PRE-CONGRESS COURSE 8

Practical applications of clinical and basic science genetics to reproductive medicine

ASRM Exchange course
Munich - Germany, 29 June 2014

eshre
SCIENCE MOVING
PEOPLE
MOVING SCIENCE

Practical applications of clinical and basic science genetics to reproductive medicine

Munich, Germany
29 June 2014

Organised by
American Society for Reproductive Medicine (ASRM)

Contents

Objectives

Couples presenting for reproductive care may be at risk for disorders that either are predictable or are yet unknown. Others present with genetic disorders of reproduction that have implications for their care and potentially their children. Finally for others, reproductive technology may provide new risks. This pre-congress course will focus on contemporary genetic issues that are encountered in the practice of reproductive medicine. Topics will include screening for genetic disease, identifying genetic causes of reproductive failure, use of assisted reproductive technologies (ART) for treatment of individuals with reproductive genetic disorders, and potential adverse genetic outcomes of ART.

Learning objectives

At the conclusion of this course, the participant should be able to:

1. Propose appropriate genetic testing for couples presenting with infertility
2. Discuss adverse genetic outcomes of ART
3. Counsel patients with Turner syndrome and Klinefelter syndrome about reproductive options and outcomes
4. Screen for genetic causes of reproductive failure

Target audience

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

Scientific programme

Chairman: Rebecca Z. Sokol - U.S.A.

09:00 - 09:30	Screening couples for genetic disease presenting for reproductive care. Screening for at-risk and not at-risk individuals ***Joe Leigh Simpson - U.S.A.***
09:30 - 09:45	Discussion
09:45 - 10:15	Genetics of premature ovarian insufficiency ***Marcelle Cedars - U.S.A.***
10:15 - 10:30	Discussion
10:30 - 11:00	Coffee break
11:00 - 11:30	Epigenetic modifications due to environmental factors ***Linda C. Giudice - U.S.A.***
11:30 - 11:45	Discussion
11:45 - 12:15	Genetic and epigenetic factors affecting embryo development and implantation ***Christos Coutifaris - U.S.A.***
12:15 - 12:30	Discussion
12:30 - 13:30	Lunch break
13:30 - 14:00	Turner Syndrome: Reproductive options and outcomes ***Richard Reindollar - U.S.A.***
14:00 - 14:15	Discussion
14:15 - 15:00	Klinefelter Syndrome: Reproductive and hormonal options and outcomes ***Rebecca Z. Sokol - U.S.A.***
15:00 - 15:15	Discussion
15:15 - 15:30	Coffee break
15:30 - 16:00	Sperm aneuploidy and ART ***Dolores J. Lamb - U.S.A.***
16:00 - 16:15	Discussion
16:15 - 16:45	Imprinting disorders and ARTc ***Joe Leigh Simpson - U.S.A.***
16:45 - 17:00	Discussion

Screening couples for genetic disease presenting for reproductive care. Screening for not at-risk and for at-risk individuals.

JOE LEIGH SIMPSON, M.D., FACOG, FACMG

Senior Vice President
for Research and Global Programs
March of Dimes Foundation, New York, USA

President, International Federation Fertility Societies (IFFS)

ESHRE 2014

Educational Objectives

- **Be able to state single gene disorders for which heterozygote screening must be offered to all couples**
- **Be able to explain to patients the rationale for undergoing PGD aneuploidy testing (PGS)**
- **State sensitivity of detecting trisomy 21 and 18 on the basis of cell free fetal DNA in maternal blood**

SCREENING DURING PREGNANCY

- **Screening <u>asymptomatic</u> individuals to detect couples at risk *(two heterozygotes for same mutant allele).* Appropriate only if disorders are relatively common, usually in a given ethnic group**
- **Screening ≠ Testing**

PREGNANCY SCREENING TRADITIONAL RECOMMENDATIONS

Blacks: Sickle Cell disease
Jewish: Tay-Sachs disease
Other
Italian and Greek: β-thalassemia
Asian: α-thalassemia
All: Cystic fibrosis, Spinal muscular atrophy (SMA)

ETHNIC DIFFERENCES: HETEROZYGOSITY IN THE ASHKENAZIM (2009)

Tay-Sachs	1/30	} 1/6	} 1/4
Gaucher	1/13		
Cystic Fibrosis	1/25		
Canavan	1/40		
Nieman-Pick	1/90		
Familial Dysautonomia	1/30		

2009: DNA testing

CYSTIC FIBROSIS

United States:

Affected	30,000
Carriers	8,000,000
• Caucasians	1/2,500
• African-Americans	1/18,000
• Asian-Americans	1/90,000

CYSTIC FIBROSIS TRANSMEMBRANE GENE: REGULATOR *(CFTR)*

- **Gene spans 250 kb**
- **27 exons**
- **Mature mRNA 6,500 bases**
- **Encodes a chloride ion channel of 1,480 amino acids *(CFTR)***
- **Three-nucleotide deletion of codon 508 (*phenylalanine*) in 70% Caucasians: ΔF508**

RECOMMENDED CORE MUTATION PANEL FOR GENERAL POPULATION CF CARRIER SCREENING *(2001)*

Standard Mutation Panel

ΔF508	ΔI507	G542X	G551D	W1282X	N1303K
R553X	621+1G>T	R117H	1717-1G>A	A455E	R560T
R1162X	G85E	R334W	R347P	711+1G>T	1898+IG>A
2184delA	1078delT	3849+10kbC>T	2789+5G>A	3659delC	I148T
3120+1G>A					

Grody WW, et al., Genet Med. 3:149-154, 2001

HETEROZYGOTE FREQUENCIES *(CF)* BY ETHNIC GROUP

Ethnic Group	Heterozygote Frequency	% of Heterozygote Detectable	Likelihood of being Heterozygote Despite Negative Screen
Ashkenazi Jewish	1/24	94%	1 in 400
European Caucasian	1/25	88%	1 in 208

HETEROZYGOTE FREQUENCIES *(CF)* BY ETHNIC GROUP

Ethnic Group	Heterozygote Frequency	% of Heterozygote Detectable	Likelihood of being Heterozygote Despite Negative Screen
African American	1/65	65%	1 in 186
Hispanic American	1/46	72%	1 in 164
Asian American	1/94	49%	1 in 184

WHY NOT SCREEN YOUR GENOME?

Genome Wide Screening for Disease Susceptibility

1. **Identify mutations**
2. **Identify disease-associated alleles**
3. **Cumulative calculation of risks. Odds ratio based on disease-associated alleles**

Genome Wide Screening for Disease Susceptibility

Sample Results

- Prostate cancer without family history 5 x
- Prostate cancer with family history 9 x
- Colon Cancer 2 x
- Resistance or sensitivity to warfarin none
- Type II Diabetes 2 x↓
- Huntington Disease not present
- Alzheimers (APOε4; +/- family history 3 x

SCREENING FOR CHROMOSOMAL ABNORMALITIES

DOWN SYNDROME DETECTION THROUGH NONINVASIVE SCREENING

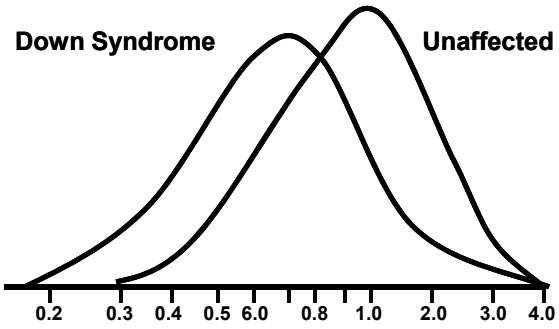
- Second trimester:
  - Three analytes ~ 69%
  - Four analytes ~ 81%
- First trimester *(11 weeks)* ~ 87%
- First trimester *(12 weeks)* ~ 85%
- First plus second ~ 92-95%

(contingent, stepwise, integrated)

5% procedure rate (false positive)

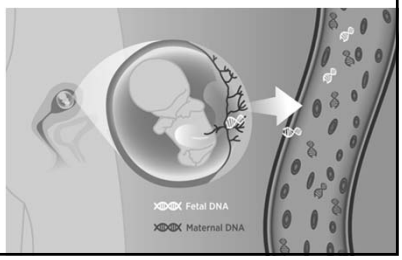
Malone, NEJM, 2005

MoM DISTRIBUTION
Maternal Serum Alpha Feto Protein



CELL-FREE DNA IN MATERNAL BLOOD

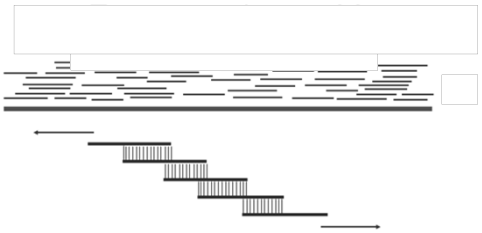
- Cell-free DNA (cfDNA) are short DNA fragments
- In pregnancy, cfDNA from both the mom and fetus are in maternal blood
- Amount of fetal cfDNA present is a small fraction of the maternal cfDNA



NEXT GENERATION SEQUENCING

- Many DNA fragments sequenced concurrently (massive parallel genomic sequencing) and compared to reference genome
- Cloned bacteria or yeast vectors unnecessary
- Completely automated and rapid / much lower cost

FRAGMENT ASSEMBLY

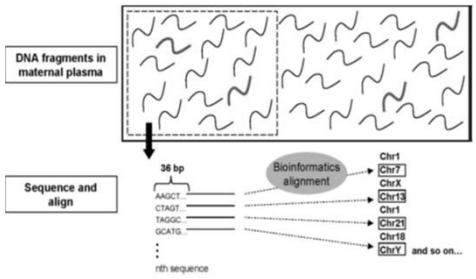


- Overlap reads and extension to reconstruct the original genomic region
- Given reference genome now known, comparison can be done directly to sample

CELL FREE FETAL DNA FOR ANEUPLOIDY DETECTION

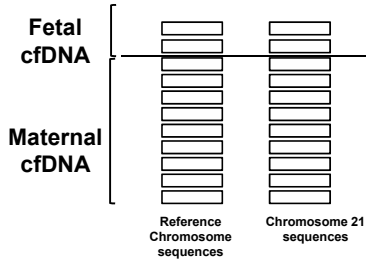
- Strategy: Increased trisomy 21 transcripts (maternal and fetal) in maternal blood of trisomic pregnancies compared to maternal blood of euploid (normal) pregnancies. Massive Parallel Genomic Sequencing (MPGS) [Massive Parallel Shotgun Sequencing – MPSS]
- Quantitative rather than qualitative difference must be shown for numbered transcripts.

Assessing Fetal 21 Transcripts by Parallel Genomic Sequencing (maternal and fetal transcripts)



Chiu, Lo, PNAS, 2008.

Fetal Trisomy Detection With cfDNA

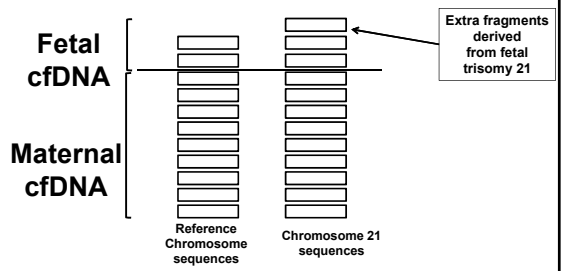


- Each bar represents thousands of cfDNA fragments
- Counting of chromosome cfDNA fragments done by DNA sequencing

Aneuploidy Detection

- Determine total chromosome 21 transcripts (maternal and fetal) in trisomic and non-trisomic pregnancy
- If 5% of cell free DNA in maternal blood is fetal, trisomic pregnancies should have 2.5% greater 21 transcripts than normal pregnancies

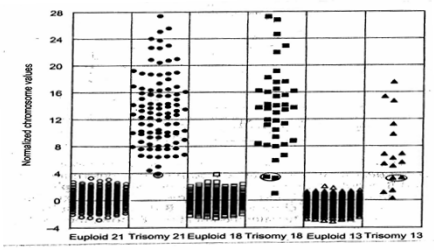
Fetal Trisomy Detection With cfDNA



- The overabundance of chromosome 21 cfDNA fragments in trisomy 21, although small, can be measured with DNA sequencing

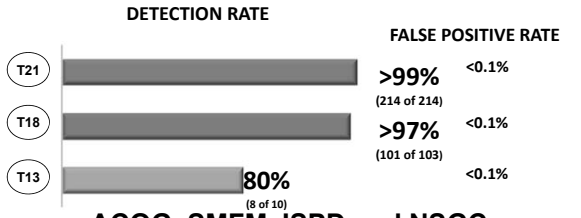
DETECTION OF TRISOMIES (Verinata)

MPGS: (MELISSA:Verinata) Massively parallel sequencing normalized chromosome values compared with karyotype classifications for chromosomes 21, 18, and 13. Circles display classifications for chromosome 21, squares display classifications for chromosome 18, and triangles display classifications for chromosome 13. Unclassified samples with trisomy karyotypes have been circled. Bianchi. Genome-Wide Fetal Aneuploidy Detection. Obstet Gynecol 212.



CLINICAL PERFORMANCE

Studied in over 6,000 patients, including >2,000 average-risk women



ACOG, SMFM, ISPD and NSGC recommend use in high-risk pregnancy

1. Sparks, A.B., Wang, E.T., Struble, C.A., Barrett, W., et al. Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy. Prenat Diagn (2012);32(1):3-9. doi: 10.1002/pd.2922. Epub 2012 Jan 6.; 2. Ashoor, G., Syngelaki, A., Wagner, M., Birdir, C., Nicolaides K.H., Chromosome-selective sequencing of maternal plasma cell-free DNA for first trimester detection of trisomy 21 and trisomy 18, *Am J Obstet Gynecol.* (2012), doi: 10.1016/j.ajog.2012.01.029.; 3. Sparks, A.B., Wang, E.T., Struble, C.A., Barrett, W., et al, Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy. *Prenat Diagn* (2012);32(1):3-9. doi: 10.1002/pd.2922. Epub 2012 Jan 6.; 4. Norton, M., Brar, H., Weiss, J., Karimi, A., et al. Non-Invasive Chromosomal Evaluation (NICE) Study: Results of a Multicenter, Prospective, Cohort Study for Detection of Fetal Trisomy 21 and Trisomy 18, *Am J Obstet Gynecol.* (2012), doi:10.1016/j.ajog.2012.05.021.; 5. Nicolaides KH, Syngelaki A, Ashoor G, et al. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. Am J Obstet Gynecol 2012;207:374.e1-6.; 6. Ashoor G, Syngelaki A, Nicolaides KH, et al. Trisomy 13 detection in the first trimester of pregnancy using a chromosome-selective cell-free DNA analysis method, *ULTRASOUND Obstet Gynecol*, (2012), DOI: 10.1002/uog.12299.

Preimplantation Genetic Diagnosis (PGD): Testing

- **When is PGD applicable?**
  - **single gene**
  - **chromosomes**
- **What pitfalls exist when applying new technologies to PGD?**

Time To Achieve Pregnancy Without PGD

(Balanced Translocation Heterozygotes)

	Cumulative Live birth rate	Time to pregnancy
Sugiura-Ogasawa (2004)	68%	- 16 year follow up
Goddijn (2004)	70%	- 6 year (mean)
Stephenson & Sierra (2006)	71%	- 4 year (mean)

PGD recommended given time to achieve pregnancies naturally. ASRM/SART Practice Committees ***Fritz & Schattman, Fertil Steril 90: 892, 2008.***

REDUCTION IN MISCARRIAGES IN RECURRENT PREGNANCY LOSS

maternal age	cycles	☆% loss expected	% loss after PGD (FISH)	
<35	85	26%	13%	p=0.09
≥35	143	39%	13%	p<0.001
Total	228	33%	13%	p<0.001

☆Logistic regression that takes into account maternal age, number, prior abortions

Brigham formula Hum Reprod 14: 2868, 1999

Munné et al. (2008) ASRM abstract and Fisher et al. (2010)

PDG Aneuploidy Testing (PGS) Methods of Analysis: 2013

Stage:

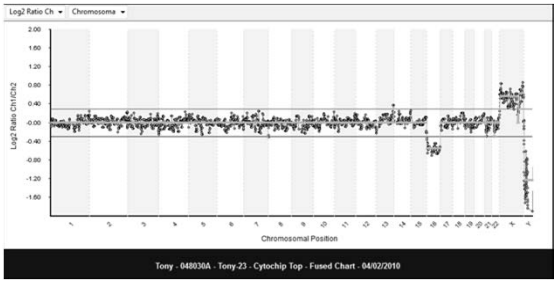
Trophectoderm biopsy (5-10 cells) at 5-6 days or polar body biopsy, but not cleavage stage embryo at 3 days.

