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 enjoy 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 Disorders 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
• 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

• 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
How do we apply genetics in OB / GYN Practice - basics to the advances

Indian Fertility Society & Sig Applied Genetics

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SECRETARIAT
Indian Fertility Society
302, 3rd Floor, Kailash Building, Kasturba Gandhi Marg, Connaught Place, New Delhi - 110001
Venue and Dates

CHANDIGARH
7th Oct 2018
Local Coordinator
Dr Umesh Jindal

PUNE
Nov 2018
Local Coordinator
Dr Mamta Dighe

CHENNAI
11th Aug 2018
Local Coordinator
Dr PM Gopinath
## List of contributors

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<th>Topic</th>
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<td>Dr. Anupam Gupta</td>
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<td>Dr. Sheetal Jindal</td>
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<td>Dr. Ashima</td>
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## Programme for the day

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<td>12:00 - 13:00</td>
<td>Registration &amp; Lunch</td>
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<td>13:00 - 13:15</td>
<td>Welcome and Introduction to the program</td>
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<td>13:15 - 13:35</td>
<td>Genetics in the clinic – the time has come</td>
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<td>13:35 - 13:45</td>
<td>Discussion</td>
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<tr>
<td>13:45 - 14:05</td>
<td>Genetic evaluation in infertility</td>
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<td>14:05 - 14:15</td>
<td>Discussion</td>
</tr>
<tr>
<td>14:15 - 14:35</td>
<td>Aneuploidy Screening: The how, what and when</td>
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<tr>
<td>14:35 - 14:45</td>
<td>Discussion</td>
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<tr>
<td>14:45 - 15:15</td>
<td>Tea</td>
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<tr>
<td>15:15 - 15:35</td>
<td>Emerging Technologies in Genetic Diagnosis – application in clinical practice</td>
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<tr>
<td>15:35 - 15:45</td>
<td>Discussion</td>
</tr>
<tr>
<td>15:45 - 16:00</td>
<td>Does ART predispose to genetic disorders?</td>
</tr>
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<td>16:00 - 17:00</td>
<td>Panel discussions</td>
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<td>63</td>
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<td>5</td>
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1. Genetics in the clinic: The time has come!
What are we Addressing?

Burden of Genetic Disorders in India

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Estimated cases per year</th>
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<tbody>
<tr>
<td>Congenital malformations</td>
<td>495,096</td>
</tr>
<tr>
<td>G6PD deficiency</td>
<td>390,000</td>
</tr>
<tr>
<td>Down syndrome</td>
<td>21,412</td>
</tr>
<tr>
<td>β-Thalassaemia</td>
<td>9,000</td>
</tr>
<tr>
<td>Sickle cell disease</td>
<td>5,200</td>
</tr>
<tr>
<td>Amino acid disorders</td>
<td>9,760</td>
</tr>
</tbody>
</table>

Community Genet 2002:5:192-196

Estimated cases with Malformations

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

Transfer of Bench technology to bed side

Diagnosis
Prevention
Treatment

And therefore awareness is the key to application

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

<table>
<thead>
<tr>
<th>Presenting Complaint/Referral indication</th>
<th>Premarital</th>
<th>Pre-conceptional</th>
<th>Prenatal</th>
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<tbody>
<tr>
<td>Amenorrhea</td>
<td></td>
<td></td>
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<tr>
<td>Genital ambiguity</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>Infertility</td>
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<tr>
<td>Consanguinity</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Family history of genetic disorder</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Recurrent pregnancy loss</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Previous child with genetic disorder</td>
<td>0</td>
<td>0</td>
<td></td>
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<tr>
<td>Abnormal Screening results</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal USG</td>
<td>0</td>
<td></td>
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</tbody>
</table>
Scenario 1

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.g.s - tuberous sclerosis, neurofibromatosis, Huntington chorea
  - Autosomal recessive disorder
e.g.s - thalassemia, spinal muscular atrophy, deafness
  - X linked disorder
e.g.s - 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
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 variants require prenatal testing

