua Puri
Emerging Technologies in Genetic Diagnosis – applicationin clinical practice	Dr. Michael Richardson
Does ART predispose to genetic disorders?	Dr. Manisha Vajpeyee Dr. Geeta Goswami
Panel Discussion : Spectrum of genetic tests in the IVF clinic – clinical scenarios and expert discussions:	Dr. Anupam Gupta Dr. Sheetal Jindal Dr. Ashima

Programme for the day

Time	Topic
12:00 - 13:00	Registration & Lunch
13:00 - 13:15	Welcome and Introduction to the program
13:15 - 13:35	Genetics in the clinic – the time has come
13:35 - 13:45	Discussion
13:45 - 14:05	Genetic evaluation in infertility
14:05 - 14:15	Discussion
14:15 -14:35	Aneuploidy Screening: The how, what and when
14:35 - 14:45	Discussion
14:45 - 15:15	Tea
15:15 - 15:35	Emerging Technologies in Genetic Diagnosis – application in clinical practice
15:35 - 15:45	Discussion
15:45 - 16:00	Does ART predispose to genetic disorders?
16:00 - 17:00	Panel discussions

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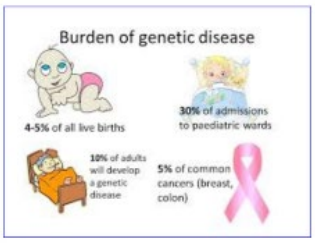
1. Genetics in the clinic : The time has come!

What are we Addressing?

"Congratulations, it's a Versace!"



Burden of Genetic Disorders in India

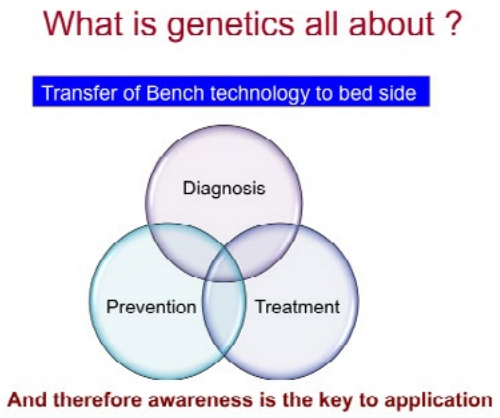
Disorder	Estimated cases per year
Congenital malformations	495,096
G6PD deficiency[1]	390,000
Down syndrome	21,412
β-Thalassaemia	9,000
Sickle cell disease	5,200
Amino acid disorders	9,760

Community Genet 2002;5:192–196

Estimated cases with Malformations

Malformations	Cases per 10,000	Estimated births
Neural tube defects	36.3	88,532
Talipes	14.5	35,364
Polydactyly	11.6	28,291
Hydrocephalus alone	9.5	23,169
Cleft lip and/or cleft palate	9.3	22,681
Congenital heart disease	7.1	17,316
Hypospadias	5.0	12,194
Tracheo-oesophageal fistula	3.7	9,023
Diaphragmatic hernia	2.6	6,341
Anorectal atresia/stenosis	2.4	5,853
Microcephaly	2.2	5,365
Cleft palate alone	1.7	4,146
Intersex and bilateral cryptorchidism	1.6	3,902
Intestinal atresia/stenosis	1.2	2,926
Anophthalmia/microphthalmia	1.0	2,438

What is genetics all about ?



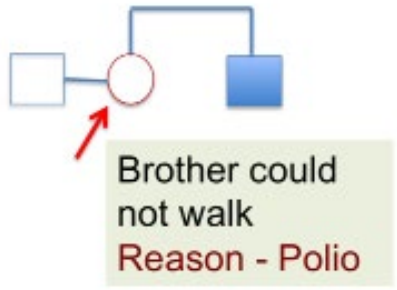
Indications for Genetics Evaluation in Obstetric Practice

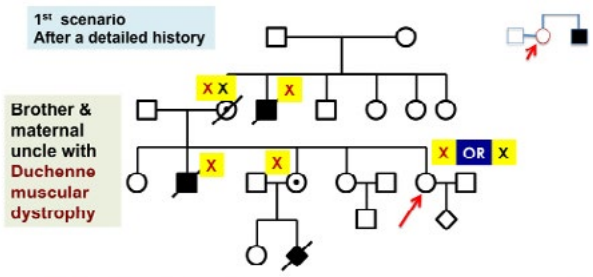
- Advanced Parental Age
- Previous Child with: Dysmorphism, Autism, Malformations, Intellectual Disability, Deafness, Albinism, Thalassemia, Short Stature, Neuromuscular disorder, Cerebral Palsy, Metabolic Defect, Hemophilia
- Consanguinity
- Unexplained stillbirths/ neonatal deaths
- Pregnancy: Aneuploidy risk, USG abnormality, teratogen exposure, IU infection
- Primary Amenorrhea / Recurrent Pregnancy Loss
- Premature ovarian failure with family history

Clinical presentation in OBG clinic

Presenting Complaint /Referral indication	Premarital	Pre-conceptional	Prenatal
Amenorrhea	0	0	
Genital ambiguity	0		
Infertility		0	
Consanguinity	0	0	0
Family history of genetic disorder	0	0	0
Recurrent pregnancy loss		0	0
Previous child with genetic disorder		0	0
Abnormal Screening results			0
Abnormal USG			0

Scenario 1





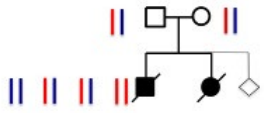
1. X linked disorder
2. There is a 50 % chance that she is a carrier
3. Test her carrier status
4. Definite Diagnosis of the disorder essential before prenatal testing

Family history of a genetic disorder
Can ascertain the inheritance from the pedigree

- **Consanguinity**
- **History of a genetic condition**
  - **– familial breast cancer**
  - **– risk for offspring – autosomal dominant disorder**
    e.gs – tuberous sclerosis, neurofibromatosis, Huntington chorea
    - autosomal recessive disorder
    e.gs – thalassemia, spinal muscular atrophy, deafness
    - X linked disorder
    e.gs – hemophilia, Duchenne muscular dystrophy

Scenario 2

Remember the recurrence risk is for each conception



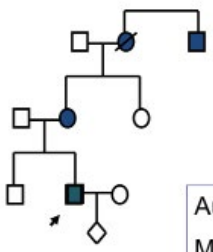
- Two children died in neonatal period
- Dx – Spinal Muscular Atrophy, autosomal recessive disorder
- Recurrence risk in each conception – 25% for disease and 75% normal fetus
- Third pregnancy - she was counseled that PND is not required. "Affected will not be born each time and the baby will be normal" Neonate was affected with SMA

How does family history help us in the Clinic

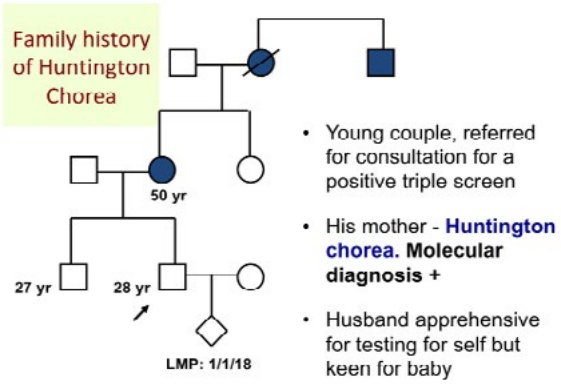
Scenario 3

Understanding recurrence risks

3rd scenario



Autosomal dominant disorder
Multiple generations affected
50% risk of occurrence in the offspring



- Young couple, referred for consultation for a positive triple screen
- His mother - **Huntington chorea. Molecular diagnosis +**
- Husband apprehensive for testing for self but keen for baby

- In addition to counseling for risk of recurrence
- Remember to determine the genetic basis of the disorder in the family before conception
- This is ideal
- Otherwise at the very FIRST visit

Reporting of genetic tests takes time
And pregnancy does not wait

Carrier Screening in the Preconception Period / 1st Visit

- Carrier screening for thalassemia / hemoglobinopathies

– Complete blood count – MCV <80 fl; MCH < 27 pg
– Hb high performance liquid chromatography [HPLC] & quantification of HbA2 & F
– If woman is a carrier, screen husband
– If both partners are carriers, risk to fetus of thalassemia major
– Identify mutation in HBB gene in couple before CVS
– Screening of at risk relatives [siblings, cousins] of the couple for carrier status

What interactions of beta globin gene and hemoglobin variantsrequire prenatal testing

Hemoglobin variants	Prenatal Diagnosis
β thal & β thal	Y
Hb E & β thal	Y
Hb S & β thal / Hb δβ / Hb S / Hb D Punjab / Hb C / Hb E	Y
δβ & HbS / β thal	Y
Hb Bart and HbH	Y
Hb O Arab & β thal	Y
Hb D Punjab & β thal	N
HbS & HPFH	N
HPFH /HPFH	N
Hb C & β thal	N

Carrier Screening

- Screening in the preconception period
- Carrierscreeningforthalassemia/hemoglobinopathies

– Complete blood count – MCV <80 fl; MCH < 27 pg
– Hb high performance liquid chromatography [HPLC] & quantification of HbA2 & F
– If woman is a carrier, screen husband
– If both partners are carriers, prenatal diagnosis

- Spinal muscular atrophy
- Fragile X – premature ovarian failure with elevated FSH < 40yrs, male relatives with ID
- Expanded carrier screening panels – appropriate genetic counseling

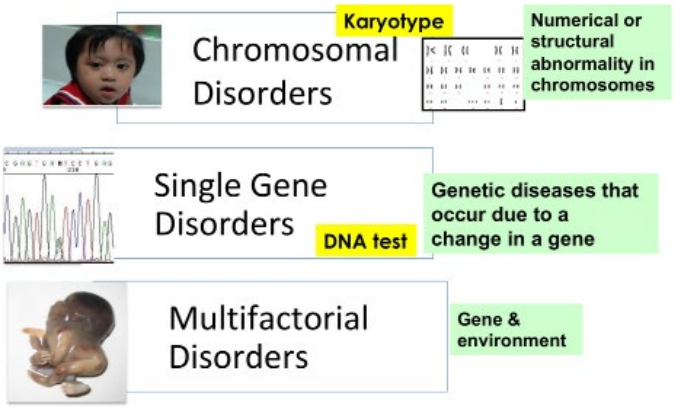
Infertility and genetic implications

- Male factor infertility – oligospermia / azoospermia
  - Genetic factors contribute to 2.2 -10% cases
  - Chromosomal disorders – sex chromosome
  - Y microdeletions
  - Cystic fibrosis – CBAVD
- Female factor and associated POF
- Birth defects – Slightly increased risk of BD; RR 1.32
- Impact of specific ART procedures on the epigenome and its consequences for the offspring - more data unfodling

M. Macek Jr. et al. 2017
European Journal of Human Genetics

Spectrum of genetic tests

Categories of Genetic Disorders



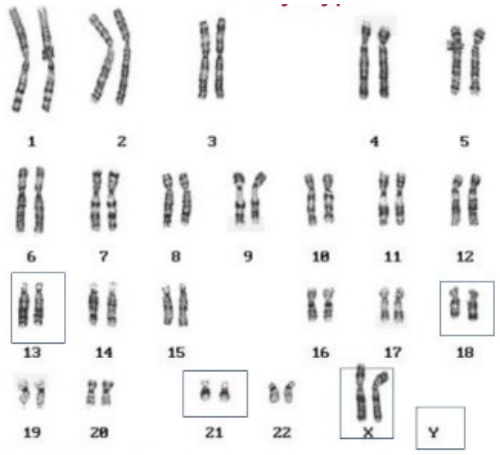
Indications of Testing for Chromosomal Disorders

- Recurrent pregnancy loss
- Infertility
- Antenatal detected fetal anomalies
- Previous child with chromosomal disorder
- Translocation carrier
- Primary amenorrhea

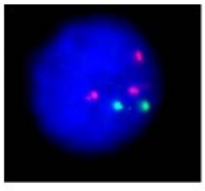
How to Choose the best test for Chromosomal Disorders

- Karyotype
- FISH - Fluorescence in situ hybridization
- QF PCR - Quantitative Fluorescence
- Polymerase Chain Reaction
- Chromosomal microarray

Karyotype

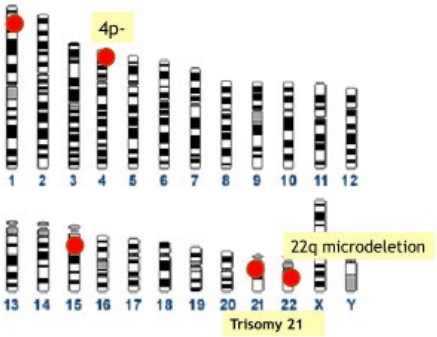


Fluorescent in situ hybridization



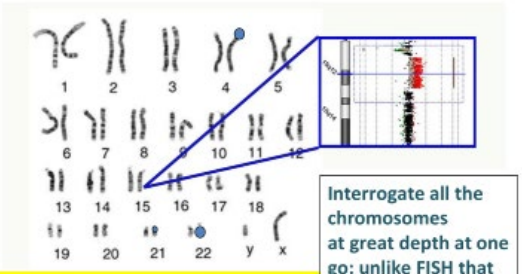
FISH is a targeted test done for a specific suspected syndrome

Probes that hybridize to a specific point on the chromosome



Karyotype / FISH / Chromosomal Microarray

FISH is a targeted test done for a specific suspected syndrome



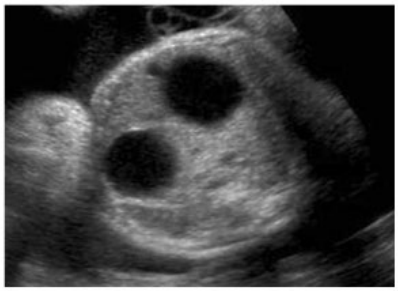
Interrogate all the chromosomes at great depth at one go; unlike FISH that will interrogate only one point on a chromosome

To look for gains and losses throughout the human genome

Scenario 5

5th scenario Normal Level II anomaly scan

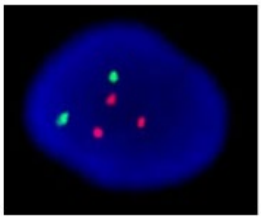
28 weeks, polyhydramnios, double bubble



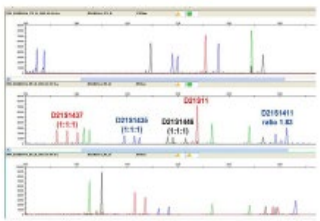
Possibility –Duodenal atresia

30% chance of Down syndrome

FISH - Fluorescent in situ hybridization

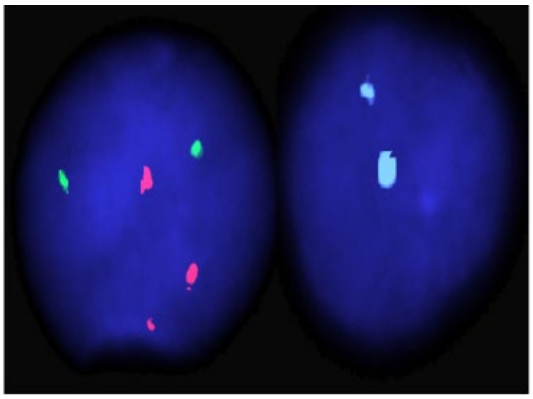


Qf PCR



Scenario 4

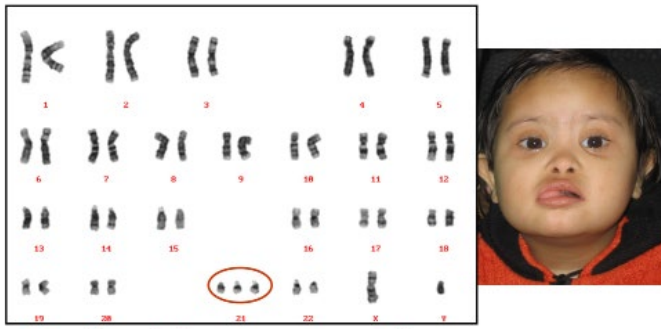
- 36 year old multigravida
- Quadruple test performed at 19 weeks after level II scan showed an absent nasal bone
- Screen risk - 1 in 150
- Came for amniocentesis at 22 weeks



FISH results

What is Fetal Aneuploidy?

Chromosomal disorders with abnormalities of number



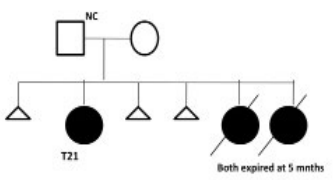
Fetal chromosomes - Karyotype

How does it present to obstetrician?

- Positive screening test for Down syndrome
  - First trimester- CUB
  - Second trimester- Triple/Quadruple
- Soft markers/malformations/IUGR on ultrasound
- Woman with previous child with Down syndrome
- Family history of Down syndrome
- Advanced maternal age

Choice of diagnostic test

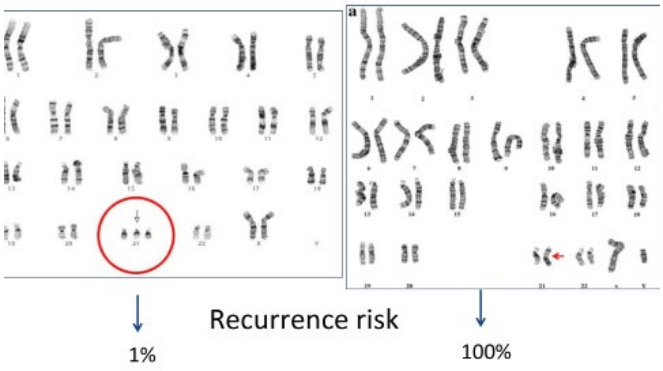
- Invasive fetal sampling: Amniocentesis/Chorionic villus sampling
- Culture of cells for karyotyping: 10-14 days
- Rapid aneuploidy diagnosis: result in 1-2 days
- FISH
- Other methods: QFPCR,MLPA



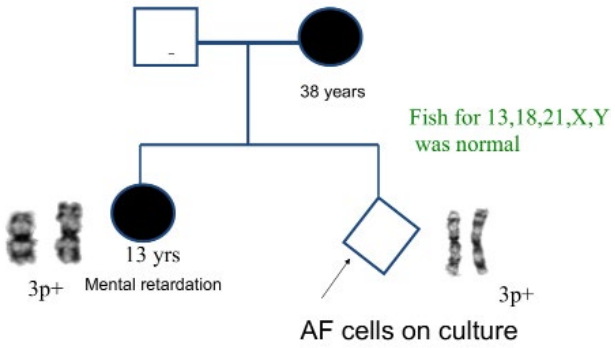
Father : 46,XY
Mother : 46,XX, t (21;21)
Daughter : Translocation 21, 46,XX, t(21;21)

Diagnosis: Translocation Down's

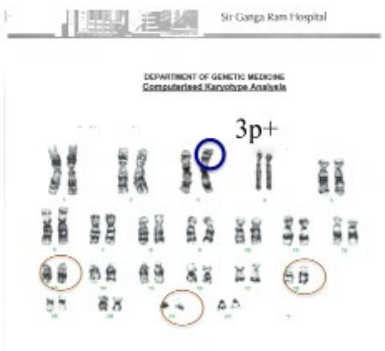
Types of Down Syndrome



What should be done in this case ?



