PRE-CONGRESS COURSE 13

Precision medicine:
Paving the way to new reproductive disease taxonomies, diagnostics and therapeutics.

American Society for Reproductive Medicine exchange course
London - UK, 7 July 2013
Precision medicine: Paving the way to new reproductive disease taxonomies, diagnostics and therapeutics

London, United Kingdom
7 July 2013

Organised by
The American Society for Reproductive Medicine
Contents

Objectives, target audience and learning objectives

Programme

Speakers' contributions

<table>
<thead>
<tr>
<th>Title</th>
<th>Author/Location</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision medicine: what it is and its relevance to infertility phenotypes, diagnosis and treatment</td>
<td>Linda C. Giudice - U.S.A.</td>
<td>9</td>
</tr>
<tr>
<td>Genetics and phenotypes in male infertility: databases and new diagnostics/therapeutics</td>
<td>Dolores J. Lamb - U.S.A.</td>
<td>23</td>
</tr>
<tr>
<td>Genetics and phenotypes in premature ovarian insufficiency and oocyte aging: databases and new diagnostics/therapeutics</td>
<td>Antonio Pellicer - Spain</td>
<td>42</td>
</tr>
<tr>
<td>Genetics and phenotypes in polycystic ovary syndrome: databases and new diagnostics/therapeutics</td>
<td>Roger Lobo - U.S.A.</td>
<td>59</td>
</tr>
<tr>
<td>Genetics and phenotypes in endometriosis: databases and new diagnostics/therapeutics</td>
<td>Serdar E. Bulun - U.S.A.</td>
<td>77</td>
</tr>
<tr>
<td>Genetics and phenotypes in hypothalamic amenorrhea: databases and new diagnostics/therapeutics</td>
<td>Richard Reindollar - U.S.A.</td>
<td>88</td>
</tr>
<tr>
<td>Genetics and phenotypes of embryos in culture: databases and new diagnostics for embryo selection</td>
<td>Antonio Pellicer - Spain</td>
<td>98</td>
</tr>
<tr>
<td>Resources of patient information</td>
<td>Craig Niederberger - U.S.A.</td>
<td>117</td>
</tr>
</tbody>
</table>

Upcoming ESHRE Campus Courses

Notes
Objectives

This Pre-Congress Course will focus on the meaning of precision medicine and discuss its application to male and female infertility and to classification, diagnosis, and targeted therapies for diseases of the reproductive tract.

Target audience

Reproductive Endocrinologists, Andrologists, General Gynecologists, Urologists, and Allied Reproductive Health Professionals

Learning objectives

At the conclusion of this course, participants should be able to:
1. Define precision medicine and discuss its relevance to integrating patient genetic, clinical, and disease information, and translating this to development of diagnostics and targeted therapies in reproduction
2. List the types and sources of data and information available to put into knowledge networks and the role of biomedical informatics in these processes.
3. Describe resources (databases), genetic abnormalities, and clinical phenotypes are involved in male and female infertility and reproductive disorders, including, polycystic ovary syndrome, premature ovarian insufficiency, endometriosis and hypothalamic amenorrhea
Scientific programme

Chairman: Linda C. Giudice - U.S.A.

09:00 - 09:30  Precision medicine: what it is and its relevance to infertility phenotypes, diagnosis and treatment
Linda C. Giudice - U.S.A.

09:30 - 09:45  Discussion

09:45 - 10:15  Genetics and phenotypes in male infertility: databases and new diagnostics/therapeutics
Dolores J. Lamb - U.S.A.

10:15 - 10:30  Discussion

10:30 - 11:00  Coffee break

11:00 - 11:30  Genetics and phenotypes in premature ovarian insufficiency and oocyte aging: databases and new diagnostics/therapeutics
Antonio Pellicer - Spain

11:30 - 11:45  Discussion

11:45 - 12:15  Genetics and phenotypes in polycystic ovary syndrome: databases and new diagnostics/therapeutics
Roger Lobo - U.S.A.

12:15 - 12:30  Discussion

12:30 - 13:30  Lunch

13:30 - 14:00  Genetics and phenotypes in endometriosis: databases and new diagnostics/therapeutics
Serdar E. Bulun - U.S.A.

14:00 - 14:15  Discussion

14:15 - 14:45  Genetics and phenotypes in hypothalamic amenorrhea: databases and new diagnostics/therapeutics
Richard Reindollar - U.S.A.

14:45 - 15:00  Discussion

15:00 - 15:30  Coffee break

15:30 - 16:00  Genetics and phenotypes of embryos in culture: databases and new diagnostics for embryo selection
Antonio Pellicer - Spain

16:00 - 16:15  Discussion

16:15 - 16:45  Resources of patient information
Craig Niederberger - U.S.A.

16:45 - 17:00  Discussion
Precision Medicine: What it is and its relevance to infertility phenotypes, diagnosis and treatment

ASRM-ESHRE Pre-congress Course #13
Linda C. Giudice, MD, PhD
University of California, San Francisco
July 7, 2013

Disclosures

• American Society for Reproductive Medicine, President 2012-2013
• World Endometriosis Society 2014, President
• World Endometriosis Research Foundation 2011-2014, Board of Directors
• Quest Diagnostics, Academic Associate
• Funding
  • National Institutes of Health
  • California Institute for Regenerative Medicine

Learning Objectives

• At the conclusion of this lecture, learners will know:
  • What Systems Biology and Precision Medicine are.
  • What their relevance is to Reproductive Medicine (today’s session).
  • How can we prepare for Precision Medicine.
  • What the Reproductive Medicine global community can do to bring Reproductive Precision Medicine to our patients and their families.
**What is Systems Biology?**

- The study of the interactions among components of a biological system and how these give rise to function and behavior.
- Involves a cycle of theory, data (experimental, human), computational modeling, and experiments to describe quantitatively cells or cell processes.

**How Old is Systems Biology?**

- 1952 Nobel Prize in Physiology or Medicine awarded to von Bertalanffy for his work in systems theory.
- 2010: Human microbiome project.
- 2015: Human epigenome project.

**Systems Biology**

- "omics" + high throughput experiments + bioinformatics
  
  - genome
  - proteome
  - transcriptome
  - epigenome
  - methylome
  - acetylome
  - phenome
  - metabolome
  - glycome
  - interactome
  - cardiolome
  - reactome
  - Roome
  - lipome
  - toxicome
  - exposome
  - microbiome
  - other omes...
Systems Biology
Lots of Data!

Challenge
Standardization of specimen collection, data collection and annotation, analyzing and pulling together the "omics" to understand biology at a higher level with its complex collection of networks and pathways and to translate it to clinical medicine.

What is Precision Medicine?
What is Precision Medicine?

- A broad term for interventions targeted to individuals based on their risk to provide a more coherent and focused approach to health care.

What is Precision Medicine?

- Personalized health care includes
  - preventive interventions
  - diagnostic interventions
  - therapeutic interventions
  - surveillance
- With risk defined through
  - genetics/omics
  - clinical presentation
  - socioeconomic status
  - environmental exposures (diet, stress, chemicals, pollution, water quality)

What is Precision Medicine?

- Prominent technology-focused definition - relies on use of molecular testing to define risk, e.g., genetics, genomics, proteomics, metabolomics, other
What is Precision Medicine?

- Goals include greater effectiveness and efficiency of health care delivery as well as improved health outcomes and quality of life

Who Will Do This?
Multidisciplinary Teams

- traditional basic scientists
- traditional clinical scientists
- quantitative scientists
  - computational biologists
  - statisticians
  - mathematicians
  - computer scientists
  - physicists
- clinicians
- nurses
- genetic counselors
- nutritionists
- environmental scientists
- community members

We are the Stakeholders

<table>
<thead>
<tr>
<th>Community</th>
<th>Goals</th>
<th>Stakes / Needs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbiological Researchers</td>
<td>Improved disease mechanisms</td>
<td>Increased productivity, decreased infection prevalence</td>
</tr>
<tr>
<td>Drug Discovery</td>
<td>Better personalized care</td>
<td>Increased productivity, decreased cost</td>
</tr>
<tr>
<td>Public Health Officials, Public Health Advisors, Health Care Industry</td>
<td>Tailored disease management</td>
<td>Increased productivity, decreased cost</td>
</tr>
</tbody>
</table>

National Academy of Sciences Press 2011
Some Examples of Precision Medicine

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ner-2/neu receptor</td>
<td>Select Herceptin (trastuzumab) for breast cancer Rx</td>
</tr>
<tr>
<td>BRCA1/2</td>
<td>Breast and ovarian cancer inherited risk, prophylactic tamoxifen and surgery</td>
</tr>
<tr>
<td>Transcriptional Profile</td>
<td>Avoid chemotherapy in breast cancer patients with low risk of recurrence</td>
</tr>
<tr>
<td>(21 genes)</td>
<td></td>
</tr>
<tr>
<td>CYP2D6/CYP2D19</td>
<td>Guide prescribing/adjust dose of ~ 25% of commonly used drugs</td>
</tr>
<tr>
<td>VKOR/CYP2C9</td>
<td>Dosing of warfarin</td>
</tr>
</tbody>
</table>

Adapted from L. Schulman, WHF 2013

Some Examples of REPRODUCTIVE Precision Medicine

<table>
<thead>
<tr>
<th>Item</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applications for diagnostics,</td>
<td></td>
</tr>
<tr>
<td>prognostics, therapeutics)</td>
<td></td>
</tr>
<tr>
<td>Oocyte quality</td>
<td>secretome</td>
</tr>
<tr>
<td>implantation potential</td>
<td>follicular fluid G-CSF</td>
</tr>
<tr>
<td>Sperm quality</td>
<td>genetics/genomics</td>
</tr>
<tr>
<td>Embryo</td>
<td>preimplantation genetic diagnosis</td>
</tr>
<tr>
<td></td>
<td>secretome</td>
</tr>
<tr>
<td></td>
<td>CGH, whole genome sequencing</td>
</tr>
<tr>
<td>Uterine receptivity</td>
<td>genomics/proteomics</td>
</tr>
<tr>
<td>Gonadal aging</td>
<td>genomics/genetics</td>
</tr>
<tr>
<td>Reproductive diseases</td>
<td>genomics/genetics/proteomics</td>
</tr>
<tr>
<td>and syndromes</td>
<td></td>
</tr>
</tbody>
</table>

Precision Medicine – Why Now?

Diagnostic and computational technologies have improved.

In the Past – Macro level testing

- tests differentiated disease from non-disease +/-
- disease defined by location, size, pathology/histology/cytology
- treatment by sensitivity – limited
Precision Medicine – Why Now?

Diagnostic and computational technologies have improved.

Now – Molecular level testing

• disease defined by individual biology and/or DNA of tumor or virus
• tests to subcategorize disease
• pathway analyses
• predict outcomes of specific therapeutics
• screen for adverse events
• monitor disease

An example of disease classification and targeted therapy TODAY

Today, in contrast to 30 years ago, we can classify and treat lung cancers based on mutations in gene genes/pathways and no longer on histology or location.
Disease networks reveal similarities among diseases not heretofore recognized, opening new avenues of targeted therapies.

Figure 2. Disease network.

doi:10.1371/journal.pone.0004346
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0004346

Precision Medicine – Future
Diagnostic and computational technologies have improved.

In the Future – Precision Medicine
• multiple technology platforms needed for higher analytic validity
• multifactorial testing for common, complex diseases
• multi-gene signatures as standard for prognosis
• new sample types – tubal fluid, follicular fluid, peritoneal fluid, urine, saliva, breath, other
• increased use of diagnostic imaging

How Do We Prepare for Precision Medicine?