<table>
<thead>
<tr>
<th>Hemoglobin variants</th>
<th>Prenatal Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>β thal &amp; β thal</td>
<td>Y</td>
</tr>
<tr>
<td>Hb E &amp; β thal</td>
<td>Y</td>
</tr>
<tr>
<td>Hb S &amp; β thal / Hb δβ / Hb S / Hb D Punjab / Hb C / Hb E</td>
<td>Y</td>
</tr>
<tr>
<td>δβ &amp; HbS / β thal</td>
<td>Y</td>
</tr>
<tr>
<td>Hb Bart and HbH</td>
<td>Y</td>
</tr>
<tr>
<td>Hb O Arab &amp; β thal</td>
<td>Y</td>
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<tr>
<td>Hb D Punjab &amp; β thal</td>
<td>N</td>
</tr>
<tr>
<td>Hbs &amp; HPFH</td>
<td>N</td>
</tr>
<tr>
<td>HPFH /HPFH</td>
<td>N</td>
</tr>
<tr>
<td>Hb C &amp; β thal</td>
<td>N</td>
</tr>
</tbody>
</table>

Carrier Screening

- Screening in the preconception period
- 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, 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 unfolding

M. Malek Jr. et al. 2017
European Journal of Human Genetics
Spectrum of genetic tests

Categories of Genetic Disorders

- Chromosomal Disorders
  - Karyotype
  - Numerical or structural abnormality in chromosomes

- Single Gene Disorders
  - DNA test
  - Genetic diseases that occur due to a change in a gene

- Multifactorial Disorders
  - Gene & environment

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
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

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

![Karyotype Image]

Recurrence risk

- 1%
- 100%

What should be done in this case?

![Pedigree Image]

- Fish for 13, 18, 21, X, Y was normal
- 3p+ Mental retardation
- 38 years
- 13 yrs
- AF cells on culture

Karyotype Report

![Karyotype Image]
How do we apply genetics in OB / GYN Practice - basics to the advances

Chorionic villus sample – FISH and microarray

- FISH study was normal
- Chromosomal Microarray – duplication of 1.4 Mb on 7q11.23 (72726572 – 7413332 bp)
- Genes: (ELN – Aortic dilatation) & GTF21 – behavior abnormalities, intellectual disability variable autism

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%

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
• 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

Concern: Ongoing pregnancy with a 50% risk of recurrence

- 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 pregnancy

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 / QF-PCR – 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 differentiate fetal and maternal tissue*
• 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
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

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

Puri RD. Fetal dysmorphology. JOFM

Value of Fetal Autopsy

Diagnostic yield of fetal autopsy (n=530/903: 58.8%)

<table>
<thead>
<tr>
<th>Isolated abnormality of amniotic fluid (n=48)</th>
<th>IUGR (n=44)</th>
<th>IUD (n=300)</th>
<th>NIHF (n=70)</th>
<th>Malformations (n=435)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=37</td>
<td>n=33</td>
<td>n=209</td>
<td>n=42</td>
<td>n=209</td>
</tr>
<tr>
<td>77%</td>
<td>75%</td>
<td>69.6%</td>
<td>55.2%</td>
<td>45%</td>
</tr>
</tbody>
</table>

Definitive cause identified in
### 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

<table>
<thead>
<tr>
<th>Chromosomal Disorders</th>
<th>Single gene disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVS / Amniotic fluid / Blood in Heparin</td>
<td>CVS / Blood in EDTA Amniotic fluid</td>
</tr>
</tbody>
</table>

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.

- 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,XXX) are eight times more common in infertile than fertile men.
- Klinefelter syndrome (47,XXX) 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).

Translocations

- 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.
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 a 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)...

---

Middle East Fertility Society Journal (2010) 15, 139–145
Rare cases of Male Infertility or Syndromes where Infertility is a Minor manifestation

- Miotonic dystrophy
- S-enzyme deficiency
- Steroidogenic enzymes deficiency (21-α-hydroxylase and others)
- Bardet-Biedl
- Noonan
- Prader-Willi
- Cerebellar ataxia with hypogonadotropic hypogonadism
- Fanconi anaemia
- Prune-Belly
- Homozygous β-thalassaemia
- 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.