Karyotype Report

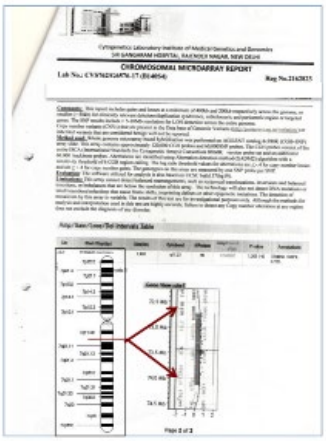


Chorionic villus sample – FISH and microarray

- FISH study was normal
- Chromosomal Microarray –

duplication of 1.4 Mb on 7q11.23 (72726572 – 74133332 bp)

- Genes: (ELN – Aortic dilatation) & GTF21 – behavior abnormalities, intellectual disability variable autism)


CHROMOSOMAL MICROARRAY REPORT

Important points to note about microarray

- It will identify all abnormalities detected by a karyotype and may identify additional abnormalities of chromosomes
- Not identify all genetic disorders

Diagnostic value of CMA after normal karyotype

- Fetuses with USG detected anomaly: 6 -13% (for pathogenic CNVs)
- Without USG anomaly but in indications like AMA, positive screen, anxiety: 1-2%

Prenat Diagn 2015, 35, 801–809; NICHD trial
ACOG Committe Opinion 682; Obstet Gynecol 2016;128:e262-e268
SMFM Consult Series 41 Am J Obstet Gynecol. 2016;215:2-9

Recommendations based on good & consistent scientific evidence

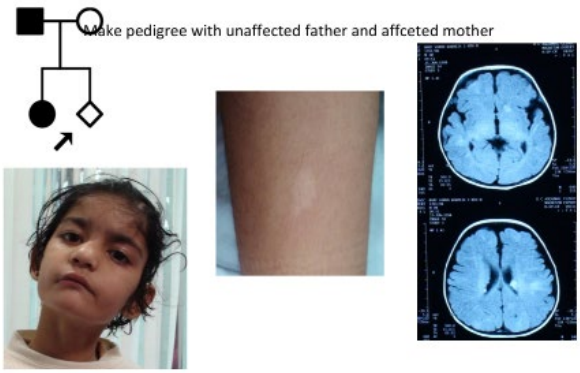
1. Recommended that chromosomal microarray analysis be made available to any patient choosing to undergo invasive diagnostic testing.
2. Chromosomal microarray recommended as the primary test (replacing conventional karyotype) for patients undergoing prenatal diagnosis for the indication of a fetal structural abnormality
3. If a specific aneuploidy is suggested by the anomaly – do karyotype +/- FISH before array .
4. Early amniocentesis[14 wks] not recommended

ACOG Practice Bulletin 162. May 2016

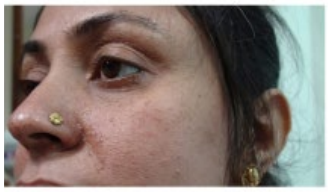
When an Invasive Procedure is Performed in Pregnancy

- FISH not an appropriate standalone test
- FISH – looks at only 5 chromosomes if done for aneuploidy
- Combine with karyotype OR microarray analysis

Child with Mental Retardation

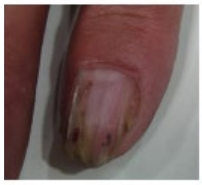


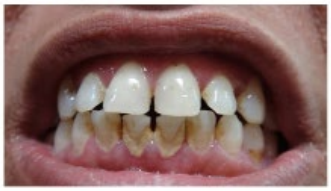
Liquor abnormalities



Tuberous Sclerosis

Autosomal Dominant

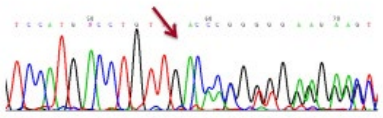




Concern: Ongoing pregnancy with a 50% risk of recurrence

- How are we going to check the fetal status for the disorder - Tuberous Sclerosis.
- Before doing prenatal diagnosis the gene mutation causing the disease in the affected child has to tested and identified.
- Only then can the CVS be done to check for the one mutation identified in the affected child

- Tuberous Sclerosis suspected
- Two genes – TSC 1 and TSC2 cause TS
- TSC2 commonest gene
- Sequence the gene in affected child



TSC2 gene - exon 11 del c at 1167

NOW CVS to check the fetus for the above mutation
Fetus at high risk of suffering from Tuberous Sclerosis that was the cause of mental retardation in this family

Family history of Mental Retardation or any suspected genetic disorder

Single case – Genetic or Non Genetic

Examine affected child

Make a diagnosis

Confirm the Dx by genetic test

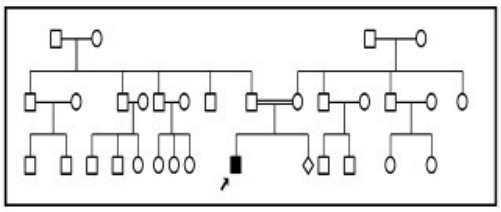
Re ascertain risk in the fetus for the specific disorder

Then CVS and prenatal testing

When should prenatal diagnostic testing be offered?

Positive screening test
But many indications beyond Down syndrome screening

Situation of application of NGS for Prenatal Diagnosis

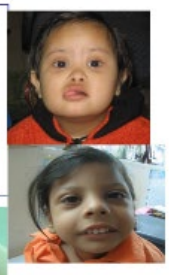


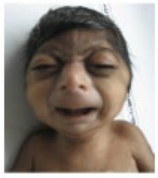
- First child had microcephaly and intellectual disability
- Referred at 11 wks of gestation for prenatal diagnosis

What is the cause of ID & microcephaly in the child ?

Can be one of multiple causes

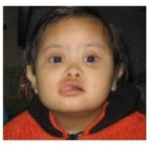
- Chromosomal disorders – Down syndrome
- Metabolic disorders – SLOS, Krabbe, MLD
- Malformation syndromes – Seckel, cDL
- Isolated ID & microcephaly





We did not have a diagnosis in the affected child

Prenatal testing not possible without a definite diagnosis in the affected



Examine the affected child,
Discuss & counsel the family,

- Molecular testing for all indicated genes in the affected child by NGS
- WDR62: Hom.c.1104_1104 delC in exon 9 p.Asp368fsX6
- Turn around time: 8 weeks
- Needs validation and further testing
- Best done preconception or early preganncy

Which patients are at increased risk of a fetal genetic disorder & need Prenatal Diagnosis?

1. Parental carrier of chromosome rearrangement
   – 5–30% risk of having offspring with unbalanced chromosomes in the future [identified through affected child]
   – 0-5% when identified for other reasons
2. Parental aneuploidy or aneuploidy mosaicism
3. Prior child with structural birth defect
4. Parental carrier of a genetic disorder – Thalassemia, TS
   – To ensure that any testing for recurrence is informative, a diagnosis established by molecular testing of the affected imp.
5. Previous fetus or child with autosomal/sex aneuploidy
6. Fetal structural abnormality

What laboratory tests on fetal sample?

Dependent on Indication of the test

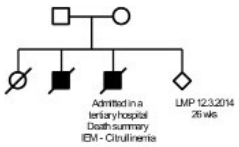
1. Chromosomes – targeted or all
   - FISH / QfPCR – targeted for specific chromosome
   - Karyotype – 46 chromosomes at a resolution of 5 Mb
   - Microarray – 46 chromosomes at resolution of 50-200 kb
2. Genes – testing for the specific mutation that causes the disease
3. Fetal malformation
   - omphalocele – chromosomes, BW syndrome gene
   - Increased NT – chromosomes, panel of genes

What is the best sample for prenatal testing

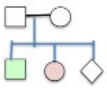
- Early gestation – Chorionic villi
- Molecular tests – Chorionic villi
- Chromosomes - amniotic fluid / CV
- Fetal cord blood – molecular tests / chromosomes / enzyme analysis / fetal hematology / NIHF

Alert for all DNA based analysis

Maternal contamination to be done to differentiat fetal and maternal tissue



- Two children died due to Citrullinemia
- Amniocentesis done at 18 wks for a positive Quadruple test
- 26 wks referred for prenatal testing for Citrullinemia



1st– operated for anal stenosis
2nd– sensorineural hearing loss
Current pregnancy 18 weeks
Evaluation so far:
- 12 wks scan but no NT
- Dual marker test without NT
- Triple test at 17 wks and USG
- TORCH at 3 mths : Rubella IgG & CMV IgG +ve at 122 and 170 U/ml IgMs - ve

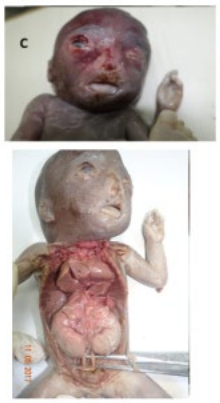
Repeated at 17 wks in view of high IgG
And then referred for counseling in view of previous affected child

- A request for a wider perspective
- Situations to avoid
- Look at the whole picture
- All fetal diagnosis are not with antenatal scans or Down syndrome screening

- 2nd gravida
- 1st preg – b/l enlarged, echogenic kidneys
- echogenic enlarged cystic kidneys, increased NFT (8mm), unossified nasal bone and polyhydramnios
- Discontinue pregnancy

- TRIO whole Exome
- ALG9 gene - p. Asn356Lys
- Novel
- Pathogenic by ACMG criteria
- Correlation of phenotype

Importance of Fetal autopsy and deep phenotyping



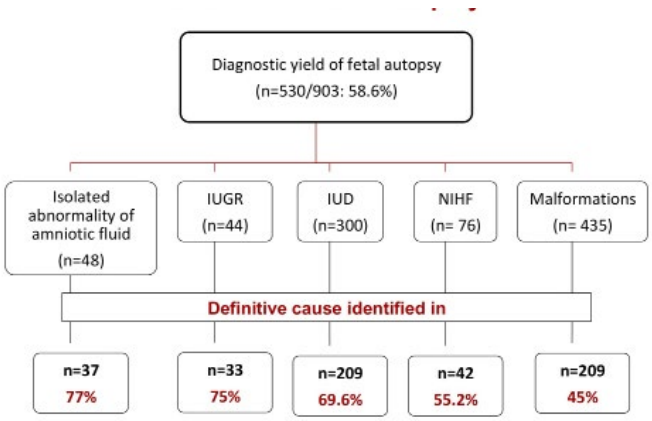
Hypertelorism,
beaked nose, flat facial profile
low-set posteriorly rotated ears
hypoplastic alae nasi,
microretrognathia,
short extremities with ulnar deviation of the hands,
deformed feet, rounded pelvis, flat vertebral bodies

Process of Evaluation for an antenatally Diagnosed Malformation

History	• Pregnancy history: drugs, viral infections, irradiation, diabetes mellitus, mode of conseption, serial antenatal scans, invasive procedures performed • Family history: consanguinty, previous affected sibling /relative, identify a mode of inheritance
Examination	• Single anomaly: malformation / deformation/ dysplasia / disruption • Multiple anomalies: multiple malformation syndrome / association / sequence • Photographs
Investigations	• Chromosomal analysis • Metabolite analysis • Molecular analysis • Fetal MRI • Radiographs after delivery
Making a diagnosis	• Synthesis of information • Gestalt diagnosis • Use of databases, search engines, books, published literature

Puri RD. Fetal dysmorphology .JOFM

Value of Fetal Autopsy



Indication tailored protocol for fetal autopsy

In All Cases
- Antenatal and family history
- Counseling for autopsy utility
- Antenatal sampling (AF/ cordocentesis) when possible
- Radiographs where skeletal abnormalities are present
- DNA storage

Puri et al.
Is the Diagnostic Yield Influenced by the Indication of Fetal Autopsy ?
AJMG Aug 2016 170(8)

Intrauterine Death	1. Placenta 2. Conventional autopsy 3. Chromosomal analysis 4. Kleihauer Betke test 5. Radiographs
Intra Uterine Growth Retardation	1. Placenta 2. Conventional autopsy 3. Geneticist opinion 4. Chromosomal analysis
Non Immune Hydrops Fetalis and Cystic Hygroma	1. Chromosomal / array cGH 2. Storage disorders testing 3. Intrauterine infections 4. Conventional autopsy
Malformations	1. Chromosomal / array cGH 2. Geneticist opinion 3. Conventional autopsy
Isolated abnormalities of Amniotic Fluid	1. Placenta 2. Conventional autopsy 3. Geneticist opinion 4. Chromosomal

Message

- Examine every fetus terminated or spontaneously aborted
- Record findings / take photographs / radiographs / store samples
- EDTA blood for DNA tests and heparin blood for chromosomes

Genetic Investigations

Chromosomal Disorders	Single gene disorders
Samples	
CVS / Amniotic fluid / Blood in Heparin	CVS / Blood in EDTA Amniotic fluid
FISH	ion

If pregnancy is discontinued
- Fetal Autopsy
- Save Samples

A Final Request for all
Universal Newborn Screening

- Universal Newborn screening
- Hypothyroidism is a must
- Other basic minimum protocol for NBS
    - Congenital adrenal hyperplasia
    - Galactosemia
    - G6PD deficiency
    - Biotinidase deficiency

Take Home Messages

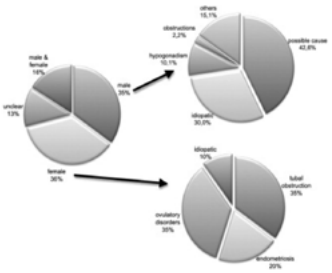
- Holistic approach to pregnancy
- Think beyond Down syndrome
- Never forget thalassemia
- One jacket does not fit all
- Choose the test appropriately
- Refer early if genetic disorders suspected
- Prenatal testing needs identification of the genetic mutation first
- Quality control of all tests performed

THANK YOU

2. Genetic evaluation in infertility

Infertility

- Infertility is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse.



Background

- Genetic tests are now available to explore the cause of the infertility and assess the risk of a given couple to transmit its genetic characteristics.
- This allows at-risk couples to take an informed decision when electing for a medically assisted reproduction.
- It also allows the professionals to offer a prenatal diagnosis when appropriate.
- Up to now, approximately 300 gene mutations, of which 70 are syndromes, are known to cause reproductive disorders

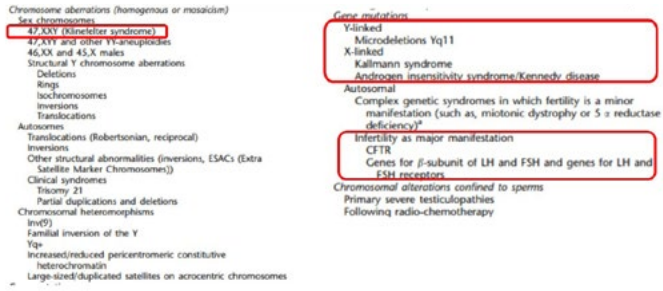
Background

- Several scientific studies have consistently shown that infertile couples have an increased frequency of chromosomal anomalies in both partners, independent of the cause of infertility.
- Genetic testing should thus always begin with a classical chromosome analysis.
- For this purpose, a whole blood sample of at least 2 ml diluted with heparin is required. For an optional molecular genetic analysis, a whole blood sample of at least 5 ml supplemented with EDTA is necessary.

Male Infertility

- Male infertility has been classified on the basis of the seminal analysis. although infertility and alterations of seminal characteristics are not synonymous.
- However, this classification is in keeping with the clinical practice, since the patients candidate to ART are often classified according to semen analysis.
- Genetic diagnosis and genetic counselling should always be part of an extensive evaluation of these patients, and basic clinical analysis should precede any genetic analysis

Genetic Causes Of Male Infertility



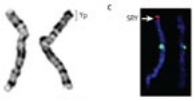
Chromosomal Aberrations

- Karyotype analysis of all numeric (gains/losses) and structural abnormalities (most frequently Robertsonian, but also reciprocal translocations, inversions) of entire chromosomes is associated with approximately 6% of all male infertility.
- Four percent of men receiving ICSI for male subfertility have chromosomal abnormalities, the majority of which involve sex chromosomes.
- Karyotypic abnormalities are identified in 3% to 5% of severely oligozoospermic (often translocations) and 14% to 19% of men with NOA (most frequently nonmosaic Klinefelter syndrome, 47,XXY)
- Are eight times more common in infertile than fertile men.
- Klinefelter syndrome (47,XXY) is identified in one in 600 males among the general population.

Klinefelter's Syndrome

- Klinefelter's syndrome (KS), 47, XXY, is the most common chromosomal abnormality.
- Clinicians should consider KS in all infertile men with azoospermia as nonmosaic KS accounts for 11% of cases, whereas mosaic KS (10% total) accounts for about 0.5% of the severely oligospermic population.
- Most KS men are never diagnosed due to a combination of the low awareness of the condition, the prevailing misconception that all have the classic textbook phenotype (tall, gynecomastia, florid hypogonadism), and the failure of clinicians to do a genital examination during routine health care.
- In reality KS has highly variable clinical features; many appear well virilized at first glance and have a wide range of school and workplace achievement that overlaps the general population.
- The only invariant finding of nonmosaic KS is that of small testes (2– 4 ml).

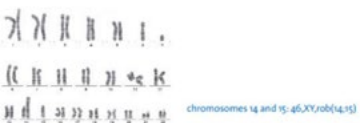
- 46,XX is another possible but rare karyotype (1:20,000 live births) identified in azoospermic men, often resulting from translocation of the distal tip of Y chromosome short arm (containing SRY gene) to the distal tip of the X chromosome short arm. The remaining Y chromosome is not present, including AZF regions, and therefore spermatogenesis is absent making TESE not possible.
- Yq loss also occurs in isodicentric Y chromosome which may be unstable and also lack AZF regions.
- Additionally, abnormalities of X chromosome linked genes (i.e., androgen receptor Xq11.2-12) may exhibit a spectrum of androgen insensitivity based on specific mutation and CAG repeat length and can result in 46,XY azoospermic males.