TOWARD PRECISION MEDICINE
Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease
http://www.nap.edu/catalog.php?record_id=13284
Board on Life Sciences
NATIONAL RESEARCH COUNCIL
NATIONAL ACADEMY OF SCIENCES
National Academy of Sciences Press 2011
Integrate practices and policies across 3 planes

Transdisciplinary science:
Merge physics/chemistry/engineering/computation theory, concepts, methods into biomedical research

Biomedical continuum:
Build seamless links between basic discovery, translation, clinical care, patients, citizens

Stakeholder synergy:
Cooperate across .edu, .com,

Building a Biomedical Continuum
Integrate rapidly expanding range and detail of biological, behavioral and experiential information to:
• Facilitate basic discovery
• Drive development of a more accurate and precise classification of disease
• Build an “information commons” and “knowledge network” that correlate and align disparate information
• Extend acquisition of molecular and phenomic data from lab to point of care settings.
• Reach toward “precision medicine”

Precision Medicine Platform
• basic discovery
• omic medicine
• computational health sciences
• digital health innovation
• clinical discovery
• knowledge networks
Basic Discovery

• Conduct innovative investigations of fundamental biological processes

• Develop instrumentation or software that improve rates or resolution of analysis

• Establish new connections between ideas or observations thought previously to be unrelated

Omic Medicine

• Collect and analyze human subject genomic and other omic data in validated, secure environment

• Coordinate nextgen sequencing and extend to other omics

• Ensure HIPAA-compliant data storage and access

• Develop educational programs: genomic medicine, genomic tumor board, certificate or masters in Precision Medicine, training in bioinformatics and computational sciences

• Ensure genomic literacy among trainees and work force

Computational Health Sciences

• Aggregate and integrate: computer science, bioinformatics, hardware/software engineering, basic/clinical/population science

• Research developing and using computational tools and approaches

• Education: establish learning programs/tools to build computational literacy
## Digital Health Innovation

- Motivate, partner, develop, implement, validate tools that collect, aggregate, analyze and report health information in the course of normal life
- Aggregate expertise of engineers and computer scientists with basic/clinical/population scientists
- Organize clinical trials of dHealth devices, apps and technologies

## Clinical Discovery

- Conduct innovative clinical trials that integrate clinical and molecular information
- Build virtual data warehouse to advance selection of disease targets and patient cohorts
- Identify and address barriers to clinical discovery across clinical operations, data management, bioinformatics, regulatory, patient privacy, clinical care

## Knowledge Networks

- Primary enabling tool for precision medicine, nucleated from neurological disease, cancer, reproduction
- Build integrated, visualizable access to basic, translational, clinical, population data locally, nationally, globally
- Combine expertise and infrastructure resources of computer scientists, bioinformaticians, commercial partners, clinical and basic scientists
Research Education Initiatives

- Workforce and diversity
- Team science; design science
- Rethinking how and what we teach
- Internships for career exploration

Building a Biomedical Knowledge Network for Basic Discovery and Medicine
Global Alliances

• Goals for successful Reproductive Precision medicine:
  – Develop extensive collaborative and cooperative interactions in three broad, overlapping areas of biosciences:
    – High performance computing
    – Omics
    – Imaging
• Enhance research collaborations, resources, funds; research and education outreach
• Developing institutional/organizational data coordination, criteria and standards

• A simple blood test on the way to the doctor’s office that gives a definitive diagnosis.
• An ideal medication identified based on one’s genetic makeup and perfectly formulated to avoid side effects for those who are susceptible.
• Using genetic data from every patient to gain insight into genetic variations in diseases and accelerate drug development to create more precise therapies, faster and less expensively.
• Applying these approaches to improve the lives of 7B people worldwide, at lower cost.

Precision Medicine
Today’s ASRM-ESHRE Pre-congress Course!

• Genetics and phenotypes in male infertility (Lamb)
• Genetics and phenotypes in premature ovarian insufficiency and oocyte aging (Pellicer)
• Genetics and phenotypes in PCOS (Lobo)
• Genetics and phenotypes in endometriosis (Bulun)
• Genetics and phenotypes in hypothalamic amenorrhea (Reindollar)
• Genetics and phenotypes of embryos in culture (Pellicer)
• Resources of patient information (Niederberger)

databases and new diagnostics/therapeutics
We are entering a New Age of Science and Medicine

where one size does not fit all!
Thank You!

References


Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease, Board of Life Sciences, National Academy of Sciences Press, 2011
http://www.nap.edu/catalog.php?record_id=13284

Learning Objectives

- To understand the concept of Personalized Medicine
- To know and understand the use of the Genomic Medicine Toolbox
- To understand the examples of epigenetics and personalized medicine in urology/andrology

“Variability is the law of life, and as no two faces are the same, no two bodies are alike, and no two individuals react alike, and therefore behave alike under the abnormal conditions we know as disease.”

Sir William Osler, 1892
What is Personalized Medicine?

- Tailoring of medical treatment to meet the individual characteristics of each patient.
  - It does not literally mean the creation of drugs or medical devices that are unique to a patient.
- The ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease or their response to a specific treatment.
  - Preventive or therapeutic interventions can then be concentrated on those who will benefit, sparing expense and side effects for those who will not.

Assumption:

The more specifically we define diseases and the patients that are affected by them, the more able we will be to treat them effectively.

Personalized Medicine

- Natural progression of good clinical practice that is the foundation of good healthcare provision reflects a continuous process of refinement through stratified medicine.
Fundamentals: The Cell is an Information Machine

- Human cells have 23 pairs of chromosomes
- Genetic code: 3.1 billion base pairs
- 35,000 genes identified so far

Personalized Medicine Strategies Are Based Upon The Central Dogma of Molecular Biology

How Do the “Omics” Relate To The Central Dogma?

- Genomics/DNA
- Transcriptome /RNA
- Proteomics /Protein
- Metabolomics /Metabolome
Genetic Aberrations in Infertile Men

- Abnormal karyotype accounts for ~19% of Non-obstructive Azoospermia (NOA)
- Y-chromosome microdeletions (AZF regions) are found in ~8% of men with NOA
- More than 200 gene defects identified in human male infertility but are not routinely tested
- With the exception of the CFTR gene in men with congenital bilateral absence of the vas deferens

The Personalized Medicine Toolbox

<table>
<thead>
<tr>
<th>Data Set ('omic Approach)</th>
<th>Technology Platform or Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human genome sequence</td>
<td>SNPs, CNVs (10–15 million)</td>
</tr>
<tr>
<td>(genomics)</td>
<td></td>
</tr>
<tr>
<td>Gene Expression Profiles</td>
<td>Microarrays of 25,000 gene transcripts</td>
</tr>
<tr>
<td>(transcriptome)</td>
<td></td>
</tr>
<tr>
<td>Proteome (proteomics)</td>
<td>Protein profiles of specific protein products (100,000)</td>
</tr>
<tr>
<td>Metabolome (Metabolomics)</td>
<td>Metabolic profiles (1000 to 10,000 metabolites)</td>
</tr>
</tbody>
</table>

What is a Genomic Disorder?

- Genomic Disorders Result from a Gene Dosage Abnormality That is a Consequence of a Rearranged Segment (a gain or deletion) of the Genome
- Example: Y chromosome microdeletion
Y Chromosome Microdeletions are a Copy Number Variation

- AZF Region encompasses more regions than originally thought
- Deleted in Azoospermia/DAZ is located in AZFc region
- Deletion of AZFa or AZFb predicts a poor likelihood for sperm retrieval

DNA Microarray
- A multiplex technology used in molecular biology and in medicine, consisting of an arrayed series of thousands of microspots of DNA oligonucleotides
- Each spot (feature) contains picomoles of a specific DNA or oligonucleotide sequence
  - Short section of gene or other DNA segment
  - Hybridizes with genetic material to be investigated (target) which is differentially colored
    - Infertile patient vs. normal control
  - Probe-target hybridization is detected and quantified
Concept of aCGH: Gender Mismatch In Analysis

All probes annealing to both samples
Normalized for over-lapping probes
Final result

aCGH of a Man with Y Microdeletion

Y Chromosome Microdeletion Assay

A PCR-based assay that amplifies small regions of only the Y chromosome
Array Comparative Genomic Hybridization (aCGH) Confirmed Y Chromosome Microdeletions

- Confirmed a loss of AZFa in this NOA man
- Confirmed a loss of AZFbc in this NOA man

But.... Exact Patient Diagnosis Can Be Analogous to the Elephant and the Blind Men

The Blind Men and The Elephant

Jain, Buddhist, Sufi Muslim, Hindu Versions

It was six men of Indostan To learning much inclined, Who went to see the Elephant (Though all of them were blind).

Diagnosis Can Depend Upon Your Clinical Perspective and “Which Part of the Elephant You See”

aCGH Identified Other CNVs in Men with Y Chromosome Microdeletions

Gain in PAR1
Loss in PAR2
Gain in PAR1

aCGH 385K Custom X-Y aCGH 135K
Men with Y-Microdeletions Display Previously Unrecognized CNVs in PAR1 and PAR2 Genes That Are Absent in Men Without Y-Microdeletions

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No PAR1</th>
<th>PAR1</th>
<th>PAR2</th>
<th>PAR1/PAR2</th>
<th>PLCXD1</th>
<th>GTPBP6</th>
<th>PPP2R3B</th>
<th>SHOX</th>
<th>CD99</th>
<th>SPRY3</th>
<th>VAMP7</th>
<th>IL9R</th>
</tr>
</thead>
<tbody>
<tr>
<td>without AZF deletions</td>
<td>Run in array 35 0 0 0 0 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with AZF deletions</td>
<td>Run in array 22 1 2 2 3 Gains 3 Gains 3 Gains 2 Gains 4 Loss 4 Loss 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASSayed by qPCR</td>
<td>Gain 65 4 3 8 7 Gain 5 Gain 1 Gain 7 Gain 5 Gain 3 Gain 6 Gain 6 Gain 6 Gain 6 Gain 6 Gain 11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAR1</td>
<td>23%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAR2</td>
<td>23%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The microduplication in PAR1 includes the SHOX gene

Co-Existing Genomic Syndromes: SHOX and Y Chromosome Microdeletions

- Haploinsufficiency of the SHOX gene is associated with shortness
  - 3.2% of patients with isolated short stature
  - 89% of patients with Leri-Weill-dyschondrosteosis
  - Langer-mesomelic-dysplasia
- SHOX duplications are associated with variable height
  - Proportional to the number of transcriptional enhancers within the duplicated region; patients with larger duplications and an increased number of enhancers are taller

What is SHOX syndrome?

- Haploinsufficiency/Mutations
  - Leri-Weill
  - Mesomelic short stature
  - Madelung deformity of the wrists
  - Bowed radius
- Homozygous Mutations
  - Langer mesomelic dwarfism
- Duplication
  - Tall Stature/ Short Stature

[Source:
http://emedicine.medscape.com/article/943343-overview
Azoospermic Men with Duplication in SHOX Have Abnormal Stature

<table>
<thead>
<tr>
<th>Patient</th>
<th>3658</th>
<th>65937</th>
<th>61783</th>
<th>74532</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>43</td>
<td>35</td>
<td>32</td>
<td>47</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>African White White White</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (inches)</td>
<td>60</td>
<td>66</td>
<td>63</td>
<td>76</td>
</tr>
<tr>
<td>Height (percentile)</td>
<td>&lt;3</td>
<td>10</td>
<td>&lt;3</td>
<td>&gt;95</td>
</tr>
<tr>
<td>Y microdeletion</td>
<td>AZF b,c AZF b,c AZF b,c AZF b,c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testis Histopathology</td>
<td>SCO/MA SCO/MA SCO/MA N/A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Medical Problems</td>
<td>None Diabetes Hypertension Cataracts</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Importance for Men With Y Chromosome Microdeletions

- The molecular karyotype confirmed Y chromosomal microdeletions in subjects
- The gene dosages changes were found in 25% of men with Y microdeletions
- SHOX Syndrome was an unrecognized co-existing genomic syndrome in some men with Y chromosome microdeletion
- Stature abnormalities

But…. Exact Patient Diagnosis Can Be Analogous to the Elephant and the Blind Men

The Blind Men and The Elephant

- The UROLOGIST sees the patient as an infertile male with Y chromosome microdeletions who may or may not have sperm on testis biopsy
- The MEDICAL GENETICIST sees a patient with SHOX syndrome
Male Infertility Gene Discovery

The Common Reverse Genetics Approach Used Today

- Genetic Manipulation Of Gene of Interest
- Male Infertility
- Transgenesis
- Targeted Deletion
- ENU Mutagenesis
- Conditional KO
- Phenotyping
- Mutation Analysis Of Patients and Controls

Functional Genomics/Forward Genetics Approach

- Infertile Male
- Genetic Manipulation Of Gene of Interest
- Transgenesis
- Targeted Deletion
- ENU Mutagenesis
- Conditional KO
- Phenotyping
- Genetic or Genomic Analysis Of Patients and Controls
- Infertile Mouse?
Hypothesis

- Subtle chromosome aberrations (<5-10 Mb) are associated with male infertility, including those caused by genitourinary birth defects
- The availability of a high density, genome-wide CGH microarray will reveal unrecognized genetic abnormalities

Copy Number Variation (CNV)

- A different form of genetic variation
- Gains and losses of relatively large fragments of DNA
- A major source of genetic diversity
- Homologous recombination errors during meiosis in the parent
  - Not a mutation

Copy Number Variant (CNV): Gene Dosage Changes

1 copy = Normal
2 copies = Normal
0 copy = Unbalanced = Abnormal
1 copy = Unbalanced = Abnormal
2 copies = Unbalanced = Abnormal
Can We Use This Approach To Identify Previously Unrecognized Genomic Defects in Men With NOA?