Epigenetic Testing

- Epigenetic testing helps reveal specific markers in a sperm’s epigenome that have been associated with infertility, poor IVF outcomes, a development of embryos.
- The Episona SEED Test
- Catsper
- ID3
**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 in male infertility, allowing new genetic factors to be discovered.

**Materials and methods:** PubMed was searched for combinations of the following terms “male,” “genetics,” “related,” “sequencing,” “whole-genome sequencing,” “whole-exome sequencing,” “next-generation sequencing,” “azoospermia,” “oligospermia,” “spermatogenesis,” “hypergonadotropic,” 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 deficits. Combined, these studies uncover variants in 26 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.

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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>
<thead>
<tr>
<th>Genetic causes Of Female Infertility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatic discordant (lack of similarity)</td>
</tr>
<tr>
<td>Chromosomal aberrations (translocations, deletions, inversions)</td>
</tr>
<tr>
<td>Infection</td>
</tr>
<tr>
<td>Immunological</td>
</tr>
<tr>
<td>Male factor</td>
</tr>
<tr>
<td>Other</td>
</tr>
</tbody>
</table>

Genetic causes Of Female Infertility
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 menstruation (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

<table>
<thead>
<tr>
<th>Infertility disorder</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycystic ovary syndrome</td>
<td>SMAR, CYP11, CYP17, CYP19, HSD17B1, HSD17B3, OCT1, ACTRI, ACTRIA-B, FS, INHA, INHBA-B, INHBC, LHCG, FSHR, MADH4, AM, AMCR, DB, OBR, LHCGR, LGD3, BMP15, NR5A1, EIF2B2, EIF2B5, HSD17B4, FSHR, LHCGR, FSHR, VDR, EPHX1, LAMN, GSK3A</td>
</tr>
<tr>
<td>XX gonadal dysgenesis</td>
<td>FSHR, BMP15, NR5A1, EIF2B2, EIF2B5, HSD17B4, HARS2, FSHR, LHCGR, BMP15</td>
</tr>
<tr>
<td>Periarchetral syndrome</td>
<td>ESR1, HED1, HSDD1, BMP15, LHCGR, FSHR, LHCGR, BMP15, LHCGR, FSHR, LHCGR</td>
</tr>
<tr>
<td>Premature ovarian failure</td>
<td>FSH, FSHB, BMP15, LHCGR, FSHR, LHCGR, BMP15, LHCGR, FSHR, LHCGR, BMP15</td>
</tr>
<tr>
<td>Rare causes of female infertility, or syndromes in which infertility is a minor manifestation</td>
<td>Galactosemia, Mucopolysaccharidoses, Mitotic dysplasia, Prader-Willi, 21 alpha-hydroxylase, 17 alpha-hydroxylase and other steroidogenic enzymes deficiency, Aromatase defect, Homozygous beta-thalassemia, Cystic fibrosis, Hemochromatosis, DAX1 gene mutations</td>
</tr>
</tbody>
</table>
Guidelines for the appropriate use of genetic tests in infertile couples

<table>
<thead>
<tr>
<th>Condition</th>
<th>Genetic Test</th>
<th>Karyotyping</th>
<th>Array CGH</th>
<th>Routine Cytogenetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klinefelter Syndrome</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Turner Syndrome</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Aneuploidies</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Balanced Translocations</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Single copy deletions</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Recurrent miscarriage</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Note: ART: assisted reproductive techniques; RNA: genome-wide association study.
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.
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 syndrome, 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?
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

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

Low risk population

Diagnostic test is definitive; expensive, associated with a risk

Selects a High Risk Population

Diagnostic Vs Screening test

<table>
<thead>
<tr>
<th>Diagnostic test</th>
<th>Screening test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definitive diagnosis (does the patient have disease)</td>
<td>Provides risk estimates (does the patient need sp. testing)</td>
</tr>
<tr>
<td>Done on high risk population</td>
<td>Done on healthy population</td>
</tr>
<tr>
<td>Expensive</td>
<td>Cheap, quick</td>
</tr>
<tr>
<td>Complex and sophisticated</td>
<td>Easy</td>
</tr>
</tbody>
</table>

Evolution of Screening for Trisomy 21
Maternal Age Related Risks

![Graph showing risk percentage across maternal ages for various conditions.]