Analysis:

Array Comparative Genome Hybridization (Array CGH)

- **All 24 chromosomes**

Array CGH and Missing Chromatid 16 (Polar Body)



PGD Aneuploidy Testing Using Array CGH

- Technically less demanding than FISH and less subjective
- Accumulating data show increased pregnancy rates using array CGH

	Blastocyst Transfers	
	Array	No array
Schoolcraft, 2012	60.8%	40.9%
Yang, 2012	69.1%	41.7%

RCT: Advanced Maternal Age

(Rubio et al., 2013)

- Maternal age 41 – 44 years
- Day 3 FISH: 13, 15, 16, 17, 18, 21, 22, X, Y
- Day 5 transfer

	PGD	No PGD
Livebirth Rate:	30/93 (32.3%)	12/43 (15.5%)
Odds Ratio:	2.585 [CI 1.26-5.29]	

Single Embryo (Array CGH) vs Double Embryo (Morphology)

	N	Preg.	Twins
Single Embryo, Array CGH	89	60.7%	0
Two Embryos, Morphology	86	65.1%	53.4%

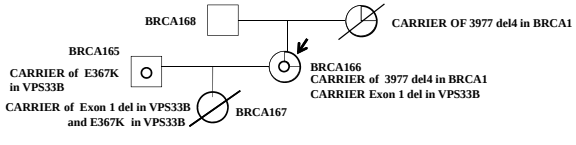
Forman, et al, 2013

PGD Testing for Single Gene Disorders

- At risk for Autosomes Dominant for Autosomal Recessive Disorder
- Single gene disorders in which nondisclosure desired (adult onset disorders like Huntington disease). Avoids parent learning their own genotype while also avoids transmitting (if present) to their offspring.
- Very high risk – if 2 different disorder in kindred; successive terminations likely statistically.

BRCA 1 AND ARTHROGRYPOSIS (ARC)

06/ 2013



ARC, Arthrogryposis, renal, cholestasis (VPS 33B)

BRCA1 AND ARC

	Mode	Affected
BRCA1	Dominant	50%
ARC	Recessive	25%
Total Abnormal Conceptions		62.5%

PGD FOR STEM CELL TRANSPLANTATION

Genetic Disorders *(25% risk)*

- β-thalassemia; Fanconi anemia
  - Non-functioning bone marrow
  - Older affected case treated with stem cells from umbilical cord blood of newborn sibling
  - HLA match ~95% successful
  - PGD Results (Turkey) 40/44 successful (kahraman)
  - Non HLA match ~60% successful
  - Impractical to expect genetically normal embryo that is also HLA compatible

LIKELIHOOD OF TRANSFERRABLE EMBRYO

Autosomal Recessive

HLA Compatible = 1/4
Normal (Autosomal Recessive): 3/4
Thus, 1/4 • 3/4 = 3/16

Approach: PGD with transfer embryos both HLA compatible and lacking mutation in homozygous form

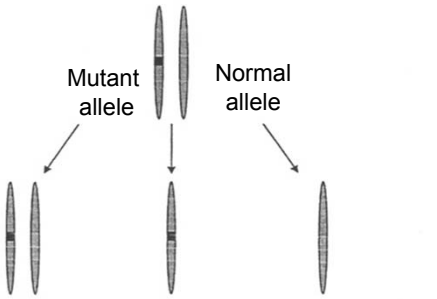
REPRODUCTIVE GENETICS INSTITUTE (Chicago)

- **Single gene cases >3300**
- **Pregnancy rate 30-35%**
- **Diagnostic errors 3 (liveborns or prenatal samples)**

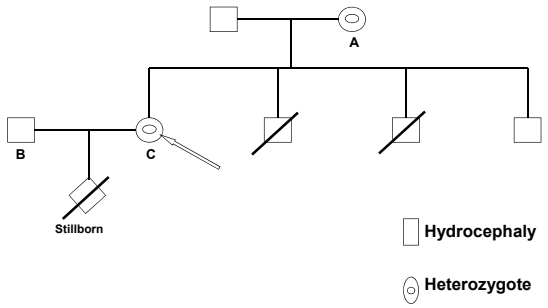
WHOLE GENOME AMPLIFICATION

- **Single cell 6 pg DNA**
- **Must be amplified (polymerase chain reaction, PCR) may allow assay.**
- **Efficiency not 100%.**
- **Pitfall: Allele drop out reflecting less than 100% amplification.**

Clinical Consequences of Allele Drop Out in Heterozygous Cell



X-Linked Hydrocephaly



STR markers initially tested and determined by PCR: Phase Unknown

Markers order	B	C	A
DXS8086	239	241, 241	241, 241
DXS8069	115	117,121	117, 117
DXS7423	138	147, 152	147, 147
DXS9929	131	131, 140	131, 131
DXS8103	138	143, 143	143, 143
DXS8061	119	117, 119	119, 111
DXS8087	154	156, 158	158, 158
Mutated gene	N	G452R, N	G452R, N
DXS1073	202	202, 193	193, 199
F8 Intron 1A	112	121, 112	114, 121
F8 Intron 9A	204	206, 206	206, 206
F8 Intron 13(CA)	97	97, 106	106, 105
DXYS154	176/182	175, 178	178, 178
SRY	Y	-	-

Determining Phase of Linked Markers

Red colored alleles are linked to the mutant allele
Black colored alleles are linked to the normal allele
N= normal

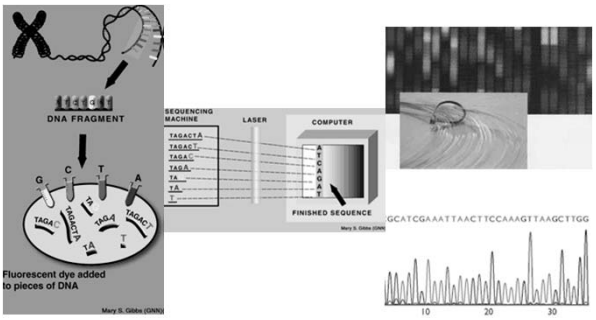
Markers order	B	C	A
DXS8086	239	241/241	241/241
DXS8069	115	117/121	117/117
DXS7423	138	147/152	147/147
DXS9929	131	131/140	131/131
DXS8103	138	143/143	143/143
DXS8061	119	119/117	119/111
DXS8087	154	158/156	158/158
Mutated gene	N	G452R/N	G452R/N
DXS1073	202	193/202	193/199
F8 Intron 1A	112	121/112	121/114
F8 Intron 9A	204	206/206	206/206
F8 Intron 13(CA)	97	106/97	106/105
DXYS154	176/182	178/175	178/178
SRY	Y	-	-

Diagnosis by Linked Markers (STRs): Blastomere analysis for X-Linked Hydrocephaly

Marker	Blastomere1	Blastomere 2	Blastomere 3	Blastomere 4	Blastomere 5	Blastomere 6	B	C	A
DXS8069	115/121	115/117	115/121	115/117	117	121	115	117/121	117/117
DXS7423	138/152	138/147	138/152	138/147	147	152	138	147/152	147/147
DXS9929	131/140	131	131/140	131	131	140	131	131/140	131/131
DXS8061	119/117	119	119/117	119	119	117	119	119/117	119/111
DXS8087	154/156	154/158	154/156	154/158	158	156	154	158/156	158/158
Mutated gene	N/N	N/G452R	N/N	FA	G452R	FA	N	G452R/N	G452R/N
DXS1073	202	202/193	202	202/193	193	202	202	193/202	193/199
F8 Intron 1A	112	112/121	112	112/121	121	121	112	121/112	121/114
F8 Intron 13(CA)	97	97/106	97	97/106	106	106	97	106/97	106/105
DXYS154	182/175	176/178	176/175	182/178	178	178	176/182	178/175	178/178
SRY	-	-	Y	-	Y	Y	Y	-	-

Red colored alleles are linked to the mutant gene
Black colored alleles are linked to the normal gene
N= normal
FA= Failed amplification

Next Generation Sequencing (automated)



Next Generation Sequencing (Pitfalls)

- **Expectation that 98% alignment with reference genome. Thus, "allele dropout" still could exist.**

<u>Solutions</u>

1. **Increased amplification polymerase chain reaction PCR upon amplification can introduce errors, creating and thus masquerading mutations if in a high proportion reads. Thus, increasing number of PCR cycles no panacea.**
2. **Linked markers still needed until clinical series proves otherwise.**

Other Limitations using NGS

- Unable to test for dynamic mutations trinucleotide repeats (fragile X; myotonic dystrophy)
- Unable to take into account pseudogenes (e.g. 21-hydroxylase adrenal hyperplasia)

CONCLUSION (1)

1. All couples should be offered DNA heterozygote screening for single gene disorders, the precise disorders depending on ethnic backgrounds
2. Universal screening to identify pregnancies at risk for aneuploidy is recommended, now using cell free fetal DNA in maternal blood

CONCLUSION (2)

3. PGD Aneuploidy Testing for chromosomal abnormalities has been proved efficacious, using array CGH
4. PGD to detect single gene disorders is accurate and can be applied widely, but must use linked markers given allele drop out

Genetics of Primary Ovarian Insufficiency (POI)

Marcelle I. Cedars, M.D.
Professor and Director
Division of Reproductive Endocrinology and Infertility
UCSF

Primary ovarian insufficiency (POI) Definition

- Age < 40 years
- Amenorrhea > 4 months
  - after normal cycles
- FSH in menopausal range x 2
  - at least 1 month apart
- 1% of women under the age of 40
- Varying and unpredictable
  - 50% may cycle again
  - 5-10% conceive and delivery

POI: Etiology

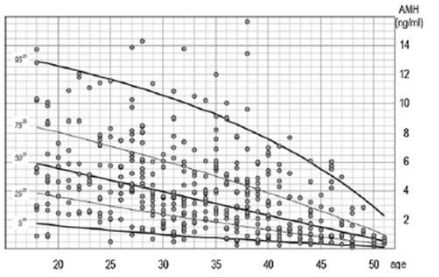
- Up to 90% - no immediate etiology identified
- Follicular dysfunction
  - e.g. FSH receptor mutation
- Follicular depletion

- Iatrogenic
- Auto-immune
- Genetic

POI: Diagnosis

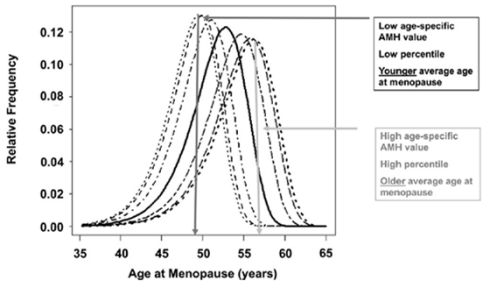
- On average, women with a diagnosis of POI have seen 3 physicians prior to diagnosis!
- Differential diagnosis for secondary amenorrhea
  - Polycystic ovary syndrome
  - Hypogonadotropic, hypogonadism
  - Hyperprolactinemia
  - Hypergonadotropic, hypogonadism - Primary ovarian insufficiency

POI: can we predict? Normative AMH data



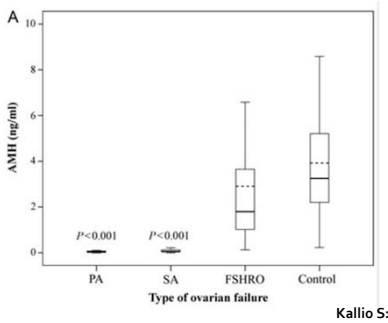
LaMarca A; European J Obstet Gynecol & Reprod Biol 2012

Impact of AMH on age at menopause



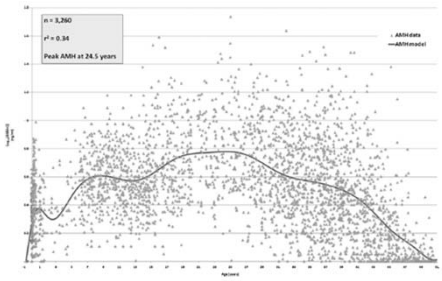
Broer SL, J Clin endocrinol Metab 2010

POI: Anti-mullerian hormone (AMH)



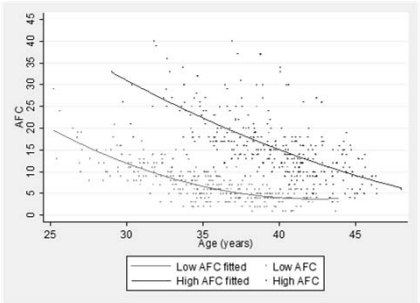
Kallio S: Hum Reprod 2012

AMH: conception to menopause

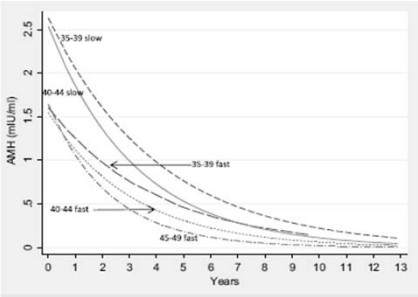


Kelsey TW: PLoS ONE 2011

POI: can we predict it?



Rate of change – AMH and time to menopause



Freeman EW, Fertil Steril 2012

Why search for genes in POI?