Chromosome Microarray at Baylor College of Medicine

Genomic Syndromes Are Far More Common Than Initially Recognized and Affect All Parts of the Body and Untold Diseases/Syndromes

Experimental Approach

- Infertile NOA men without Y microdeletions n=82
- Fertile men n=70

DNA Extraction
- aCGH Nimblegen
- 135K, 385K, 720K

Analysis of CNV using Nexus and SignalMap

Secondary confirmation of copy number: qPCR or FISH

Recapitulate phenotype in mouse model
Microdeletion and Microduplication aCGH Analysis Reveals Genes of Interest Identified in Infertile Men

<table>
<thead>
<tr>
<th>Patient</th>
<th>gDNA Cells</th>
<th>Chromosome Region</th>
<th>Gene</th>
<th>Band</th>
<th>Size</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1516</td>
<td>Y</td>
<td>chr6:124,474,59</td>
<td>E2F1</td>
<td>q23.3</td>
<td>2520</td>
<td>Required for spermatogenesis in fox</td>
</tr>
<tr>
<td>1519</td>
<td>Y</td>
<td>chr20:31,627,04</td>
<td>E2F1</td>
<td>q23.3</td>
<td>2062</td>
<td>20F1 overexpression and deletion – testicular toxicity in mice</td>
</tr>
<tr>
<td>1230</td>
<td>Y</td>
<td>chr19:47,656,87</td>
<td>E2F1</td>
<td>q23.3</td>
<td>46120</td>
<td>Highly expressed in testis, interacts with STAT3</td>
</tr>
<tr>
<td>5792</td>
<td>Y</td>
<td>chr9:115,179,74</td>
<td>E2F1</td>
<td>q23.3</td>
<td>17923</td>
<td>Involved in spermatogenesis</td>
</tr>
<tr>
<td>5802</td>
<td>Y</td>
<td>chr7:35,100,14</td>
<td>E2F1</td>
<td>q23.3</td>
<td>23131</td>
<td>Highly expressed in testis interstitial cells</td>
</tr>
<tr>
<td>1516</td>
<td>Y</td>
<td>chr16:55,189,32</td>
<td>E2F1</td>
<td>q23.3</td>
<td>2062</td>
<td>20F1 overexpression and deletion – testicular toxicity in mice</td>
</tr>
</tbody>
</table>

Duplication of 20q11.22 that Includes E2F1 Identified in NOA Male

Confirmation of E2F1 Microduplication by qPCR and Detection of Additional Infertile Men with Microdeletions or Microduplications of E2F1

Mouse models of e2f1 over-expression and targeted deletion and the human male infertility patients display a similar testicular pathology
Testicular Histopathologies in Mice

Overexpressing or Lacking E2F1

Testicular Atrophy in Mice

Lacking E2F1

Testicular Atrophy in Mice

Overexpressing E2F1

Yamasaki, et al., Cell 85:537-548, 1996

Holmberg, et al., Oncogene 17:143-155, 1998

CNVs in E2F1 Rarely Occur in the General Population

• Signature Genomic Laboratories’ data from 26,706 tested individuals indicate CNVs ranging from 1.8-9.5Mb that include E2F1 in only 3 individuals (0.011%).

• DECIPHER, indicates that from three individuals have a CNV in E2F1 (ranging from 2.2-9.8Mb) our of 5404 subjects deposited on this database because they have CNVs

• Gains in 20q11 leading to increased E2F1 expression reported in colon, esophagus, melanoma and prostate tumors

Other Male Infertility CNVs

• Deletion of DPY19L2 causes abnormal sperm head elongation and acrosome formation

• X Chromosome Gains and Losses: DMD, SLC25A3, SAGE1, AFF2, VAMP7, PLCXD1, GTBP6, PPP2R3B, ASMT, DHRSX, HDHD1, ST5, VCX, PNPLA, MIR651, KAL1, GLRA, FANC8, MOSPD2, ASB9, ASB11, PIGA, SSK3, 2C4H2, ZC3H12BB, UPRT, ZDHHC15
Other Male Infertility CNVs

- Many other genes with unknown mechanism in fertility: THRAP3, SLC25A4, NBF4, SYT6, TSSC1, FAM82A1, PLSCR2, SERF1A, SERF1B, SMN1, SMN2, NAIP, GTF2H12, SIRT4, CMK1B, STRC/CATSPER, CLEC18B
- Genes in Oligospermia and SCO Men: EPHA3, SH3TC1, ANKS1A, NRG3, CYP219, LRPI, MIR1228, PRMT7, SMPD3, SLFN11, SLFN12, SLFN13, KCNG2, PQLC1, PLEC, MIR661, DDX11, OVOS2, ANKS1B

Single Nucleotide polymorphisms or SNPs

- Minor variations in the genetic sequence that differ between members of a species or even between paired chromosomes in an individual.
  - Common SNPs: occur in at least 1% of a population.
  - May be specific to an ethnic group or between individuals of a geographic region.
  - Rare SNPs with minor allele frequencies of less than 1%.

SNP Categories And Their Action on mRNA Structure, Stability or Processing Ultimately Affecting Protein Expression, Amount, Structure and/or Function

<table>
<thead>
<tr>
<th>GENERAL POPULATION FREQUENCY (i.e., 90% of the population)</th>
<th>SINGLE NUCLEOTIDE POLYMORPHISM (SNP) (i.e., 10% of the population)</th>
</tr>
</thead>
</table>
How Are SNPs Measured?

- Direct sequencing
  - Known SNPs such as CFTR in CBAVD
- SNP Arrays
  - Similar technology again spanning the genome
  - Discovery

Types of SNPs

- Not Synonymous SNP
  - Amino Acid Sequence Change
  - mRNA Structure Or Stability
- Synonymous SNP
  - No Amino Acid Sequence Change
  - Translation Kinetics
- Regulatory SNP
  - Protein Expression Or Function Change
  - Alternative mRNA Or Protein Splicing

Congenital Bilateral Absence of the Vas Deferens
A Genital Form of Cystic Fibrosis
Congenital Bilateral Absence of the Vas Deferens (CBAVD)

Cystic Fibrosis Transmembrane Conductance Regulator Gene (CFTR)

Over 1300 different mutations identified in CFTR to date

Detection is a major concern
- If just 30 of the common CFTR mutations are analyzed a negative result does not ensure that mutations are not present
- The 5T allele is common in CBAVD
  - Alters mRNA stability and splicing efficiency

Assume all men with CBAVD have mutation in CFTR
Test the female partner for CFTR mutations
### Genes

#### Nature of the variants

**Genetic Variants linked to male infertility**

- **CFTR Deletion of 3 nucleotides**
  \[ \Delta F508 \]

- **Repeat Length variant of a polypirimidine tract (allele 5T)**
  \[ \text{Repetition of 9-13 TG nucleotides} \]

- **Androgen Receptor Polymorphic sequence of CAG triplets coding for polyglutamines in the first exon**
  \[ \text{Length of a GGN segment} \]

- **Insl3-Lgr8**
  \[ \text{2 not synonymous SNP T222P and R223K in Lgr8} \]

**Genetic Variants with a potential link to male infertility**

- **Folate-Related Enzyme Polymorphisms**
  - MTFHR (C677T)
  - Methionine synthase (A2756G)
  - Methionine synthase reductase (A666G)

- **DAZL SNP in exon 3 (A386G)**

- **FSHR Estrogen Receptor**
  - Xba I and Pvu II intron 1 variants

- **Variable repeated elements (TA)n within the promoter region**

- **SNP C325G in exon 4**

- **Region 11p15 SNPs in ZnF214, ZnF215, HNRNP GT, NALP14 c-kit and KIT ligand SPO11 Mei1 Hrb, TC10, GOBC**

**Genetic variants with no association to male infertility**

- **TNP1 Protamines (PRM1 and PRM2)**

- **Harp1 gene**

### Whole Exome/Whole Genome Sequence Analysis

- **Recessive HYDIN mutations cause primary ciliary dyskinesia**

### Potential Biomarkers In Infertile Men

<table>
<thead>
<tr>
<th>Gene</th>
<th>Name</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>XNP</td>
<td><strong>–</strong></td>
<td>Xq11.23</td>
</tr>
<tr>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
</tr>
<tr>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
</tr>
<tr>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
</tr>
<tr>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
</tr>
<tr>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
</tr>
<tr>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
</tr>
<tr>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
</tr>
<tr>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
</tr>
<tr>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
</tr>
<tr>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
<td><strong>–</strong></td>
</tr>
</tbody>
</table>
Conclusions

- Advances in genomics will improve the diagnosis of the infertile male
  - Y Chromosome microdeletions
  - SHOX
  - E2F1
  - Other dosage sensitive genes

- Next generation sequencing provides in depth information about mutations and SNPs spanning the genome

- Patients will benefit from additional insights into the genetic defects causing their infertility
OBJECTIVES

After this presentation, the participants should:

- Be familiar with the concept and characteristics of POI
- Know the most relevant causes of POI
- Understand the genetics mechanisms leading to POI
- Be able to perform an adequate work-up
- Be familiar with current fertility preservation techniques for patients at risk of POI
- Manage women with POI

POI: DEFINITION

CLINICAL FINDINGS

- Primary amenorrhea in a 15-yr old girl; or secondary amenorrhea > 4 months
- Women <40 yrs of age
- Serum FSH >40 mIU/ml (2 recordings separated 1 month)
- Serum E2 <50 pg/ml
Primary ovarian insufficiency
Premature menopause
Premature ovarian failure
Hypergonadotropic amenorrhea
Hypergonadotropic hypogonadism

NOT A PERMANENT CONDITION
• 50% will have intermittent and unpredictable ovarian function
• Women have recovered ovarian function even 8 yrs after amenorrhea
• 5–10% have spontaneous pregnancies

TV ultrasound in idiopathic POI

Follicles in 40% of cases

Mehta et al Fertil Steril 1992; 57: 56
Biopsies in idiopathic POI

NOT A PERMANENT CONDITION

- 60% NO follicles
- 9% numerous follicles
- 30% few follicles
- 1% oophoritis

Van Kesteren and Schoemaker, Hum Reprod Update 1999; 5:483

Mechanisms of POI

1. Ovarian follicle dysfunction
   - Signaling defect
   - Enzyme deficiency
   - Autoimmunity
   - Associated with insufficient follicle number

1. Ovarian follicle depletion
   - Insufficient initial follicle number
   - Spontaneous accelerated follicle loss

Mechanisms of POI

1. Ovarian follicle dysfunction
   - Signaling defect:
     - FSHr mutation
     - LHr mutation
     - Pseudohypoparathyroidism type 1a (GNAS)
   - Enzyme deficiency:
     - CYP17A1
     - CYP19
   - Autoimmunity
     - Lymphocytic oophoritis
     - Polyglandular autoimmune syndrome
     - Polyendocrinopathy-candidiasis-ectodermal dysplasia
     - Associated with insufficient follicle number
     - Luteinized graafian follicles
Mechanisms of POI

2. Ovarian follicle depletion
- Insufficient initial follicle number
- Blepharophimosis, ptosis, epicanthus inversus synd.
- 46, XY gonadal dysgenesis
- Spontaneous accelerated follicle loss
- Turner syndrome
- Trisomy or polysomy X
- Macrodeletions Xp or Xq
- Autosomal or X translocations

ETIOLOGY OF POI: Genetic mutations

<table>
<thead>
<tr>
<th>X CHROMOSOME GENES</th>
<th>AUTOSOMAL CHROMOSOMES GENES</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHLHB9</td>
<td>FSHB</td>
</tr>
<tr>
<td>BMP15</td>
<td>MSH5</td>
</tr>
<tr>
<td>DACH2</td>
<td>BRCA1</td>
</tr>
<tr>
<td>DIAPH2</td>
<td>MRE11</td>
</tr>
<tr>
<td>FMR1</td>
<td>ATX1</td>
</tr>
<tr>
<td>POF1B</td>
<td>NOS1</td>
</tr>
<tr>
<td>XIST</td>
<td>ATM</td>
</tr>
<tr>
<td>XPNPEP2</td>
<td>Rad51</td>
</tr>
</tbody>
</table>

De Vos et al. Lancet 2010; 376:911-21

AGE

Doble-strand Break (DSB)