Performance based Measures

- **Sensitivity of the test** - % of 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

![Graph showing risk of Down syndrome pregnancy at term.]

Risk of Down syndrome pregnancy at term
Which Test?

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

CffDNA after 10 weeks of gestation

Factors affecting screening performance

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

![Graph showing marker values change with gestational weeks]

- Marker values change with gestation trimester.

- Gestational age measured by Ultrasound.
- Use CRL measure from 1st trimester scan.

---

Markers and Disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>PAPP A</th>
<th>Fb hCG</th>
<th>AFP</th>
<th>E3</th>
<th>IA</th>
</tr>
</thead>
<tbody>
<tr>
<td>T 21</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>T 18</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T 13</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex chromosome</td>
<td>↓</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triplody</td>
<td>↓</td>
<td>↓</td>
<td>↑↑</td>
<td>↓</td>
<td></td>
</tr>
</tbody>
</table>


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 be beneficial 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 ‘healthy baby’
  - 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?

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

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

2nd trimester screen
- 15 – 20th weeks

Specifics for Assisted Reproduction

- Impact of ART on DS screening are controversial
- Maternal serum markers for DS screening are significantly modified in ART and ovum donation
- The 1st trimester screen for DS could be influenced by mode of conception, particularly IVF & ICSI, but data on this are still conflicting
- Egg donation – age of the donor at the time of harvesting to be taken into account. Not the age of the recipient woman.
- Date of embryo transfer used in analysis
- Gestational age was also estimated by first trimester-CRL
- Oocyte donation, the donor’s maternal age
- Results on the impact of assisted reproductive technology (ART) on maternal serum Down syndrome screening are controversial.
- Variations: a decrease of PAPP-A in ART pregnancies and increase of hCGβ in


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 cfDNA 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 antenatal 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

- 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

<table>
<thead>
<tr>
<th>Test</th>
<th>Advantages</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>USG - dating &amp; NT + PAPP-A &amp; fHCG</td>
<td>12 wks: Confirmation of GA. Major malformation may be detected. Chorionicity of twins.</td>
<td>Preferred for 1st trim visits NT expertise</td>
</tr>
<tr>
<td>AFP + HCG + uES + IVF scan</td>
<td>17–18 wks: One visit for anomaly &amp; screen Chr. abnormal fetuses abort naturally</td>
<td>20 wks limit for confirmatory test if reqd.</td>
</tr>
<tr>
<td>cfDNA</td>
<td>After NT scan: Non invasive</td>
<td>Only for 5 chromosomes, expensive</td>
</tr>
</tbody>
</table>

References

- J Obstet Gynaecol Can 2017;39(9):805e817
THANK YOU
4. Emerging Technologies in Genetic Diagnosis
Application in 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

Methods for PGT-A
PGT-A Improves live birth rates

Effect of next-generation sequencing in preimplantation genetic testing on live birth rates:
- Analyzed live birth rates in frozen embryo transfer (FET) cycles where embryo ploidy status was determined PGT-A using Ion Torrent NGS.
- 112 cycles received PGT-A (47% success).
- 65 cycles in the control group with no PGT-A
- Control group consisted of 65 patients who underwent the
- Results showed:
  - Live birth rate per cycle was higher in PGT-A group compared to controls (47% vs 33.5%)
  - Pregnancy loss rates were lower in PGT-A group (8.4% vs 26.5%)
  - 16 cycles had no embryo suitable for transfer after PGT-A

Liaris et al., Fertility and Development 2016

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

<table>
<thead>
<tr>
<th>Study</th>
<th>Mosaic Embryos</th>
<th>Duplicated Embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Implantation rate (%)</td>
<td>On-Going Pregnancy Rate (%)</td>
</tr>
<tr>
<td>Green 2015</td>
<td>44</td>
<td>33</td>
</tr>
<tr>
<td>Pragade 2017</td>
<td>49</td>
<td>28</td>
</tr>
<tr>
<td>Murrey 2017</td>
<td>53</td>
<td>40</td>
</tr>
<tr>
<td>Spivak 2016</td>
<td>58</td>
<td>40</td>
</tr>
</tbody>
</table>