Translocations

Translocations

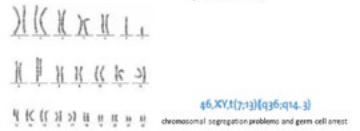
- Robertsonian



chromosomes 14 and 15: 46,XY,rob(14;15)

high risk of infertility, abnormalities in offspring, and spontaneous abortions

- Balanced Reciprocal

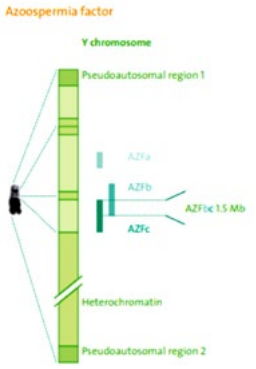


46,XY,t(7;13)(q36;q14..3)

chromosomal segregation problems and germ cell arrest

- In patients with non-obstructive azoospermia or oligozoospermia, there is an increased incidence of deletions in the long arm of the Y chromosome, region Yq11.21-23.
- This region is also known as the azoospermia factor (AZF) region and is subdivided into the subregions AZFa, AZFb, AZFc
- Deletions of AZFa result in the Sertoli Cell Only Syndrome (SCOS), i. e. azoospermia with complete loss of germ cells in the testes------ TESE will not yield sperms
- Deletions of AZFb cause spermatogenic arrest.
- AZFc deletions cause a variable phenotype ranging from oligozoospermia with all meioses stages to SCOS.
- Deletions in the AZFc region as the single causative of infertility allow for treatment with in vitro fertilization via intracytoplasmic sperm injection after TESE.

Yq microdeletions are the most common identifiable genetic cause of spermatogenic failure



CFTR Screening

- The CF transmembrane conductance regulator (CFTR) gene (7q31.2) encodes an epithelial chloride channel for which more than 1200 different mutations are known.
- CF is a serious autosomal recessive condition with birth incidence of about 1:2500 and a cumulative carrier frequency of one in 25. Preconceptual detection of carrier status allows preventative strategies to be used.
- Almost all CF males have absent vasa.
- Bilateral congenital absence of the vas (BCAV), in isolation, is a frequent cause of obstructive azoospermia (OA) in apparently healthy men

CFTR

- In CF/BCAV, the Wolffian duct derivatives (seminal vesicles, ejaculatory ducts, vasa, epididymal body/tail) appear to atrophy during fetal life , giving the
- classic presentation of OA with normal testis volume;
- thin/absent scrotal vasa; and
- a low volume,
- low fructose,
- acidic ejaculate.

CFTR screening is indicated whenever suggestive physical finding and/or unexplained OA is present.

AR Mutations

- Several hundred mutations of AR have been described with resultant phenotypes ranging from testicular feminization to partial androgen insensitivity syndrome to male infertility.
- The AR has an essential role in transducing androgen action on spermatogenesis, and whereas missense mutations have been associated with an isolated male infertility phenotype
- the prevalence rate is low and assessment is rarely performed
- Clinical presentations indicative of subtle AR mutations include clinical evidence of androgen deficiency despite raised serum LH and testosterone levels.

Hypogonadotrophic Hypogonadism

- a clinical syndrome characterized by low sex steroid and low gonadotropin levels resulting from a defect in the normal pulsatile secretion pattern of GnRH from the hypothalamus.
- Clinically, HH can be present with or without anosmia, the latter known as Kallmann's syndrome. Mutations of genes involved in the migration and/or function of the GnRH-secreting neurones are found in over 50% of the familial cases of HH and, more rarely, in sporadic cases .
- **Gene responsible was KAL1,** which encodes for a protein of the extracellular matrix, anosmin-1. Successively, more genes were found to be mutated in HH with or without anosmia (Following Table)…

Table 2 Genes mutated in Isolated Hypogonadotropic Hypogonadism and in Kallmann syndrome (4).

Acronym	Name	Location	Gene ID	Function
GNRHR	Gonadotropin-releasing hormone receptor	4q21.2	2798	Receptor for the gonadotropin-releasing hormone
KISS1	KiSS-1 metastasis-suppressor (metastin)	1q32	3814	Ligand of GPR54: stimulation of GnRH secretion
GPR54	G protein-coupled receptor 54	19p13.3	84634	Receptor for Kiss-1: stimulation of GnRH secretion
KAL1	Kallmann syndrome 1 sequence (anosmin-1)	Xp22.32	3730	Possible function in neural cell adhesion and axonal migration
FGFR1	Fibroblast growth factor receptor 1	8p11.2-p11.1	2260	Binds both acidic and basic fibroblast growth factors
FGF8	Fibroblast growth factor 8	10q24	2253	Member of the fibroblast growth factor (FGF) family involved in organogenesis
PRKR2	Prokineticin receptor 2	20p12.3	128674	G protein-coupled receptor for prokineticins
PRK2	Prokineticin 2	3p13	60675	Chemoattractant for neuronal precursor cells in the olfactory bulb
CHD7	Chromodomain helicase DNA binding protein 7	8q12.2	55636	Expressed in undifferentiated neuroepithelium and in mesenchyme of neural crest origin
GnRH1	Gonadotropin-releasing hormone	8p21 p11.2	2796	Stimulation of LH and FSH secretion

Middle East Fertility Society Journal (2010) 15, 139–145

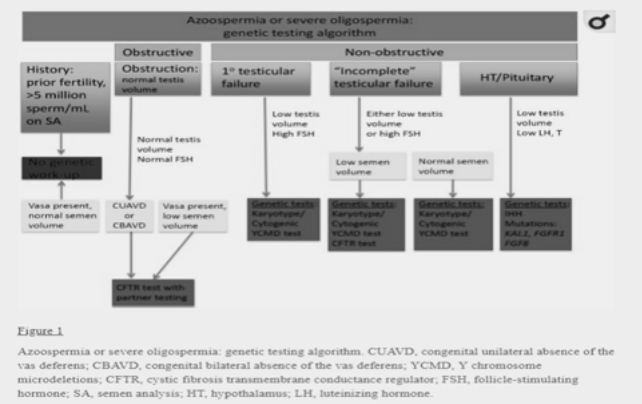


Figure 1

Azoospermia or severe oligospermia: genetic testing algorithm. CUAVD, congenital unilateral absence of the vas deferens; CBAVD, congenital bilateral absence of the vas deferens; YCMD, Y chromosome microdeletions; CFTR, cystic fibrosis transmembrane conductance regulator; FSH, follicle-stimulating hormone; SA, semen analysis; HT, hypothalamus; LH, luteinizing hormone.

CFTR Screening

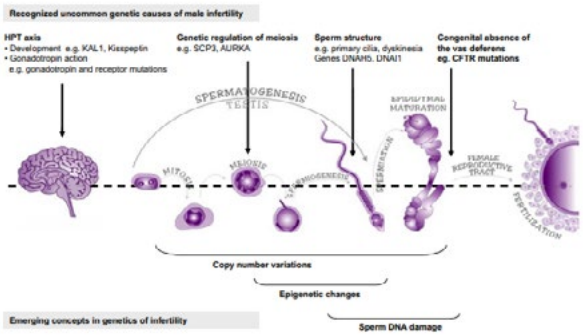
Rare cases of Male Infertility or Syndromes where Infertility is a Minor manifestation

Miotonic dystrophy
5α-reductase 2 deficiency
Steroidogenic enzymes deficiency (21 α-hydroxilase and others)
Bardet-Biedl
Noonan
Prader-Willi
Cerebellar ataxia with hypogonadotropic hypogonadism
Fanconi anaemia
Prune-Belly
Homozygous β-thalassemia
Hemochromatosis

Epigenetics

- **Whereas not strictly genetic, changes in the human epigenome are increasingly being associated with human male infertility.**
- **Epigenetic mechanisms include the way in which the genome is packed and thus the ability for genes to be activated. Epigenetic changes can be inherited across cell divisions or across generations and can have a profound effect on an individual's phenotype.**
- **It is clear that homozygous mutations in key epigenetic regulators affect male fertility more overtly than most biological systems and**
- **that sperm from some infertile men have an abnormal epigenome**

Known, but rare, and emerging causes of human male infertility.



List of aberrant epigenetic modification reported in male infertility.

Genes/proteins	Aberration and male infertility
MTHFR	DNA hypermethylation results in poor semen quality and infertility
PAX8, NTF3, SFN, HRAS	DNA hypermethylation associates with poor sperm concentration, motility and morphology
JHM2DA	Knockout results in loose packaging of DNA and may cause infertility
IGF2, H19	Low methylation associates with low sperm concentration
RASGRF1	Hypermethylation at the imprinted locus associates with poor semen parameters
GTL2	Hypermethylation at the imprinted locus associates with poor semen parameters
PLAG1, D1RAS3, MEST	Hypermethylation at the imprinted loci associates with poor semen parameters
KCNQ1, LIT1, SNRPN	Hypermethylation at the imprinted loci associates with poor semen parameters

Epigenetic Testing

- **Epigenetic testing helps reveal specific markers in a sperm's epigenome that have been as with infertility, poor IVF outcomes, a development of embryos.**
- **The Episona SEED Test**
- **Catsper**
- **ID3**

Table 1 Promising sperm markers of male infertility based on so far published literature

Approach type	Main outcomes	Ref.	Advantages (+)/disadvantages (-)
Semen analysis	Macroscopic and microscopic evaluation of semen according WHO guidelines	[2]	(+) Established reference values (-) High operator variability (-) Poorly predictive of fertility
Genetic and epigenetic	NGS: Found a set of sperm RNA elements required to achieve live births	[47]	(+) Broad-spectrum analysis
	miRNA: Alteration of 5 miRNAs in subfertile and NOA subjects compared to controls	[46]	(-) Lack of validation (-) Not independently predictive of fertility
	DNA methylation: Different methylation pattern between fertile and infertile subjects	[42,43]	(-) Too early for diagnostic purpose
	sDF: Discrimination between fertile and infertile subjects	[35,36,38]	(+) Presently adopted in many ART laboratories (+) Prediction of fertility independent from semen quality (-) Employment of different techniques to detect sDF (-) Lack of agreement on cutoff values
Proteomic	> 6000 proteins (histone variants, transcription factors, zinc finger proteins, receptors, proteins related to metabolism, structure and motility, carriers)	[80,95-98]	(+) Broad-spectrum analysis (-) Isolation of spermatozoa (-) Low available sperm material in oligozoospermic subjects (-) Intra- and inter-variability of proteomic profiles
PTMs	Phosphorylation: Reduced tyrosine phosphorylation in asthenozoospermic subjects	[101]	(+) Higher biological relevance compared to gene or protein expression *per se*
	Ubiquitination: Sperm quality control system	[104]	(-) No target proteins identified
	Sumoylation: Marker of defective sperm	[107,108]	(-) Too early for diagnostic purpose
Ion channels	Slo3: Involved in hyperpolarization during sperm capacitation	[111,112]	(+) Analysis free from confounders
	Catsper: Involved in sperm progressive and hyperactivated motility	[123]	(-) Skilled personnel and advanced instruments are required (-) Too early for diagnostic purpose

PTMs: Post-translational protein modifications; WHO: World Health Organization; NGS: Next-generation sequencing; NOA: Non-obstructive azoospermia sDF: Sperm DNA fragmentation; ART: Assisted reproduction technique.

Arab Journal of Urology (2018) 16, 53–64

Arab Journal of Urology
(Official Journal of the Arab Association of Urology)
www.sciencedirect.com

ETIOLOGY
ORIGINAL ARTICLE

A systematic review on the genetics of male infertility in the era of next-generation sequencing

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Epigenetics

Abstract ***Objectives:*** To identify the role of next-generation sequencing (NGS) in male infertility, as advances in NGS technologies have contributed to the identification of novel genes responsible for a wide variety of human conditions and recently has been applied to male infertility, allowing new genetic factors to be discovered.

Materials and methods: PubMed was searched for combinations of the following terms: 'exome', 'genome', 'panel', 'sequencing', 'whole-exome sequencing', 'whole-genome sequencing', 'next-generation sequencing', 'azoospermia', 'oligospermia', 'asthenospermia', 'teratospermia', 'spermatogenesis', and 'male infertility', to identify studies in which NGS technologies were used to discover variants causing male infertility.

Results: Altogether, 23 studies were found in which the primary mode of variant discovery was an NGS-based technology. These studies were mostly focused on patients with quantitative sperm abnormalities (non-obstructive azoospermia and oligospermia), followed by morphological and motility defects. Combined, these studies uncover variants in 28 genes causing male infertility discovered by NGS methods.

Conclusions: Male infertility is a condition that is genetically heterogeneous, and therefore remarkably amenable to study by NGS. Although some headway has been made, given the high incidence of this condition despite its detrimental effect on reproductive fitness, there is significant potential for further discoveries.

Guidelines…

- The main goal of the Guidelines is to promote the appropriate use of the available genetic tests.
- Using these Guidelines, together with careful genetic counselling would provide a better diagnosis and management of the infertile couple.
- However, it should be kept in mind that genetic tests are part of the diagnostic workup of the infertile couple, and therefore other investigations should be performed first.
- The Guidelines have been prepared not to include all the genetic causes of infertility, but only those clinically relevant, both in terms of prevalence and risk of transmission

Guidelines for the appropriate use of genetic tests in infertile couples

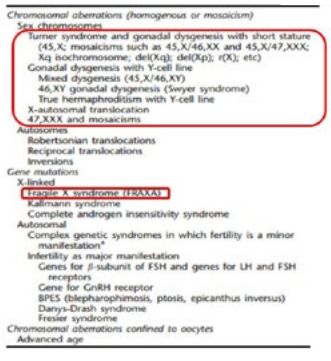
Table 2 Genetic tests in male infertility

	Azoospermia	*Severe oligozoospermia (sperm count < 10 × 10⁶/ml)*	*Moderate oligozoospermia (sperm count 10–20 × 10⁶/ml and normozoospermia)*
Karyotype	During diagnostic workup Prior to ART	During diagnostic workup Prior to ART	After 1 year of sexual intercourse aimed at pregnancy Prior to ART
Microdeletions of the Y chromosome long arm	During diagnostic workup (non obstructive) Prior to ART	During diagnostic workup Prior to ART	–
CFTR	During diagnostic workup (CBAVD) Prior to ART	During diagnostic workup (CUAVD) Prior to ART	–
KAL1	During diagnostic workup (HH)	–	–
Androgen receptor	Suggested: During diagnostic workup (high ASI)	Suggested: During diagnostic workup (high ASI)	–
5 α-reductase 2	Suggested: Selected clinical cases	Suggested: Selected clinical cases	–
Aneuploidy analysis on spermatozoa by FISH		Not suggested Eventually during diagnostic workup After radio-chemotherapy	–

ART: assisted reproduction techniques; ASI: androgen sensitivity index; CBAVD: congenital bilateral absence of vas deferens; CUAVD: congenital unilateral absence of vas deferens; HH: hypogonadotropic hypogonadism.

Genetic causes Of Female Infertility

Genetic causes Of
Female Infertility



)2) 10, 303 ± 312

Chromosomal Aberrations

- **Turner syndrome. The karyotype 45,X,** which causes Turner syndrome, is a common chromosomal abnormality in females. It affects 1/2500 live-born girls.
- Diagnosis is often delayed until the first years of school when growth retardation becomes apparent.
- Some are only diagnosed when presenting with primary ovarian failure, mostly as primary amenorrhea. The patient's intelligence is normal.
- The **47,XXX karyotype** has an incidence of 1 in 1000. Two thirds of the carriers have a clinically normal phenotype. One third has learning difficulties and psychotic disorders. **Premature ovarian failure with infertility has been described for carriers of this chromosomal abnormality**

Polycystic ovary syndrome (PCOS)

- A complex and heterogeneous endocrine condition that affects 5%–10% of women. PCOS is marked by hyperandrogenism, hyperinsulinemia, insulin resistance, and chronic anovulation
- Current literature dealing with the genetics of PCOS is inconsistent and inconclusive
- PCOS is influenced by obesity, and obesity itself has complex genetic associations. PCOS susceptibility genes are believed to be involved in sex hormone regulation, insulin sensitivity, and steroid biosynthesis

Premature Ovarian Failure (POF)

- A condition thought to be genetically determined
- It is defined as a primary ovarian defect characterized by absent menarche (primary amenorrhea) or premature depletion of ovarian follicles/ arrested folliculogenesis before the age of 40 years (secondary amenorrhea).
- Many genes and CNVs implicated but due to considerable heterogeneity in POF no routine genetic screening can be recommended so far beyond karyotype.
- FMR1 gene screening is also recommended. 6.5% of women with POF carry a FRAXA Furthermore, premutation has been shown to be associated with low response to ovarian stimulation during in vitro ART.

XX gonadal Dysgenesis

- Complex molecular signalling pathway in sex determination in the mammalian embryo.
- PGC-Migration-Gonad development from Bipotential Gonad
- XX female gonadal dysgenesis (XX-GD). XX-GD is genetically heterogeneous, but phenotypically identified by the **presence of gonadal streaks, lack of spontaneous pubertal development, primary amenorrhea, uterine hypoplasia, and hypergonadotropic hypogonadism**
- Mutations in FSHR, BMP15, NR5A1, EIF2B2, EIF2B5, HSD17B4, and HARS2 have been reported in XX-GD

List Of Genes Implicated in Female Infertility

	Infertility disorder	Genes
1	Polycystic ovary syndrome	*StAR, CYP11, CYP17, CYP19 HSD17B1-3, HSD3B1-2, ACTR1, ACTR2A-B, FS, INHA, INHBA-B, INHC, SHBG, LHCGR, FSHR, MADH4, AR, MC4R, OB, OBR, POMC, UCP2-3, IGF1, IGF1R, IGFBP11-3, INS VNTR, IR, INSL, IRS1-2, PPARG LHCGR, FSHR VDR EPHX1, LMNA, GSK3A*
2	XX, gonadal dysgenesis	*FSHR, BMP15, NR5A1, EIF2B2, EIF2B5, HSD17B4, HARS2 PSMC3IP*
	Perrault syndrome	*HSD17B4 LARS2, HARS2*
3	Premature ovarian failure	*FSH, FOXO3A, FOXL2, BMP15 TSHB, ADAMTS16 PCSK1, DBH FMR1*

Rare causes of female infertility, or syndromes in which infertility is a minor manifestation

Galactosemia
Mucopolysaccaridoses
Miotonic dystrophy
Prader-Willi
21 α-hydroxilase, 17 α-hydroxilase and other steroidogenic enzymes deficiency
Aromatase defect
Homozygous β-thalassemia
Cystic fibrosis
Hemochromatosis
DAX1 gene mutations

Guidelines for the appropriate use of genetic tests in infertile couples

Table 3 Genetic tests in female infertility

	Amenorrhoea (primary and secondary, including POF) and oligomenorrhoea with hypergonadotropinism	*Hypogondatropic hypogonadism*	*Apparently normal*	*Recurrent foetal loss*
Karotype	During diagnostic workup Prior to ART	–	After 1 year of sexual intercourses aimed to pregnancy Prior to ART	During diagnostic workup
FRAXA	During diagnostic workup Prior to ART	–	Prior to ART (poor responders	–
KALI	–	During diagnostic workup	–	–
CFTR	–	–	Prior to ART	–

ART: assisted reproduction techniques; POF: premature ovarian failure.