DSB repair genes
BRAC1
MRE11
Rad51
ATM

Genetic mechanisms of POI
Effect of cancer on ovarian function

Table 1

<table>
<thead>
<tr>
<th>Case w/o Tumors (n = 141)</th>
<th>Case w/ Tumors (n = 144)</th>
<th>Control (n = 150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2 levels (pg/mL)</td>
<td>E2 levels (pg/mL)</td>
<td>E2 levels (pg/mL)</td>
</tr>
<tr>
<td>Oocytes</td>
<td>Oocytes</td>
<td>Oocytes</td>
</tr>
<tr>
<td>MII oocytes</td>
<td>MII oocytes</td>
<td>MII oocytes</td>
</tr>
<tr>
<td>13.7 (10.7 - 15.8)</td>
<td>13.7 (10.7 - 15.8)</td>
<td>13.7 (10.7 - 15.8)</td>
</tr>
<tr>
<td>1.6** (0.5 – 3.7)</td>
<td>1.6** (0.5 – 3.7)</td>
<td>1.6** (0.5 – 3.7)</td>
</tr>
<tr>
<td>1.6** (0.5 – 3.7)</td>
<td>1.6** (0.5 – 3.7)</td>
<td>1.6** (0.5 – 3.7)</td>
</tr>
</tbody>
</table>

*P<0.005  **P<0.05


Etiology of POI

- Unknown mechanism (63%)
- Structural and numerical abnormalities of the X chromosome (13%)
- Iatrogenic (12%)
- Gen mutations (6%)
- Autoimmunity (4%)
- Systemic or syndromic disorders (2%)

Etiology of POI: Turner Syndrome

- Largest subgroup with POI
- Oocyte depletion within 10 yrs of life
- Fertility Preservation measures may be taken
- 45,X/46,XX menstruation can continue for several yrs
ETIOLOGY OF POI: Genetic mutations

- FMR1 premutation is the most common known genetic cause of POI
- Expansion to >200 CGG repeats leads to Fragile X syndrome
- Alleles with repeat length 59-199 inestable premutation state
- Risk of POI inversely correlated with number of repeats

ETIOLOGY OF POI: Systemic/syndromic diseases

- Ataxia telangiectasia (ATM)
- Type 1 blepharophimosis ptosis-epicanthus inversus syndrome (FOXL2)
- Galactosaemia

ETIOLOGY OF POI: Autoimmunity

- Polyglandular syndrome
- Dry-eye syndrome
- Myasthenia gravis
- Rheumatoid arthritis
- Diabetes mellitus
- Systemic lupus erythematosus
ETIOLOGY OF POI: Iatrogenia

- Ovarian surgery
- Exposure to viral agents
- Exposure to environmental toxic agents: smoking
- Chemotherapy (alkylating agents)
- Radiotherapy

DIAGNOSIS OF POI

- Exclude pregnancy
- Iatrogenia: surgery, Ca treatment
- Exclude:
  - Autoimmune diseases
  - Systemic/syndromic diseases
  - Other conditions: malnutrition, varicella, malaria, TBC

- Serum FSH, E2, and AMH
- Transvaginal ultrasound
- Karyotyping
- FMR1 premutation testing
- Antiperoxidase and anti-21 hidroxylase antibodies
PREVENTION OF POI

Serum AMH levels in 926 healthy infants, girls, adolescents and adult women

Hagen et al. J Clin Endocrino Metab 2010; 95:5003-10

PREVENTION OF POI

172 Turner syndrome

Hagen et al. J Clin Endocrino Metab 2010; 95:5003-10

PREVENTION OF POI

Loh and Maheshwari. Hum Reprod 2011; 26:2925-32
Fertility preservation in women with cancer

- Medical protection of the gonads
- Ovarian cortex freezing/transplantation
- Oocyte/embryo vitrification

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Nonneological</th>
<th>Oncological</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>36.7 ± 4.2</td>
<td>31.9 ± 5.1*</td>
</tr>
<tr>
<td>Previous children (%)</td>
<td>11.3%</td>
<td></td>
</tr>
<tr>
<td>Days from hCG to oocytes retrieved (%)</td>
<td>14.6 ± 1.6</td>
<td>11.4 ± 2.5*</td>
</tr>
<tr>
<td>Total no. of oocytes</td>
<td>7.225</td>
<td>4.154</td>
</tr>
<tr>
<td>Total no. of metaphase II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total FSH/MG with metaphase (%)</td>
<td>1.459 ± 0.40</td>
<td></td>
</tr>
<tr>
<td>Total FSH/MG (%)</td>
<td>3.038 ± 3.37*</td>
<td>1.841 ± 1.42*</td>
</tr>
<tr>
<td>Breakdown (%)</td>
<td>18.7 ± 5.29*</td>
<td></td>
</tr>
<tr>
<td>Breakdown at triggering (%)</td>
<td>22.14 ± 5.08</td>
<td>1.369 ± 1.37*</td>
</tr>
</tbody>
</table>

* PCO:ol

**Note:** SP = sterility preservation, MG = metaphase II.
### Ovarian Cortex Transplantation

**Ovarian cortex Orthotrasplantation program (Established 2005)**

- **N**: 663 (Dec 1st, 2012)
- Breast Ca 55%  
  - Hodgkin 21%  
  - Others 24%
- MEAN AGE 29 yrs (range 11-39 yrs)
- Previous CHT: YES 15%; NO 85%

### Management of POI

- **Ovarian follicle depletion**
- **Iatrogenia**
- **Ovarian follicle dysfunction**

**Fertility Preservation actions**
- **ART**
- **OOCYTE DONATION**

**Expected management**
- **Ovulation Induction**
- **ART**

<table>
<thead>
<tr>
<th>No</th>
<th>Age</th>
<th>Chem</th>
<th>AMH</th>
<th>Pat Age</th>
<th>Prev</th>
<th>Previous CHT</th>
<th>AMH</th>
<th>Age</th>
<th>Pat Age</th>
<th>Previous CHT</th>
<th>AMH</th>
<th>Age</th>
<th>Pat Age</th>
<th>Previous CHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>SÍ</td>
<td>0.8</td>
<td>39</td>
<td>SÍ</td>
<td>YES</td>
<td>36</td>
<td>SÍ</td>
<td>0.8</td>
<td>YES</td>
<td>36</td>
<td>SÍ</td>
<td>0.8</td>
<td>YES</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>SÍ</td>
<td>0.3</td>
<td>36</td>
<td>SÍ</td>
<td>YES</td>
<td>34</td>
<td>SÍ</td>
<td>0.3</td>
<td>YES</td>
<td>34</td>
<td>SÍ</td>
<td>0.3</td>
<td>YES</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>SÍ</td>
<td>1.0</td>
<td>34</td>
<td>SÍ</td>
<td>YES</td>
<td>32</td>
<td>SÍ</td>
<td>1.0</td>
<td>YES</td>
<td>32</td>
<td>SÍ</td>
<td>1.0</td>
<td>YES</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>SÍ</td>
<td>0.3</td>
<td>39</td>
<td>SÍ</td>
<td>YES</td>
<td>36</td>
<td>SÍ</td>
<td>0.3</td>
<td>YES</td>
<td>36</td>
<td>SÍ</td>
<td>0.3</td>
<td>YES</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>SÍ</td>
<td>1.3</td>
<td>30</td>
<td>SÍ</td>
<td>YES</td>
<td>28</td>
<td>SÍ</td>
<td>1.3</td>
<td>YES</td>
<td>28</td>
<td>SÍ</td>
<td>1.3</td>
<td>YES</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>SÍ</td>
<td>0.3</td>
<td>44</td>
<td>SÍ</td>
<td>YES</td>
<td>40</td>
<td>SÍ</td>
<td>0.3</td>
<td>YES</td>
<td>40</td>
<td>SÍ</td>
<td>0.3</td>
<td>YES</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>SÍ</td>
<td>0.3</td>
<td>36</td>
<td>SÍ</td>
<td>YES</td>
<td>33</td>
<td>SÍ</td>
<td>0.3</td>
<td>YES</td>
<td>33</td>
<td>SÍ</td>
<td>0.3</td>
<td>YES</td>
</tr>
</tbody>
</table>
Weekly control: US, FSH, Inh A&B, E2

2 mg/día E2

6 weeks

Follow-up to pregnancy

38 ovulatory cycles
18 with estradiol
20 without estradiol

Welt et al J Clin Endocrinol Metab 2005; 90: 826

Expectant management of POI

24 women ovulating (49%)
18 anovulation (37%)
7 inactive ovaries (14%)

6 weeks

2 mg/día E2

Welt et al J Clin Endocrinol Metab 2005; 90: 826

All ovulatory women FSH<40 UI/l (26.4 ± 7.7)

Welt et al, 2005

Follow-up to pregnancy

Welt et al J Clin Endocrinol Metab 2005; 90: 826

38 ovulatory cycles
18 with estradiol
20 without estradiol

Expectant management of POI

All ovulatory women

FSH<40 UI/l (26.4 ± 7.7)

Welt et al J Clin Endocrinol Metab 2005; 90: 826

Author Regimen Patients Ovarian function Ovulation Pregnancy

Nelson et al (1994) aGnRH-hMG / aGnRH-hMG 45 50% 10%

Surrey-Cedars (1990) eGnRH-hMG / EP-hMG 20 87%


Van Kasteren et al (1995) aGnRH+ hMG / aGnRH+ hMG 30 33% / 25% 20% / 0%

Van Kasteren et al (1999) DXM-hMG/hMG 36 0 / 0%

Anasti et al (1994) Danazol/EP 46 48% / 46% 17% / 9%

Ovulation Induction in POI

Page 55 of 139
CONCLUSIONS

- POI is NOT a permanent condition and patients should be advised accordingly.
- Whole-genome sequencing and genome-wide association studies have identified, and will continue to identify, genes involved in POI.
- Some gene-related mechanisms have been elucidated providing the basis for POI prevention.
- Turner syndrome, FMR1 premutation and autoimmunity are frequent causes of POI.
- Iatrogenia needs Fertility Preservation actions.
CONCLUSIONS

- Serum AMH is only partially useful
- GnRHa during chemotherapy, oocyte vitrification and ovarian cortex transplantation are the current Fertility Preservation methods with proven success
- Reducing serum FSH with estrogens and/or inducing ovulation may lead to some ovulatory cycles, but rarely to pregnancies
- Oocyte donation is the treatment of choice in the worst Case scenario

ACKNOWLEDGMENTS

Daniela Galliano
Juan Garcia-Velasco
Javier Domingo
Ana C. Cobo
Carlos Simón

Suggested readings

- De Vos et al. Lancet 2010; 376:911-21
- Del Mastro et al. JAMA 2011; 306: 269-276
- Hagen et al. J Clin Endocrinol Metab 2010; 95:5003-10
- Welt et al J Clin Endocrinol Metab 2005; 90: 826-30
Genetics and phenotypes in PCOS: databases and new diagnostics/therapeutics

Roger A. Lobo M.D
Columbia University
New York, USA
Past President ASRM

No conflicts of interest to disclose

Precision Medicine in PCOS

• The hope: define disorder by underlying molecular and other factors in addition to clinical signs and symptoms – to affect treatment and outcomes
  Genetics
  Endocrine features (AMH, LH?)
  Ovarian morphology
How do you begin to address this?

Challenges

• Can we assume PCOS is one disorder
• PCOS is characterized by its heterogeneity – is this varied expression or is it really several overlapping disorders
• If it is the latter, will genetics help us?

NIH Office of Disease Prevention Evidence-based Methodology Workshop on
POLYCYSTIC OVARY SYNDROME (PCOS)
DECEMBER 3-5, 2012
Natcher Conference Center

The 2012 NIH Evidence-based Methodology Workshop on Polycystic Ovary Syndrome (PCOS) will seek to clarify:
• Benefits and drawbacks of using the Rotterdam Criteria for diagnosis
• PCOS causes, predictors, and long-term consequences
• Optimal prevention and treatment strategies.
The so called “NIH” definition

NIH 1990

- Chronic anovulation
- Clinical and/or biochemical signs of hyperandrogenism (with exclusion of other etiologies, e.g., congenital adrenal hyperplasia)

(Both criteria needed)

“New” definition of PCOS – the Rotterdam consensus conference

- European focus on ovarian morphology accepted
- PCOS diagnosed by Polycystic Ovaries on US; MENSTRUAL IRREGULARITY; and/or HYPERANDROGENISM - 2/3 sufficient for diagnosis
- Human Reproduction 19:41, 2004
- Fertil Steril 81:19-25, 2004

Androgen Excess Society guidelines: endorsing the importance of androgen excess

- Hyperandrogenism: hirsutism and/or hyperandrogenemia
- Ovarian Dysfunction: oligo-anovulation and/or polycystic ovaries
- Exclusion of other disorders

The non hyperandrogenic phenotype possible in Rotterdam

- How often does it occur? – minority (16% in Dewaily study – JCEM 2006; 91:3922-27)
- Do these women resemble classic PCOS at all?