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

Gcota et al., NEJM 2019; Pragade et al., Human Genetics 2017; Murrey et al., Fertility and Sterility 2017; Spivak et al., Fertility and Sterility 2017
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 aneuploid – usually non viable
- Lower panel: Errors in mitosis during embryonic cell divisions lead to a mixture of euploid and aneuploid
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 disease 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 condition
  - Mild previous pregnancy due to presence of genetic condition
  - Consanguinity

- Selects any genetic condition where a specific gene or mutation is known to cause that condition can be screened for:
  - For example:
    - Tay-Sachs disease
    - Cystic fibrosis
    - Duchenne muscular dystrophy
    - Huntington’s disease
    - Poly cystic kidney disease
    - Spinal muscular atrophy
    - Sickle cell anemia
PGT-A and PGT-M Combined

PGT-M by NGS

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

Non-Invasive PGT-A (niPGT-A)
Non-Invasive iPGT-A Methods

Blastocoelic Fluid

- Blastocoelic Fluid (BF) - natural medium supporting the development of the ICM
  - Presence of DNA found in BF (Fahimi et al. 2013)
- Mapi (Fertility & Sterility, 2015)
  - Compared success between TE, BF, and BF using ISS embryo
  - 1% amplication failure rate from 116 samples (65/116)
  - BF were process for 24 chromosome screening
- Data from Z2 variants
- 78 embryos used to compare with TE (90), blastomere (90) or PBS (48) biopsy data
  - 41% full concurrence with TE biopsy
  - 16% partial concurrence with TE biopsy
- Comments
  - No mention of source of DNA
  - Was BF collected before or after TE biopsy
  - Extent of inactivation was not addressed
  - Fresh or cryopreserved

Blastocoelic Fluid

- Todd et al (Fertility & Sterility, 2015)
  - Compared BF to blastomere and whole embryo
  - 80 embryos had amplification
  - 39% below norm
  - Full concurrence = 40%
  - Partial concurrence = 17%
  - Concurrence = 90%

DNA isolated from the BF were discordant to the ICM-TE in 53% 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 Shiplane et al. 2014
  - Xu et al. (2016) looked at the use of spent media from 42 blastocysts and compared results to WIE:
    - 100% WGA
    - Concordance rate of 62.7% (39/62)
    - Full concordance = 91.3% (57/62)
    - Partial concordance = 61% (46/62)
    - Sensitivity = 52.2% Specificity = 54.0%
  - They since used the method on patients with successful outcomes

- Forster et al. (2017)
  - Used 22 samples of spent culture media (60) and compared to Polar Body biopsy
    - WGA rate at 64% (48/75)
    - 72% concordance rate (53/76) for embryo aneuploidy
    - 37.1% Full concordance (51/138)
    - 44.4% Partial concordance (81/138)
    - 49% concordance between chromosomes collected

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 seeded or aneuploidy samples
  - Compared Spent Media to TE (spicy to SS embryos)
    - 6.6% amplification issues
    - Full concordance = 88.4% (28/32)
    - Partial Concordance = 27.8% (14/51)
  - Discordance = 66.7% (5/8)
  - Mainly due to maternal contamination as shown by STR analysis
    - Suggest changes in methodology

- After modifying culture conditions / assay
  - Increased concordance to ~84%
  - Discordance to ~6%

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

- Kuznetsova et al. (2017) collected and combined BF and spent media from day 5/6 blastocysts and compared to TE and WIE biopsy
  - Cut 2P with lower and allowed BF to seed into media (20 frozen embryos)
  - BF and media collected after TE biopsy (19 fresh ovarian embryos)
  - 100% amplification rate