Phosphorus
Diagnostics

The Female Infertility Panel is a comprehensive next-generation sequencing (NGS) panel that analyzes genes associated with increased risks for female infertility, including primary ovarian insufficiency, polycystic ovary syndrome, sex chromosome aneuploidy, ovarian hyperstimulation syndrome, and thrombophilia related pregnancy loss.

Included Gene(s) (17)

BMP15	*CYP19A1*	*FOXL2*	*FSHR*	*GDF9*	*GNRHR*	*LHCGR*	*NR5A1*	*ZP1*
CYP17A1	*FMR1*	*FSHB*	*GALT*	*GNAS*	*KISS1R*	*NOBOX*	*STAG3*	

Emerging Evidence Gene(s) (8):

Emerging evidence genes can also be included. These genes do not have a clear association with primary ovarian insufficiency, but emerging evidence suggests that they may play a role in disease pathogenesis.

EIF2B2	*EIF2B3*	*FIGLA*	*LHB*	*POF1B*	*POLG*	*PSMC3IP*	*WT1*

CENTOGENE

Diagnostic strategy

WHOM TO TEST?		TESTING STRATEGY
	Go for testing	
Men	▶	Male infertility panel: AR, CATSPER1, CFTR, FSHR, LHCGR
Men/Women	▶	Global infertility panel: AR, CATSPER1, CFTR, FSHB, FSHR, HESX1, LHB, LHCGR, NR5A1, POU1F1, SRY
Women	▶	Female infertility panel: BMP15, CYP21A2, FSHR, LHB, LHCGR, ZP1

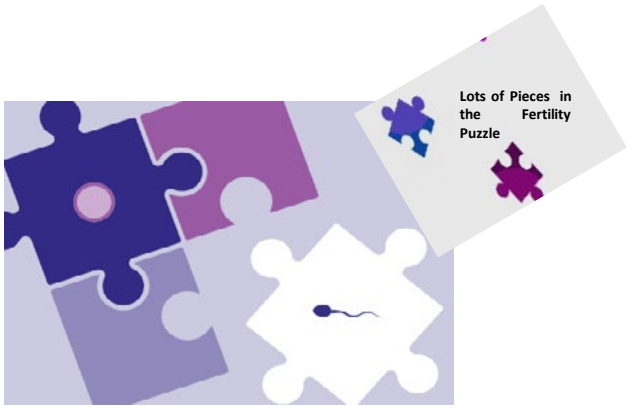
Turnaround time: 25 days

Infertility panel
GENETIC TESTING

CooperGenomics
a CooperSurgical company

FertilityMap

FertilityMap℠ is a large-scale, IRB-approved research study aimed at uncovering the links between genomics and infertility. The FertilityMap study pools together genetic information from CarrierMap testing, alongside personal and family medical history information and fertility-related clinical information, with the goal of developing predictive algorithms to inform infertility diagnosis, prognosis, and treatment. Participation in the FertilityMap study is available to patients undergoing CarrierMap testing at our participating partner IVF centers.



Concluding Remarks

- Infertility is genetically heterogeneous; scores of distinct genes cause grossly identical phenotypes when mutated in mice.
- This likely explains why genome-wide association studies (GWAS) have not been effective even in stratified cohorts,
- Even if associations could be readily obtained, identification and validation of causative variants remain problematic
- Novel research into the mechanisms of infertility may provide future therapeutic targets and tangible biomarkers in bringing patient care into the era of precision medicine.


THANK
YOU !

3. Aneuploidy Screening: The how, what and when

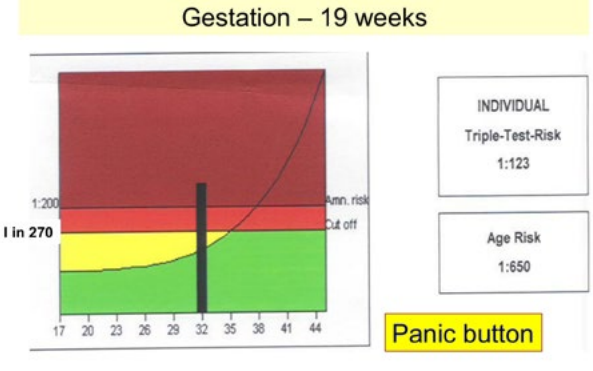
What all can we screen for in pregnancy

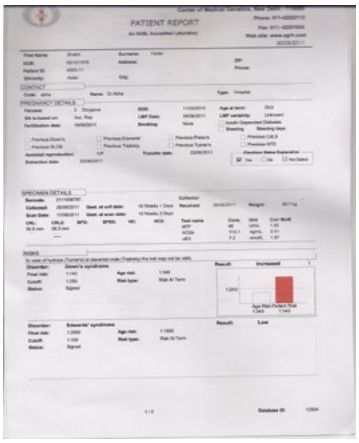
- Major genetic disorders & congenital disabilities with increased morbidity and mortality - for reducing the burden of genetic disorders
- Primary prevention & secondary prevention
- Screening to identify a high risk population
- Prenatal definitive diagnosis
- Common disorders – thalassemia, Down sdrome, Neural tube defect

Cover in my talk

- Aneuploidy screening basics
- Serum and USG markers markers with
- Relevance
- Options of screening
- Special aspects in ART conceptions

Positive Triple Test: what next?





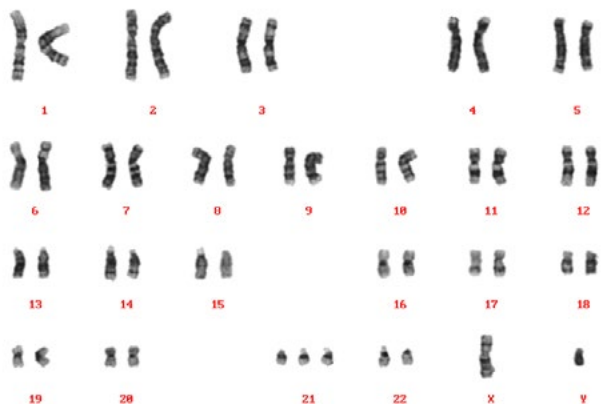
- Doctor asked to repeat
- Why ?
- Because it was positive
- Current report negative
- So now what ?

Discomforting situation

Frantic
"sleepless nights"

What do we have here ?
- Gestation – 21 weeks
- One test positive and one test negative
- Hysterical family

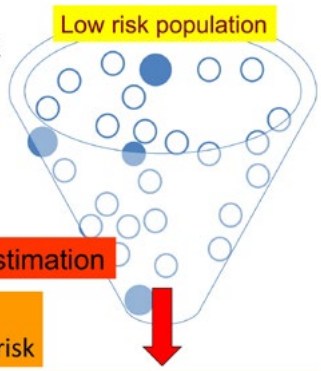
Screening Test is for Fetal Aneuploidies



Verma IC: Community Genetics 2002;5(3):192– 6

What is a screening test ? Screening versus Diagnostic test

Identifies a group at high risk for a specific disorder from an unselected population so as to justify a subsequent diagnostic test



Screening test gives a risk estimation

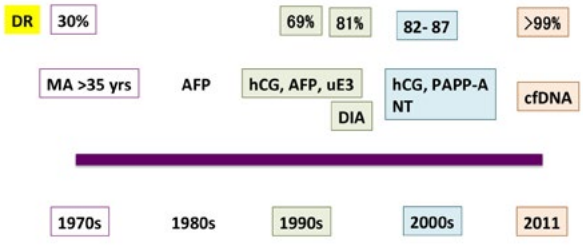
Diagnostic test is definitive; expensive, associated with a risk

Selects a High Risk Population

Diagnostic Vs Screening test

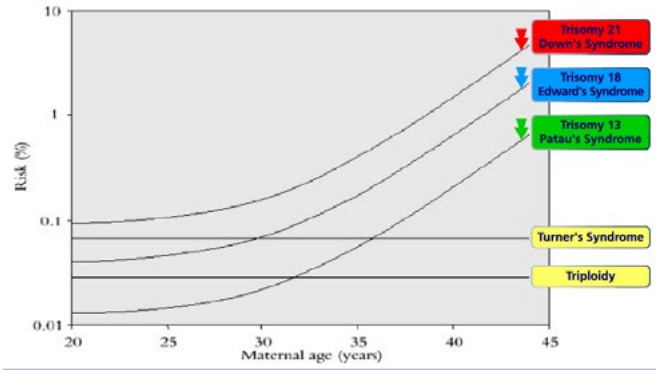
Diagnostic test	Screening test
Definitive diagnosis (?does the patient has disease)	Provides risk estimates (? does the patient needs sp. testing)
Done on high risk population	Done on healthy population
Expensive	Cheap , quick
Complex and sophisticated	Easy

Evolution of Screening for Trisomy 21



Harris S et. al. Semin Fetal Neonatal Med. 2018 Apr;23(2):85-93

Maternal Age Related Risks



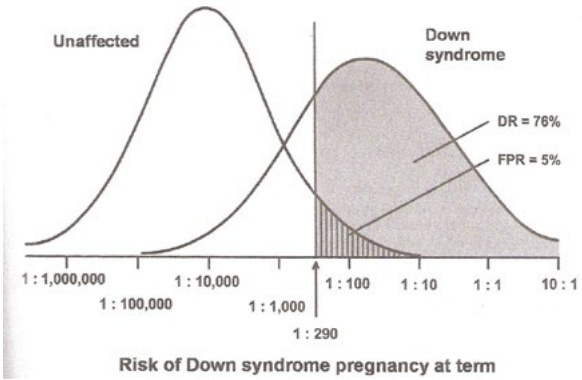
Performance based Measures

- Sensitivity of the test - % with disease who are identified / true positives
- Specificity of the test - True negatives

Characteristics inherent in the test

- Odds of being affected given a positive result (OAPR) / PPV
- Negative Predictive value

Down syndrome screening – Defining a Cut-off value



Which Test ?

2nd Trimester Screen 15 - 21 weeks	First Trimester Screen 11 – 13 +5 weeks
Quadruple / Triple Test	Pregnancy-associated plasma protein A (PAPP-A)
MSAFP levels	Serum free β-human chorionic gonadotropin (fβ-hCG)
uE3 levels	DR – 83% (FPP 5%)
hCG levels	AFP/USG in 2nd trim. for NTD
Inhibin A	
DR – 75–80% FPP 3-5%	
Adv. – risk for ONTD	

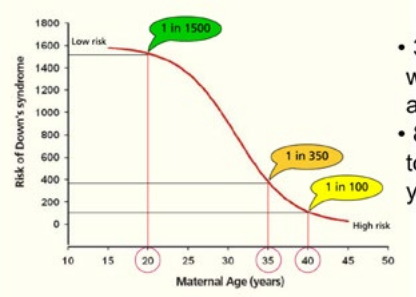
CffDNA after 10 weeks of gestation

Factors affecting screening performance

Maternal characteristics
- Correct date of birth
- Maternal Weight
- Racial origin
- Smokimg

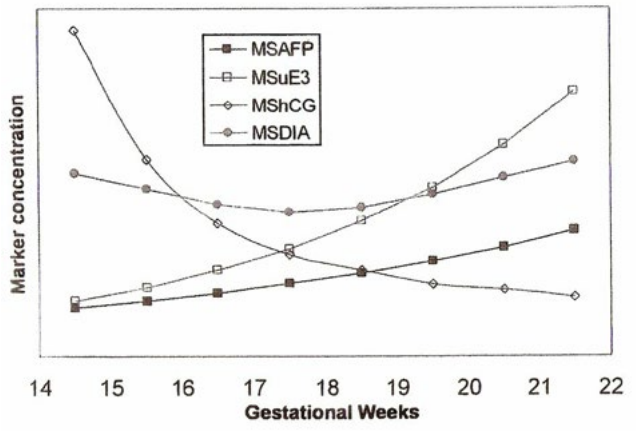
IVF / Twin
Gestational age by ultrasound – CRL in first trimester
Machine and reagents used
MoMs

Maternal age related baseline risk



SGRH data:
- 300 children with DS analyzed
- 80% were born to mothers < 35 yrs

Marker values change with Gestation



Marker values change with Gestationtrimester

- **GestahtiCoGn al age measured by Ultrasound**
- **Use CRL measure from 1st trimester scan**

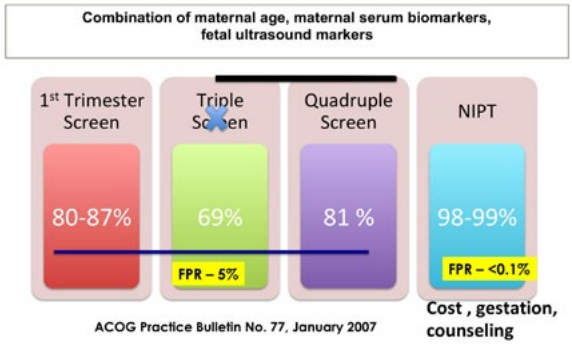
Markers and Disorders

Disorder	PAPP A	Fb hCG	AFP	E3	IA
T 21	↓	↑	↓	↓	↑
T 18	↓	↓			
T 13	↓	↓			
Sex chromosome	↓	N			
Triploidy	↓ ↓ ↓	↑↑ (paternal) ↓ ↓ (maternal)			

Newer Markers

- Serum placental growth factor (PLGF)
- α-fetoprotein (AFP)
- Measured in the same sample and by the same automated machines used for free β-hCG and PAPP-A at little extra cost, would bebeneficial in screening for trisomies
- Useful in first-trimester screening for pre-eclampsia, fetal growth restriction and preterm birth

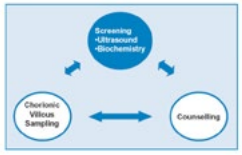
Is that the appropriate screening protocol ?



Screening Tests - Pretest Counseling

- Screening test, Not a diagnostic test
- Risk of aneuploidy
- Positive / negative implications
  - A negative result does not guarantee a 'healthybaby'
  - A positive result does not mean that the baby has a problem, BUT further testing (ultrasound & CVS or amniocentesis) would be offered
- Detection rate / false positive

When to perform Aneuploidy screen?



OSCAR- One Stop Clinic for Risk assessment
12 weeks
Detection rate - 90% ;
FPR 5%

2nd Option
- Biochem at 9-10 wks
- NT scan at 12 weeks
- Detection rate – 93%

3rd Option
PAPP-A at 9 wks
fbHCG & USG at 12 wks
DR 95%

2nd trimester screen
- 15 – 20^{+5} weeks

Specifics for Assisted Reproduction

- Impact of ART on DS screening are controversial
- Maternal serum markers for DS screening aresignificantly modified in ART and ovum donation
- The 1st trimester screen for DS could beinfluenced by mode of conception, particularlyIVF & ICSI, but data on this are still conflicting
- Egg donation – age of the donor at the time ofharvesting to be taken into account. Not the age of the recipient woman .

Cavoretto P et al. Prenat Diagn. 2017 Jun;37(6):540-555

- In all types of ART pregnancies - PAPP-A is significantly lower compared to spontaneous conceptions
- Extent of this difference is about 25%
- fβ-hCG are significantly higher, in particular within the ICSI subgroup where the extent of this difference is about 10%
- May be responsible for higher false positive results

Cavoretto P et al. Prenat Diagn. 2017 Jun;37(6):540-555

- Date of embryo transfer used in analysis
- Gestational age was also estimated by firsttrimester-CRL
- Oocyte donation, the donor's maternal age
- Results on the impact of assisted reproductivetechnology (ART) on maternal serum Down syndrome screening are controversial.
- Variations: a decrease of PAPP-A in ART pregnancies and increase of hCGβ in
- Oocyte donation pregnancies. Bonnin A, Prenat Diagn.

Cavoretto P et al. Prenat Diagn. 2017 Jun;37(6):540-555

Guidelines from Professional Societies

- ACMG - recommends that cfDNA should be made available to all pregnant women and that screening for clinically significant microdeletions may also be offered
- SMFM - cfDNA microdeletion screening should not be routinely offered

Detection of other chromosomal & genetic disorders by screening

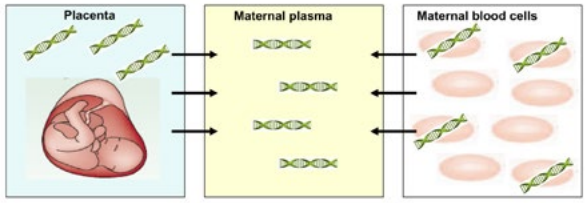
- Chromosomal – T 13, 18, XO, Triploidy
- Genetic conditions – X linked ichthyosis, Disorders of cholesterol metabolism - SLOS, congenital adrenal hypoplasia, Zellweger
- The presence or absence of soft markers or anomalies in the 18- to 20-week ultrasound can be used to modify the a priori risk of aneuploidy established by age or prior screening

Aneuploidy Screening in Multiple Gestation

- Chorionicity needs to be identified
- Dichorionic twins – each fetus has an independent risk
- It is not possible to determine the contribution of each individual fetus to the analyses values
- Risk is calculated for each fetus based on maternal age & fetal NT: advantage is calculation of specific risk for each fetus
- Sensitivity – 87%[MC]; 86%[DC] Specificity – 95%
- Higher order multiple gestation – NT

Genetics in Medicine (2014) 16, 594–600

Cell - free DNA (cf DNA)



- Fetal DNA comes from the placenta
- Mat. blood contains fetal & maternal cfDNA
- 2–20% of total cfDNA is fetal
- Fetal cfDNA reliably detected after 7 wks gestation
- Fetal cfNDA undetectable within hours postpartum

Pre test Counseling NIPT

- Case & family history reviewed to decide if patient should be offered invasive testing or NIPT
- Baseline ultrasound exam – NT / 2nd trimes.
- Information given reg. trisomy 21/ 18, 13, X & Y
- Not diagnostic, but a high efficiency screening test.
- Only tests for specific chromosomes
- Does not exclude other abnormalities
- Affected by maternal obesity, maternal disease & transplantation history, cotwin demise

Professional society guidelines

- Pre-test counseling
- All women :Option of invasive diagnostic testing
- Women may decline aneuploidy screening/testing
- First trimester screening (NT, PAPP-A, and hCG) : Acceptable, cost effective
- Nasal bone: optional
- Adherence to strict standards and maintenance of quality
- First visit in T2: offer multiple marker screening
- Post-test counseling: Risk communication, Need for diagnostic test, risk of procedure

Pre test Counseling NIPT

- Turn around time – 2 weeks
- Results - low risk & high risk
- High risk - confirmation with amniocentesis or CVS
- Low risk – pregnancy to be followed with routine ante-natal care.