Box-and-whisker plots showing the distribution of individual values for BMI (A), waist circumference (B), and SHBG (C) in controls and in patients with phenotypes A-D


Official definition of the Polycystic Ovary

- Either 12 or more follicles, 2-9 mm diameter, or increased ovarian volume >10 c.c.
- Only one ovary necessary
- Increased stroma and follicle orientation not required

Balen AH. Human Reproduction Update 2003; 9: 505-14
The diagnosis of PCOS (Rotterdam) is dependent on assessment of ovarian morphology

- Subjective – operator dependent
- Age (teens to older women)
- Ethnicity
- Rotterdam criteria: follicular numbers (≥12) ovarian volume (≥10 c.c.), one ovary (≥7 c.c.) Ovarian stroma deemed unimportant

Example of median ovarian section with outlined ovarian and stroma areas A1 is the total area and A2 is the stroma area

ROC for A and T: S/A AUC – 0.73, p<0.01
The influence of menstrual irregularity on the prevalence of IR

- Increased insulin response to oral glucose and decline in glucose with insulin (Kitt)
  abnormal only in PCOS with menstrual irregularity; not in hyperandrogenic women with regular cycles


### Hyperandrogenic PCOS – Anovulatory versus Ovulatory phenotype

<table>
<thead>
<tr>
<th></th>
<th>Olig</th>
<th>Reg cycle</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>11</td>
<td>19</td>
<td>31</td>
</tr>
<tr>
<td>Age (years)</td>
<td>27±1.0^ 7</td>
<td>20±1.1</td>
<td>21±1.2^ 2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76±2.6</td>
<td>75±2.6</td>
<td>72±2.6</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>24.6±0.4</td>
<td>26±0.6</td>
<td>24.3±0.7</td>
</tr>
<tr>
<td>Waist-Hip ratio</td>
<td>0.82±0.01</td>
<td>0.79±0.03</td>
<td>0.79±0.03</td>
</tr>
<tr>
<td>Fasting insulin (mU/L)</td>
<td>5.4±1.0</td>
<td>5.5±0.5</td>
<td>5.5±0.1</td>
</tr>
<tr>
<td>Fasting glucose (mM)</td>
<td>4.4±0.1</td>
<td>4.8±0.01</td>
<td>4.8±0.01</td>
</tr>
<tr>
<td>Insulin sensitivity (mU/mM)</td>
<td>107±2</td>
<td>182(12.0)</td>
<td>105±2</td>
</tr>
</tbody>
</table>

Robinson S. Clin Endocrinology 1993; 39: 351-55

### Similar insulin responses to glucose challenge in controls and Ovulatory PCOS

Robinson S. Clin Endocrinology 1993; 39: 351-55
Normal insulin sensitivity and glucose tolerance in Ovulatory PCOS

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>PCOS (n=10)</th>
<th>Controls (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>87.2 ± 5.9</td>
<td>82.8 ± 5.4</td>
</tr>
<tr>
<td>Insulin</td>
<td>58.1 ± 4.8</td>
<td>51.3 ± 4.1</td>
</tr>
<tr>
<td>Fat</td>
<td>1.7 ± 0.5</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>Urine</td>
<td>2.7 ± 0.2</td>
<td>2.5 ± 0.2</td>
</tr>
</tbody>
</table>

Evolving Cardio-Metabolic risks with various phenotypes relating to PCOS

<table>
<thead>
<tr>
<th>Androgens</th>
<th>Normal</th>
<th>Elevated</th>
<th>Normal</th>
<th>Elevated</th>
<th>Normal</th>
<th>Elevated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles</td>
<td>Normal</td>
<td>Normal</td>
<td>Irreg (ANOV)</td>
<td>Normal (OVULATE)</td>
<td>Irreg (ANOV)</td>
<td>Irreg (ANOV)</td>
</tr>
<tr>
<td>Ovaries</td>
<td>Normal</td>
<td>Normal</td>
<td>PAO/PCO</td>
<td>Normal (OVULAT)</td>
<td>PAO/PCO</td>
<td>Normal (OVULAT)</td>
</tr>
</tbody>
</table>

NIH Workshop Panel Recommendations

- "The name "PCOS" is a distraction and an impediment to progress"
- "The right name will enhance recognition of this major public health issue for women..."
- No new name suggested
- Recommend "maintaining the broad inclusionary diagnostic criteria of Rotterdam"
- "Specific phenotypes should be reported explicitly in all research studies and clinical care"
**Best potential targets for diagnostics/therapeutics**

- Genetic susceptibility
- AMH
- LH (not circulating levels)
- Ovarian morphology

**Genetics and PCOS: the beat goes on**

- Familial associated well known – thought to be autosomal dominant (Cooper HE 1968; Givens JR 1988; Legro RS 1988)
- Realized to be not monogenic
- 50 or more candidate genes studied (Urbanek M 2002)
- Multiple polymorphisms studied in different populations
- PCOS susceptibility locus – Fibrillin -3 mapped to 19p13.2 close to D19S884
  Urbanek M. J Clin Endocrinol Metab 2005; 90:6623-9

**Genetics and PCOS: the beat goes on**

- GWAS findings
- Confirmation in other populations
- Next Generation Sequencing (NGS)
- And the beat goes on looking at susceptibility loci in a complex multigenic heterogeneous disorder
Family studies of women with PCOS: traits of PCOS in family members

- Male: Brothers with exaggerated 17 OHP to GnRHa (45%) Petermann TS 2004; Impaired glucose, IR, Metabolic Syndrome (Baillargeon JP 2007, Covello AD 2009; Elevated DHEAS Legro RS 2002; more CV events in Fathers Taylor MC 2011
- Female: Daughters with CV and Metabolic risk factors Battaglia C 2009, Sir-Petermann T 2007; Sisters with metabolic risks Diamanti-Kandarakis 2004; more CV events in Mothers Cheang KI 2008

Prepubertal metabolic changes in daughters of women with PCOS

Challenges in uncovering the genetics of PCOS

- Many phenotypes possible
- Environmental/Life style variables
- Genetic heterogeneity
- Unknown gene-gene and gene-environmental influences
- Small sample size
Finding a genetic cause for PCOS: A bit of a fishing expedition

GWAS best for common variants with small effects

GWAS – multiple SNPs over the genome SNPs “associated” with a disease – affected versus controls
GWAS studies – Han Chinese have used Rotterdam criteria – most women are non-hyperandrogenic by European standards

One “confirmatory” study in an European population has characterized patients by “NIH” criteria

GWAS from Han Chinese showing susceptibility loci on 2p16.3, 2p21, 9q33.3

GWAS 2 combination 1+2= 8226 cases; 7578 controls (not a very hyperandrogenic cohort): 8 risk loci

Chen Zi-Jiang, Nature Genetics 2010; doi: 10.1038/ng.732

Shi Yongyong, Nature Genetics 2012; doi:10.1038/ng.2384
Family-based analysis of susceptibility
loci for polycystic ovary syndrome on
chromosome 2p16.3, 2p21 and 9q33.3

Muthgarasan P. J Clin Endocrinol Metab 2013; 98: E185-E190

Best potential targets for
diagnostics/therapeutics

- Genetic susceptibility
- AMH
- LH (not circulating levels)
- Ovarian morphology
Less of a decline in AMH over time in WHO 2


Log ovarian volume (A) and follicle number (B) as a function of age in PCOS (black circles) and control subjects (open squares) and postmenopausal PCOS (red circles) and control subjects (red squares).

Alsamarai S et al. JCEM 2009;94:4961-4970

Small study
AMH cut off: 33 pmol/L; follicles: 13.
Would only help diagnose 47% of "suspected" group - B

Casadei L. Gynecol Endocrinol 2013, online 1-6
Assessments of AMH and ovarian morphology require a nomogram constructed by age and to control for ethnicity.

**Best potential targets for diagnostics/therapeutics**

- Genetic susceptibility
- AMH
- LH (not circulating levels)
- Ovarian morphology

Fine mapping of 2p16.3 (LHCGR, FSHR)

PCOS (905) of European ancestry (956 con)

dark red: strong Linkage Disequilibrium
Human Theca and Granulosa increased ovarian expression

LH upregulates AMH and down regulates AMHR-2 (granulosa cells) only in anovulatory PCOS

Best potential targets for diagnostics/therapeutics

- Genetic susceptibility
- AMH
- LH (not circulating levels)
- Ovarian morphology (probably present in 90-95% of women diagnosed clinically)
Example of median ovarian section with outlined ovarian and stroma areas. A1 is the total area and A2 is the stroma area.

The isolated asymptomatic Polycystic Ovary

Subtle CV risk factor abnormalities in some normal women with polycystic ovaries (PAO)

Why the Ovary may be so important in PCOS


The Syndrome (PCOS) Develops When: One or More “Insults” Persist

PAO

Insulin
P450 17α
Ovarian dysgenesis
IGFBP1
Other


PCOS: Diagnosis & Pathophysiology

Diagnostic and therapeutic targets

- Genetics – susceptibility genes
  LH/FSH dynamics? insulin/metabolic and ovarian susceptibility factors conspicuously absent
- AMH – biomarker to replace ovarian morphology? Age/ethnic variability
- Ovarian Morphology – diagnostic criteria not agreed to – but could be considered a marker
- Treatment targets: LH, Ovary/AMH, insulin

Practical Considerations

- Know your phenotype – diagnostic and therapeutic approaches based on phenotype
- The term PCOS in its broadest sense (Rotterdam) may be used
- Not all women with PCOS should be treated in the same way
- A unifying therapeutic target may be the ovary for the majority of women
ENDOMETRIOSIS: PHENOTYPES AND GENETICS

Serdar Bulun, MD
JJ Sciarra Professor and Chair
Department of Ob/Gyn
Northwestern University

PHENOTYPES
PERITONEAL ENDOMETRIOSIS

OVARIAN ENDOMETRIOMA

RECTOVAGINAL NODULE

OVARIAN STEROIDS

postmenopausal uterus

E2

P

implantation

donor embryo

EPITHELIUM

DNA SYNTHESIS PROLIFERATION

basement membrane

ER

DNA SYNTHESIS PROLIFERATION

DNA SYNTHESIS PROLIFERATION

DIFFERENTIATION

OPPOSE E2 ACTION

STROMA

PR

PARACRINE FACTORS

ER-dependent factors

PR-dependent factors

HSD17B2

E2

E1


ER

ER-dependent factors

PR

PR-dependent factors

STROMA

DNA SYNTHESIS PROLIFERATION

DIFFERENTIATION

OPPOSE E2 ACTION

PROGESTERONE RECEPTOR MODULATORS

PROGESTINS
(Progestrone Agonists)

PROGESTERONE
antagonists

R5020

PROGESTERONE

progestogenic activity

Mixed agonist/antagonists

J867

CDB4124

CDB2924

RU486

MPA

R5020

PROGESTERS

mixed agonist/antagonists

J867

CDB4124

CDB2924

RU486

MPA

R5020

PROGESTIN

progestogenic activity

ANTIPROGESTERS

AGONIST

AGONIST ANTAGONIST

ANTIPROGESTIN

PROGESTERONE

progestogenic activity

ANTIPROGESTIN

PROGESTIN

progestogenic activity
progesterone on basal promoter

KLF11 and distal regulatory regions

FAILURE TO ACTIVATE BENEFICIAL GENES

symptoms persist

AND POSSIBLY MANY OTHER GENES

symptoms improve

Yin, et al, Can Res, 2010

Bulun SE, NEJM 2009

Cheng Y, JCEM, 2008

GENETICS
Endometriosis can be inherited in a polygenic manner; its incidence in relatives of affected women is up to seven times the incidence in women without such a family history.


There is evidence of linkage to chromosomes 7 and 10, but no relevant genes in these regions appear to have yet been identified.

Treloar SA et al, Am J Hum Genet, 2005

---

**GWAS**

**CDKN2BAS**  
**WNT4**  
**NFE2L3**  
**HOXA10**  
**GREB1**  
**VEZT**  
**IL1A**  
**FN1**  
**CHD5**  
**ESR1**

---

**CDKN2BAS**

- CDKN2BAS (ANRIL) encodes the cyclin-dependent kinase inhibitor 2B antisense RNA (also known as the antisense non-coding RNA in the INK4 locus).
- 3 tumor suppressor genes, CDK4/5 in the P16 (P14), ARF (P14), CDKN2B (P14) in this locus. CDKN2BAS is transcribed on the antisense side of these important tumor suppressor genes.