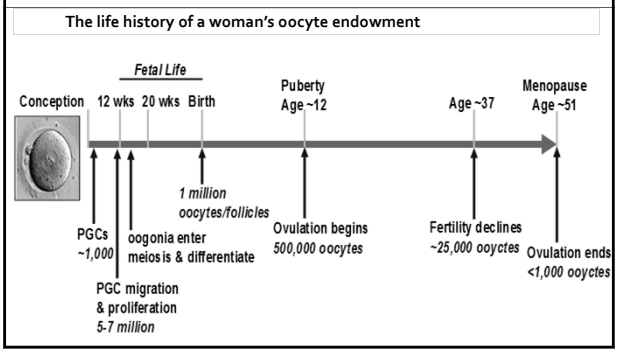
- Identification of causative genes would allow:
  - Prospective identification of at-risk women
  - Prospective counseling regarding fertility chances with aging to allow reconsideration of family planning
  - Counseling regarding fertility preservation – oocyte/ovarian cryopreservation
  - Testing of other female relatives

Natural menopause

- Average age 51.4 years
- Heritability 30-85%

- 15-30% of POI is familial

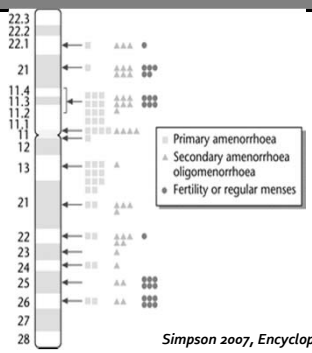
Reproductive Aging - Quantity



Genetic syndromes

- Genetic Syndromes associated with general aging
  - Fanconi Anemia (*FANCA*)
  - Werner syndrome (*WRN*)
  - Bloom syndrome (*BLM*)
  - Ataxia telangiectasia syndrome (*ATM*)
- X chromosome imbalances
  - Including Fragile X

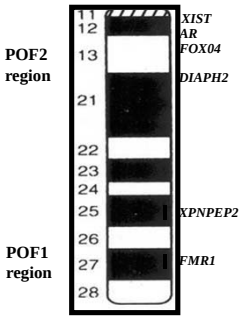
X chromosome



Simpson 2007, Encyclopedia of Life Sciences

X chromosome: long arm

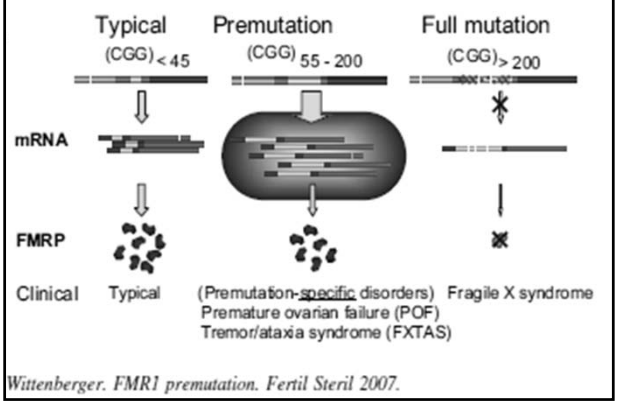
- POF2 (proximal) & POF1 (distal) often invoked but at arguable validity.
- "POF2" extends from Xq 13.3 to 21.1 and harbors *DACH2, DIAPH2 and POF1B.*
- POF1 extends from Xq26-q28 and is home to *XPNPEP2* on Xq25 and the *FMR1* gene (Xq27.3)



X-linked genes

PGRMC1 Xp22	1/67 (1.5%) Swedish and Italian
(Progesterone Receptor Membrane Component)	
AR Xq12 (Androgen Receptor)	2/100 (2%) Indian
FOX04 Xq11.3	0/116 Tunisian
DACH 2 Xq21.3	2/257 (0.8%) Italian

Expression of Fragile X gene FMR-1

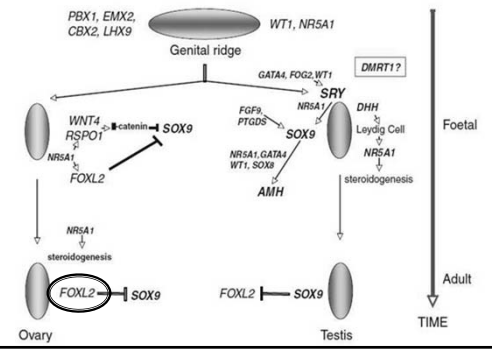


Wittenberger. FMR1 premutation. Fertil Steril 2007.

FMR1 Xq27 and POF

- Premutation (55-200 CGG Repeats) may expand into full mutation (>200 repeat)
- 10-15% of premutation carriers manifest POF.
- Premutation occurs in 1-5% sporadic POF and 10-15% familial POF.
- Prevalence of POF increases as $(CGG)^n$ increases to 80-99, but plateaus thereafter. POF not increased in full mutation.

Gonadal differentiation



Blepharophimosis-Ptosis-Epicanthus - BPES

- FOXL2 is a master regulatory of ovarian differentiation and primordial follicle activity, suppressing SRY.
- Expressed in human eyelids and oocytes in humans.
- Mutations in Forkhead Transcription Factor (Forkhead bOX) explain BPES.
- FOXL2 mutations uncommon in isolated POF.

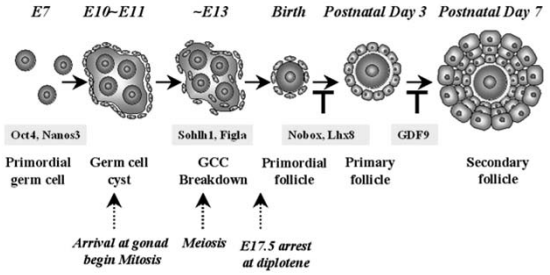
Forkhead transcription factors and non-syndromic POF

FOXL2 (3q23)	0/118 0/80 0/120	Chinese Indian Italian
	2/70 (2.9%)	New Zealand; Slovak

Other forkhead transcription factors

FOX01 (13q14.1)	1/90 (1.1%)	New Zealand; Slovak
FOX03 (6q21)	4/302 (1.3%) 15/114 (13.2%) 3/150 (2%)	European Chinese French
	2/60 (3.3%)	New Zealand; Slovak
FOX04 (Xq13.1)	0/116	Tunisian

Genes expressed in early mouse oogenesis



Collaborative studies: Shandong University, Baylor College of Medicine, Florida International University

Drs. Qin, Chen, Simpson, Rajkovic, Kovanci, Zhao (2005)

FIGLA

- *Figla* knock-out mice
  - Normal germ cell migration and proliferation
  - No primordial follicle development
  - Loose all oocytes shortly after birth
  - Regulates zona pellucida glyco-proteins
- Haplo-insufficency has been show to cause accelerated oocyte loss in humans

Zhao, Chen, Qin, Shi, Wang, Choi, Simpson, Rajkovic,2008

SOHLH

- Represses primordial follicle activation
- SOHLH 1 – induces oocyte specific genes including *Zp1 and Zp3*
- SOHLH 1 and 2 regulate *kit and gdf-9*
- SOHLH 1 and 2 regulate *Lhx8* and NOBOX

Newborn Ovarian Homebox (NOBOX)

- Homeobox transcriptional regulator binds TAATTG, including GDF9 (member of transforming growth factor gene family)
- Murine knockouts lack follicles
- Expressed in human oocytes from primordial follicle through metaphase II

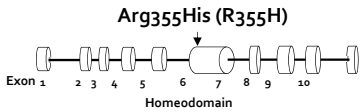
NOBOX in humans

96 U.S. Caucasian women, 2 missense mutations (Arg 355 His; Arg 360Alu)

Not present in 96 Caucasian controls

Both mutations in conserved region-functional studies

Arg 355 His disrupts binding DNA and, hence, dominant negative effect.



Qin, Choi, Zhao, Simpson, Chen, Rajkovic. Amer Jour Hum Genet 81:576-581, 2007

KIT

- LHX8, SOHLH1 and SOHLH2 upregulate *Kit* expression, as deficiency in these transcriptional regulators cause a severe down-regulation in *Kit* expression
- Point mutations (in mice) have fewer germ cells and depletion of germ cells shortly thereafter
- Limited human data

ATK1

- Promotes primordial follicule activation
- Acts through MTOR – a mechanistic target of rapamycin
- Potential for therapeutic intervention
  - Concern: ubiquitous nature of this pathway
- No human correlate with POF identified

FOX 03

- FOXO3 represses primordial follicle activation and its absence leads to widespread activation
- Mice lacking *Pten*, a negative regulator of AKT1, have elevated FOXO3 phosphorylation, primordial follicle activation
- FOXO3 was found to act upstream of galactose-1-phosphate uridylyltransferase (*Galt*)

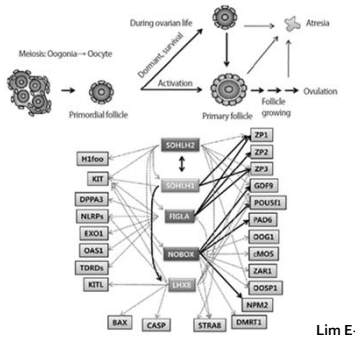
Oocyte-specific transcription factors

NOBOX 7q35	0/200 Chinese 0/30 Japanese 1/96 (1%) Caucasian 12/178 (6.7%) Mixed: (Caucasian, Sengalese, Bauta)	
FIGLA 2p13.3	2/100 (2%) Chinese	
POU5F1 6p21.31	1/115 (0.9%) Chinese	
(Downstream target of NOBOX)		
LHX8 1p31.1	0/95 Chinese	

Transforming growth factors

TGFBR3 1p33 (Receptor)	2/112 (1.8%) 1/133 (0.8%) 0/54	Chinese Indian New Zealand
GDF9 5q31.1 Dimerizes with BMP15	1/200 (0.5%) 6/127 (4.7%) 2/284 (0.7%)	Chinese Indian Caucasian (mostly)
NOG 17q22 (TGF Antagonist)	1/100 (1%)	French

POI: the role of transcriptional factors



Lim E-J: Clin Exp Reprod Med 2012

Steroid and related pathway genes

PGRMC1 (Progesterone Receptor Membrane Component 1)	Xp22	1/67 (1.5%)	Swedish; Italian
GPR3	1p36.1	2/100 (2%)	Chinese
NR5A1 (SF1)	9q33	0/82	Caucasian
Reduces CYP11 and CYP 19		5/356 (1.4%)	Asian, Caucasian, Mediterranean
		1/81 (1.2%)	Tunisian
		3/180 (1.7%)	French
		2/28 (7.1%)	Indian, Senegalese, African

Conclusions: candidate gene searches

- Genetic heterogeneity (many different genes). Varies in frequency by ethnic group and usually infrequent if present (1 or 2%)
- Approximately 25% of non-syndromic POF have identified perturbations

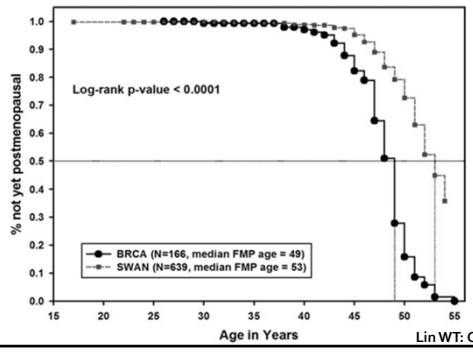
Conclusions: candidate gene searches

- Molecular heterogeneity: different nucleotide alterations within same gene
- Multiple molecular mechanisms can be causative but involvement of DNA binding and transcription factors pivotal

POI: BRCA 1 and 2

- DNA repair genes
- Women with known mutations have increased risk for breast and ovarian cancer –
- BUT – also primary ovarian insufficiency
- Increased risk for DNA damage
- Accelerated loss

BRCA 1/2 and menopause



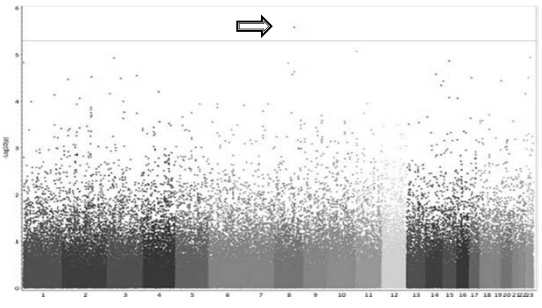
Lin WT: Cancer 2013

Genome-wide association study

- 800 POF cases with FSH levels > 40 MIL/ml. Case control design
- Two independent replicates
- 8q22.3, most significant ($p < 10^{-5}$ to 10^{-6})

Qin, Zhoo, Xu, Shi, Lu…Simpson, Chen, Hum Molec. Genet 21:430, 2012

Genome-wide association study for POF (Negative log10 unadjusted P-values)



8q22.3 and POF

- "Gene desert" region. No genes detected using repository with location of all known genes.
- Significance $<10^{-8}$, but still could connote important ovarian regulatory region. Analogous findings in male sex-determining region upstream of SOX9 (17q24).
  - Deletion in XY individuals → female
  - Duplication in XX individuals → male (testes)

Non-coding DNA and enhancers

- Vast majority (98.5%) of the human genome does not code for proteins.
  - Non-coding regions could be important for structural integrity.
  - Non-coding regions contain stretches of DNA that bind proteins and RNA molecules, bringing them into juxtaposition to cooperatively regulate function and level of expression of other (protein-coding) genes

Non-coding DNA: promoters and enhancers

- >200,000 enhancers (DHSs) identify sites of DNA binding for transcription. Enhancers identified as DNase I hypersensitive sites (DHSs) readily accessible to enzymatic cleavage as result of the displacement of nucleosomes
- Transcription factors sculpt chromatin landscape for generation of organ-specific mRNA → gene products.
- Cell-type-specific enhancers are often located far away from promoters

POI: future investigations mi RNA

- miRNAs are short (22 nucleotides), non-coding, endogenous RNA molecules that regulate gene function
- 1/3 of human genes have conserved miRNA targets
- Recent study identified a SNP associated with POF

Rah HC: Menopause 2013

Conclusions

- POF genes with mutations detected number ~ 20, explaining ~ 25% of cases.
- Heterogeneous genetically. For any given gene, only 1-2% of POF cases explained, save fragile X premutation (FMR1).
- Additional genetic explanations for POF will become evident as different ethnic groups and different candidate genes are sequenced.

Conclusions (cont)

- Genes responsible for ovarian failure not necessarily predictable based on known endocrine function.
- Whole genome association studies and whole exome/genome sequencing will reveal novel genes not imagined at present.
- Perturbation in regulatory regions (promoters; enhancers) likely to explain other cases and 8q22.3 locus.

Current evaluation for genetic etiologies – non-syndromic

- Family history
- Fragile X
- Karyotype

- Microarray analysis to detect submicroscopic chromosome abnormalities
- Whole exome/genome sequencing

Epigenetic Modifications Due to Environmental Factors

ASRM-ESHRE Exchange Pre-Congress Course 8:
Practical Applications of Clinical and Basic Science Genetics to Reproductive Medicine

Linda Giudice, MD, PhD
University of California, San Francisco

29th Annual Meeting, ESHRE
Munich
June 29-July 2, 2014

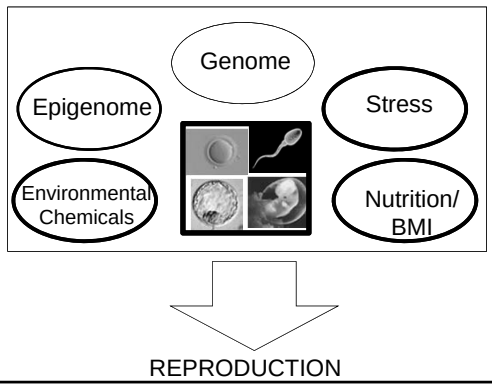
Disclosures

- Quest Diagnostics, Academic Associate
- American Society for Reproductive Medicine, President 2012-1013
- World Endometriosis Society 2014, President
- World Endometriosis Research Foundation Board of Directors, 2011-2014
- March of Dimes Scientific Advisory Board, Member, 2013-2016
- Low Cost IVF Foundation, Board Member, 2011-2015

Learning Objectives

- Review determinants of reproduction
- Understand what epigenetics is
- Learn about the evidence linking environmental factors and epigenetics to health that can affect reproduction and reproductive processes directly

Determinants of Reproduction



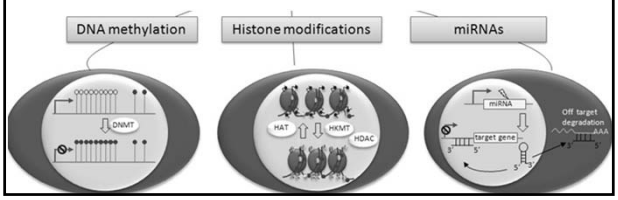
The Epigenome

"epi" (Gr.(επι) over, outside of, around)

- Epigenetics is the study of heritable changes in gene expression ***not*** caused by changes in the DNA sequence.
- Jean Baptiste Lamarck (1744-1829) formulated the theory that the environment shapes genes and these changes are passed on to offspring.
- Charles Darwin (1809-1882) postulated that genes are not changed by the environment and formulated natural selection.
- Word coined by G.H. Waddington 1942

The Epigenome

- Mechanisms
  - DNA methylation and chromatin remodeling
  - Histone modifications and chromatin remodeling
  - Small interfering RNAs (e.g., miRNAs)



Epigenetics and Biological Processes

- Reprogramming in early development
- X-inactivation
- Cancer
- Obesity
- Biobehavioral
- Reproduction

- Mostly DNA methylation at CpG sites investigated
  CpG site methylation = usually gene repression
  CpG site demethylation = usually gene expression

Developmental origins of health and disease (DOHaD)

Human Disease Trends

- Over the past 50-100 years marked changes in chronic diseases and changes in reproductive potential have been observed, including:
  - Increases in
    - obesity
    - diabetes
    - cancers (e.g., breast, prostate)
  - Declining age at puberty and menarche

Stress, Diet, Environmental Chemicals: Indirect Effects on Reproduction?