<table>
<thead>
<tr>
<th>Concordance at embryo level (20 Fresh)</th>
<th>Concordance at embryo level (19 Freshes)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concordance</strong></td>
<td><strong>Concordance</strong></td>
</tr>
<tr>
<td>Full Concordance</td>
<td>Full Concordance</td>
</tr>
<tr>
<td>10.98% (19/19)</td>
<td>10.98% (19/19)</td>
</tr>
<tr>
<td>Partial Concordance</td>
<td>Partial Concordance</td>
</tr>
<tr>
<td>5.36% (10/19)</td>
<td>5.36% (10/19)</td>
</tr>
<tr>
<td>Discordant</td>
<td>Discordant</td>
</tr>
<tr>
<td>3.68% (7/19)</td>
<td>3.68% (7/19)</td>
</tr>
</tbody>
</table>

- Data indicates niPGT-A gives a similar level of overall concordance but may be 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 2019)
- Use a combination of BF and spent culture media
- Wash to get amplifiable DNA from Dulbecco's or BF alone
- Used to export multiple indicator and abort BF to apply into culture media

- Study included 40 embryos
  - Combined BF and spent media with human fetal cells (FBCs)
  - Amplification in 1 ECB sample, 1 BA and 1 TE: 1 BA: 2 EB: 1 TE: 2 BA: 1 BA
  - In 50% cases of 10 embryos, no components TE and SE
  - In 20% cases, TE, BA: 1 BA: 1 BA: 1 BA: 1 BA

Using PCC to determine whether from BF as the standard
- Sensitivity: 83.3% Specificity: 78.5%
- ECB: Sensitivity: 93.3% Specificity: 66.4%

<table>
<thead>
<tr>
<th>Component</th>
<th>ECB</th>
<th>BA</th>
<th>SA</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBC</td>
<td>15%</td>
<td>15%</td>
<td>4%</td>
</tr>
<tr>
<td>BA</td>
<td>15%</td>
<td>15%</td>
<td>4%</td>
</tr>
<tr>
<td>TE</td>
<td>15%</td>
<td>15%</td>
<td>4%</td>
</tr>
<tr>
<td>SE</td>
<td>15%</td>
<td>15%</td>
<td>4%</td>
</tr>
</tbody>
</table>

niPGT-A Summary

- Uncertainties about source of cell-free DNA
- Cell apoptosis: normal or abnormal cells
- ECM in TE, combination
- Material 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 cells
- High sensitivity ~95% in concordance studies with TE and SE

- Easy to perform
- No need for biopsy-trained embryologists
- Reduce embryological time
- No need of expensive lasers
- No damage to embryos

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)
How do we apply genetics in OB / GYN Practice - basics to the advances

Indian Fertility Society & Sig Applied Genetics

NIPT with NGS – Massively Parallel Shotgun Sequencing (MPSS)

Challenges with NIPT

- Fetal fractions
  - Affected by gestational age, maternal BMI, type of aneuploidy
  - Low fetal fractions associated with increased risk for aneuploidy
- Twins/surrogacy/donors
- False Positives
  - Placental mosaicism
  - Vanishing twin
  - Maternal sex chromosome abnormality
- Neoplasia – apoptosis of cancer cells, aneuploidy common
- NIPT in Average Risk Pregnancies and rare conditions

Advances in Non-Invasive Prenatal Testing

- Fetal Cells for NIPT
- Fetal Exomes
- De Novo Panels (PreSeek)
- NIPD for Monogenic Disorders (de novo and paternally inherited mutations)
- More Specific
- More Informative
- More Diagnostic
Fetal Cells

- 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 Revealed” system to capture trophoblasts and the nucleated RBC (nRBC)
- Gibson-based, nanostructured miniaturized tool using immunoaffinity
- Captured both Trophoblasts and nRBC
- WGA followed by NGS and compared to karyotype of trisomies and routine SNP data
- Confirmation of fetal aneuploidy by SNP analysis
- After validation of system on 24 samples ran a verification on 5 samples.
- 3 with trisomy
- 2 normal
- In all cases results by NGS were in agreement with karyotyping and SNP data
- Notable advantage of being able to detect aberrations in all chromosome arms and microsatellites
- nRBC is a direct reflection of fetal genome

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 qPCR
- Compared with NGS on invasive samples
- Isolated ~12 cells per sample (25 ml blood)