Uno et al, Nat Gen 2010  
Racine et al, Nat Gen, 2011  
Ryffel et al, Nat Gen, 2012  
Aboal et al, J Hum Genet, 2010  
Falconer et al, J Hum Genet, 2010  
Wang et al, BMJ Open, 2013  
Sundqvist et al, J Hum Genet, 2013  
Pagliardini et al, J Med Gen, 2013
WNT4

- WNT4, which encodes wingless-type MMTV integration site family, member 4, and which plays a role in the development of the female genital tract from the Müllerian (paramesonephric) duct that develops into the fallopian tubes and uterus.
- The loss of Wnt4 in knockout mice is known to lead to complete absence of the Müllerian duct and its derivatives.
- WNT4 is expressed in normal peritoneum, suggesting that endometriosis may arise through metaplasia using developmental pathways involved in the development of the female genital tract.
- It is possible that genetic variants in WNT4 might contribute to endometriosis susceptibility through abnormal cell growth in female genital tract.

Uno, et al, Nat Gen 2010

STEM CELLS & EPIGENETICS
SF1 and ERβ GENE PROMOTERS

Xue Q, et al, JCEM 2007

PGE2
SF1
COX-2
estradiol
estrone
HSD17B2
retinoic acid
PR
HERα

Bulun SE, NEJM, 2009

NORMAL ENDOMETRIOSIS
ROLE OF EPIGENETICS IN MECHANISM OF ENDOMETRIOSIS

ENDOMETRIUM
SF1/ERβ
on
ENDOMETRIOSIS
SF1/ERβ
off
ENDOMETRIUM

MeCP2
Co-repressing complex
unmethylated CpG island
methylated CpG island

Menses
Painful menses
peritoneum
methylated promoter
unmethylated promoter

FAILURE OF CELLS TO IMPLANT
IMPLANTATION OF ENDOMETRIOC CELLS
Genetics and Phenotypes in Hypothalamic Amenorrhea: Databases and New Diagnostics/Therapeutics

Richard H. Reindollar, M.D.
Professor and Chair, Department of Obstetrics and Gynecology
Geisel School of Medicine at Dartmouth

I have no conflicts of interest.

Learning Objectives

At the conclusion of this lecture, the participants should be able to:

1. Describe the phenotype of patient with hypothalamic amenorrhea, irreversible vs. reversible.
2. Discuss types of genes found to be mutant as causes.
3. Utilize gene mutation data to predict certain phenotypes and counsel patients regarding pattern of inheritance.
4. Discuss future directions of testing and treating patients with hypothalamic amenorrhea based on genotype.
Hypothalamic Amenorrhea

- Irreversible hypogonadotropic hypogonadism
  - Previously termed IHH (idiopathic or isolated)
  - Normosmic vs. Anosmic (Kallmann)
- Acquired and Reversible hypogonadotropic hypogonadism
  - Usually known as hypothalamic amenorrhea

Disorders of Reproduction: Early Observations
Hypogonadotropic Hypogonadism
Earliest Descriptions

- 1943 Hurxhall: 7 cases of hypogonadism, 3 "eunuchoids" in one family
- 1944 Kallmann: primary eunichoidism associated with anosmia, first described in males.
- Consistent initial phenotype: irreversible hypo/hypo
- Variable phenotype (heterogeneity): no or partial secondary sexual development, anosmia and normosmia, midline facial defects (cleft lip/palate, dental agenesis), neurologic defects (ataxia, synkinesia, visual impairment, deafness), renal agenesis (in males), cardiac defects.
Frequent blood sampling studies (FSH/LH pulse studies):
• Apulsatile gonadotropin secretion [majority]
• Sleep entrained pulsatility*
• Decreased pulse frequency*
• Decreased pulse amplitude*
Amenorrhea Hypogonadotropic Hypogonadism
Reindollar et al. Delayed Puberty 1989; Reindollar et al. Adult Onset Amenorrhea 1986

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Pubertal</th>
<th>Adult Onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reversible</td>
<td>39</td>
<td>184</td>
</tr>
<tr>
<td>Physiologic delay</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Systemic illness</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Eating disorders</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>Hypothalamic: non specific</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>Pituitary disorders</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Hypopituitarism</td>
<td>9</td>
<td>27</td>
</tr>
<tr>
<td>Reversible</td>
<td>37</td>
<td>7</td>
</tr>
<tr>
<td>Idiopathic Hypogonadotropic</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>Hypogonadism (IHH)</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Congenital CNS defects</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Acquired Anatomic Lesions</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Undiagnosed malig tumor</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Empty sella</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Craniofacial anomalies</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Unclassified malig tumors</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>76 (27%)</td>
<td>111 (43%)</td>
</tr>
</tbody>
</table>

Gene Mutations Causing Hypogonadotropic Hypogonadism

- KAL1 gene mutations (1992): XR, anosmic, males only, unilateral renal agenesis (50%)
- Currently mutations in 25+ genes found etiologic
- Genes affected involve GnRH production, processing, neuronal development, secretion, effect at receptor, as well as other genes involved in various aspects of hypothalamic and/or pituitary function or peptide hormones produced
- Ligand/receptor gene partners (one or other mutated) identified:
  KAL1, FGFR1/FGFR2, LEP/LEPR, GNRHR, GNRHR, PROK2/PROK, KISS1/KISS1R
  TAC3/TACR3
- Digenic Disease exists

Anosmia vs. Normosmia Genes

Anosmia (KS)

KAL1 (this gene affects GnRH neuron migration)

Normosmia (nHH)

GNRH1/GNRHR
KISS1/KISS1R
LEP/LEPR
TAC3/TACR3

Either KS or nHH

KAL1/FGFR1/FGFR2/H65ST1
PROK2/PROK2R
(nov gene affect with anosmic migration)

CHD7, NF2, WDR4, SFM4

Page 93 of 139
Gene Mutations Associated with Kallmann syndrome or normosmic IHH (Hypothalamic Defects)

- **GnRH production**
  - (GNRH1 gene)
- **GnRH processing**
  - (PCSK1 gene)

*Most Common (with GNRHR)*

+ Least Common

- **GnRH neuronal development**
  - (FGFR1*, FGF8, PROK2, CHD7*, KAL1* genes)
- **GnRH secretion**
  - (GPR54 gene)

Gene Mutations Associated with IHH (Pituitary Defects)

- **GnRHR gene**
- **LHX3 gene**
- **LHX4 gene**
- **LH β gene**
- **FSH β gene**

Gene Mutations Associated with IHH (Hypothalamic and Pituitary Defects)

- **Leptin gene**
- **Leptin receptor gene**
- **NROB1 (DAX 1)**
Contemporary Ideas about IHH

- Pubertal IHH (even with gene mutations, e.g. FGF1) is reversible in 10% of males after long periods of treatment.
- Female reversible forms exist.
- Adult onset IHH is reported in males (with gene mutations).
- Adult hypothalamic amenorrhea may be caused by the same mutations.
- Adult hypothalamic amenorrhea is caused in some patients by hypoleptinemia.


- IHH diagnosed approximately age 18 years
- She and mother have anosmia
- Off and on hormones through the years; when off hypoestrogenic amenorrhea
- Stopped hormones age 35, 1 spontaneous menses, became pregnant and had SAB.
- Subsequent hypoestrogenic amenorrhea
- 6 common K5 genes studied were normal


- Background: 14 mutations (13 missense and 1 truncation) of GNRHR gene identified in nHH patients.
- Investigators previously showed that 5 of the missense mutations (studied in Cos-7 cells) can be rescued with GnRH peptidomimetic antagonist (acts as folding template to stabilize misfolded mutants restoring function).
- Current study demonstrated that the approach rescued 11 of the 13 GNRHR missense mutations.
- This approach suggests therapeutic opportunities in the future.
### A Genetic Basis for Functional Hypothalamic Amenorrhea


- 55 women with hypothalamic amenorrhea
  - 6 months secondary amenorrhea
  - Low or normal gonadotropins and normal head imaging
  - Low serum estradiol
  - One or more of:
    - Excessive exercise (> 5 hours weekly) (n = 25), loss of more than 15% body weight (n = 20), subclinical eating disorder (by Eating Attitudes Test) 5n > 20, but NOT anorexia.
    - Mean age menarche 13.5 (13 menarche ≥ 15 years), 22.4 yrs at dx,
    - Family history of delayed puberty (n = 6) and hypothalamic amenorrhea (n = 9).
  - Genes evaluated: KAL1, GNRHR, GPR54, FGFR1, FGF8, PROK2, PROKR2.
  - 6 heterozygous mutations (FGFR1, PROKR2, GNRHR, KAL1) found in 7 or 55 patients; none in cohort of normal menstruating patients.

---

### Leptin is an effective treatment for hypothalamic amenorrhea

Chou SH, PNAS 2011;108:6585-6590.

- RCT of metreleptin (replacement doses) over 36 weeks.
- 19 women with HA, hypoleptinemia
  - 7 of 10 fixed subjects, 2 of 9 placebo subjects developed menstruation (p = 0.0046)
    - Leptin levels increased from 4.55 ng/ml to 59 ng/ml
    - Menses after 4 – 32 weeks, earlier in metreleptin subjects
    - All menstruating maintained cycling, although irregular
    - 4 of metreleptin subjects ovulatory progesterone levels
    - 5 of 7 metreleptin subjects completed study continued to have menses in follow up at 52 weeks.

---

### Long-term metreleptin treatment increases bone mineral density and content at the lumbar spine of lean hypoleptinemic women

Sienkiewicz e. Metabolism 2011;60:1211

- 20 strenuously exercising women with hypoleptinemia (leptin < 5 ng/ml) and hypothalamic amenorrhea of 6 months’ duration.
- 13 randomized to metreleptin for 3 months, 9 to placebo
- Metreleptin increased BMC (p = 0.34) and BMD (p = 0.69) at 9 months (n = 11) at lumbar spine.
- Metreleptin increased BMC (p = 0.49) and BMD (p = 0.02) at 2 years (n = 4) at lumbar spine by 4 – 6%
How could gene databases for hypothalamic amenorrhea, reversible or irreversible, direct care?

**Evaluation**
- 25% of nHH patient will have mutation in FGFR1 (10%), CHD7 (6%), TAC3 (6%), GNRHR (5%).
- FGFR1 (10%) and CHD7 (6%) most common genes for KS.
- KAL1 considered in males but not females; has 50% chance of unilateral renal agenesis.
- FGFR3 may result in cleft lip/palate, dental agenesis, and synkinesia.
- GNRHR may have variable phenotype but no additional abnormalities.
- Mutation-based/hypo-connected hypogonadism may be found in adult-onset disease.

**Counseling**
- KAL1 and MBD2, XR. Most AR (N genes <2), FGFR1 and CHD7 AD (N genes >6).
- Some previously thought irreversible, may become reversible with treatment (FGF1).

**Treatment**
- GNRHR mutation patients won’t respond to pulsatile GnRH.
- Leptin RX may become treatment of choice for some women with hypothalamic amenorrhea.
- Some GNRHR mutations may be rescued by a single agent — and treated!

**References**
(Included with slides)
Genetics and Phenotypes of embryos in culture: Databases and new diagnostics for embryo selection

Prof. Antonio Pellicer
Instituto Valenciano de Infertilidad (IVI)
University of Valencia
apellicer@ivi.es
www.ivi.es

DISCLOSURE

- IVI is a minor shareholder in Unisense Fertilitech A/S.
- This work has not received any financial support from any commercial entity and the instrumentation, disposables and utensils belong to IVI.