Invasive testing

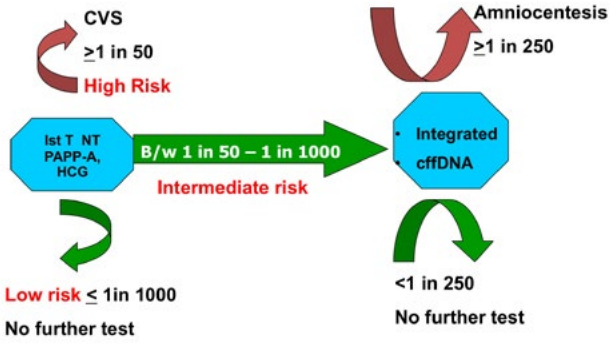
- Family history of a monogenic disorder
- Recurrent pregnancy loss – karyotype not done
- Increased NT in 1st trimester
- Fetal malformations present / increased NFT

Committee Opinions on NIPT

COMMITTEE OPINION ACMG ispd RCOG

- Patients at increased risk of aneuploidy can be offered testing with cell free fetal DNA
- While the test result is much more accurate than existing screening strategies, it is still not a diagnostic assay.
- Report fetal fraction
- No-call / low FF cfDNA result be counseled about the increased risk of aneuploidy and offered diagnostic testing

Contingent screening



Prenatal screening for genetic disorders: Suggested guidelines for the Indian Scenario

Test		Advantages	Comment
USG - dating & NT + PAPP-A & fb hCG	12 wks	Confirmation of GA. Major malformation may be detected. Chorionicity of twins.	Preferred for 1st trim visits NT expertise
AFP + hCG + uE3 + InhA & simultaneous anomaly scan	17 – 18 wks	One visit for anomaly & screen Chr. abnormal fetuses abort naturally	20 wks limit for confirmatory test if reqd.
cffDNA	After NT scan	Non invasive	Only for 5 chromosomes Expensive
Combination of above based on local expertise and availability • Contingent model			

Indian J Med Res 146, December 2017, pp 689-699

References

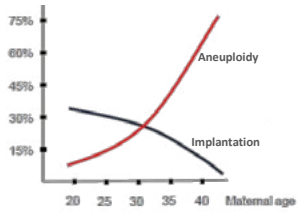
- No. 261-Prenatal Screening for Fetal Aneuploidy in Singleton Pregnancies. J Obstet Gynaecol Can 2017;39(9):e380-e394
- Society for Maternal-Fetal Medicine (SMFM) Publications Committee. Consult series 36: prenatal aneuploidy screening usingcell-free DNA. Am J Obstet Gynecol. 2015;212:711–716.
- Gregg AR, Skotko BG, Benkendorf JL, et al. Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics. Genet Med. 2016;18:1056–1065
- Norton ME, Biggio JR, Kuller JA, et al. Society for Maternal-FetalMedicine (SMFM) Publications Committee. Consult series 42: the role of ultrasound in women who undergo cell-free DNA screening. Am J Obstet Gynecol. 2017;216:B2–B7.
- Post AL, Mottola AT, Kuller JA. What's New in Prenatal Genetics? A Review of Current Recommendations and Guidelines. Obstet Gynecol Surv. 2017 Oct;72(10):610-617
- J Obstet Gynaecol Can 2017;39(9):805e817

THANK YOU

4. Emerging Technologies in Genetic Diagnosis Applicationin clinical practice

Aneuploidy is main cause for IVF failure

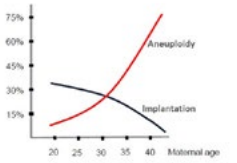
- **Genetic abnormalities are common and explain most implantation failures and miscarriages**
- Aneuploidy is almost always lethal (failed implantation / miscarriage)
- Aneuploidy increases with age, implantation rate decreases
- High % of transferred embryos do not implant



Data from >2000 oocytes analyzed by Reprogenetics UK

Chromosomal Aberrations - Numerical

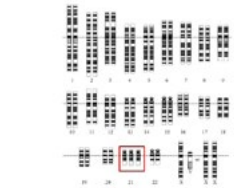
- Genetic abnormalities are common and explain most implantation failures and miscarriages
  - Aneuploidy is almost always lethal (failed implantation / miscarriage)
  - Aneuploidy increases with age, implantation rate decreases
  - High % of transferred embryos do not implant



Data from >2000 oocytes analyzed by Reprogenetics UK
Dagan Wells, Ion World, November 2013

Methods for PGT-A

- Aneuploidy
  - Gain or loss of whole or large segments of chromosomes
  - Most chromosome aneuploidy is not compatible with life
    - Exceptions:
      - Trisomy 21 (Down Syndrome)
      - Trisomy 18 (Edwards Syndrome)
      - Trisomy 13 (Patau Syndrome)
      - Sex Chromosome Aneuploidy
        - Monosomy X (Turners Syndrome)
        - 47, XXY (Kleinfelter's Syndrome)
        - 47, XXX (Triple X Syndrome)
        - 47, XYY

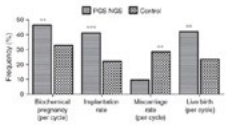


PGT-A Improves live birth rates

Effect of next-generation sequencing in preimplantation genetic testing on live birth ratio

Joanna Liss [A,B], Ewa Pastuszek [A,C], Sebastian Pukszta [A], Eva Hoffmann [D], Waldemar Kuczynski [E,F], Artur Lukaszuk [A,G] and Krzysztof Lukaszuk [A,C,H]

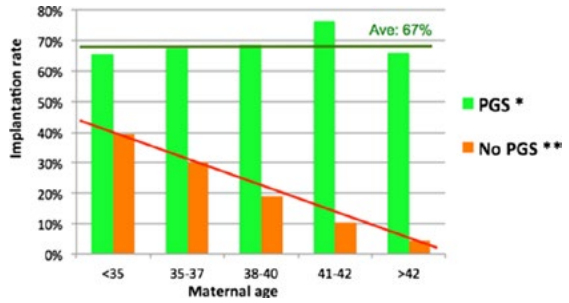
- Analysed live birth ratios in frozen embryo transfer (FET) cycles where embryo ploidy status was determined PGT-A using Ion Torrent NGS.
  - 112 cycles included PGT-A (TE Biopsies)
  - 85 cycles in the control group with no PGT-A
  - Control group consisted of 85 patients who underwent the
- Results showed:
  - Live birth rate per cycle was higher in PGT-A group compared to controls(42.0% vs 23.5%)
  - Pregnancy loss rates were lower in PGT-A group (9.6% vs 28.6%)
  - 18 cycles had no embryo suitable for transfer after PGT-A



Average age ~35 in both groups

Liss et al., Reproduction, Fertility and Development 2018

PGT-A – Improvement in All Age Groups



- PGT-A removes the negative effect of maternal age on implantation rates
- Improves implantation rate in all age groups

Transfer of mosaic embryos

Study	Mosaic Embryo		Euploid Enbryo	
	Implantation rate (%)	On Going Pregnancy Rate (%)	Implantation rate (%)	On Going Pregnancy Rate (%)
Greco 2015	44	33		
Fragouli 2017	40	26	55	45
Munné 2017	53	40	70	63
Spinella 2018	38	30	55	46

Transfer of mosaic embryos can lead to healthy live births but is likely to lead to lower implantation rate and higher rates of miscarriage.

Greco et al., NEJM 2015; Fragouli et al., Human Genetics. 2017; Munné et al., Fertility and Sterility 2017; Spinella et al., Fertility and Sterility 2017

PGDIS Guidelines for transfer of Mosaic Embryos

PGDIS Guidelines for transfer of Mosaic Embryos

PGDIS Newsletter, July 19, 2016

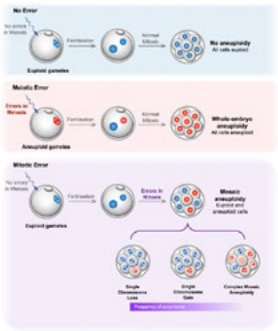
PGDIS POSITION STATEMENT ON CHROMOSOME MOSAICISM AND PREIMPLANTATION ANEUPLOIDY TESTING AT THE BLASTOCYST STAGE

http://www.pgdis.org/docs/newsletter_071816.html

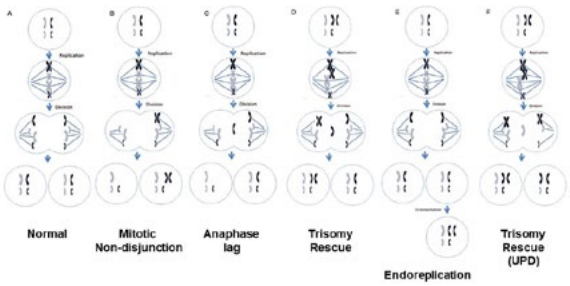
- **Recommendations for the laboratory (If reporting mosaic aneuploidies)**
  - 1. For reliable detection of mosaicism, ideally 5 cells should be biopsied
  - 2. Only a validated NGS platform that can quantitatively measure copy number should be used for measurement of mosaicism in the biopsy sample. Ideally, a NGS methodology that can accurately and reproducibly measure 20% mosaicism in a known sample.
  - 3. For reporting embryo results, the suggested cut-off point for definition of mosaicism is:
    - >20%, so lower levels should be treated as normal (euploid),
    - > 80% abnormal (aneuploid),
    - between 20-80% mosaic (euploid-aneuploid mosaics).
- **Suggested guidelines to prioritize mosaic embryos for transfer**
  - The following can be used as a guide by the clinician (or a genetic counselor if available) when a mosaic embryo is being considered for transfer:
    - 1. Embryos showing mosaic euploid/monosomy are preferable to euploid/trisomy, given that monosomic embryos (excepting 45, X) are not viable
    - 2. If a decision is made to transfer mosaic embryos trisomic for a single chromosome, one can prioritize selection based on the level of mosaicism and the specific chromosome involved

Aneuploidy in the preimplantation embryo

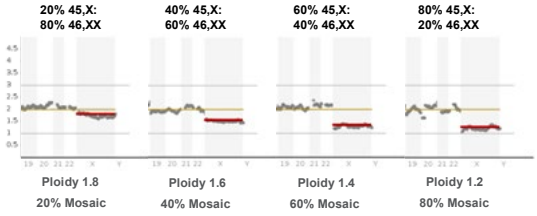
- Aneuploidy originates from meiotic and mitotic chromosome segregation errors.
- Top panel: Normal fertilization of euploid gametes and error-free progression of meiosis-II and embryonic mitosis results in embryos in which all cells are euploid.
- Middle panel: Meiotic errors rendering gametes homogenously aneuploidy – usually non viable
- Lower panel: Errors in mitosis during embryonic cell divisions lead to a mixture of euploid and aneuploid



Mosaicism Formation



Whole chromosome monosomy X mosaic control



- **Ion Torrent software automatically calls:**
- Ploidy status
- Size of abnormality
- Whole chromosome and segmental aneuploidy
- Allows adjustment of tile size for increased accuracy of smaller abnormalities

PGT-A and PGT-M combined

Single Gene Disorder Screening

- Screening for mutations in single genes enables people with an **inheritable condition** in their family to avoid passing it on to their offspring.
  - It involves checking the genes and/or chromosomes of embryos created through IVF
- Reason for Single Gene Disorder screening.
  - Family history of genetic disorder
  - Previous affected child with a serious genetic condition
  - Previous miscarriage due to serious genetic conditions
  - Ended previous pregnancy due to presence of genetic condition
  - Consanguinity
- Virtually any genetic condition where a specific gene or mutation is known to cause that condition can be screened for.
  - For example:
    - Thalassemia
    - Charcott Marie Tooth
    - Cystic Fibrosis
    - Walker-Warburg Syndrome
    - Duchene Muscular Dystrophy
    - Hemophilia
    - Polycystic Kidney disease
    - Spinal Muscular Atrophy
    - Sickle Cell Anemia

PGT-A and PGT-M Combined

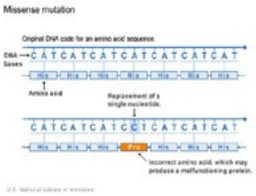
Single Gene Defect

PGT-A = ≤ 0.01 X Coverage

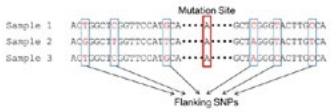
PGT-M = ≥ 20 X Coverage

PGT-M by NGS

Direct Method
Sequence across mutation of interest



Indirect Method
Use flanking markers to determine which allele has been inherited



Why you need indirect test

- **Potential problems that can cause misdiagnosis**

- Preferential amplification (PA)
- PA means the failure of one allele to reach the threshold of detection Allele Drop Out (ADO)
- The random amplification failure of one of the two heterozygous alleles whilst the other allele successfully amplifies

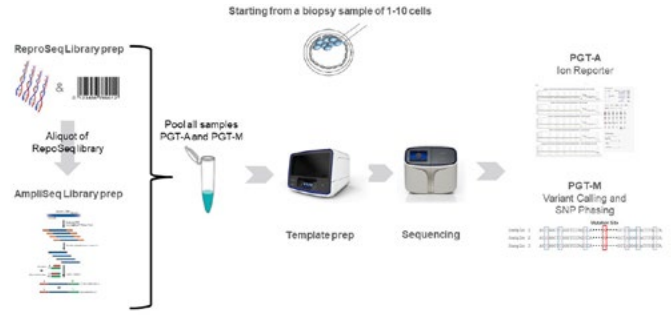
- **Published rates for both PA and ADO from WGA enzymes vary widely**

- Higher ADO from single cells

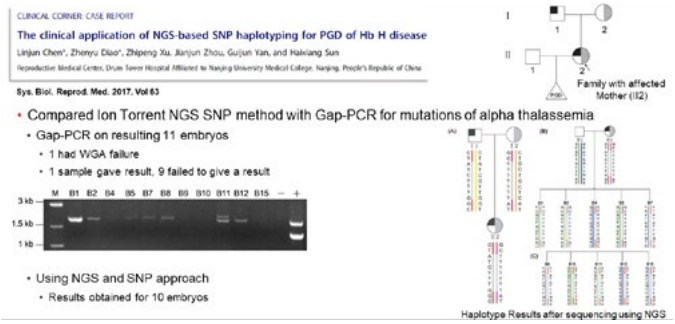
- **Informative SNP markers**

- Control for amplification issues (ADO and PA).
- Informative markers should be closely linked with target region
- Multiplex PCR reduces the chance of misdiagnosis

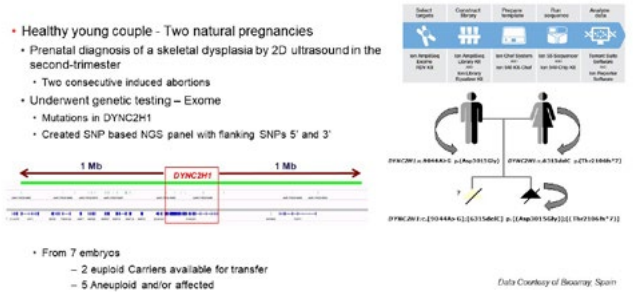
Combined PGT-A and PGT-M Workflow



NGS PGT-M



Case Study - DYNC2H1

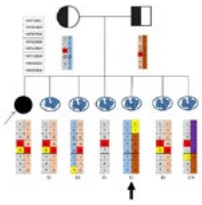


PGT-M: Non-Syndromic Hearing Loss

Successful preimplantation genetic diagnosis by targeted next-generation sequencing on an ion torrent personal genome machine platform

YAN HAO[1,2], DANIEL CHEN[1,2], ZHIGUO ZHANG[1,2], PENG ZHOU[1,2], YUNXIA CAO[1,2], ZHAOLIAN WEI[1,2], XIAOYING XU[1,2], HUILI CHEN[1,2], WUJING ZOU[1,2], MINGHONG LI[1,2], DONGHUI H[1,2] and XIAOJIN HE[1,2]

- Hearing loss can place a heavy burden on the patient and patient's family.
- Incidence ~2% worldwide
  - ~30,000 new borns/yr in China
  - ~77% of NSHL cases are due to autosomal recessive inheritance
  - High cost of treatment and care (including cochlear implantation),
    - Prenatal diagnosis is strongly recommended.
- Couple carried separate mutations in SLC26A4
  - Ampliseq NGS Panel created with SNPs 3Mb both 5' and 3' to the gene, included SNPs in gene as well as mutations.
  - Single embryo (E7) identified as normal and transferred
    - Amniocentesis confirmed fetus did not carry either mutation

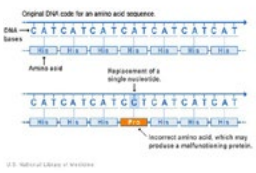


Oncology Letters, 2017

PGT-M by NGS

Direct Method
Sequence across mutation of interest

Missense mutation



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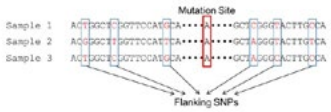
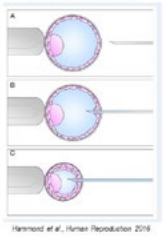


Non-Invasive PGT-A (niPGT-A)

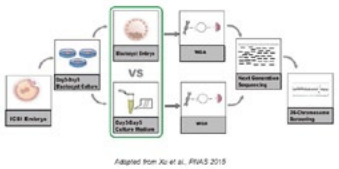
Non-Invasive iPGT-A Methods

Blastocoelic Fluid



Hammond et al., Human Reproduction 2016

Spent Culture media



Adapted from Xu et al., PNAS 2016

Blastocoelic Fluid

- Blastocoelic Fluid (BF) - Natural medium supporting the development of the ICM
  - Presence of DNA found in BF (Pallini et al 2013)
- Magli (Fertility & Sterility, 2015)
  - Compared results between TE, PB and BF using 116 embryos
  - 18% amplification failure rate from 116 samples (95/116)
  - 87 were process for 24 chromosome screening
    - Data from 82 samples
  - 70 embryos used to compare with TE (69), blastomere (36) or PB (34) biopsy data.
    - ~81% full concordance with TE biopsy
    - 16% partial concordance with TE Biopsy

- Comments
  - No mention of source of DNA
  - Was BF collected before or after TE biopsy
  - Detection of mosaicism was not addressed
  - Fresh or cryopreserved

Blastocoelic Fluid

- Tobler et al (Fertility & Sterility, 2015)
  - Studied 96 cryopreserved embryos
  - Compared BF to blastomere and whole embryo
    - 60 embryos had amplification
      - 38% failure rate
    - Full concordance = 48%
    - Partial concordance = 17%
    - Discordance = 35%

Both the molecular karyotype and ploidy (aneuploid vs. euploid) status of BF-DNA, compared with the ICM-TE of all embryos and the quantitative parameters for the diagnostic accuracy of BF-DNA to represent the ICM-TE (whole embryo).