- From 5 samples
- Confirmed invasive test results in all cases
  - 1 nT21
  - 2 nT13 Mosaic (0.5%), Full T13 by SNP
  - 3 nT2 (Mosaic), Full T2 by SNP
  - 4 ~12.4 Mb duplication
  - 5 ~Unbalanced translocation 11.4 Mb terminal deletion on chromosome 4q and a 10.1 Mb terminal duplication on chromosome 8p

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.
- Postnatal: Research for chromosomal abnormalities related to fetal anomalies detected by ultrasound.
- Analysis of DNA from products of conception to identify chromosomal abnormalities related to pregnancy loss.
- Confirmation of abnormal results found with other screening technologies.

Exon-level CNVs are critical in clinical research

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

![Genetic Diagram]

Research Case I: Waardenburg Syndrome

- Run on CMA (CytoScan HD)
  - Large deletion seen across GCH10 gene
- Waardenburg Syndrome panel run:
  - No findings
- WES
  - No sequence findings
- Run on CytoScan HD
  - A additional GCH10 findings

<table>
<thead>
<tr>
<th>Waardenburg Syndrome Genes</th>
<th>WS1</th>
<th>WS2</th>
<th>WS3</th>
<th>WS4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PAK3 (100%)</td>
<td>MITF (15%)</td>
<td>PAK3 (50%)</td>
<td>EDN1/EDNRB (20%)</td>
</tr>
<tr>
<td></td>
<td>SOD1 (15%)</td>
<td>EDN1/EDNRB (5%)</td>
<td>SOD1 (50%)</td>
<td>EDN1/EDNRB (5%)</td>
</tr>
</tbody>
</table>
Research Case I: Waardenburg Syndrome

- Nested heterozygous deletion in homozygous 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

<table>
<thead>
<tr>
<th>Specifications</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content</td>
<td>6.85 million probes empirically selected for whole-genome coverage including:</td>
</tr>
<tr>
<td></td>
<td>• 6.5 million copy number probes</td>
</tr>
<tr>
<td></td>
<td>• 300,000 SNP probes for LOH/ADH analysis as well as du/trio assessment and sample tracking</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>95% sensitivity for the detection of exonic CNVs*</td>
</tr>
<tr>
<td>Coverage</td>
<td>Total number of genes with coverage: 25,969</td>
</tr>
<tr>
<td></td>
<td>• Full coverage: 21,844</td>
</tr>
<tr>
<td></td>
<td>• Partial coverage: 4,136</td>
</tr>
<tr>
<td></td>
<td>• Exome genes for medical research (including cancer genes): 7,000</td>
</tr>
</tbody>
</table>

*Genetically validated loci - 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?

[Diagram showing the relationship between ART and underlying factors of infertility in patients]
Information about technology-related and patient-related concerns in OB/GYN practice with respect to genetics.

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

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

- TTP is reduced with successful treatment
- Increased TTP is associated with risks in terms of outcome like preterm delivery and congenital malformations
### How do we apply genetics in OB/GYN Practice - basics to the advances

**Table 1. Studies reporting on general physical health and childhood cancer in intrauterine growth retardation (IUGR) and in vitro fertilization (IVF) conceived offspring**

<table>
<thead>
<tr>
<th>Study</th>
<th>Design and study population</th>
<th>Main outcomes</th>
<th>Key findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gouger et al. (2015)</td>
<td>Consecutive cohort, IUGR children with and without IVF</td>
<td>Physical measurements, including anthropometrics, daily activity, and plantar foot arches</td>
<td>Compared to controls, children with IVF showed higher body mass index (BMI), higher waist circumference, and lower lean body mass.</td>
</tr>
<tr>
<td>Knoeber et al. (2016)</td>
<td>Retrospective cohort, IUGR children with and without IVF</td>
<td>Physical measurements, including anthropometrics, daily activity, and plantar foot arches</td>
<td>Higher levels of physical activity observed in children conceived through IVF.</td>
</tr>
<tr>
<td>Pincott et al. (2018)</td>
<td>Retrospective cohort, IUGR children with and without IVF</td>
<td>Physical measurements, including anthropometrics, daily activity, and plantar foot arches</td>
<td>No significant differences observed in physical measurements between groups.</td>
</tr>
</tbody>
</table>