Critical Windows of Susceptibility

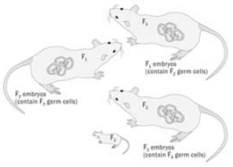
- Pre/peri-conception
- Prenatal
- Postnatal
- Childhood
- Adolescence
- Adulthood

- Considerations for chemical exposures
  - dose
  - duration
  - mixtures

Programming ("Barker Hypothesis"): developmental origins of adult health and disease. Process in which a stimulus or insult at a critical/sensitive period in development or perinatal life has permanent effects on structure, physiology, and metabolism and is ***transgenerational.***

Barker 1992

Some Definitions about Generations



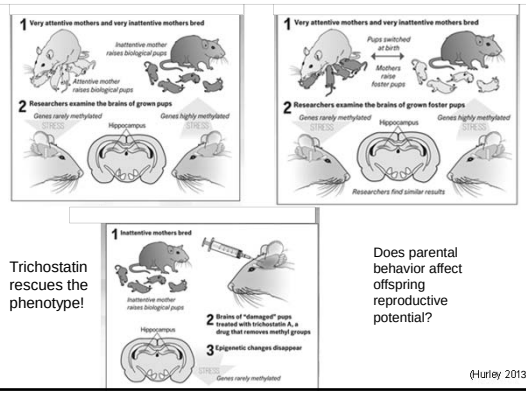
F0 generation = Parent

F1 generation = Fetus

F2 generation = Germline of fetus

F3 generation = Trans-generational (no direct exposure)

Maternal Stress Influences Fetal Brain Development via Epigenetic DNA Methylation



Trichostatin rescues the phenotype!

Does parental behavior affect offspring reproductive potential?

(Hurley 2013)

Obesity, Epigenetics, and Gene Regulation

The Agouti Mouse

Genetically identical mice but different color (brown, yellow) and size (normal, obese)

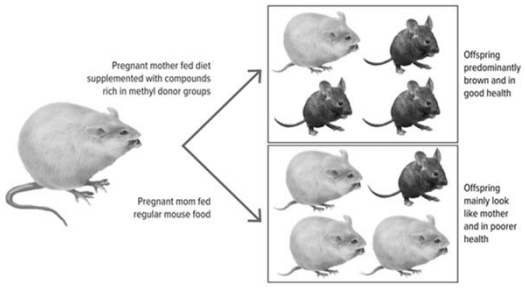
Agouti gene product binds to melanocortin R
- blocking black pigment
- involved in feeding behavior
- involved in weight set point

Normal healthy mice – agouti gene is methylated and is off.
Yellow, obese sisters – agouti gene is unmethylated and is on.
Higher risk of diabetes and cancer as adults.

(Dolinoey 2007)

Maternal Diet Influences Fetal Development

Changing Epigenetic Marks

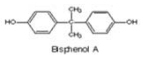


(Zeisel 2009)

Obesity, Epigenetics, and Gene Regulation

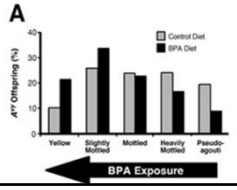
The Agouti Mouse

Environmental triggers



Bisphenol A

Bisphenol A (BPA) to pregnant dams:
- higher ratio of yellow, obese progeny than expected
- global DNA hypomethylation
- agouti gene DNA methylation sites 30% decrease



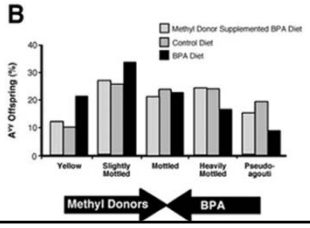
(Dolinoey 2007)

Obesity, Epigenetics, and Gene Regulation

The Agouti Mouse

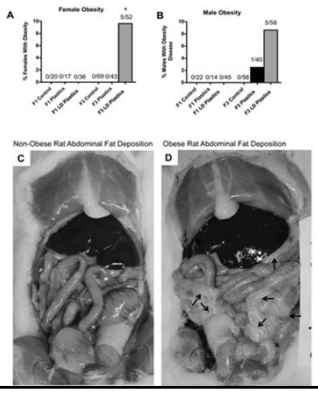
Environmental protection

Methyl donors (folic acid, Vit B12) rescue the phenotype
- increased DNA methylation
- normal ratio of pups



(Dolinoey 2007)

Obesity Develops in F3 Generation with F0 Generation Exposure to Plastics (BPA, Phthalates)



Reproductive outcomes depend on maternal obesity.

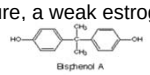
Manikkam PLoS One 2013

Environmental Chemicals: Direct Effects on Reproductive Processes

Germ cells migrate to the fetal ovary and initiate meiosis

Can chemicals affect early meiotic events?

- The only data comes from bisphenol A (BPA) exposure, a weak estrogen



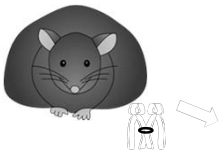
Bisphenol A

- BPA affects synapsis and recombination
  - increased oocyte loss
  - problems in creating chromosomally normal eggs

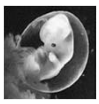
- Susiarjo et al., 2007

Maternal bisphenol A (BPA) exposure disrupts early meiotic events in the fetal ovary

- Susiarjo et al., 2007



Fetal BPA exposure increases the likelihood of chromosomally abnormal eggs and embryos in adult females



- A grandmaternal (F3) effect
  - DNA methylation?

with permission, Pat Hunt

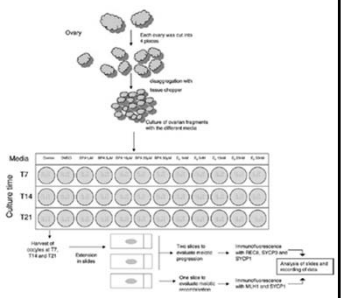
Meiotic progression and recombination are affected by Bisphenol-A during in vitro *human* oocyte development

Fetal ovarian follicles were incubated with BPA (1uM, 3-30uM) or E2 (1nM, 30nM)

Main findings BPA:
- decreased oocyte survival
- increased MLHI foci (cross-over marker)

Conclusion:
BPA can affect key events in meiotic prophase and oocyte survival in fetal ovary.

DNA methylation?



Brien-Enriquez 2011

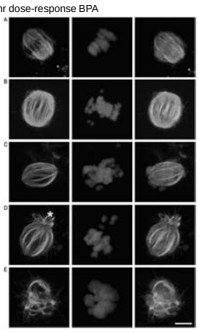
Bisphenol-A and *human* oocyte maturation *in vitro*

Discarded oocytes from IVF/ICSI 30 hr dose-response BPA

As BPA dose increased:
- decrease in % oocytes progressed to MII (P< 0.002)
- increase in % of degenerated oocytes (P< 0.01)
- Increase % oocytes that had undergone spontaneous activation (P< 0.007).

Among MII oocytes, as the BPA dose increased, there were:
- decreased incidence of bipolar spindles (P< 0.0001)
- Decreased incidence of aligned chromosomes (P< 0.02)

DNA methylation?



Machtingerd 2013

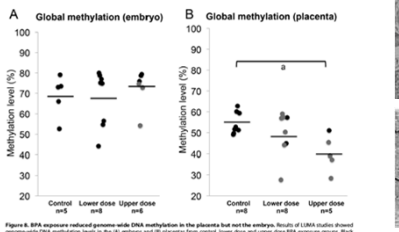
Bisphenol A Exposure Disrupts Genomic Imprinting

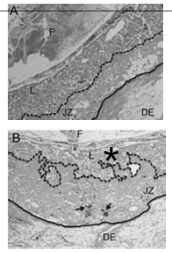
- Maternal BPA exposure during **late stages of oocyte development** and **early stages of embryo development** significantly disrupted imprinted gene expression in embryonic day (E) 9.5 and 12.5 embryos and placentas.
- Affected genes: Snrpn, Ube3a, Igf2, Kcnq1ot1, Cdkn1c, and Ascl2 (mutations and aberrant regulation of these genes are associated with imprinting disorders in humans).
- Exposure outside of the epigenetic reprogramming window did not cause significant imprinting perturbations.
- Are these changes associated with changes in DNA methylation?

Susiarjo 2013

BPA Exposure Reduces Genome-Wide DNA Methylation in the Placenta, Placentomegaly and Small Labyrinth

- Majority of affected genes were expressed abnormally in the **placenta** with DMRs including the Snrpn imprinting control region (ICR) and Igf2 DMR1.
- Exposure significantly reduced genome-wide methylation levels in the placenta, but not the embryo.





Susijaro et al 2013

Transgenerational Effects of Pesticides, EDCs, and Jet Fuel

Gestating F0 generation female rats exposed to “plastics (BPA, phthalates), EDCs, jet fuel during embryonic days 8-14 (gonadal sex determination). Incidence of adult onset disease was evaluated in F1 and F3 generation.

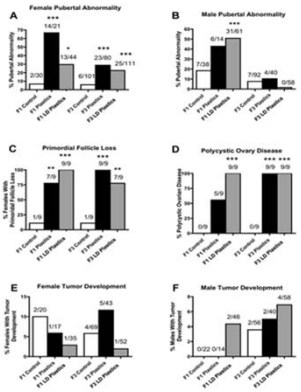
Findings:

- Significant increases in the incidence of total disease/abnormalities in F1 and F3 generation male and female animals from plastics lineages.
- Pubertal abnormalities, testis disease, obesity, and ovarian disease (primary ovarian insufficiency and polycystic ovaries) were increased in the F3 generation animals.
- Plastics lineage F3 generation sperm epigenome: 197 differential DMR (“epimutations”) in gene promoters correlating with identified pathologies.

Conclusion: A BPA/phthalate mixture can promote epigenetic trans-generational inheritance of adult onset disorders.

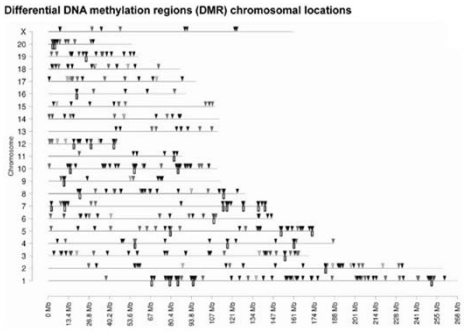
Mannikkam 2012

F0 Exposure to Plastics Results in F3 Pubertal Abnormalities, Primordial Follicle Loss, Polycystic Ovaries and Tumors



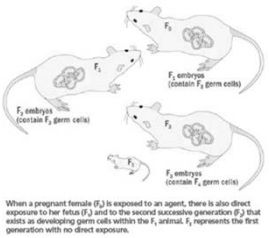
Manikkem 2013

Trans-generational Effects of Pesticides, EDCs, and Jet Fuel on DNA Methylation Regions



Mannikkam 2012

BPA and Other Chemicals Show Epigenetic Effects Across Generations



When a pregnant female (F_0) is exposed to an agent, there is also direct exposure to her fetus (F_1) and to the second succeeding generation (F_2) that exists as developing germ cells within the F_1 animal. F_3 represents the first generation with no direct exposure.

TCDD and JP-8 (jet fuel mixture) exposure of F_0 resulted in abnormalities in the F_3 generation:

- Primordial follicles decreased 30-40%.
- 2 day earlier puberty (=2 years in humans).
- 50-60% lower T levels in males.
- Global DNA methylation changes
- ? Gene expression changes?

Skinner & Colleagues PLoS One 2012

Some things to ponder…

What are the mechanisms underlying effects of parental stress, obesity, and environmental chemicals/endocrine disrupters, air pollution, and heavy metals on human reproduction?

Can we rescue the phenotype(s)?

Does the epigenome change with age?

What do we tell our patients?

What Do Obstetricians Ask About?

Why?

"Bigger fish to fry" "Wont know what to say" "Pandora's Box"

All That Matters

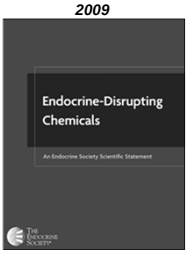
UCSF PRHE www.prhe.ucsf.edu

Strength of the Evidence

2008

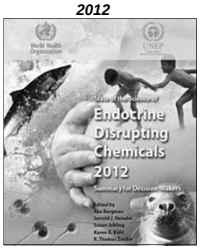
Woodruff T, Jansen S, Guillette, L, Giudice LC 2008

2009



Diamanti-Kandarakis E et al. 2009 Endocrine-Disrupting Chemicals: An Endocrine Society Scientific Statement. Endo Rev 30(4):293-342

2012



Åäke Bergman, Jerrold J. Heindel, Susan Jobling Karen A. Kidd and R. Thomas Zoeller WHO/UNEP 2012

Thank You!

References

- Baccarelli A, Bollati V. Epigenetics and environmental chemicals. Curr Opin Pediatr 2009;21(2):243-251.
- Barker DJP (1992). *Fetal and infant origins of adult disease*. London: British Medical Journal Books.
- Brien-Enriquez MA, Robles P, Camats-Tarruella N, Garcia-Cruz R, Roig I, Cabero L, Martinez F, Garcia Caldes M. Meiotic progression and recombination are affected by Bisphenol-A during in vitro *human* oocyte development. Human Reprod 2011;26:2807-2818
- Dolinoy DC. The agouti mouse model: an epigenetic biosensor for nutritional and environmental alterations on the fetal epigenome. Nutr Rev. 2008; 66(Suppl 1): S7–11.
- Hurley D. Grandma's experiences leave a mark on your genes. Discory. May 2013, 1-12.
- Machtinger R, Combelles C, Missmer S, Correia K, Williams P, Hauser R, Racowsky C. Bisphenol-A and ***human*** oocyte maturation *in vitro*. Human Reprod 2013;28:2735-2745
- Manikkam M, Guerrero-Bosagna C, Tracey R, Haque MM, Skinner MK. Transgenerational actions of environmental compounds on reproductive disease and identification of epigenetic biomarkers of ancestral exposures. PLoS One 2012;7(2):e31901
- Manikkam M, Tracey R, Guerrero-Bosagna C, Skinner MK (2013) Plastics derived endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. PLoS ONE 8(1): e55387. http://www.plosone.org
- Smith ZD, Meissner A. DNA methylation: roles in mammalian development. Nature Reviews Genetics 14, 204-220 (March 2013)**.**
- Susiarjo M, Hassold TJ, Freeman E, Hunt PA. Bisphenol A exposure in utero disrupts early oogenesis in the mouse. PLoS Genet 2007, Jan 12;3(1):e5
- Susiarjo M, Sasson I, Mesaros C, Bartolomei MS. Bisphenol A Exposure Disrupts Genomic Imprinting. PLoS Genetics 2013; 9(4): e1003401
- Zeisel SH. Epigenetic mechanisms for nutrition determinants of later health outcomes. Am J Clin Nutr 2009;89 (sup.): 1S-6S.

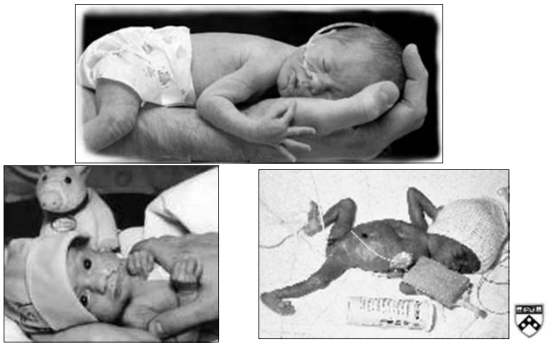
Pre-Congress PG Course No. 8
ESHRE Annual Meeting
June 29, 2014

Genetic and Epigenetic Factors Affecting Embryo Development and Implantation/Placentation: Lessons from Clinical ART

Christos Coutifaris, MD, PhD
The Nancy and Richard Wolfson Professor
of Obstetrics and Gynecology
Chief, Division of Reproductive Endocrinology and Infertility

Perelman School of Medicine
University of Pennsylvania
Philadelphia, Pennsylvania, USA

Low Birthweight and Preterm Birth

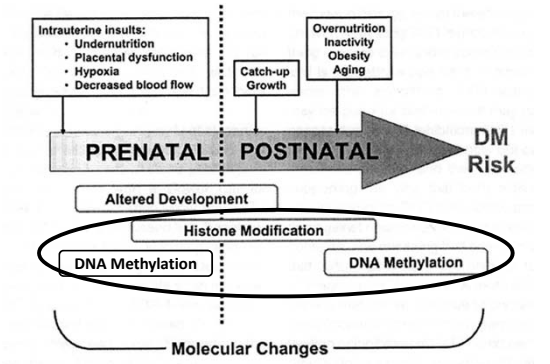


Can we identify <u>patients at risk</u> for adverse perinatal outcomes following ART?