OBJECTIVES

After this presentation, the participants should:

- Be familiar with the current methods of embryo selection
- Know the most recent advances in comprehensive chromosomo analysis (CCA) of the embryos
- Understand the basis and results of employing time-lapse to select embryos
- Be familiar with current knowledge of proteomics and metabolomics to select embryos
The present of Assisted Reproduction

Personalized Embryo Transfer (pET)

Identification/Modification of receptive endometrium

- Invasive methods: CCS (D3 or D5)
- Non-invasive methods:
  - Time-lapse
  - Proteomics
  - Metabolomics

Identification of the viable embryo

- Identification of the viable embryo
- Other non-invasive methods

Window of Implantation

- Identification of the viable embryo

Endometrial receptivity assay (ERA)

Other non-invasive methods

Personalized Embryo Transfer

Identification of the viable embryo

- Previous failed attempt(s)
- Reduced ovarian reserve
- Aged patient
- Endometriosis
- Severe male factor

- Regular IVF-ICSI (morphology)
- Time-lapse
- Proteomics
- Metabolomics
- CCS (aCGH)

Indications for Comprehensive Chromosomal Screening (CCS)

- Advanced maternal age (≥38 yrs; >40 yrs)
- Implantation failure (≥3 IVF attempts)
- Recurrent miscarriage (≥2 miscarriages)
- Severe male factor
- No indications

Polar body biopsy

Methaphase II

Chromatid stage biopsy

Blastocyst biopsy

Day 0

Day 1

Day 6

Day 6
Controversies in CCS: published RCTs

- Advanced Maternal Age (5 RCTs)

- Recurrent implantation failure (1 RCT)
  - Blockeel et al., RBM Online 2008

- Young IVF couples with risk (4 RCTs)
  - Jansen et al., Hum Reprod 2008; Mersereau et al., Fertil Steril 2008; Staessen et al., Hum Reprod 2008; Meyer et al., Fertil Steril 2008

Mosaicism in day-3 embryos

- FISH 5-7 chromosomes

- Technique pitfalls

Controversies in CCS: critical aspects

<table>
<thead>
<tr>
<th>RIF (≥ 3 IVF failures; &lt; 40 yrs)</th>
<th>Blastocyst</th>
<th>PGS</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>43</td>
<td>48</td>
<td>——</td>
</tr>
<tr>
<td>Mean Age (SD)</td>
<td>35.3 (2.9)</td>
<td>35.2 (3.1)</td>
<td>——</td>
</tr>
<tr>
<td>Ongoing implantation rate (%)</td>
<td>15/43 (27.9)</td>
<td>25/48 (52.1%)</td>
<td>p=0.0112</td>
</tr>
<tr>
<td>Live birth rates (%)</td>
<td>12/43 (27.9)</td>
<td>25/48 (52.1%)</td>
<td>p=0.0112</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AMA (41-44 years; ≥ 5 MII)</th>
<th>Blastocyst</th>
<th>PGS</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>90</td>
<td>93</td>
<td>——</td>
</tr>
<tr>
<td>Mean Age (SD)</td>
<td>41.7 (0.9)</td>
<td>41.8 (0.9)</td>
<td>——</td>
</tr>
<tr>
<td>Ongoing implantation rate (%)</td>
<td>20/152 (13.1)</td>
<td>40/114 (35.1%)</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Live birth rate (%)</td>
<td>14/90 (15.5)</td>
<td>30/93 (32.3%)</td>
<td>p=0.0099</td>
</tr>
</tbody>
</table>


CCS employing CGH arrays: 24-chromosomes screening

- Binning
- Amplification (~2 hrs) (~48%)  
- Labeling (2 hrs)
- DNA precipitation (~2 hrs)
- Hybridization (~5-12 hrs)
- Washing (~1/2 hr)
- Scanning

- Sample 1
- Sample 2
- Cy3
- Cy5

- BlueFuse Multi software

Results <24 hours
Table 1 Characteristics of patients whose embryos were randomized to assessment by morphology with aCGH (Group A) and biopsy cytogenetics only (Group B)

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 55)</th>
<th>Group B (n = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>31.2 ± 3.5</td>
<td>31.2 ± 3.7</td>
</tr>
<tr>
<td>Enroll outcome</td>
<td>10.9 ± 8.2</td>
<td>10.5 ± 8.1</td>
</tr>
<tr>
<td>Male/female</td>
<td>16:9 ± 7.8</td>
<td>16:5 ± 7.6</td>
</tr>
<tr>
<td>Oocytes matured (24h)</td>
<td>13.1 ± 6.7</td>
<td>12.8 ± 6.4</td>
</tr>
<tr>
<td>Day 3 embryos</td>
<td>12.9 ± 1.8</td>
<td>12.6 ± 1.9</td>
</tr>
<tr>
<td>Day 5 biopsies</td>
<td>8.3 ± 2.4</td>
<td>8.1 ± 2.4</td>
</tr>
</tbody>
</table>

Yang et al. Mol Cytogenet 2012, 5:24

Table 2 Comparison of laboratory findings and clinical outcomes among IVF patients undergoing SET with embryo assessment by aCGH + morphology (Group A) and blastocyst morphology alone (Group B)

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh blastocyst transfer rate (morphology assessment)</td>
<td>55 (100)</td>
<td>48 (100)</td>
<td>0.386</td>
</tr>
<tr>
<td>Grade 4</td>
<td>55 (100)</td>
<td>48 (100)</td>
<td>0.386</td>
</tr>
<tr>
<td>Grade 3</td>
<td>55 (100)</td>
<td>48 (100)</td>
<td>0.386</td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>3 (5.4)</td>
<td>1 (2.1)</td>
<td>0.177</td>
</tr>
<tr>
<td>Clinical pregnancy (C20 weeks)</td>
<td>38 (69.1)</td>
<td>20 (41.3)</td>
<td>0.009</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>1 (2.6)</td>
<td>2 (9.1)</td>
<td>0.258</td>
</tr>
</tbody>
</table>

Note: All data reported as n (%) | SET = single embryo transfer, aCGH = array comparative genomic hybridization, GA = gestational age | Fisher’s exact test

Yang et al. Mol Cytogenet 2012, 5:24

PGS with CGH arrays: 24-chromosomes screening

Gain chromosome 21

Loss chromosome 8
Incidence of chromosomal abnormalities by aCGH (Dec 2012)

<table>
<thead>
<tr>
<th>Age range</th>
<th>N. Cycles</th>
<th>N. Informative embryos</th>
<th>N. Abnormal embryos (%)</th>
<th>N. Amplification failure (%)</th>
<th>N. Chaotic embryos (%)</th>
<th>N. Partial Aneuploids (&gt;20 Mb)</th>
<th>% Abnormal chromosomes ≠ FISH</th>
<th>% Abnormal &gt;1 chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM (&lt;38 yrs)</td>
<td>150</td>
<td>175</td>
<td>30</td>
<td>197</td>
<td>5</td>
<td>14</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>RIF (&lt;38 yrs)</td>
<td>140</td>
<td>160</td>
<td>30</td>
<td>197</td>
<td>4</td>
<td>14</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>MF (&lt;38 yrs)</td>
<td>130</td>
<td>150</td>
<td>30</td>
<td>197</td>
<td>4</td>
<td>14</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>PTP (&lt;38 yrs)</td>
<td>120</td>
<td>140</td>
<td>30</td>
<td>197</td>
<td>4</td>
<td>14</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>AMA (40–44 yrs)</td>
<td>110</td>
<td>130</td>
<td>30</td>
<td>197</td>
<td>4</td>
<td>14</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

Incidence of chromosomal abnormalities by aCGH (Dec 2012)

<table>
<thead>
<tr>
<th>Age range</th>
<th>N. Cycles</th>
<th>N. Informative embryos</th>
<th>N. Abnormal embryos (%)</th>
<th>N. Amplification failure (%)</th>
<th>N. Chaotic embryos (%)</th>
<th>N. Partial Aneuploids (&gt;20 Mb)</th>
<th>% Abnormal chromosomes ≠ FISH</th>
<th>% Abnormal &gt;1 chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM (&lt;38 yrs)</td>
<td>150</td>
<td>175</td>
<td>30</td>
<td>197</td>
<td>5</td>
<td>14</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>RIF (&lt;38 yrs)</td>
<td>140</td>
<td>160</td>
<td>30</td>
<td>197</td>
<td>4</td>
<td>14</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>MF (&lt;38 yrs)</td>
<td>130</td>
<td>150</td>
<td>30</td>
<td>197</td>
<td>4</td>
<td>14</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>PTP (&lt;38 yrs)</td>
<td>120</td>
<td>140</td>
<td>30</td>
<td>197</td>
<td>4</td>
<td>14</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>AMA (40–44 yrs)</td>
<td>110</td>
<td>130</td>
<td>30</td>
<td>197</td>
<td>4</td>
<td>14</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>
Clinical results according to pure indications (Dec 2012)

<table>
<thead>
<tr>
<th></th>
<th>RM (≥ 2 miscarriages)</th>
<th>RF (≥ 3 implantation failures)</th>
<th>PTP (≥ 38 yrs)</th>
<th>AMA (≥ 40-44 yrs)</th>
<th>MF (≥ 40-44 yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>107</td>
<td>94</td>
<td>16</td>
<td>396</td>
<td>74</td>
</tr>
<tr>
<td>Mean Age (SD)</td>
<td>34.5 (2.2)</td>
<td>35.0 (2.2)</td>
<td>35.8 (1.3)</td>
<td>41.2 (1.5)</td>
<td>39.9 (2.5)</td>
</tr>
<tr>
<td>% of transfers</td>
<td>83.3</td>
<td>86.2</td>
<td>83.2</td>
<td>47.4</td>
<td>83.4</td>
</tr>
<tr>
<td>Mean embryos tr. (SD)</td>
<td>1.3 (0.9)</td>
<td>1.3 (0.7)</td>
<td>1.5 (1.0)</td>
<td>3.3 (0.6)</td>
<td>1.6 (0.7)</td>
</tr>
<tr>
<td>% of abnormal embryos</td>
<td>62.7</td>
<td>63.6</td>
<td>67.4</td>
<td>94.7</td>
<td>60.8</td>
</tr>
<tr>
<td>PR/transfer</td>
<td>55.2</td>
<td>55.6</td>
<td>38.5</td>
<td>46.8</td>
<td>65.3</td>
</tr>
<tr>
<td>% of miscarriages</td>
<td>9.1</td>
<td>2.2</td>
<td>20.0</td>
<td>9.4</td>
<td>2.0</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>62.6</td>
<td>67.6</td>
<td>30.0</td>
<td>61.2</td>
<td>64.0</td>
</tr>
</tbody>
</table>

RM: ≥ 2 miscarriages; RF: ≥ 3 implantation failures; MF: male factor; PTP: previous trisomic pregnancy; AMA: advanced maternal age

Accuracy of aCGH in day-3 and day-5 biopsies

N= 76 reanalysed abnormal embryos

Day-3 aCGH reconfirmation with FISH on day-5

<table>
<thead>
<tr>
<th>Confirmed Diagnosis</th>
<th>No confirmed</th>
<th>Day-3 aCGH</th>
<th>Confirmed Diagnosis</th>
<th>No confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed Diagnosis</td>
<td>No confirmed</td>
<td>Day-3 aCGH</td>
<td>Confirmed Diagnosis</td>
<td>No confirmed</td>
</tr>
<tr>
<td>aCGH day-3</td>
<td>74/76</td>
<td>(97.4%)</td>
<td>aCGH day-5</td>
<td>36/37</td>
</tr>
<tr>
<td></td>
<td>3/76</td>
<td>(2.6%)</td>
<td></td>
<td>1/37</td>
</tr>
</tbody>
</table>

Day-5 aCGH reconfirmation with FISH on day-5

<table>
<thead>
<tr>
<th>Confirmed Diagnosis</th>
<th>No confirmed</th>
<th>Day-5 aCGH</th>
<th>Confirmed Diagnosis</th>
<th>No confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed Diagnosis</td>
<td>No confirmed</td>
<td>Day-5 aCGH</td>
<td>Confirmed Diagnosis</td>
<td>No confirmed</td>
</tr>
<tr>
<td>aCGH day-5</td>
<td>36/37</td>
<td>(97.3%)</td>
<td>aCGH day-5</td>
<td>36/37</td>
</tr>
<tr>
<td></td>
<td>1/37</td>
<td>(2.7%)</td>
<td></td>
<td>1/37</td>
</tr>
</tbody>
</table>

Mir et al., ASRM 2011

EmbryoScope D

Time-Lapse Imaging - Blastomere Activity
Timing was classified in quartiles and grouped in two categories:
- The two central quartiles (IN)
- The rest of the data (OUT).