**Childhood cancer**

<table>
<thead>
<tr>
<th>Study</th>
<th>Main outcomes</th>
<th>Key findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lam-Croft et al. (2016)</td>
<td>Retrospective cohort</td>
<td>Risk of childhood cancer in ART vs. IVF group, but not statistically significant due to adjustment for maternal and fetal characteristics.</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sperm concentration (10^6 sperm/mL)</th>
<th>Frequency of chromosomal abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azospermia</td>
<td>18.7%</td>
</tr>
<tr>
<td>Severe oligospermia (0-5)</td>
<td>4.6%</td>
</tr>
<tr>
<td>Mild to moderate oligospermia (5-20)</td>
<td>2.6%</td>
</tr>
<tr>
<td>Normospermia (&gt;20)</td>
<td>3.0%</td>
</tr>
<tr>
<td>Total</td>
<td>6.11%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Children born in oligospermic men (n=25)</th>
<th>Children born in normospermic men (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major anomalies</td>
<td>Number</td>
</tr>
<tr>
<td>Cordoma</td>
<td>1</td>
</tr>
<tr>
<td>Syltke</td>
<td>1</td>
</tr>
<tr>
<td>Blatt</td>
<td>1</td>
</tr>
<tr>
<td>Hippo</td>
<td>1</td>
</tr>
<tr>
<td>Ngild</td>
<td>1</td>
</tr>
<tr>
<td>Neep</td>
<td>1</td>
</tr>
<tr>
<td>Table 6: Congenital anomalies in study group according to whether the latter had oligospermia</td>
<td></td>
</tr>
</tbody>
</table>

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

---

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

<table>
<thead>
<tr>
<th>Key results</th>
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<tbody>
<tr>
<td>No significant differences in HCC, height and weight between groups at one time point</td>
</tr>
</tbody>
</table>

---

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

| No significant differences in anthropometric measures between 1 and 2 y in NCC children and controls in either cohort, or adult weight at 41-45 | 49-50 year between CC and HC group |
| No significant differences in weight at 1, 1.1, 1.2 and 1.3 y in NCC children and controls in either cohort, or adult weight at 41-45 | 49-50 year between CC and HC group |

---

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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.

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
- Higher perinatal mortality
- Breeding difficulties
- Malformation in offspring
- Reduced survival
- Increased abortions
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
• 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
• 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

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.
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.
Empowering you with the innovative technologies you need is our passion—we know that the scientific advances you make help build healthier families. From preconception carrier screening through preimplantation genetic testing to prenatal and postnatal applications, our comprehensive portfolio of reproductive health solutions can help you achieve your clinical research goals and positively impact families around the world. We strive to be your trusted partner on this inspiring reproductive health journey.
Choose from our comprehensive portfolio of reproductive health solutions

Preconception carrier screening

Applied Biosystems ™ CarrierScan ™ Assay
- A single solution for expanded carrier screening
- Consolidates sequence and structural variants into a single assay for increased productivity
- Generates reliable results from empirically selected probes that provide biological validation of the most common variants
- Provides easy data analysis through powerful algorithms and curated annotations with automatic calculations for single or paired sample analysis; also enables customizable file exporting and reporting
Find out more at thermofisher.com/carrierscan

Prenatal applications

Ion ReproSeq ™ PGS kits for the Ion GeneStudio ™ S5 System
- A simple and scalable next-generation sequencing (NGS) workflow for aneuploidy analysis of embryo biopsy samples
- Rapid and cost-effective workflow for 16, 24, or 96 samples per run; 10–13 hours from cells to analyzed data
- Enhanced interpretation of results with adjustable workflows for increased sensitivity to detect segmental CNV events and low-level mosaicism calling
Find out more at thermofisher.com/reproseq

Postnatal applications

Applied Biosystems ™ CytoScan ™ XON Suite
- 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

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

ThermoFisher SCIENTIFIC
Ion ReproSeq PGS Kits for the Ion S5 System
Simple and scalable next-generation sequencing workflow for aneuploidy analysis