Can we identify <u>modifiable factors</u> that may prevent these adverse perinatal outcomes following ART?

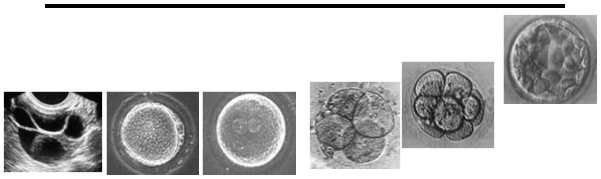
Educational Objectives

- Review exposures associated with Assisted Reproductive Technologies (ART)
- Describe the time course for the establishment of epigenetic marks during gametogenesis and embryo development
- Describe the rationale for focusing on research on implantation and placentation
- Start the discussion on understanding the molecular basis of adverse perinatal outcomes associated with some infertility treatments



Modified from Woo & Patti, Cell Metabolism, pp. 8-10, July 2008

Egg and Embryo Manipulations



Ovarian stimulation

Egg Retrieval

Insemination ICSI

Days post-insemination

1 | 2 (tx) | 3 tx | 4 | 5/6 tx

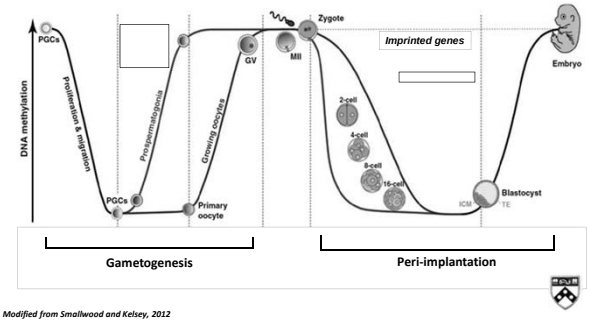
Assisted Hatching (Blastomere Bx)

Incubation conditions / Culture media

Exposures: Temperature - Light - Oxygen - Embryo manipulation/pipetting

Infertility *per se*?

Epigenetic reprogramming during mammalian development

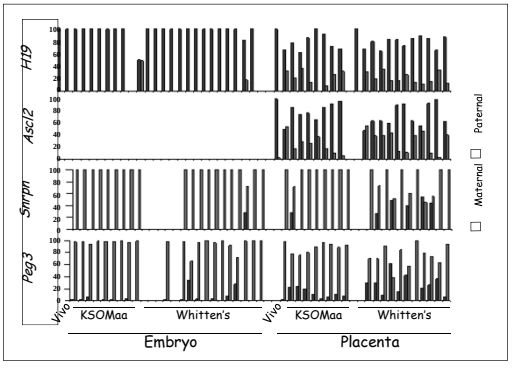


Modified from Smallwood and Kelsey, 2012

Assisted Reproductive Technologies

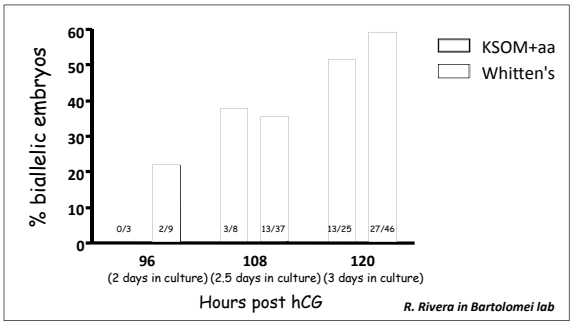
- A model to study epigenetic regulation of development in humans and experimental animals?

Loss of imprinted expression occurs primarily in the placenta after pre-implantation culture



Mann et al, 2004 Richard Schultz and Marisa Bartolomei's Laboratories

In-vitro culture of mouse embryos results in bi-allelic *H19* expression



At least 10% expression from repressed allele to be counted as bi-allelic

Mean Methylation of CpG sites

- Cord blood
  - 358 CpG sites differed (*in vivo* vs. *in vitro*)
  - 277(77%) more methylated (*in vitro*)
  - 81 less methylated
- Placenta
  - 246 CpG sites differed (*in vivo* vs. *in vitro)*
  - 154 (63%) less methylated (*in vitro*)

Katari , et al., Human Mol Genet 18:3769-78, 2009

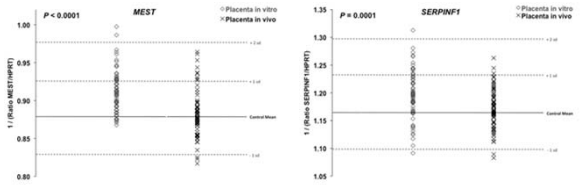
Differential methylation – **placenta**

Genes which differ between ART and Control placenta in two or more independent experiments

AMY2A	CPE	GDF3	MEG3	SERPINF1	VHL
BMP1	CYCS	GRB10	MEST	SFRP2	WNT9B
C1QBP	*DLX2*	GRPEL1	MRPL12	SHC3	WT1
CART1	DNAJA1	HYMA1	MSX1	SLIT1	
CBL	*ERBB2*	IGF1R	*MYL2*	SRC	
CD9	FABP2	IGF2AS	PAX4	STAT3	
CDC25A	FAU	IMPDH2	PAX6	TMSL3	H19
CDH5	FGF8	INS	PEG10	TNNI2	IGF2
CDKN1C	FMR1	MAPK13	*RHOC*	UBE2D1	
CGB5	GATA4	MAPRE2	SCGB3A2	UBE3A	

(Katari, Turan et al. 2009 Hum Mol Genet)

Scatter plots of transcript levels for two genes in which mean transcript levels differ in *in vitro* and *in vivo* populations



Does the expression of any of the genes correlate with birth weight?

DNA methylation profiling identifies epigenetic signatures correlated with birth weight

Turan et al., (BMC Med Genomics. 5:10, 2012)

Differential methylation – **placenta**

Genes which differ between ART and Control placenta in two or more independent experiments

AMY2A	CPE	GDE3	MEG3	SERPINF1	VHL
BMP1	CYCS	GRB10	MEST	SFRP2	WT1B
C1QBP	*DLX2*	GRPEL1	MRPL12	SHC3	WT1
CART1	DNAJA1	HYMA1	MSX1	SLIT1	
CBL	*ERBB2*	IGF1R	*MYL2*	SR	
CD			PAX4	STAT	
CDC2			PAX6	TMSL	H19
CDH			PEG10	TNN	IGF2
CDKN1C	FMR1	MAPK13	*RHOC*	UBE2D1	
CGB5	GATA4	MAPRE2	SCGB3A2	UBE3A	

Nuclear encoded mitochondrial genes

HIF1a

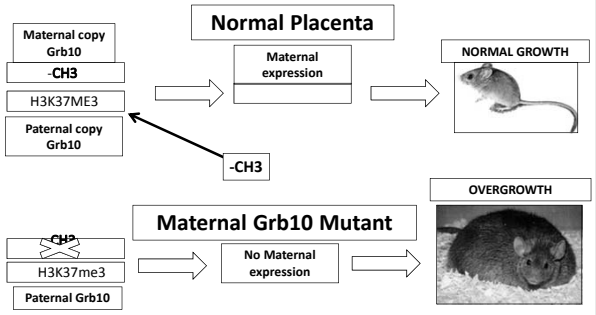
(Katari, Turan et al. 2009 Hum Mol Genet)

GRB 10 and Growth

- *A*daptor molecule that negatively regulates fetal growth
- Functions downstream of EGFR, IGF1R and IR
- Deletion of the maternal copy of Grb10 leads to 40% INCREASE in fetal weight and a 30% increase in placental weight
- Deletion of the paternal copy has no effect on fetal weight

Charalambous et al. 2010
Liu and Roth 1995

GRB10 and Growth

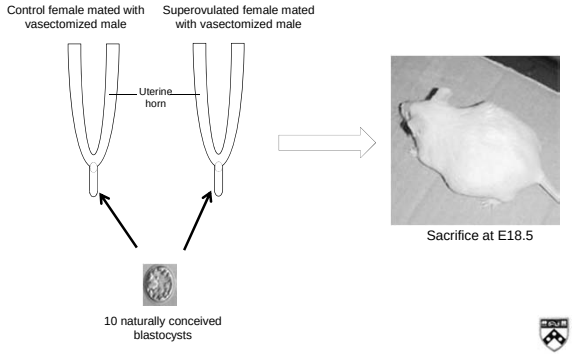


Garfield et al. Nature 2011

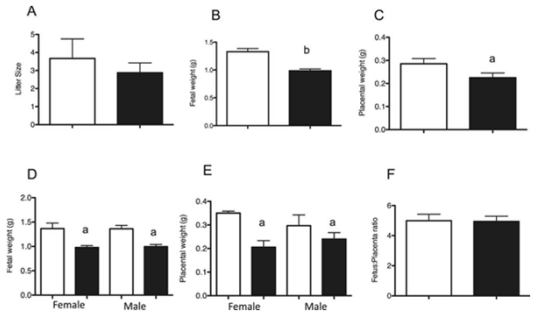
GRB 10 in placenta

- Trophoblast cells
- Endothelial cells
- Other

Experimental Model

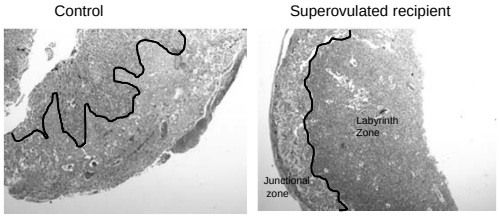


Five control (white bars) and eight SO dams (black bars) were sacrificed at E18.5.



Mainigi et al. Biol Reprod 2014;90:26

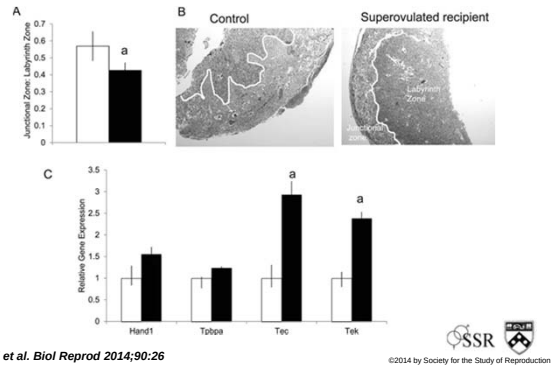
Placenta histology



Biol Reprod. 2014 Feb 13;90(2):26

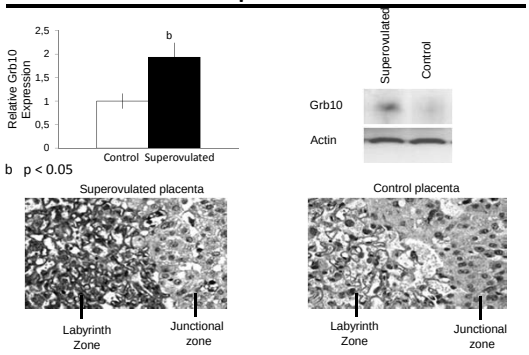
Mainigi, unpublished

Histopathological examination of placentas from control and SO recipients.



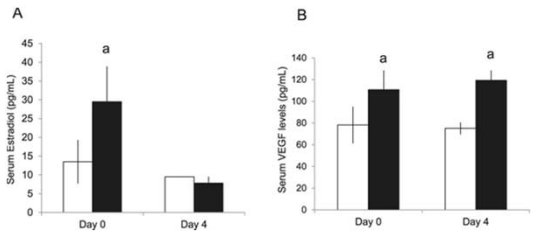
Mainigi et al. Biol Reprod 2014;90:26

Placental expression: *Grb10*

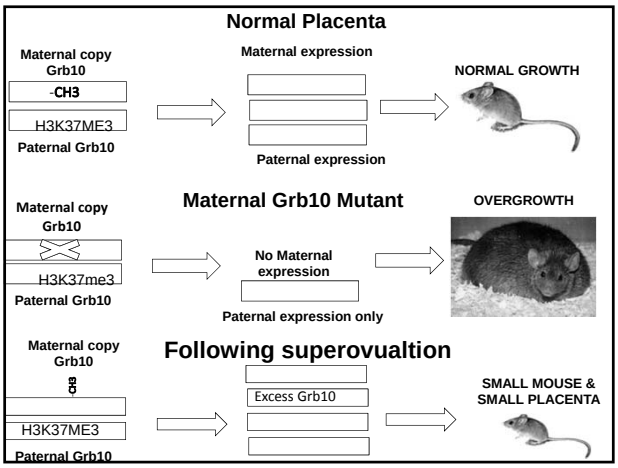


Mainigi et al. Biol Reprod 2014;90:26

Serum Estradiol and VEGF in mice following natural mating (white bar) or mating following superovulation with gonadotropins (black bar).



Mainigi et al. Biol Reprod 2014;90:26



Summary I

- Singletons
- Clinical/translational studies have suggested
  - Placental / Placentation problems (?)
  - Epigenetic regulation problems (?)
- Mouse work
  - Biallelic expression of imprinted genes
  - "Confined" to the placenta
  - Prolonged culture effects on embryo proper
  - GRB 10 and the role(s) of superovulation and VEGF

Summary II

- Human Work
  - Variability in placenta
    - (Normal vs. ART-related?)
    - Consequences?
  - Methylation Microarray data
    - Differences
    - Significance
- GRB10, MEST, GRPEL1, DNAJA1, GRINC1, MSX1, etc
- The mouse model
- Implications for long term health?

The Most Important Slide

- Nahid Turan, PhD, Sunita Katari, MD
  Raffi Chalian, MD, Mike Foster, BA, Harry
  Chatzicharalampous, MD, Erica Pocharska, BA
  Christopher Morse, MD, Emelia Bachman, MD

- Karine Chung, MD, MSCE
  Suleena Kalra, MD, MSCE

- Carmen Sapienza, PhD
  Monica Mainigi, MD
  Kurt Barnhart, MD, MSCE
  Marisa Bartolomei, PhD
  Richard Schultz, PhD

- NIH (RO1-HD048730; U54-HD068157; T32-HD40135)

Turner Syndrome: Reproductive Options and Outcomes

Richard H. Reindollar, M.D.

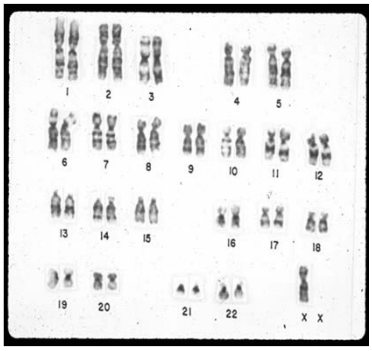
Adjunct Professor, Department of Obstetrics and Gynecology
Geisel School of Medicine at Dartmouth
Executive Director, American Society for Reproductive Medicine

Disclosure Statement:
Richard H. Reindollar, M.D. has no relevant financial relationships with any manufacturers of pharmaceuticals, laboratory supplies, or medical devices.

The material presented here reflects my own opinion, not the consensus of the ASRM.