Variable	Data
Concordant karyotypes	48% (29/60)
Discordant karyotypes	52% (31/60)
Sensitivity	0.88 (95% CI: 0.62–0.98)
Specificity	0.55 (95% CI: 0.39–0.70)
Positive predictive value	0.41 (95% CI: 0.25–0.60)
Negative predictive value	0.92 (95% CI: 0.75–0.99)

- DNA isolated from the BF were discordant to the ICM-TE in 52% of the embryos analyzed; thus, based on the data in this study the use of BF-DNA does not adequately represent the remaining embryo (ICM-TE) and should not be used as an alternative biopsy modality.

Spent Culture Media

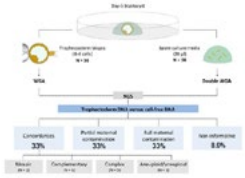
- Presence of DNA in culture media demonstrated by Stigliani et al. 2014
- Xu et al (2016) looked at the use of spent media from 42 blastocysts and compared results to WE
  - 100% WGA
  - Concordance rate of 85.7% (36/42)
    - Full Concordance = 64.3% (27/42)
    - Partial concordance = 21.4% (9/42)
    - Sensitivity = 88.2%; Specificity = 84.0%
  - Have since used the method on patients with successful outcomes

Xu et al., PNAS 2016

- Feichtinger et al., (2017)
  - Used 22 samples of spent culture media (D5) and compared to Polar Body biopsy
    - WGA rate of 82% (18/22)
  - 72% concordance rate (13/18) for embryo aneuploidy
    - 27.7% Full concordance (5/18)
    - 44.4% Partial concordance (8/18)
  - 49% concordance between chromosomes detected

niPGT-A and Maternal Contamination

- Vera-Rodriguez et al., (Human Reproduction, 2018)
  - Set out to explore the origin of DNA found in culture media
    - Found DNA quantity in spent media is very low concentration <1 cell equivalent. No difference between euploid or aneuploidy samples
  - Compared Spent Media to TE biopsy in 56 embryos.
    - 8.9% amplification failure
    - Full concordance = 5.9% (3/51)
    - Partial Concordance = 27.5% (14/51)
    - Discordant = 66.7% (34/51) – Mainly due to maternal contamination as shown by SNP analysis
      - Cumulus cells, Polar bodies
      - Suggest changes ion methodology



- After modifying culture conditions / assay
  - Increased concordance to ~84%
  - Discordance to ~9%

Non-Invasive PGT-A – Blastocoelic Fluid & Spent Media

- Kuznyetsov et al., (2017) collected and combined BF and spent media from day 5/6 blastocysts and compared to TE and WE biopsy
  - Cut ZP with laser and allowed BF to extrude into media (28 frozen embryos)
  - BF and media collected after TE biopsy (19 fresh cultured embryos)
    - 100% amplification rate

Concordance at embryo level (28 Frozen)

Concordance	niPGT		TE*
	TE*	WE	WE
Full Concordance	16 (66.7%)	22 (78.5%)	18 (75%)
Partial Concordance	5 (20.8%)	3(10.7%)	3 (12.5%)
Discordant	3 (12.5%)	3 (10.7%)	3 (12.5%)

*4 embryos did not have TE Biopsy data

Concordance at embryo level (19 Fresh)

Concordance	niPGT
	TE
Full Concordance	13 (68.4%)
Partial Concordance	3 (15.8%)
Discordant	3 (15.8%)

- Data indicates niPGT-A gives a similar level of overall concordance but maybe more representative of the future fetus as it has a higher rate of full concordance

Combined BF and spent media

- Li et al., (Scientific reports 2018)
  - Use a combination of BF and spent culture media
    - Inconsistency in getting amplified DNA from Culture Media or BF alone.
    - Used laser to pierce Zona Pellucida and allow BF to seep into culture media on D5
  - Study included 40 embryos.
    - Compared TE, Whole Embryo (WE) and combined media and blastocoelic fluid (ECB)
    - Amplification failure in 1 ECB sample, 1 WE and 1 TE = 2.5% failure rate
    - High level of partially concordant embryos compared to TE and WE
      - In 29% cases TE differed to WE
      - In 50% cases ECB differed to WE
      - In 56% cases ECB differed to TE
  - Using Ploidy as an outcome from WE as the standard
    - Sensitivity and specificity is:
      - TE: Sensitivity = 89.5%; Specificity = 73.7%
      - ECB: Sensitivity = 89.5%; Specificity = 68.4%

Concordance at embryo level

Concordance	ECB		TE
	TE	WE	WE
Full Concordance	17 (45%)	19 (50%)	27 (71%)
Partial Concordance	12 (32%)	11 (29%)	4 (11%)
Discordant	9 (24%)	8 (21%)	7 (18%)

niPGT-A Summary

- Uncertainties about source of the cell free DNA
  - Cell apoptosis; normal or abnormal cells
  - ICM or TE, combination
- Maternal contamination issues demand modified culture conditions
- Limited evidence on detection of mosaics
- Degradation of DNA may lead to false negatives/false positives, especially mosaic detection
- Damage to surrounding cells by laser may allow DNA to leak into media
- High error rate ~15% in concordance studies with TE and WE

- Easy to perform
- No need for biopsy trained embryologist
- Reduce embryologist time
- No need of expensive lasers
- No damage to embryo

Current work is very promising, needs more research to improve concordance

Non-Invasive Prenatal Testing

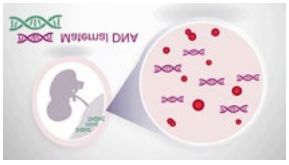
NIPT

- **Non-Invasive Pre-Natal Testing**
- A risk free DNA test on maternal blood to screen pregnancies for the most common fetal aneuploidies

- Trisomy 21 (Down syndrome),
- Trisomy 18 (Edwards syndrome)
- Trisomy 13 (Patau syndrome)
- X and Y chromosome aneuploidy
- Selected Micro-deletions

- Recommended to be offered to all pregnant women

- ACOG (2016)



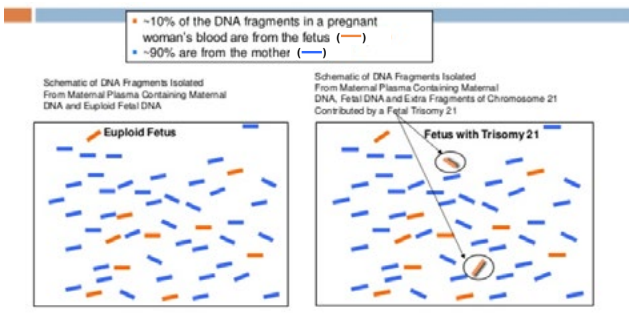
NIPT as a Screening Test

- NIPT screening using cfDNA is an excellent way to screen for common fetal aneuploidies, with distinct advantages over traditional screening methods

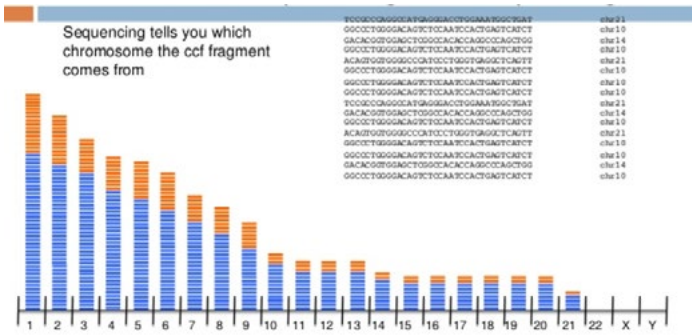
- Higher Detection Rate
- Lower False Positive Rate
- Faster Turn Around Time

- NIPT cannot replace invasive testing for diagnostic results
- NIPT results must be reported with information about false positive rates and PPV
- Expansion of cfDNA for prenatal testing to average risk pregnancies or for rarer conditions should be met with caution as the benefits will start to diminish

NIPT with NGS – Massively Parallel Shotgun Sequencing (MPSS)



NIPT with NGS – Massively Parallel Shot-gun Sequencing (MPSS)



Challenges with NIPT

- Fetal fractions

- Affected by gestational age, maternal BMI, type of an-euploidy
- Low fetal fractions associated with increased risk for an-euploidy

- Twins/surrogacy/donors
- False Positives

- Placental mosaicism
- Vanishing twin
- Maternal sex chromosome abnormality

Neoplasia – apoptosis of cancer cells, aneuploidy com-mon

- NIPT in Average Risk Pregnancies and rare condi-tions

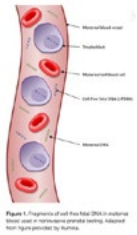
Advances in Non-Invasive Prenatal Testing

- Fetal Cells for NIPT
- Fetal Exomes
- De Novo Panels (PreSeek)
- NIPD for Monogenic Disorders (de novo and pater-nally inherited mutations)

- More Specific
- More Informative
- More Diagnostic

Fetal Cells

- cfDNA NIPT is preferred by patients and physicians over invasive testing
- cfDNA NIPT detects aneuploidy and large deletions
  - Not severe de novo mutations caused by CNVs or point mutations
- Rapid introduction into clinical care as an alternative to invasive testing
  - Major identifiable disorders (Trisomies 21, 18, 13; sex chromosome abnormalities) Represents only a small proportion of fetal genetic abnormalities that can be detected by amniocentesis and chorionic villus sampling.
- "One might even argue that cell-free NIPT has been wildly successful commercially, financially and academically, **but has been a net harm to society causing the birth of more rather than fewer infants with severe genetic disabilities, especially involving common deletion syndromes**" Art Beaudet, AJMG, 2016
- ***Use of Fetal Cells would offer a more comprehensive non-invasive test with higher diagnostic potential (cbNIPT)***



Fetal Cells in Maternal Blood

4 Fetal cell types are found in maternal blood

- Leukocytes
  - Can persist in bloodstream for >20 years post pregnancy
- Undifferentiated stem cells and progenitors
  - Rare, persist in maternal blood for many years.
- **Fetal Trophoblasts**
  - First cells which are known to cross into the maternal peripheral blood.
    - Rapidly cleared from blood
    - Placental origin
- **Fetal Nucleated red blood cells (fNRBC)**
  - Contain single nucleus
  - Distinctive shape
  - Specific antibodies
  - Fetal origin
- ~1 cell /1-10 million RBC

Challenges

- Efficient and reproducible identification of fetal cells,
- Isolation of highly pure and viable CFC
- Methods
  - Microfluidic devices
  - Immunostaining
    - Specific antibodies - specific cell surface markers
  - Manual picking using laser microdissection
    - Can be automated picking
- Companies developing technology
  - Abnova
  - Silicon Biosystems
  - Arcedi Biotech
  - RareCyte

Isolation methods

- Challenges:

- Efficient and reproducible identification of fetal cells,
- Isolation of highly pure and viable CFC

- Methods

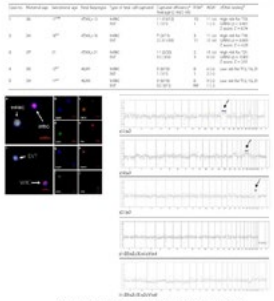
- Microfluidic devices
- Immunostaining
  - Specific antibodies - specific cell surface markers
- Manual picking using laser microdissection
  - Can be automated picking

- Companies developing technology

- Abnova
- Silicon Biosystems
- Arcedi Biotech
- RareCyte

Comparing fnRBC and Extra Villous Cytotrophoblasts

- Used "Cell Reveal™" system to capture trophoblasts and the nucleated RBC (nRBC)
  - Silicon-based, nanostructured microfluidics using immunoaffinity
- Captured bith Trophoblasts and fnRBC
  - WGA followed by NGS and compared to Karyotype of amniocentesis and routine NIPT data
  - Confirmation of fetal origin by STR analysis
- After Validation of system on 24 samples ran a verification on 5 samples.
  - 3 with aneuploidy
  - 2 Normal
  - In all cases results by NGS were in agreement with karyotyping and NIPT data
    - Added advantage of being able to detect abnormalities in all chromosomes and microdeletions
    - fnRBC is a direct reflection of fetal genome.



Huang et al. Molecular Cytogenetics (2017) 10:44
DOI 10.1186/s13039-017-0343-3

Fetal Cells: Aneuploidy, Microduplication and Mosaicism

- Fetal trophoblast cells were enriched and stained using fetal cell specific antibodies.
  - Enriched cell fraction was scanned, and fetal cells were picked using a capillary-based cell picking instrument.
  - WGA followed by aCGH
    - Compared with aCGH on invasive samples
- Isolated ~12 cells per sample (30ml blood)
- From 5 samples
  - Confirmed invasive test results in all cases
  - 1 = T21
  - 2 = T13 Mosaic (CVS) Full T13 by cbNIPT only 2 cells analysed
  - 3 = T2 (Mosaic), Full T2 by cbNIPT (shown to be confined placental mosaicism)
  - 4 = 12.4Mb duplication T21
  - 5 = Unbalanced translocation 31.4-Mb terminal deletion on chromosome 4p and a 30.1-Mb terminal duplication on chromosome 8p

Vestergaard et al, Prenatal Diagnosis 2017;37:1120–1124
DOI 10.1002/pd.5150

Chromosomal Microarray

Chromosomal Microarray Analysis

- CMA is a molecular method of analyzing **chromosomes**.
  - With a single test, CMA can detect genetic abnormalities on all **chromosomes** simultaneously
- Prenatal
  - Invasive prenatal testing Research for chromosomal abnormalities related to fetal anomalies detected by ultrasound
  - Analysis of DNA from products of conception to identify chromosomal aberrations related to pregnancy loss
  - Confirmation of abnormal results found with other screening technologies.

- Postnatal
  - Research for chromosomal abnormalities (including microdeletions or microduplications) related to:
    - Developmental delay,
    - Intellectual disabilities
    - Autism related syndromes

Exon-level CNVs are critical in clinical research

Homozygous and hemizygous CNV detection from exome sequencing data in a Mendelian disease cohort

Identification of Small Exonic CNV from Whole-Exome Sequence Data and Application to Autism Spectrum Disorder

- There is increasing evidence of the importance of exon-level copy number changes in a number of pathologies, including neurodevelopmental disease.
- Up to 40% of intragenic mutations can involve just one or two exons within a gene.
- Methods for detection should attempt good coverage of individual exons with high resolution.

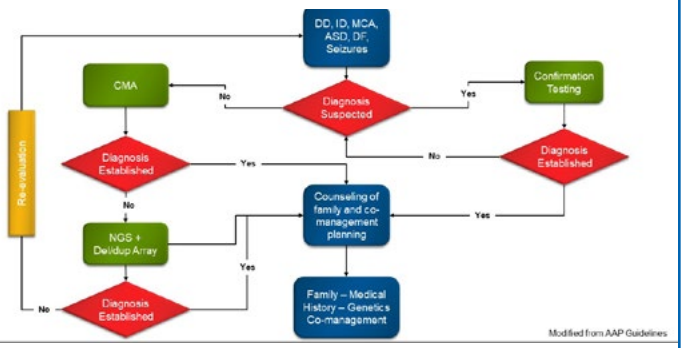
Why would an array be useful to detect exon-level CNVs?

- Single exon CNVs can be reliably detected with an exome array.
- A whole-exome array can be a useful tool in autosomal recessive disorders when there is one mutation on a gene found by sequencing and a deletion/duplication is suspected on the other allele.
- An exome array is a useful tool to confirm findings by exome sequencing.

ORIGINAL RESEARCH ARTICLE

Assessing copy number from exome sequencing and exome array CGH based on CNV spectrum in a large clinical cohort

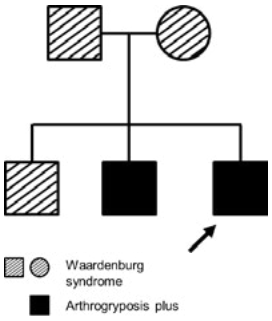
Overview



Research Case I: Waardenburg Syndrome

- US (32 weeks): fixed limbs, scalp edema, micrognathia
- Exam (32 weeks):

- White hair
- Small palpebral fissures, ears
- Hypertelorism and cleft palate
- Excessive nuchal skin
- 4-limb pterygia, Syndactyly, Clinodactyly, Absent palmar ceases, foot deformation
- Tag-like genital structure and undescended testes



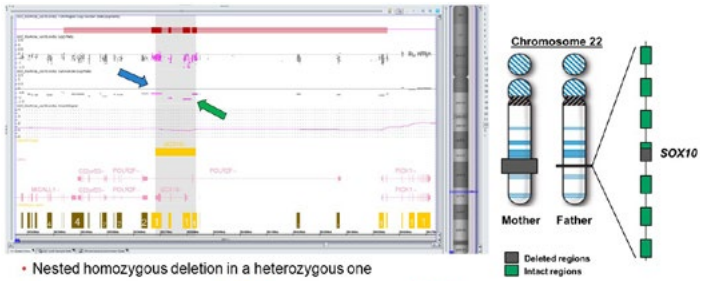
Research Case I: Waardenburg Syndrome

- Run on CMA (CytoScan HD)
  - Large deletion seen across SOX10 gene
- Waardenburg Syndrome panel run:
  - No findings
- WES
  - No sequence findings
- Run on CytoScan XON
  - Additional SOX10 findings!