Meseguer et al., Hum Reprod, 2011; 26: 2658-71

Incidence of direct division 1-3 in all embryos dividing to 3 cells

Rubio et al., Fertil Steril, 2012; 96: 1655-63
Morphology dynamics

Time dependent Multinucleation

Diameters and surfaces

Cleavage planes

Symmetry

Exclusion Criteria

Grade A
Grade B
Grade C
Grade D
Grade E
Discarded

Algorithm for embryo implantation

N=1137 embryos with known implantation (nas, neo= transferred embryos)
### Time-lapse vs regular morphology

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Median</th>
<th>Timing (hours post-insemination)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st cleavage (to 2 cells, t2)</td>
<td>28.94</td>
<td>27.88</td>
<td>24 ± 18 (ICSI)</td>
</tr>
<tr>
<td>2nd cleavage (to 3 cells, t3)</td>
<td>39.21</td>
<td>37.86</td>
<td>-</td>
</tr>
<tr>
<td>3rd cleavage (to 4 cells, t4)</td>
<td>44.71</td>
<td>42.41</td>
<td>-</td>
</tr>
<tr>
<td>4th cleavage (to 5 cells, t5)</td>
<td>51.03</td>
<td>50.51</td>
<td>-</td>
</tr>
<tr>
<td>5th cleavage (to 6 cells, t6)</td>
<td>53.36</td>
<td>52.91</td>
<td>-</td>
</tr>
<tr>
<td>6th cleavage (to 7 cells, t7)</td>
<td>59.11</td>
<td>58.64</td>
<td>-</td>
</tr>
<tr>
<td>Embryo Compaction (tM)</td>
<td>86.62</td>
<td>85.92</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>Blastocyst formation (tB)</td>
<td>104.12</td>
<td>103.02</td>
<td>120 ± 3</td>
</tr>
</tbody>
</table>

#### Morphokinetics

- **ASEBIR A**
- **ASEBIR B**
- **ASEBIR C**
- **ASEBIR D**

#### AUC

- AUC=0.63
- AUC=0.76
**Time-lapse vs Standard Incubation**

Multicenter cohort study
Stratified analysis for logistic regression model

N= 1372


**Time-Lapse vs Standard Incubation**

Logistic regression analysis
Effect of ESD on clinical pregnancy: OR=1.201 (CI95% 1.059-1.363) p=0.0043

+ 19% (IC95% 5.8-37.0%)


**Time-lapse and chromosomal status**

N= 77 ICSI PGI-cycles employing CGH arrays
N= 504 embryos

97% confirmed diagnosis (Mir et al. 2012)
### Time-lapse and chromosomal status

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Abnormal n=361</th>
<th>Normal n=143</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean [h] CI95%</td>
<td>Mean [h] CI95%</td>
<td></td>
</tr>
<tr>
<td>t2</td>
<td>26.9 26.5-27.2</td>
<td>26.4 25.9-26.8</td>
<td>0.409</td>
</tr>
<tr>
<td>t5</td>
<td>37.2 36.3-37.7</td>
<td>37.9 37.3-38.6</td>
<td>0.159</td>
</tr>
<tr>
<td>t5*</td>
<td>42.4 41.6-50.2</td>
<td>51.8 50.8-52.8</td>
<td>0.001</td>
</tr>
<tr>
<td>t2*</td>
<td>10.4 9.9-10.8</td>
<td>11.6 11.1-12.0</td>
<td>0.004</td>
</tr>
<tr>
<td>t5-t2*</td>
<td>22.6 21.8-23.3</td>
<td>25.5 24.6-26.4</td>
<td>0.001</td>
</tr>
</tbody>
</table>

### The -OMICS era

**Transcriptome**
- Transcription regulation
- Alternative Splicing

**Proteome**
- Transduction regulation
- Metabolome

**Metabolites**
Embryo secretome has been defined as the subset of proteins that are consumed or secreted by the human embryo.

Secretome could be analyzed using conditioned media by proteomic techniques.


Protein array

Secretome of the human blastocyst in sequential media

Proteins significantly increased or decreased between control media (spotted bar) and media with a blastocyst (white bar).


Secretome of the human embryo in sequential medium

Control medium

Implanted blastocyst

Non implanted blastocyst

Culture medium from 5 blastocysts

Protein array

ANÁLISIS DE DATOS

Implantome results

Significantly elevated proteins secreted in implanted (white bar) vs non implanted (black bar) human embryos

**PROTEOMICS**

Protein Network of altered proteins

*Domínguez et al. Hum Reprod 2008*

**PROTEOMICS**

Katz-Jaffe & Reynolds

*Fertil Steril 2013; 99:1073-77*

**METABOLOMICS**

Noninvasive metabolic profiling of embryo culture media using Raman and near-infrared spectroscopy correlates with reproductive potential of embryos in women undergoing in vitro fertilization

*Egan, B.S., M.D.,* *Dennis Jakway, Ph.D.,* *Michael Zorc, M.D.,* *Bing C. Xue, M.D.,* *Scott M. Rosenfield,* and *David M. Stone, Ph.D.*


- Rapid, noninvasive metabolic profiling of human embryo culture media using Raman or near-infrared spectroscopy combined with bioinformatics correlates with pregnancy outcome (69 spent media from 30 patients).
Glutamate concentrations determined by 1H NMR were significantly higher in spent culture media of embryos that resulted in pregnancy and delivery compared to those that failed to implant (from 34 CM).

Proton NMR spectroscopy predicted viability of individual embryos with a sensitivity of 88.2% and a specificity of 88.2%.

NIR metabolomic profiling of spent embryo culture media was able to distinguish viable versus non-viable embryos.
The live birth rate after embryo selection by NIR spectroscopy and morphology is not significantly different compared with the live birth rate after embryos were selected by morphology alone (from 400 CM).

**STUDY DESIGN**

*Sánchez-Ribas et al. Fertil Steril 2012; 98: 1157-64*
**METABOLOMICS**

**Problems in using metabolomics as a diagnostic tool in Embryology**

Methodological reasons:
- There are no tools such as in:
  - Genomics: automated sequencing
  - Transcriptomics: Arrays with oligos or SNPs
  - Proteomics: 20/21 aa
- Metabolites' short life time
- Important heterogeneity of metabolites

---

**CONCLUSIONS**

- ✔️ Personalized Medicine is the next step in ART
- ✔️ aCGH seems to be a reliable method for embryo selection improving pregnancy rates
- ✔️ Time-lapse is a good method of embryo selection: correlation with implantation and aneuploidy
- ✔️ Retrospective and ongoing prospective studies show significantly improved outcomes employing time-lapse
- ✔️ The proteomic profile (consumption and secretion) of the human blastocysts seems to be a promising technique to differentiate viable embryos
- ✔️ Metabolomics is not yet a reliable method

---

**ACKNOWLEDGEMENTS**

Marcos Meseguer
Carmen Rubio
Francisco Domingues
Inmaculada Sánchez
Alberto Tejera
Irene Rubio
Javier Herrero
Natalia Basil
Maria Cruz
Carlos Simón
Sources of Information

Craig Niederberger MD FACS
Clarence C. Saele Professor and Head, Department of Urology, UIC College of Medicine
Professor, Department of Bioengineering, UIC College of Engineering
Co-Editor in Chief, Fertility and Sterility

1947
Walter Brattain and John Bardeen make the first point contact transistor
Microprocessor Transistor Counts 1971-2011 & Moore's Law

1947
2012
NVIDIA GK110
7,080,000,000 transistors

1976
1 MHz clock
256 bytes RAM
No hard drive

2013
3.7 GHz quad core
16 Gbyte RAM
1 Tbyte HD
null
Clinical factors associated with live birth after single embryo transfer

Post by Stephen, MD on Thursday, November 1, 2012  |  0 Comments and 0 Reactions

Capsule:
Female age and Matroncyt expansion are associated with live birth in patients undergoing SET. Many other clinical factors including obesity do not appear to affect SET outcome.

Authors:
Jessica D. Klonowski, M.D., Amy E. T. Spuls, Ph.D., Bradley J. Van Voorhis, M.D.
Volume 18, Issue 5, Pages 1572-1596, November 2012

Abstract:

Objectives:
To identify patient, cycle, and retrieval characteristics associated with embryo implantation and live birth in patients undergoing SET.

Design:
Analysis of prospectively collected IVF database.

Setting:
Academic IVF program.

http://fertilityandsterility.org/Manuel Fernández Sánchez

Congratulations for your work. To have a mandatory policy of SET in a group of patients of a given center is very difficult and to achieve 66.8% of live birth rate after SET, even in a good prognosis of patients, are excellent results. In a recent publication, we observed that the rate of patient's refusal in a similar good prognosis patient of our private center and we found that 33% of them finally refused to perform SET. Did you measure the rate of patient’s refusal to undergo SET? Thank you very much.

Jessica Klonowski

Thank you for your comments. We actually discuss our policy for single embryo transfer qualitative and quantitative data of the new IVF visit and tell patients that if they meet these requirements they will only have one embryo transferred. The patients are not given a choice on the day of transfer if they meet the criteria and we had excellent acceptance of our policy. We reviewed our data prior to implementing the policy and we have not had any change in clinical outcome since this was started.

Michael HOB

Congratulations for another nice paper from the University of Iowa group on single embryo transfer. I appreciate the significant publications and efforts from this group in pushing the SET discussion in the US.

First, I was curious if these findings have changed your algorithm at all or is your SET policy the same after this paper? Specifically with uterine factor, it seems that only significant malformation anomalies negatively affect your live birth rates. However, these patients are a group that twin gestation may put their pregnancy at very high risk. Conversely the patients with FSH and polyps removal had good live birth rates of 42%. So it seems that severe uterine factor should perhaps still get SET from an obstetric standpoint and the "mild" uterine factor should also still get SET from a success standpoint.

Second, you point out the differences between your paper and the Abtinon paper with...
Jessica Komowick

We appreciate your insight and comments on our paper. As you point out, due to the risk of multiple gestation in patients with uterine anomalies we still perform SET on these patients despite their decreased rate of live birth. We also consider those patients with mild uterine factor to qualify for SET as subgroup analysis did not reveal a decrease in live birth in this patient population.

Regarding the TE and ICM grading, our sample size was smaller and overall pregnancy rate higher, thus making a significant difference in the outcomes based on these parameters more difficult to detect. Additionally, embryos with a C grade were included in the Abdominal analysis, where our patients would have undergone double embryo transfer with this grade. Our numbers were as follows:

Day 3 Topup Clinical Preg No Clinical Preg OR 95% CI p-value
A 228 (64%) 61 (44%) 1.04 0.66 - 1.64 0.871
B 63 (14%) 41 (27%) 0.56 0.34 - 0.94 0.02

Day 5 ICM
A 217 (59%) 61 (44%) 1.55 0.95 - 2.55 0.091
B 97 (22%) 64 (44%) 0.74 0.47 - 1.19 0.331

Nicola Hill
Thank you for the reply and especially for providing more information. It is very helpful for us!

Lucyworth

This is wonderful article highlighting the success of SET in the appropriately selected population. I think it gives really important clinical information that will help us counsel patients.

Jessica Komowick
Thank you!

---

Fertility Forum
How Clomid Works in Men

April 19th, 2015 | 567 comments

With the suspension of Cincinnati Reds pitcher Edinson Volquez for performance enhancing drug use and a swirl of rumors that the agent involved was clomiphene (also known as Clomid), I thought it timely to write about how clomiphene works and how it’s used. From what I read on the internets, there is an enormous amount of misinformation floating around out there.

To understand how clomiphene works, you need to know how the pituitary controls the making of testosterone in the testis. Testosterone is made by Leydig cells in the testis, which I explained in my last post. The pituitary releases a hormone called luteinizing hormone ("LH") that stimulates the Leydig cells to make testosterone. Testosterone is converted to the female hormone estrogen, (which I also explained in my last post) and estrogen tells the pituitary to stop making more LH. This kind of negative feedback system is common when it comes to how hormones work. It’s just like a thermostat and heater. As the room gets warmer, the thermostat sends less electricity to the heater. When the room gets colder, the thermostat sends more electricity to the heater.

FAQ

Some frequently asked questions: "I have this medical problem…"

I can’t answer personal medical questions on this site. It’s designed for general information about male fertility and potency. Medical problems are always best handled by a qualified health professional in person. One great resource is the American Society for Reproductive Medicine’s Society for Male Reproduction and Urology page and the ASRM’s find a doctor search page, (just click on the "Society for Male Reproduction and Urology (SMRU)" button in the “Find Member by Affiliated Society;” section.) Another excellent way to find a specialist who treats men with reproductive issues is to use the American Urological Association’s Society for the Study of Male Reproduction’s search engine.
estrogen becomes too elevated, other drugs, like anastrazole, can be used instead.

Reply

Bharadwaj
February 16, 2012 at 3:58 pm (Edt)

All Hormones are in the normal range but sperm count Zero and some time shows very very spers (x2) . My Doc write prescription for me.

- chorionsen 5000iu injection per week for 3 months
- Clomid One tab for 3 months.

Is that right to increase sperm counts >>>i need your advise

Reply

Response
February 16, 2012 at 7:46 pm (Edt)

I'm sorry. Babna, but I can't answer personal questions about your own health. Please read the FAQ.

Reply

Bharadwaj
February 16, 2012 at 9:00 pm (Edt)

Thanks Doc..... i would like to know one thing Doc ! Pla Is it ok to use both at
You can now register for these upcoming ESHRE Campus events:

- Application and challenges of emerging technologies in preimplantation and prenatal diagnosis
  12-13 September 2013 - Prague, Czech Republic

- Female genital tract congenital malformations: new insights in an old problem
  27-28 September 2013 - Thessaloniki, Greece

- Introducing new techniques into the lab
  4-5 October 2013 - Barcelona, Spain

- Polycystic ovary syndrome: A new look at an old subject
  25-26 October 2013 - Rome, Italy

- Infections from conception to birth: role of ART
  7-8 November 2013 - Berlin, Germany

- Endoscopy in reproductive medicine
  20-22 November 2013 - Leuven, Belgium

- From early implantation to later in life
  28-29 November 2013 - Brussels, Belgium

Mark your calendar for:

- Premature ovarian insufficiency
  6-7 December 2013 - Utrecht, The Netherlands