A 36 year old G1 pregnant patient discussed all of the options for aneuploidy screening and elected to have CVS for her singleton pregnancy. She was informed that the karyotype of 50 cells showed 45,X(25 cells)/46,XY(25 cells). You inform her of all phenotypic possibilities for this finding at prenatal diagnosis and state that the vast majority of babies exhibit:

- Turner female with bilateral streak gonads and normal female external genitalia
- Turner female with a unilateral abdominal testes, contra lateral streak gonad, and mild clitoramegaly
- Turner female with a unilateral streak gonad, a contra lateral gonad descended into a labio-scrotum, and marked clitoramegaly
- Normal male phenotype



45,X

- 1 in 2500 females;
- 99% don't survive > 28 wks;
- 15% of SAB;
- nearly 80% maternal X.

Karyotype of Patients with TS

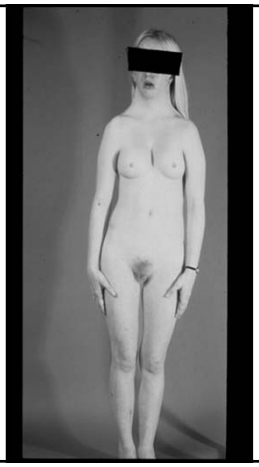
Classical Turner Syndrome (45,X)	24*	Other	
Y-cell Lines:	12	46,X,t(X:X)(qter-p22)	1*
45,X/46,XY	1	45,X/46,X,dei X(q13)	2
45,X/47,XYY	11	46,X,Xq+	1*
Structural abnormalities of X:	25*	45,X/46,X,Xq+	1
Isochromosome		45,X/46,X,r(X)	1
46,X,i(Xq)	5*	45,X/46,X,r	1
45,X/36,X,i(Xq)	9	Other X mosaic cell lines:	8
45,X/46,X,dic I(Xq)	1	45,X/46,XX	7
45,X/46,X,i(Xq)/46,i(Xq),I(Xq)	1	45,X/47,XXX	1
45,X/46,X,i(Xq)/47,X,i(Xq),i(Xq)	2		

*Single cell lines

Privation of X Chromosomal Material

- Short stature
- Turner stigmata
- Abnormal lymphatics and associated deformations
- Somatic abnormalities
- Endocrine and autoimmune abnormalities
- Ovarian failure

Height 4' 11"

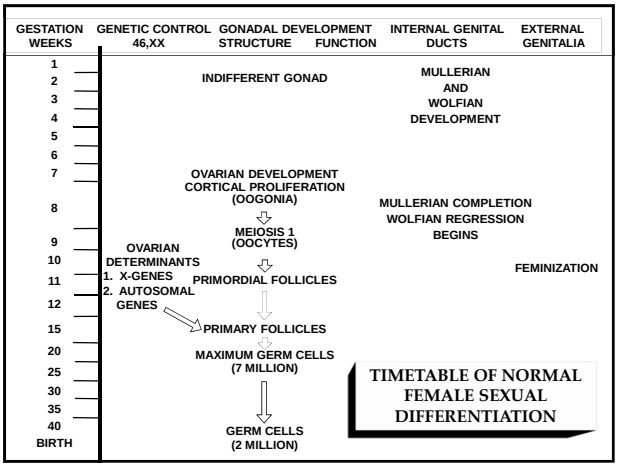


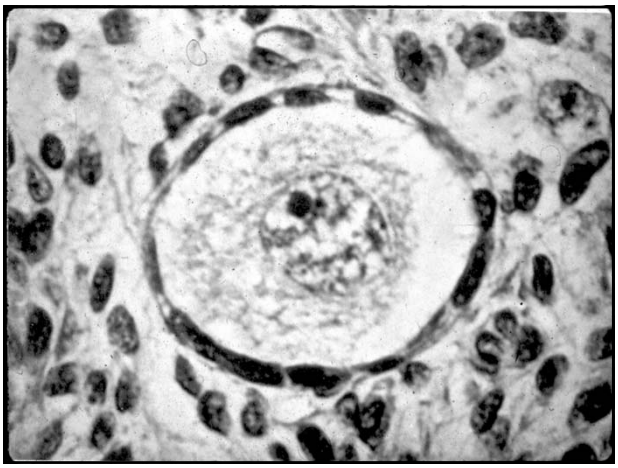
Height 4' 9"

Karyotypic-Phenotypic Correlations (?)

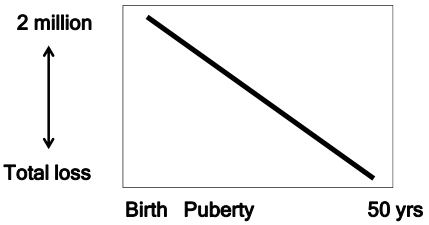
- Often thought dose dependent (i.e., findings more common with higher % 45,X cells)
- Recently when ascertainment considered, better such correlations (those found prenatally fewer findings)
- Gene imprinting suggested: parent of origin of X chromosome partially explains findings (renal abn exclusive with maternal X)

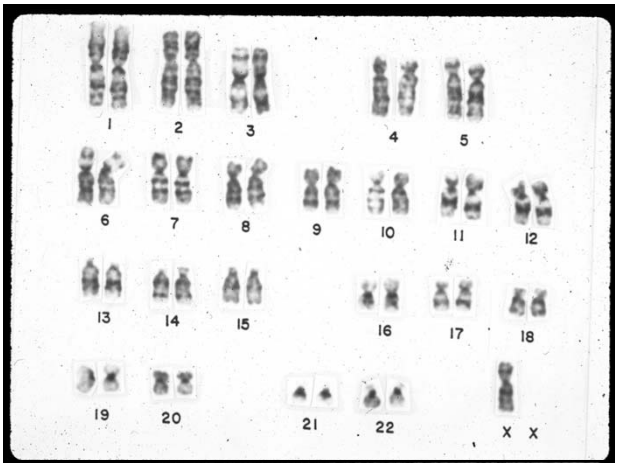
Premature Loss of Germ Cells

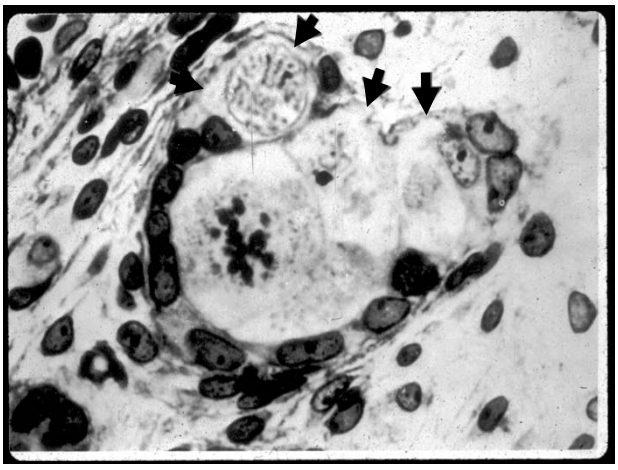




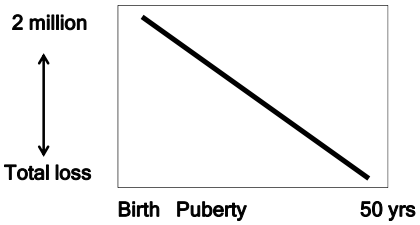
Loss of Oocytes With Age



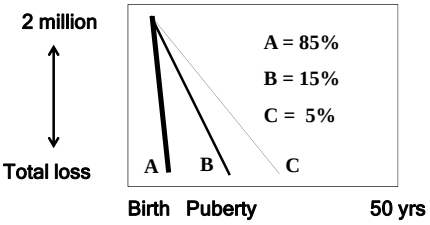


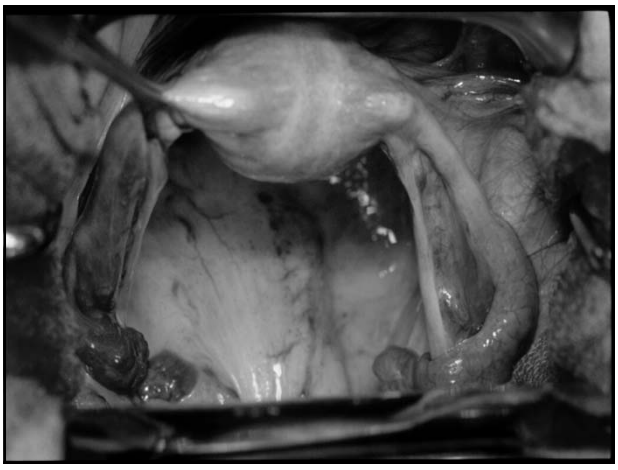


Usual Loss of Oocytes With Age



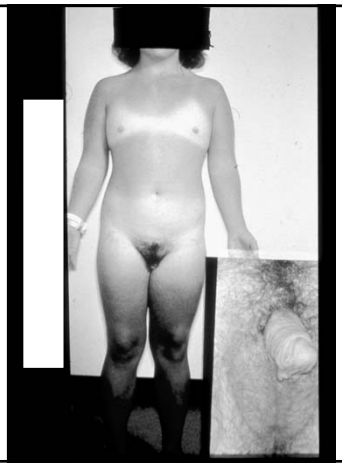
Accelerated Loss of Oocytes With Age for Women with Turner Syndrome



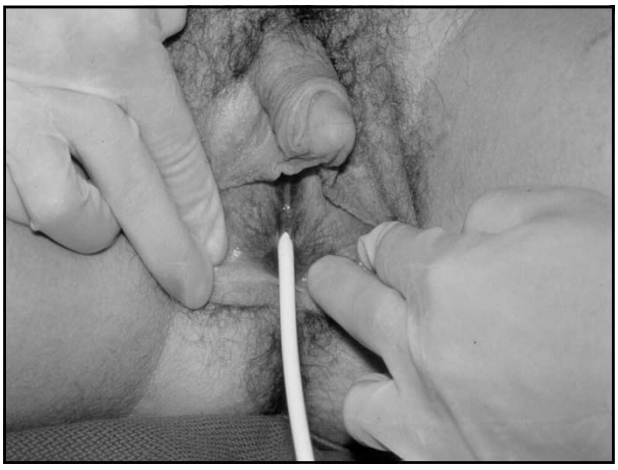


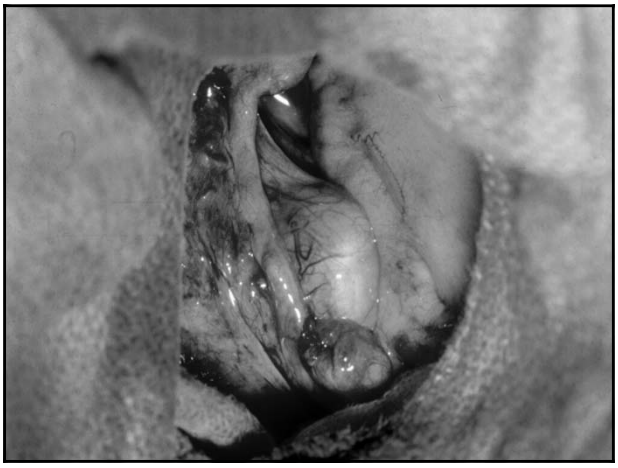
X Chromosome Loci Important in Maintenance of Germ Cell Complement

- Translocation studies
  - POF1 (Xq26-q28), candidate genes (HS6ST2, TDPF3, GPC3) and known gene (FMR1)
  - POF2 (Xq13.3-q22), candidate genes (DIAPH2)
- TGF-β Superfamily genes: bone morphogenetic protein 15 (BMP15) (Xp11.2)
- Other candidates: DEAD-box 3 (DBX), Ubiquitin-Specific Protease 9 (USP9X) both Xp11.4 (escapes inactivation)



Height: 59 inches





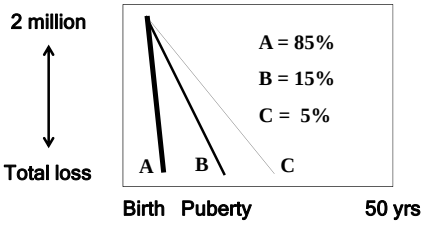
45,X/46,XY Gonadal Dysgenesis (5 phenotypes at birth)

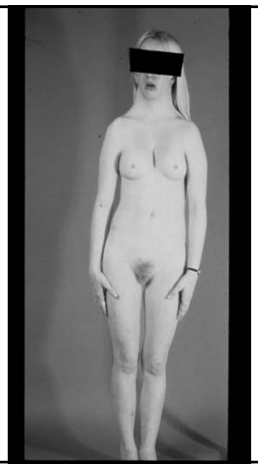
1. Bilateral streak gonads (normal female phenotype)
2. Unilateral streak, contra-lateral intra-abdominal testis (clitoramegaly)
3. Unilateral streak, contra-lateral descended testis (ambiguity)
4. Bilateral descended testes (normal male phenotype)
5. Bilateral intra-abdominal testes (normal male/ ambiguous)

The majority of 45,X/46,XY found in-utero are normal males.

- Wheeler et al, AJMG, 1988;29:565
  - 6 prenatally dx (all normal); 9 postnatally dx (7 AG, 2 primary amenorrhea)
- Hsu, LYF., Prenatal Diagnosis 1989;9:31
  - 54 prenatally dx (89.4% normal male phenotype; 6.4% asymmetric internal gonads; 2 cases ? Abn)
- Chang et al, AJHG, 1990, 46:156 (Intl survey)
  - 76 cases prenatally diagnosed (95% normal male genitalia, 4/11 testes biopsied abnormal; 5% abn, 3 hypospadias, 1 clitoramegaly. Other Turner abn in 5 cases)

Accelerated Loss of Oocytes With Age for Women with Turner Syndrome





Height 4’ 9”

What outcomes/options have been reported for TS women with ovarian function?

A. Spontaneous pregnancy
B. IVF with the patients own oocytes and pregnancy
C. Superovulation and oocyte cryopreservation for later use
D. All of the above.

Ovarian Function in TS Patients

- 85% will not have ovarian function for puberty or beyond
- 15% have limited function for pubertal development
- 5% have function for spontaneous menstrual periods of limited time (rarely beyond age 30 years)
- 1% have function for puberty, menstrual periods and pregnancy

54 Spontaneous Pregnancies in Women with Turner Syndrome

- 5 Reports - 45,X patients
- 7 Reports - 45,X/46,XX patients
- 7 Reports - 45,X/46,XX/47,XXX

Dewhurst, 1978

Spontaneous Pregnancy in Turner Syndrome Patients

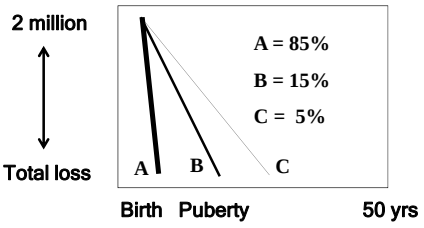
6 new patients

Review of 160 pregnancies/ 74 patients in literature

- 29% spontaneous abortion
- 7% perinatal deaths
- (approx) 20% TS
- 38% children with 46,XX or 46,XY

Tarani et al, Gynecol Endocrinol 1998;12:83

Loss of Oocytes With Age Turner Syndrome



Pregnancy in Turner Syndrome Patient Following Ovarian Stimulation and IVF

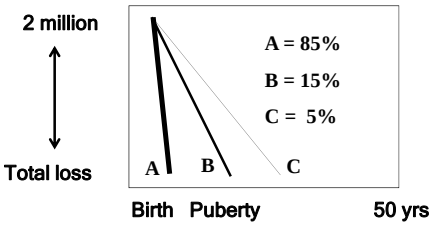
- 33 y/o known 45,X/46,XX, and regular cycles
- Turner physical findings
- Laparotomy: resection of right endometrioma, myomectomy, resection communicating left uterine horn and streak ovary
- 4 cycles infertility treatments (OI/IUI)
- IVF: E2 3095 pg/ml, 21 oocytes, 13 fertilized, 5 embryos transferred, pregnant with 46,XY
- Ovarian biopsy: 46,XX (147 cells)