Waardenburg Syndrome Genes

WS1	*PAX3* (100%)
WS2	*MITF* (~15%)
	SOX10 (~15%)
	EDN3/EDNRB (~5%)
	SNAI2 (~5%)
WS3	*PAX3*
WS4	*SOX10* (50%)
	EDN3/EDNRB (~20%)

Research Case I: Waardenburg Syndrome



- Nested homozygous deletion in a heterozygous one
- qPCR confirmed as well as maternal (larger deletion) and paternal (smaller deletion) samples

Research Case II: Craniosynostosis

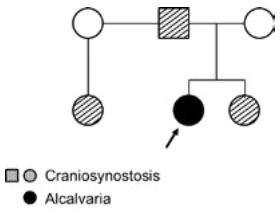
- Fetus

- Acalvaria

- Father, 1 sister and 1 half sister

- Facial appearance of Crouzon craniosynostosis

- Posterior parietal/occipital areas of calvaria not ossified



Research Case II: Craniosynostosis

- Craniosynostosis panel

- No findings

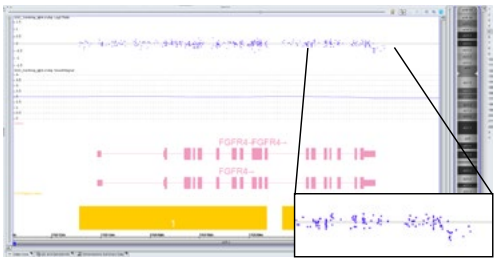
- WES

- Benign variants in paternal sample

- CytoScan XON

- Partial exonic deletion on FGFR4

- qPCR confirmed as well as maternal and paternal samples



CytoScan XON Array specifications

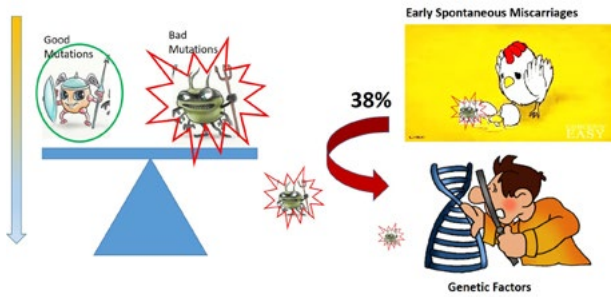
Specifications	Details
Content	6.85 million probes empirically selected for whole-genome coverage including: • 6.5 million copy number probes • 300,000 SNP probes for LOH/AOH analysis as well as duo/trio assessment and sample tracking
Sensitivity	95% sensitivity for the detection of exon-level CNVs*
Coverage	Total number of genes with coverage: 25,980 • Full coverage: 21,844 • Partial coverage: 4,136 • Exome genes for medical research (including cancer genes): 7,003 Exon-level CNV detection with an average of 15 probes per call

*Sensitivity calculated from Level 1 genes

For Research Use only

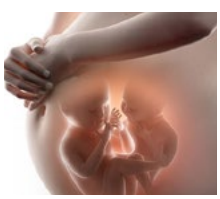
Thank You

5. Does ART predispose to Genetic Disorders?



Health risks

- Use of ART increases the risk of multiple births
- Including higher rates of caesarean sections
- Prematurity, low birth weight
- Infant death and disability
- Elevated risks of birth defects



Except for an increased incidence of premature births, these technologies are considered safe

BUT genetic risk in offspring of IVF and ICSI pregnancies is a concern

Whys and wherefores…

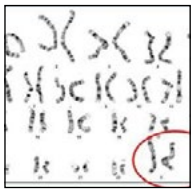
- *In vitro* mechanism in selecting or eliminating abnormal sperm may not be as rigorous as *in vivo*
- Physical injury to gametes, such as damage to the meiotic spindle, is known to occur
- Transmission of genes that cause spermatogenic failure may lead to offspring having somatic anomalies
- Altered hormonal milieu and gamete manipulation may result in delayed DNA replication, point mutation or impaired meiosis or mitosis
- Chromosomal abnormalities (despite a normal somatic karyotype) are found at a higher rate in sperm from men with severe oligozoospermia and azoospermia

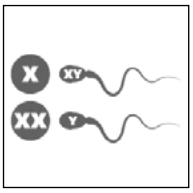
ART And Pre-existing mutations

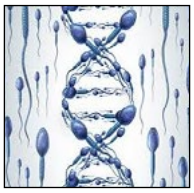
Existing genetic conditions of the parents can be transmitted to the offspring through ART, as they would with natural conception

- Three genetic conditions of special relevance to male-factor infertility are

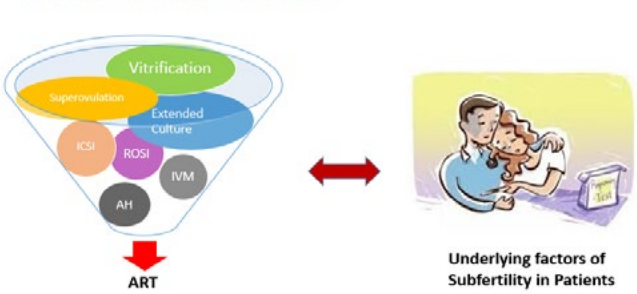
Microdeletion of the Y-chromosome
Congenital bilateral absence of the vas deferens (CBAVD)
Klinefelter's syndrome







Is it ART or the Patient?



Technology-related concerns…

- Deficiencies in culture media that could increase the risk of long-lasting epigenetic alterations
- Changes in oocytes following ovarian stimulation and endometrial preparation,
- Exposure of oocytes and embryos to biochemical contaminants in IVF culture systems,
- Bypassing of natural sperm selection during ICSI, physical damage to the ooplasm or meiotic spindle during ICSI
- Damage from cryopreservation and PGD

Patient-related concerns…

- Parental age,
- Infertility type and duration and the
- Use of gametes from an ageing population of IVF/ ICSI patients with defective genes or organelles

J Assist Reprod Genet (2011) 28:699–705
DOI 10.1007/s10815-011-9583-z

ASSISTED REPRODUCTION TECHNOLOGIES

A meta-analysis of the impact of IVF and ICSI on major malformations after adjusting for the effect of subfertility

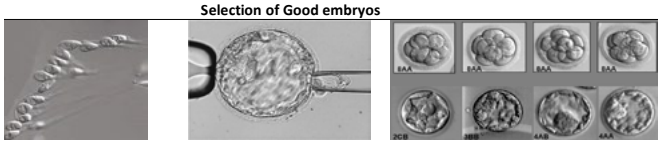
Alfred A. Rimm · Alyce C. Katayama · K. Paul Katayama

- ART does not increase the risk of major malformations as much as previously reported in 2004 study
- It is reasonable to think that subfertile patients would have underlying conditions that may predispose them to poor pregnancy outcomes
- Increasing TTP is associated with a risk of adverse outcomes in the offspring

Favor towards ART

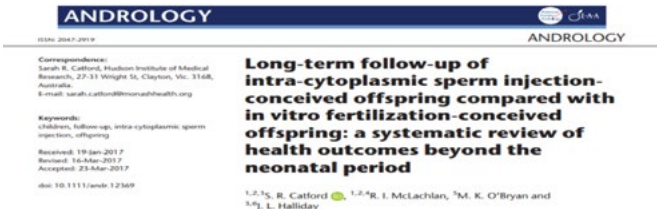
- Protective effect of ART, as observed in 5 studies in meta-analysis of 18 studies by Rimm et al., 2011

Selection of Good embryos



Sperm selection PGS/PGD Extended culture

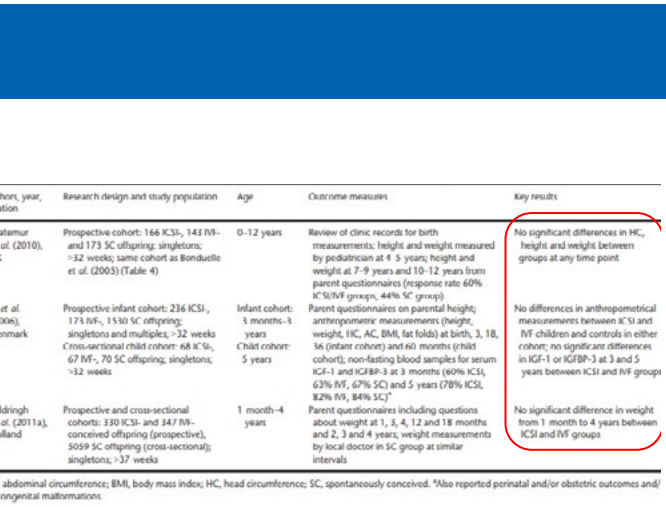
- TTP is reduced with successful treatment
- Increased TTP is associated with risks in terms of outcome like preterm delivery and congenital malformations



ANDROLOGY

ANDROLOGY

Long-term follow-up of intra-cytoplasmic sperm injection-conceived offspring compared with in vitro fertilization-conceived offspring: a systematic review of health outcomes beyond the neonatal period



Authors, year, location	Research design and study population	Age	Outcome measures	Key results
Basatemur *et al.* (2010), UK	Prospective cohort: 166 ICSI-, 143 IVF- and 173 SC offspring; singletons; >32 weeks; same cohort as Bonduelle *et al.* (2005) (Table 4)	0–12 years	Review of clinic records for birth measurements; height and weight measured by pediatrician at 4 5 years; height and weight at 7–9 years and 10–12 years from parent questionnaires (response rate: 60% ICSI/IVF groups, 44% SC group)	No significant differences in HC, height and weight between groups at any time point
Kai *et al.* (2006), Denmark	Prospective infant cohort: 236 ICSI-, 173 IVF-, 1530 SC offspring; singletons and multiples; >32 weeks Cross-sectional child cohort: 68 ICSI-, 67 IVF-, 70 SC offspring; singletons; >32 weeks	Infant cohort: 3 months–3 years Child cohort: 5 years	Parent questionnaires on parental height; anthropometric measurements (height, weight, HC, AC, BMI, fat folds) at birth, 3, 18, 36 (infant cohort) and 60 months (child cohort); non-fasting blood samples for serum IGF-1 and IGFBP-3 at 3 months (60% ICSI, 63% IVF, 67% SC) and 5 years (78% ICSI, 82% IVF, 84% SC)*	No differences in anthropometrical measurements between ICSI and IVF children and controls in either cohort; no significant differences in IGF-1 or IGFBP-3 at 3 and 5 years between ICSI and IVF groups
Woldringh *et al.* (2011a), Holland	Prospective and cross-sectional cohorts: 330 ICSI- and 347 IVF-conceived offspring (prospective), 5059 SC offspring (cross-sectional); singletons; >37 weeks	1 month–4 years	Parent questionnaires including questions about weight at 1, 3, 4, 12 and 18 months and 2, 3 and 4 years; weight measurements by local doctor in SC group at similar intervals	No significant difference in weight from 1 month to 4 years between ICSI and IVF groups

AC, abdominal circumference; BMI, body mass index; HC, head circumference; SC, spontaneously conceived. *Also reported perinatal and/or obstetric outcomes and/or congenital malformations

Table 4 Studies reporting on general physical health and childhood cancer in intra-cytoplasmic sperm injection (ICSI)- and in vitro fertilization (IVF)-conceived offspring

Authors, year, location	Research design and study population	Age (years)	Outcome measures	Key results
General physical health				
Bonduelle *et al.* (2005), Belgium, UK, Denmark, Sweden, Greece	Cross-sectional cohort: 540 ICSI-, 437 IVF- and 538 SC offspring; singletons; >32 weeks; same cohort as Barnes *et al.* (2004) (Table 3)	5	Parent interview; physical examination including anthropometric data, visual acuity and pure tone audiometry[a]	Compared to SC group, ICSI and IVF children more likely to have significant childhood illness (24% ICSI, 17% IVF, 17% SC; $p < 0.001$), need surgery (24% ICSI, 22% IVF, 14% SC; $p < 0.001$) esp. genitourinary surgery (5% ICSI, 3% IVF, 1% SC; $p = 0.005$), require medical therapy (11% ICSI, 9% IVF, 5% SC; $p < 0.001$) and be admitted to hospital (31% ICSI, 28% IVF, 20% SC; $p < 0.001$); no difference in physical examination between groups; no difference in outcomes between countries
Knoester *et al.* (2008a), Holland	Retrospective cohort: 81 ICSI- and 81 IVF-conceived offspring, 87 ICSI- and 85 SC offspring; singletons; any gestation	5–8	Parent questionnaire; physical examination including biometrical data and vision[a]	Higher rate of physical therapy in IVF vs. ICSI group (OR 2.6, 95% CI 1.0–6.6); unexplained increased frequency of vomiting in IVF vs. ICSI group; no difference in general health, growth or hospitalizations between ICSI and IVF or SC groups
Pinborg *et al.* (2004a), Denmark	Retrospective cohort: 2117 ICSI offspring (1282 singletons, 833 twins), 6406 IVF offspring (3848 singletons, 2558 twins) and 10,239 SC twins; any gestation; same cohort as Pinborg et al. (2004a,b) (Table 2)	2–7	Review of registry data for hospital admissions, mean number of days in hospital, outpatient appointments, diagnoses and operations performed	No difference in hospitalizations and surgical procedures between ICSI and IVF children; no difference in hospitalizations and surgical procedures between IVF/ICSI twins and SC twins
Childhood cancer				
Lerner-Geva *et al.* (2016), Israel	Retrospective cohort: 9042 ART vs. 211,763 SC children, ICSI vs. IVF (numbers not disclosed in study); singletons and multiples; any gestation	9–11	Cancer diagnoses via linkage with the Israel National Cancer Registry	Elevated risk for overall cancer in ART vs. SC group, but not statistically significant after adjustment for maternal and infant characteristics (RR 1.42, 95% CI 0.85–2.37); significantly increased risk for retinoblastoma (RR 6.18, 95% CI 1.22–31.2) and renal cancer (RR 3.25, 95% CI 1.67–6.32) in ART group but small numbers; no difference in risk of cancer between ICSI and IVF (OR 0.76, 95% CI 0.32–1.81)

Most studies have assessed the risks of ART by comparing the outcomes of ART-conceived pregnancies to naturally conceived pregnancies.

There is emerging evidence that underlying maternal or paternal subfertility might be an important factor in obstetric, neonatal and childhood outcomes in the ART population.

Favor towards ART

Association of somatic chromosomal abnormalities and semen spermatozoa concentration

Sperm concentration (10^6 sperm/mL)	Frequency of chromosomal abnormalities
Azoospermia	18.7%
Severe oligospermia (0-5)	4.6%
Mild to moderate oligospermia (5-20)	2.8%
Normospermia (>20)	3.0%
Total	6.11%

Gekas et al., Hum Reprod 2001;16:82-90

Outcome in the second year of life after in-vitro fertilisation by intracytoplasmic sperm injection: a UK case-control study

Children born to oligozoospermic men (n=121)				Children born to non-oligozoospermic men (n=87)			
Major anomalies	Number	Minor anomalies	Number	Major anomalies	Number	Minor anomalies	Number
Coloboma	1	Prominent ear	4	Exomphalos	1	Prominent ear	1
Scrotal fusion	1	Undescended testis	1	Congenital cataract	1	Haemangioma	1
Bilateral duplex ureters	1	Umbilical hernia	1			Talipes equinovarus	2
Hypospadias	2	Haemangioma	2			Preauricular pit	1
Thyroglossal cyst	1	Talipes equinovarus	2				
Hip displasia	2	Accessory nipple	1				
		Clindoactyly	1				
		Polydactyly	3				
		Syndactyly	1				
		Congenital skin aplasia	1				

Table 6: **Congenital anomalies in study group according to whether the father had oligozoospermia**

Sutcliffe et al.,THE LANCET • Vol 357 • 2001

Authors, year, location	Research design and study population	Age	Outcome measures	Key results
Basatemur *et al.* (2010), UK	Prospective cohort: 166 ICSI-, 143 IVF- and 173 SC offspring; singletons; >32 weeks; same cohort as Bonduelle *et al.* (2005) (Table 4)	0–12 years	Review of clinic records for birth measurements; height and weight measured by pediatrician at 4·5 years; height and weight at 7–9 years and 10–12 years from parent questionnaires (response rate 60% ICSI/IVF groups, 44% SC group)	No significant differences in HC, height and weight between groups at any time point
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AC, abdominal circumference; BMI, body mass index; HC, head circumference; SC, spontaneously conceived. *Also reported perinatal and/or obstetric outcomes and/or congenital malformations.

A lower antral follicle count is associated with infertility

Mitchell P. Rosen, M.D., H.C.L.D.,[a] Erica Johnstone, M.D.,[b] Carolyne Addanan-Andersen, B.S.,[a] and Marcelle I. Cedars, M.D.[a]

[a] Division of Reproductive Endocrinology and Infertility, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, California; and [b] Utah Center for Reproductive Health, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Utah, Salt Lake City, Utah

Objective: To determine whether infertile women have lower antral follicle counts (AFC) than age-matched normal women.
Design: Case-control.
Setting: Academic center.
Patient(s): A total of 881 infertile women and 771 women from the community.
Intervention(s): Antral follicle count and basal hormone measurements.
Main Outcome Measure(s): Median AFCs and FSH levels were compared between the two groups within 5-year age strata by using the median test. A subanalysis was performed by identifying women in the control group with a history of attempting conception without success (subfertile group) and with a spontaneous conception in fewer than 12 months resulting in a live birth (fertile group). Age-specific AFC percentiles were calculated and compared within strata determined by age at the time of attempted conception.
Result(s): AFCs were significantly lower in infertile women than in control women across age groups up to 40 years of age. Average FSH levels were significantly higher in the younger-age infertile group versus the community. AFC percentiles differ significantly between fertile and subfertile women within the community up to 40 years of age.
Conclusion(s): Decreased AFC in infertile women suggests that factors affecting the size of the remaining follicle pool in younger women also affect oocyte quality and the likelihood of conception. (Fertil Steril® 2011;95:1950–4. ©2011 by American Society for Reproductive Medicine.)

Key Words: Antral follicle count, decreased ovarian reserve, DOR, unexplained infertility, subfertile, FSH

Fertility and Sterility Vol. 95, No. 6, May 2011

Syndrome	Cases (number)	ART	Loss of imprinting (gene)	Country	Reference
Cases with analysis of underlying imprinting defect					
Beckwith-Wiedemann	6	IVF and ICSI	*KCNQ1OT1*	UK	7
	7	IVF and ICSI	*KCNQ1OT1 and H19*	USA	6
	6	IVF and ICSI	*KCNQ1OT1*	France	8
Angelman	1	ICSI	*SNRPN*	Norway	9
	2	ICSI	*SNRPN*	Germany	10
Cases without analysis of underlying imprinting defect					
Beckwith-Wiedemann	1	ICSI		Belgium	2
	1	IVF and ICSI		-	11
	1	IVF and ICSI		-	12
	1	IVF		Netherlands	13
	1	IVF		UK	14

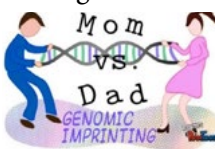
IVF= in-vitro fertilisation. ICSI=intracytoplasmic sperm injection.

Cases of apparent imprinted gene diseases associated with assisted reproductive technology (ART)

Gosden et al., THE LANCET • Vol 361 • June 7, 2003

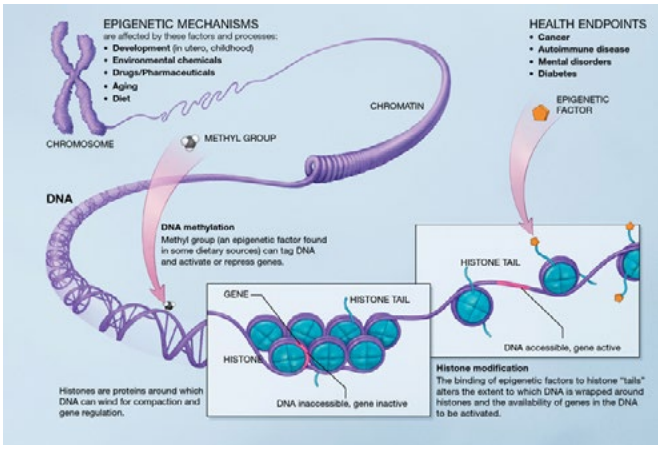
What is Imprinting?

- Genomic imprinting is one example where Mendel's laws are not obeyed
- Maternal and paternal genomes are not functionally equivalent; a number of genes may have modifications, specific to the parent of origin, and are said to be imprinted
- Imprinted genes show preferential expression from a specific parental allele; More than 100 such genes are known and are expressed according to their sex cell lineage



How are genes Imprinted?