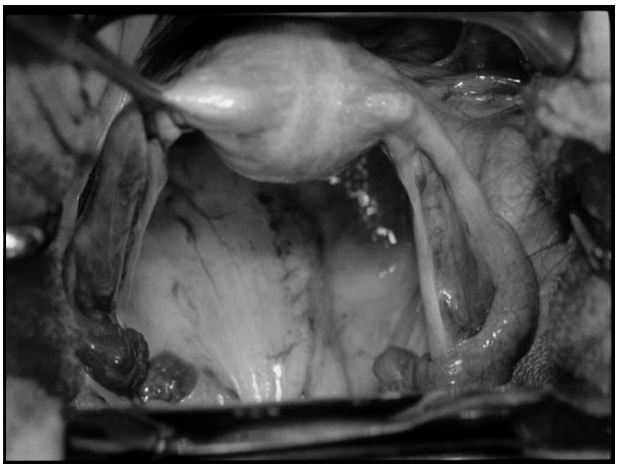
Dilkoff et al, JARG, 1996

Oocyte Freezing in Rare Menstruating Patients

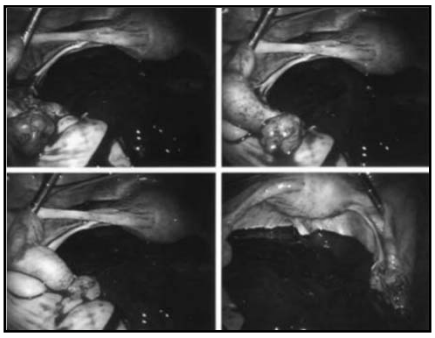
- Kanoussi et al, J Reprod Med 2008;53(3):223
  28 yo mosaic TS, COH, 15 oocytes retrieved , 13 cryopreserved.
- Huang et al, Hum Reprod 2008;23:336
  16 yo 45,X/46,XX, ovarian biopsy, 11 oocytes, 8 matured in-vitro and cryopreserved

Loss of Oocytes With Age Turner Syndrome





Ruptured Ectopic Pregnancy in Patient with Turner Syndrome Having Achieved Pregnancy Through Donor Oocyte



All of the following scenarios for Turner patients who become pregnant have been reported

EXCEPT:

A. Patient known to have hypertension and dilated aorta allowed to become pregnant by donor oocyte and died from aortic rupture.
B. Patient with triplet pregnancy from donor oocyte died at 24 weeks gestation from aortic rupture.
C. Patient found to have dilation of aorta during donor oocyte pregnancy who died three years after delivery.
D. Patient with spontaneous pregnancies at ages 36 and 38 years, who dissected during the third trimester of her second pregnancy and was subsequently diagnosed with mosaic Turner syndrome.
E. Patient with no known risk factors unexpectedly developed dissection and rupture of the aorta and died during singleton donor oocyte pregnancy.

Largest IVF/Donor Oocyte Series for Turner Syndrome Patients

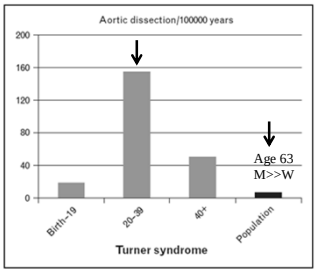
- 17/29 women had 28 pregnancies (U/S) (42%/cycle)
- 10 women: 1 pregnancy; 4 women: 2 pregnancies; 2 women: 3 pregnancies; 1 woman: 4 pregnancies (only 1 with viable pregnancies)
- 2 triplets; remaining singletons
- 50% spontaneous abortion; 1 ectopic
- 13 (19.1%) “take home” baby rate/cycle

Khastgir, 97

Aortic Dilation, Dissection and Rupture in Non-pregnant Turner Syndrome Patients

- Rev Fac Cien Med Cordoba, 1961
- J Thorac Cardiovasc Surg, 1964
- Am J Med, 1965
- Am J Cardiol, 1969
- J Pediatr, 1971
- J Pediatr, 1975
- JAMA, 1972
- Circulation, 1978
- Am J Dis Child, 1979
- Am Heart J, 1982
- Postgrad Med J, 1982
- J Med Genet, 1983
- Clin Cardiol, 1984
- J Pediatr, 1986

Incidence of aortic dissection in Turner syndrome by age group



This is a summary of Danish epidemiological data. The frequency of aortic dissection is greater in women with Turner syndrome at all ages compared with the general population (500- 2000 X), with the highest incidence in young adults.

Bondy. Curr Op Cardiol 2008 23:519-526

85 Cases Of Aortic Dissection

Carlson and Silberbach, J Med Genet 2007;44:745

- 1961 – 2006
- Average age 30.7 yrs (usual in females is 68 years, male to female ration 3.2:1)
- Karyotype: 45,X (39/49); mosaics (10/49)
- Dissection: proximal 55%; distal 23%
- CHD 69% (51/74): C (24/51), BAV (15/51), both (9/51)
- Hypertension 54%
- No risk factors 21%
- Cystic medial necrosis (22/22)

85 Cases Of Aortic Dissection

Carlson and Silberbach, J Med Genet 2007;44:745

Symptoms

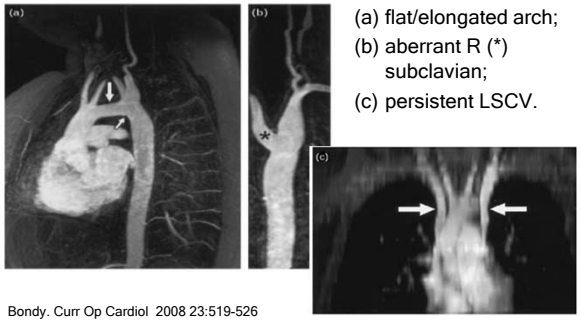
- Abdominal pain
- “heart burn”
- Back or shoulder pain
- Change in phonation (traction on recurrent laryngeal nerve)

TS NIH Consensus Study Group

- Baseline echocardiogram for CHD
- Frequent BP checks
- In absence of CHD or HTN, MRI measurements of aorta (T-1 images, level of R pulmonary artery, perpendicular to long axis of AA)
- Repeat echocardiogram or MRI q 5 – 10 years

JCEM 2007;92:10 - 25

Elongated transverse arch of the aorta and associated anomalies in a woman with Turner syndrome revealed by Gdenhanced 3D MRA



(a) flat/elongated arch;
(b) aberrant R (*) subclavian;
(c) persistent LSCV.

Bondy. Curr Op Cardiol 2008 23:519-526

Ascending Aorta Measurements: Historical Thresholds for Repair

- 5.5 cm general population
- 5.0 cm Marfan syndrome
- 4.5 cm Loeys-Dietz syndrome

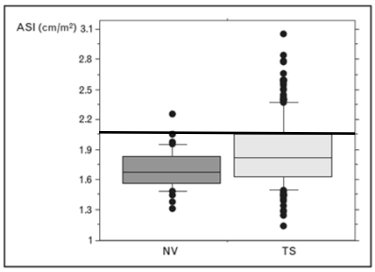
Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association
Learn and Live

Aortic Dilatation and Dissection in Turner Syndrome
Lea Ann Matura, Vincent B. Ho, Douglas R. Rosing and Carolyn A. Bondy
Circulation 2007;116;1663-1670; originally published online Sep 17, 2007;
DOI: 10.1161/CIRCULATIONAHA.106.685487
Circulation is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 75514

 Print ISSN: 0009-7322. Online ISSN: 1524-4539

Ascending aortic diameters normalized for body surface area in 160 adults with Turner syndrome and age-matched control women



This normalization termed aortic size index or ASI showed that mean ascending aortic diameter is significantly increased in TS, and that a group of women with TS have extremely elevated values. (Solid Bar 95% Normal Population)

Bondy. Curr Op Cardiol 2008 23:519-526

Aortic Dilation and Dissection in Turner Syndrome

- MRI evaluation of aorta 166 TS women
- When normalized to body surface area, aorta measurements abnormal in 32% of TS women (> 95 percentile)
- Aortic size < 5 cm may be at increased risk for TS patients (in subset > 3.5 cm: 3.69, 4.63, 4.78 ruptured)
- Aortic size index > 2.0 cm/m^2 close surveillance (> 2.5 highest risk)

Circulation 2007;116:1663

Donor Oocyte for Turner Syndrome: 4 Deaths During Pregnancy

- Two deaths reported in *Letter to Editor*, Fertil Steril, 1997: one hypertension and slightly dilated aorta.
- One death reported as case report, 1998: hypertension; dx of preeclampsia; died postoperative.
- One death, 2001, reported in pregnancy experience with 50 coarctation patients.

Mosaic Turner Syndrome Patient with postpartum aortic dissection and survival

- DeBakey type IIIb aortic dissection (not involving proximal aorta)
- Two weeks post cesarean section for eclampsia
- No aortic dilation or other cardiovascular malformation
- Distal extension and uncomplicated dissection: treated medically

Weytjens, C et al., J Cardiovasc Surg 2000;41:295-7

Total (≥ 10) Aortic Dissection Cases Pregnancy Related

Carlson Silverbach Literature Review
(J Med Genet 2007;44:745)
- 6 cases during pregnancy (1 with prior spontaneous pregnancy)
- 1 case, 1 year after ART pregnancy

Boissonnas CC, et al (Fertil Steril 2009;91:929.e5)
- 1 case, missed BAV, found 16 weeks, dissection/ death 38 weeks

Bondy C, Rosing D, Reindollar R (Fertil Steril 2009; eLetter to Editor)
- 1 patient, dilation during pregnancy, dissection/ death 3 years later

Unpublished Cases (at least one)

A Survey of 259 Donor Oocyte Programs

MF Karnis, A Zimon, S Lalwani, L Timmreck, S Klipstein, and RH Reindollar, F&S 2003;80:498

- 134 (52%) responses
- 146 Turner patients
- 72 (49%) patients screened with echocardiography
- 6 (8.3%) abnormal echocardiography

A Survey of 259 Donor Oocyte Programs

MF Karnis, A Zimon, S Lalwani, L Timmreck, S Klipstein, and RH Reindollar, F&S 2003;80:498

146 Turner patients

- 101 pregnancies (1 spontaneous abortion)
- 17 multiples, all delivered at least 1 live-born
- Live birth rate: 65% (94/144)
- 7% SAB rate

A Survey of 259 Donor Oocyte Programs: Estimated Death Rate

MF Karnis, A Zimon, S Lalwani, L Timmreck, S Klipstein, and RH Reindollar, F&S 2003;80:498

101 pregnancies / 52% of US programs

- estimated 194 pregnancies (max) / 100% US programs
- Estimated death rate: 4/194 (2%)

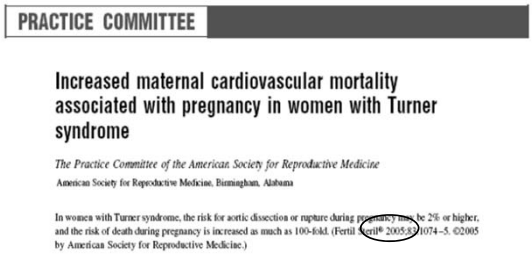
Survey by French Study Group for Oocyte Donation

Chevalier N, Letur H, Lelannou D et al. and the French Study Group for Oocyte Donation. JCEM 2011;96(2):260-267

93 TS pregnancies identified > 20 weeks gestation (DO)

- 2 died of aortic dissection and rupture (2.2%)

American Society of Reproductive Medicine (ASRM) Practice Guidelines



PRACTICE COMMITTEE

Increased maternal cardiovascular mortality associated with pregnancy in women with Turner syndrome

The Practice Committee of the American Society for Reproductive Medicine

American Society for Reproductive Medicine, Birmingham, Alabama

In women with Turner syndrome, the risk for aortic dissection or rupture during pregnancy may be 2% or higher, and the risk of death during pregnancy is increased as much as 100-fold. (Fertil Steril® 2005;83:1074–5. ©2005 by American Society for Reproductive Medicine.)

Unpublished Concerns

- National Turner Syndrome Society Meeting (8/08)
- C Bondy
  - Evidence during pregnancy of progressive dilation of aorta
  - Deaths after pregnancy
  - MRI evaluation of aorta is more sensitive than cardiac echo
  - Unpublished deaths during pregnancy
- Silverbach
  - Unpublished deaths during pregnancy
- Reindollar
  - Unpublished deaths during pregnancy

ASRM Practice Guidelines: Suggested Revisions (Bondy C, Rosing D, Reindollar R; Fertil Steril, 1990 eLetter to Editor)

1. Women without known CHD require full CV evaluation with MRI at center with expertise (particular attention to AV, aortic diameter, vascular tree)
2. Absolute contraindications to pregnancy
   - known CHD or hypertension
   - aortic size index > 2 cm/m^2
3. TS women may have latent vasculopathy, increasing risk during or following pregnancy in absence of absolute contraindications.

ASRM Practice Committee Report
(2008 Recommendations)

Specific recommendations for *surveillance* in women with Turner syndrome *during pregnancy* include:

- Treatment of hypertension.
- Periodic echocardiography and consultation with a cardiologist.
- Women in stable condition having an aortic root diameter less than 4 cm may attempt vaginal delivery under epidural anesthesia.
- Women exhibiting baseline or progressive aortic root dilation should have an elective cesarean delivery prior to the onset of labor under epidural anesthesia.

ASRM Practice Committee Report
(2008 Recommendations)

SUMMARY

- The risk of death during pregnancy may be increased as much as 100-fold for women with Turner syndrome.
- In general, Turner syndrome is a relative contraindication to pregnancy.
- Cardiology consultation and careful screening for cardiac abnormalities is a prerequisite for any planned attempt at pregnancy via oocyte donation.
- An echocardiogram revealing any significant abnormality represents an absolute contraindication to attempting pregnancy in a woman with Turner syndrome.
- Women with Turner syndrome having a normal echocardiogram who intend to attempt pregnancy require careful observation and frequent formal reevaluation throughout gestation.

French Government Requested Recommendations

-After death of 2 French TS women during pregnancy

-Joint report of practice committees of FCOG, FCS, FCCSS, FSAIC. Euro J Ob Gynecol Repro Biol, 2010; 152: 18 - 24.

Recommendations

- Check up before pregnancy (multifaceted including PE, endocrine tests, LFTs, Renal FTs, cardiac echo, mandatory MRI,)
- Contraindications to Pregnancy:
  - Hx of aortic surgery - aortic dilatation > 25 mm/m2 or 35mm
  - Hx of dissection - aortic coarctation
  - Coarctation - uncontrolled hypertension
  - Hx of BAV surgery (BAV in absence of aortic dilatation)

French Government Requested Recommendations (cont.)

- Detailed Counseling
- In absence of contraindications, DO allowed with:
  - yearly US and, if > 10% increase A dilation, MRI
  - SET
  - Cardiac echo end of 1st and 2nd trimester and monthly 3rd trimester
  - MRI if increase in A size >10%
  - If dissection: < 25 weeks, emergency surgery; > 25 weeks cesarean followed by aortic root surgery
  - If increased size 10% or > 25mm/m2: admit, 25 - 34 wks: lung maturation followed by cesarean
  - Cardiac echo 5 - 8 days post delivery

Turner Syndrome: Unanswered Questions

- What is the real maternal mortality rate? (Some think likely greater than our 2% estimate)
- Is aortic dissection and rupture of the same pathophysiology as in Marfan syndrome or is there a vasculopathy specific to Turner syndrome that increases the risk?
- Are there phenotypes of TS that have different risks?
- Does the physiology of pregnancy damage the aorta (and other vessels) and increase the risk of subsequent rupture?
- Should MRI be performed more frequently?
- Should Turner syndrome be an absolute contraindication to pregnancy?

TS Deaths Following "Successful" Pregnancy

- Gravholt CH, Landin-Wilhelmsen K, Stochholm K et al. (Cardiol Young, 2006)
  1patient, died 1 year after ART twin pregnancy
- Bondy C, Rosing D, Reindollar R (Fertil Steril 2009; eLetter to Editor)
  1 patient, dilation during pregnancy, dissection/ death 3 years later

Obstetric and neonatal outcome after oocyte donation in 106 women with TS: A Nordic cohort study. Hagman et al, Hum Reprod Advance Access, February 2013.