- At any imprinted locus, only one allele is active and the inactive one is marked epigenetically, that is, **there is a stable alteration in DNA other than the sequence itself**
- Epigenetic modifications include histone acetylation, cytosine methylation or both and essentially alter chromatin organisation
- **Methylation** is one of the best-studied epigenetic modifications of DNA and all imprinted genes show differences in methylation patterns between maternal and paternal alleles
- Loss of imprinting can involve hypomethylation or hypermethylation, depending on the gene



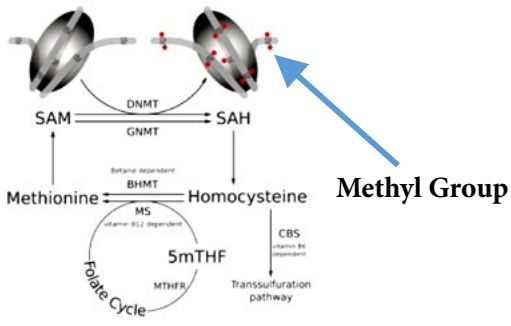
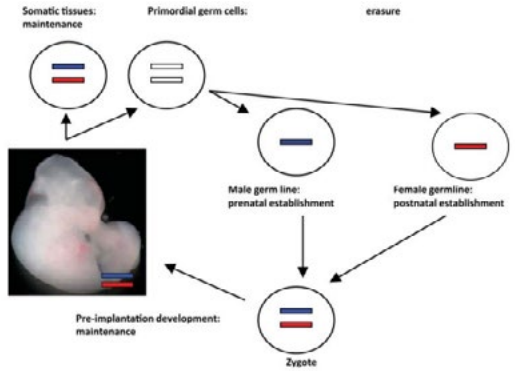


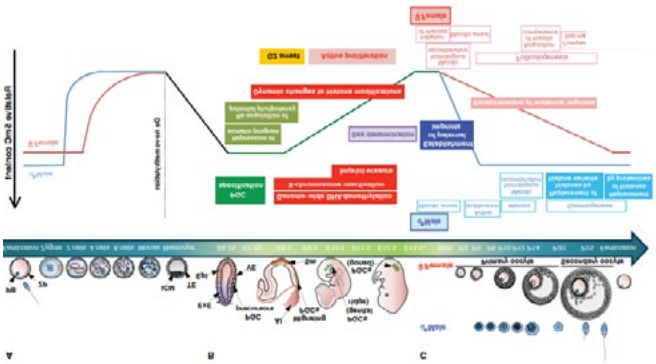
Figure 1. Methionine cycling provides methyl groups for DNA methylation during epigenetic imprinting. Methyl groups originate from the

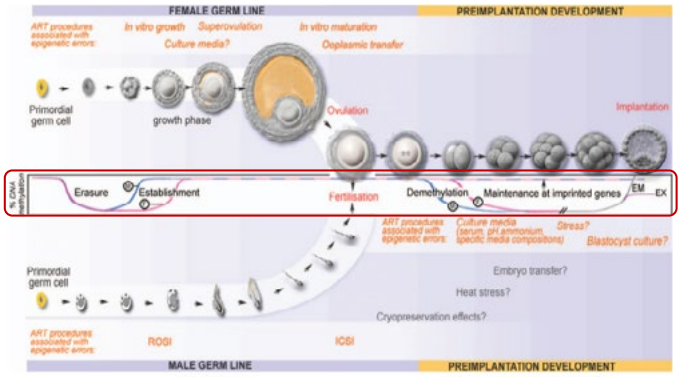
Hoiejmakers et al., Mol. Reprod. Dev. 83:94–107 (2016)

Imprinting occurs at two stages;

- Gametogenesis and embryonic development.
- Imprints are established during the development of the germ cells







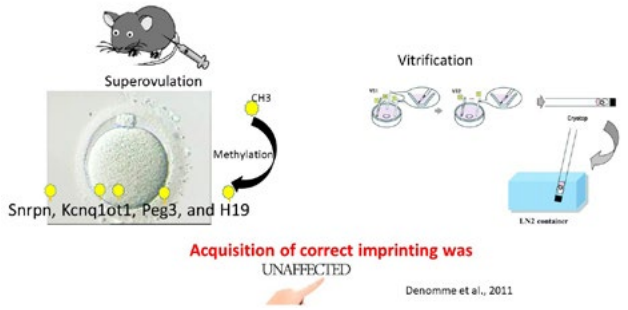
ART and Imprinting: Animal studies

- In vitro culture may affect embryo outcome was initially made in ruminants
- This proposed link was confirmed when it was found that sheep with "large offspring syndrome" showed both lack of expression and aberrant methylation of Igf2r (Young et al., 2001)

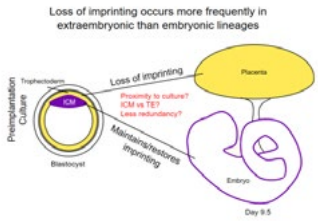
Developmental abnormalities in *in vitro* produced livestock

Large Offspring Syndrome

•Increased prenatal loss	• Higher perinatal mortality
•Large placentas	• Breathing difficulties
•Large fetuses	• Reluctance to suckle
•Polyhydramnios	• Skeletal anomalies
•Parturition problems	• Large organs
	• Cerebellar dysplasia



- In contrast to the oocyte, disrupted DNA methylation was consistently observed in ART conceived mouse embryos and it was dose dependent
- Embryo culture in five different commercial media systems resulted in loss of imprinted methylation at different levels



Human Molecular Genetics, 2015, Vol. 24, No. 24 6975–6985
doi: 10.1093/hmg/ddv400
Advance Access Publication Date: 23 September 2015
Original Article

ORIGINAL ARTICLE

The cumulative effect of assisted reproduction procedures on placental development and epigenetic perturbations in a mouse model

Eric de Waal[1,†], Lisa A. Vrooman[1,†], Erin Fischer[1], Teri Ord[2], Monica A. Mainigi[2], Christos Coutifaris[2], Richard M. Schultz[3] and Marisa S. Bartolomei[1,*]

- First study to evaluate the effect of NSET with and without superovulation on placental development and epigenetic profiles of both the placenta and its associated foetus
- Collectively, their results suggest that ART can induce biallelic expression of imprinted genes in both foetal and placental tissues from fully developed concepti, but epigenetic defects occur at a much higher frequency in IVF derived term placentae

Compromised oocyte quality and assisted reproduction contribute to sex-specific effects on offspring outcomes and epigenetic patterning

Laura Whiteden, Josée Martel, Sophia Rahimi, J. Richard Chaillet, Donovan Char, Jacquetta M. Trasler

Human Molecular Genetics, Volume 25, Issue 21, 1 November 2016, Pages 4649–4660,

A proof of principle experiment to test if alterations in oocyte quality, that emulate conditions that might be found in older infertile women, could make embryos more susceptible to adverse effects of ART

- Using a mouse model of females with approximately half of normal DNMT1o levels in their oocytes, demonstrated that compromised oocyte quality and ART techniques interact to exacerbate both developmental and epigenetic outcomes in a sex-specific manner following ART
- Only minor changes in genomic imprints were observed in the embryo, suggesting relatively robust mechanisms for ensuring proper imprint patterning, the placenta was more sensitive to imprinting defects

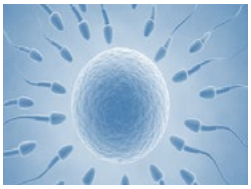
ART and Imprinting: Human Studies

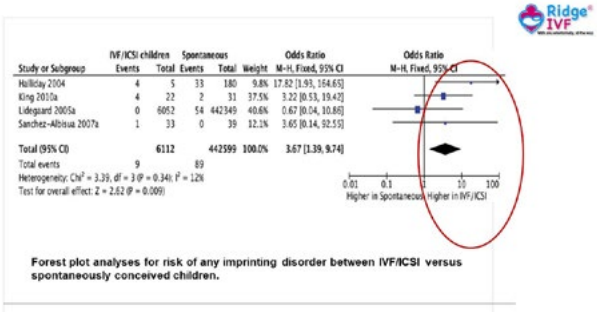
Human Reproduction Update, Vol.20, No.6 pp. 840–852, 2014
Advanced Access publication on June 24, 2014 doi:10.1093/humupd/dmu033

human reproduction update

A systematic review and meta-analysis of DNA methylation levels and imprinting disorders in children conceived by IVF/ICSI compared with children conceived spontaneously

Gabija Lazaraviciute[1], Miriam Kauser[1], Sohinee Bhattacharya[1], Paul Haggarty[2], and Siladitya Bhattacharya[1,*]





Forest plot analyses for risk of any imprinting disorder between IVF/ICSI versus spontaneously conceived children.

The results in relation to methylation within individual imprinted genes were inconclusive, largely because of the small numbers of studies and their heterogeneity

- The variability in ART protocols and the rarity of imprinting disorders complicate determining the causative relationship between ART and an increased incidence of imprinting disorders
- Compelling experimental data from animal studies also suggest a link between increased imprinting disorders and ART

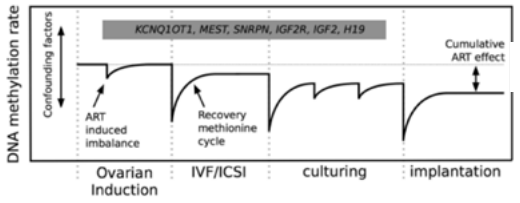
RESEARCH ARTICLE

Human Oocyte-Derived Methylation Differences Persist in the Placenta Revealing Widespread Transient Imprinting

Marta Sanchez-Delgado[1], Franck Court[2], Enrique Vidal[3], Jose Medrano[4], Ana Monteagudo-Sánchez[1], Alex Martin-Trujillo[1], Chiharu Tayama[5], Isabel Iglesias-Platas[6], Ivanela Kondova[7], Ronald Bontrop[7], Maria Eugenia Poo-Llanillo[4], Tomas Marques-Bonet[8,9,10], Kazuhiko Nakabayashi[5], Carlos Simón[4], David Monk[1]*

Sanchez-Delgado M, et al. (2016) PLoS Genet 12(11).

- Hundreds of CpG island sequences that maintain methylation on their maternal allele in blastocysts and placenta indicative of incomplete reprogramming
- sperm-derived methylation is reprogrammed by the blastocyst stage of development
- Oocyte-derived gDMRs in placenta are largely restricted to primates, being most abundant in human; No placenta-specific maternal methylation was observed in mouse.



- Disruption in methionine cycling by ART induces an altered DNA methylation state of imprinted genes
- Alterations in the DNA methylation rate due to ART treatments (i.e., ovarian induction, IVF/ICSI, embryo culturing, and implantation) occur temporally since the methionine cycle is able to buffer fluctuations due to the presence of feedback loops in the methionine and folate cycle
- Confounding factors (i.e., parent subfertility and advanced parental age) determine the initial level of DNA methylation before ART treatment

Fertil Steril. 2012 Jan;97(1):147-53.e7. doi: 10.1016/j.fertnstert.2011.10.027. Epub 2011 Nov 23.

Defects in imprinting and genome-wide DNA methylation are not common in the in vitro fertilization population.

Oliver VF[1], Miles HL, Cuffield WS, Hofman PL, Ludgate JL, Morison IM.

Journal of Assisted Reproduction and Genetics
https://doi.org/10.1007/s10815-018-1173-x

REVIEW

Comprehensive meta-analysis reveals association between multiple imprinting disorders and conception by assisted reproductive technology

Victoria K. Cortessis[1,2] · Moosa Azadian[1] · James Buxbaum[3] · Fatimata Sanogo[1] · Ashley Y. Song[1] · Intira Sriprasert[1] · Pengxiao C. Wei[1] · Jing Yu[1] · Karine Chung[2] · Kimberly D. Siegmund[1]

Received: 14 December 2017 / Accepted: 23 March 2018

Couples can currently be counseled that while frequency may be several fold higher following ART, absolute risk is low.

REVIEW

Health outcomes of children born after IVF/ICSI: a review of current expert opinion and literature

BCJM Fauser [a,*], P Devroey [b], K Diedrich [c], B Balaban [d], M Bonduelle [e], HA Delemarre-van de Waal [f], C Estella [g,h], D Ezcurra [i], JPM Geraedts [j], CM Howles [k], L Lerner-Geva [l], J Serna [i], D Wells [m], Evian Annual Reproduction (EVAR) Workshop Group 2011

Reproductive BioMedicine Online (2014) 28, 162– 182

- IVF-conceived children have lower birthweights and higher peripheral fat, blood pressure and fasting glucose concentrations than controls.
- Growth, development and cognitive function in assisted-conception children are similar to controls.
- **The absolute risk of imprinting disorders after assisted reproduction is less than 1%.**
- A direct link between assisted reproduction and health-related outcomes in assisted-conception children could not be established.

Concluding Remarks

- in-vitro culture and number of ART procedures should be optimized to ensure fidelity of genomic imprinting during preimplantation development.
- Subfertility and ART interact.
- New research addressing epigenetic state of gamete DNA in the etiology of both male- and female-factor subfertility would be further justified
- Well-controlled, large-scale, multicentre, prospective, long-term epidemiological studies are required. Without ruling out the infertility factor itself from the analysis as a potential source of imprinting defects.
- There is a need for careful follow up of IVF/ICSI-conceived children into adulthood to determine long-term health-related consequences.

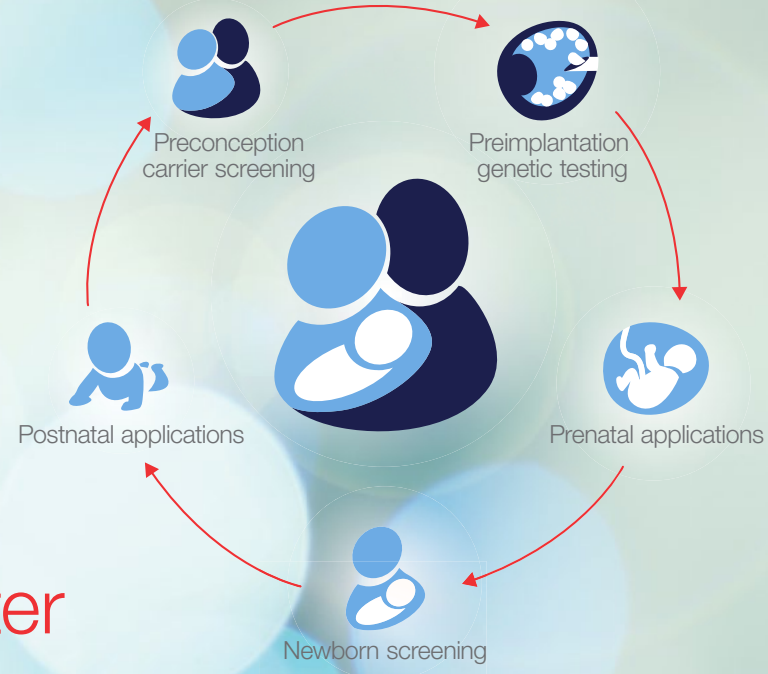
Take Home Message

Although results from animal studies indicate that ARTs are associated with epigenetic alternations, great caution is recommended in extrapolating these findings to human embryology. At present, data obtained in humans are inconclusive.

One should also keep in mind that the **incidence of imprinting disorders is reassuringly low and the great majority of children conceived through ARTs are developing normally.**

reproductive health

applied biosystems ion torrent



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Find out more at thermofisher.com/cytoscan

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- An exon-level microarray designed to comprehensively detect single-exon deletions and duplications in a cost-effective manner
- Complements NGS mutation analysis to reliably confirm CNVs
- Simple and streamlined variant analysis and reporting flexibility with gene panel or gene-level tier options

Find out more at thermofisher.com/cytoscanxon

Postnatal applications

Find out more at thermofisher.com/appliedbiosystems or thermofisher.com/iontorrent

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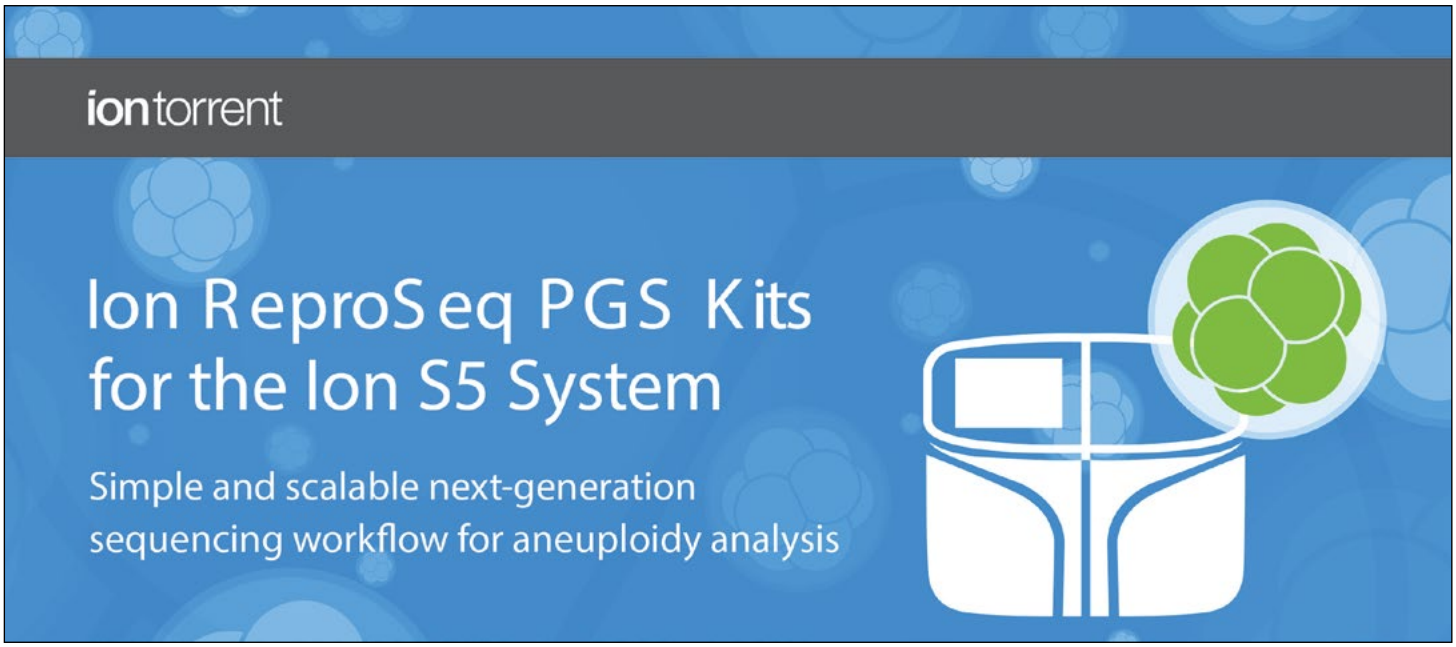

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