106 TS women, 122 deliveries and 131 newborns after DO from Finland, Denmark, Sweden , 1992 – 2011. SET 70%, twins 7.4%

- Main finding: TS pregnancies carry substantial risk, particularly 35% of pregnancies hypertensive disorders (20% pre-eclampsia)
- Potentially life threatening complications 3.3%: AD (1); tricuspid/ MV regurgitation (1); HELLP in patient with mechanical valve; heart failure (1); post-partum hysterectomy from hemorrhage.
- CV outcomes:
  - 10 pregnant with CVD;
  - one previously normal CV developed mild LVD;
  - one previously normal CV exam- chest pain several days before delivery, CT misread as normal, acute symptoms at 20 days, aortic dissection, successful surgical correction 1.5 years later.
- 22% hypothyroidism
- 2.3% perinatal mortality

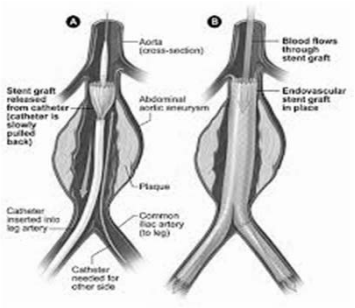
Morbidity and Mortality after childbirth in women with Turner karyotype. Hagman et al, Hum Reprod Advance Access, April 2013.

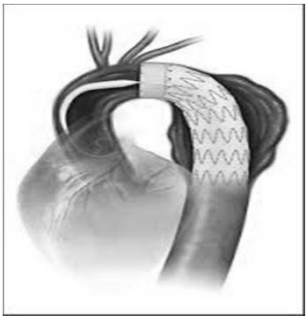
Swedish population study, registry, 124 TS women, birth between 1973 and 2010 vs. control group

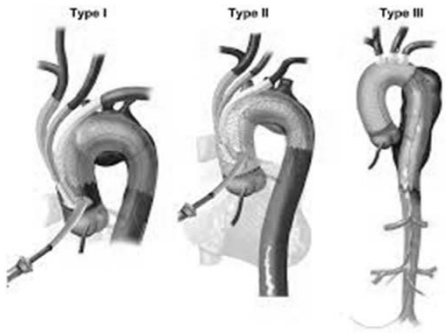
- No mortality with childbirth (? effect of low risk and need for larger study population, enforced guidelines)
- Morbidity from CV disease vs. control group
  - Before pregnancy increased (HR 3.83; 95% CI 1.02 -14.43
  - During pregnancy or within 1 year of delivery increased (HR 5.78; 95% CI 1.94-17.24)
  - ≥ 1 years after pregnancy similar (HR1.91; 95% CI 0.74-4.96)
- Among TS patients, morbidity similar ≤ 40 years with and without childbirth (HR 1.02; 95% 0.53-2.25)
- Of Interest, thyroid disease increased during or within 1 year of pregnancy (HR 5.78; 95% CI 1.94-17.24)

Turner Syndrome: How Do We Reduce the Risk?

- Registry for Turner syndrome patients undergoing donor oocyte badly needed (All French TS DO pregnancies registered)
- In absence of hard data, provide nondirective counseling, and **IF** proceed, do so with extreme caution
  - 2% MM is likely underestimate
  - Pregnancy may or may not place patient at higher risk for later rupture
  - Turner syndrome alone may be absolute contraindication
- MRI needed in everyone
  - Variant vascular anatomy may be important finding regarding risk
  - Serial imaging of ascending aorta necessary
- Cardiac consultation should never be for clearance, rather for assessment
- Surgical option for protection in future.


A
Aorta (cross-section)
Stent graft released from catheter (catheter is slowly pulled back)
Abdominal aortic aneurysm
Plaque
Catheter inserted into leg artery
Common iliac artery (to leg)
Catheter needed for other side
B
Blood flows through stent graft
Endovascular stent graft in place





Thoracic Endovascular Aortic Repair (TEVAR) for the treatment of aortic diseases: a position statement from the European Association for Cardio-Thoracic Surgery (EACTS) and the European Society of Cardiology (ESC), in collaboration with the European Association of Percutaneous Cardiovascular Interventions (EAPCI)†

Martin Grabenwo¨ ger1, Fernando Alfonso2, Jean Bachet3, Robert Bonser4, Martin Czerny5*, Holger Eggebrecht6, Arturo Evangelista7, Rossella Fattori8, Heinz Jakob9, Lars Lo¨nn10, Christoph A. Nienaber11, Guido Rocchi12, Herve` Rousseau13, Matt Thompson14, Ernst Weigang15, and Raimund Er

European Heart Journal Advance Access published May 4, 2012

"Indications and contraindications for TEVAR"

TEVAR for TAA

"In asymptomatic TAA patients TEVAR is indicated (by consensus) when the maximum diameter of the aneurysm exceeds 5.5 cm or if rapid expansion (.5 mm in 6 months) occurs.24,25 In certain morphologic situations which are considered prone to rupture, e.g. saccular aneurysms, TEVAR may be justified at a diameter of less the above referenced 5.5 cm. Comorbidities and age of the patient have to be considered,26 and it may be appropriate to set a larger aortic diameter threshold in patients with increased operative risk."

European Heart Journal Advance Access published May 4, 2012

Reproductive Options/Outcomes in Women with Turner Syndrome

- Spontaneous pregnancy is a rare occurrence
  - ≥ 40% normal live-born children
  - Approx 20% children with TS
  - ≥ 30% spontaneous abortion rate
- Successful oocyte freezing and IVF for menstruating patients (one report, each)
- "Successful" donor oocyte with real but unknown risk of aortic dissection, rupture, and death.
- Donor oocyte with gestational carrier
- Adoption

References on Slides of Presentation

Klinefelter Syndrome: Reproductive and Hormonal Options and Outcomes

Rebecca Z. Sokol, M.D.,M.P.H.
Professor
Medicine
Obstetrics and Gynecology
Keck School of Medicine
University of Southern California

Disclosure

Rebecca Z. Sokol , M.D., M.P.H.

Nothing to Disclose

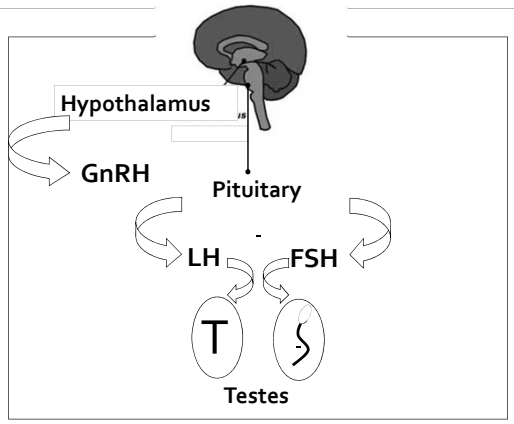
Learning Objectives

At the conclusion of this presentation, the participant should be able to:

1. Discuss the pathophysiology, genetics, and associated medical conditions of Klinefelter Syndrome
2. List the signs, symptoms and laboratory results associated with Klinefelter Syndrome
3. Discuss the treatment of patients with Klinefelter Syndrome

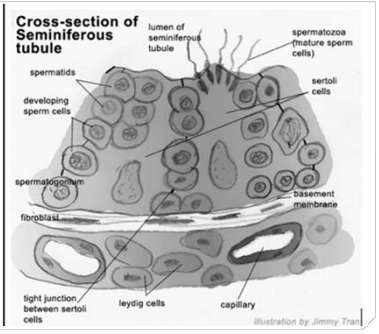
Overview

- Overview of Klinefelter Syndrome
- Evaluation of patients with KS
- Controversies in the treatment of KS



Testicular Compartments

- Germ Cells
- Sertoli Cells
- Leydig Cells



Klinefelter Syndrome

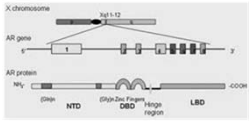
- Most common cause of primary testicular failure
- Incidence 1.2-1.5/1000 male births)
- Under-diagnosed
- May be increasing

Genetics of Klinefelter Syndrome

- Non-disjunction during meiosis
- 53% paternal, 42% maternal
- 5% post zygotic mitosis
- XXY or XXY mosaic
- XXXY maternal origin
- XYXY paternal origin
- Short arm of X chromosome likely region of KS genes

KS and the Androgen Receptor Gene

- X-linked
- Differences in AR sequence found in CAGn in exon 1
- Normal length 9-37



KS and the Androgen Receptor Gene

- CAGn polymorphism modulates androgen action
- Preferential inactivation of the more functional short CAGn allele in KS
- Long allele associated with lower androgenic activity
  - Taller, gynecomastia, osteoporosis, learning disabilities

Symptoms

- Learning disabilities
- Behavioral problems
- Delayed puberty
- Poor coordination
- Head aches/hot flushes
- Weak muscle strength
- Increased BMI/fat
- Fractures
- Decreased libido
- Erectile dysfunction
- Infertility

Signs

- Broad range of presentation from normal body type to eunichoid
- Female body habitus
- Gynecomastia
- Decreased body hair
- Small penis
- Skeletal disorders
- Poor muscle strength
- Anemia
- Low sperm count
- Small testes

Vast array of phenotypes

- Midfacial hypoplasia
- Narrow shoulders
- Scoliosis and kyphosis
- Pectus carinatum or excevatum
- Pes planus and narrowing of forearm
- Clinodactyly

Associated Medical Issues

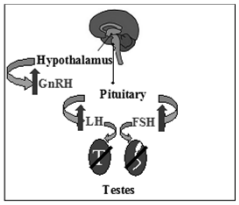
- Motor, cognitive, behavioral dysfunction
- Cancer
- Vascular and Cardiac
- Endocrine/Metabolic
- Autoimmune
- Mortality RR 1.63 (CI 1.40-1.91)

Laboratory testing

- Pre puberty
  - Karyotype
  - Educational testing
- Puberty and Adulthood
  - Karyotype
  - T, LH, FSH, E2
  - Androgen receptor studies
  - SA
  - Educational testing

Klinefelter Syndrome

- Low testosterone
- Elevated
  - LH and FSH
- Oligospermia or Azoospermia



Treatment with Testosterone

Preparations

- Oral
- IM
- Patch
- Gel
- Skin Implants

Controversies

- Timing
  - At 3 mo
  - Pre-pubertally
  - Pubertal age
  - Adulthood
- Dose
  - High vs low
  - Topical vs IM

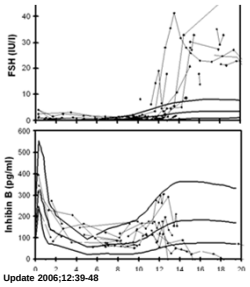
Hormone and germ cell changes with age

Testosterone declines with age

- Prenatal
  - Unclear if T increases at 12 wks
- Neonatal
  - "Mini-puberty" at 3mo may be absent
- Pre-puberty
  - T does not increase in majority of pts
  - T increases in some
- Puberty
  - T decreases by 15 yrs in majority
- Adulthood
  - T low in 80%
  - E2 elevated in most

FSH &LH Increase and Inhibin Decreases With Age

- Longitudinal study
- 36 untreated KS boys



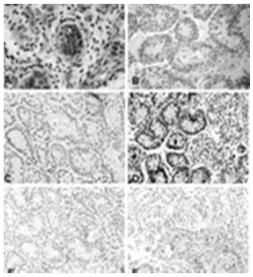
Aksglæde L et al. Hum. Reprod. Update 2006;12:39-48

Testicular Histology Changes With Age

- Germ cell migration to genital ridge is normal
- Infant testicular histology near normal
- Mitotic proliferation is reduced as testis develops neonatally
- Spermatogonia start to decrease prepubertally
- Apoptosis occurs at puberty
- Sertoli cells decline around puberty
- Adults have extensive fibrosis & hyalinization of seminiferous tubules and hyperplasia of interstitium

Testicular Histology Changes With Age

- A. Fetal testis
  - Germ cells
- B. 4 yr old boy
  - Still with germ cells
- C-E. 10-12 yr old boys
  - Decreasing germ cells
- F. 14 yr old boy
  - No germ cells
  - Degeneration of tubules



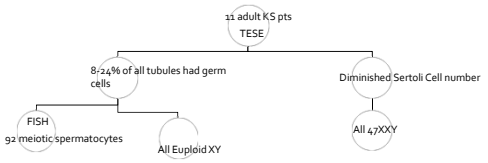
Human Repro Update 2006; 12:39-48

Etiology of Testicular Degeneration

- Increased expression of genes on the extra X chromosome
- Intratesticular hormone imbalance
- Abnormal apoptotic activity of Sertoli and Leydig Cells
- Defects in spermatogonial stem cells

Fertility of KS Patients

What is the Source of the Fertile sperm?



Sciurano et al Hum Reprod 2009; 24-2353-60

What is the Source of the Fertile sperm?

- The sperm originate from clones of spermatogonia that have randomly lost one of the X chromosomes
- Suggests that the sperm that are "lost" after puberty are the 47XXY sperm that never had the potential to become haploid
- Thus harvesting after puberty is futile

TESE/Micro-TESE and ICSI for Adults

- Sperm retrieval 29-57%
- Pregnancy rate 50%
- No predictive factors of sperm retrieval
- 2-3X increase in autosomal and sex chromosome abnormalities in KS sperm than in controls
- Offspring usually with normal karyotype
- Genetic Counseling recommended

Should aggressive fertility management be offered to peripubertal boys? PRO

- Some studies report progressive decline in spermatogonia
- Puberty is associated with accelerated germ cell depletion
- Some TESE studies reported a correlation between sperm aspirated and younger age of patient
- Science is evolving

TESE for Adolescent KS patients

Successful testicular sperm retrieval in adolescents with Klinefelter syndrome treated with at least 1 year of topical testosterone and aromatase inhibitor

Akanksha Mehta, M.D., Alexander Bolyakov, M.Sc., Jordan Roosma, Peter N. Schlegel, M.D., and Darius A. Paduch, M.D., Ph.D.

Department of Urology, Weill Cornell Medical College, New York, New York

Should aggressive fertility management be offered to all peripubertal boys? CON

- There are known negative effects of TESE/ micro-dissection
- There is no evidence that the sperm cells harvested will increase fertility potential
- Data suggest that it is only sperm that have haploid potential that are the "fertile" sperm harvested and may be preprogrammed in utero.
- There are no clinical parameters available to detect patients who might benefit
- Successful recovery of sperm by TESE in adults is 50%

It is Not all About the Testes

Etiology of KS Cognitive Phenotype

KS Cognitive Phenotype

- Executive dysfunction
  - Poor judgment & decision making
  - Poor self-control
  - Poor problem solving & reaoning
  - Don't learn from their mistakes

KS Cognitive Phenotype

- Learning disabilities
  - Language and language-based learning
    - Delayed early speech
  - Auditory processing and memory
  - Less difficulty with non verbal and spatial testing

Left Hemisphere Dysfunction

- Cerebral blood flow
  - KS: symmetric in temporal & parietal(language)
  - Controls: asymmetric
- MRI
  - Reduction of left temporal gray matter
  - Increase in lateral ventricle volume
    - Correlated with poor language skills

KS Cognitive Phenotype

- Not clear of role of testosterone exposure
- KS animal model
  - Rate of learning slower in XXY mice
  - Decreased X inactivation + Decreased T
- CAGn (trinucleotide repeat in exon 1) Polymorphism
  - Higher functioning with shorter length

Other Medical Conditions

Tumors

- Mediastinal germ cell tumors
  - Incidence in general population is 1.13-1.53
  - Incidence in KS is estimated at 60X
- Breast Cancer
  - Controversial
  - Incidence in KS is 3.7%-7.5%
  - 50X that of the general male population

Vascular and Cardiac Disease

- Phlebitis and thrombosis
  - HR 5.29 (95% CI 3.29-8.5)
- Pulmonary embolism
  - HR 3.60 (95% CI 1.92-6.74)
- Ischemic heart disease
  - HR 1.71 (95% CI 1.28-2.29)

Autoimmune Diseases

- SLE
- Sjogren syndrome
- Rheumatoid Arthritis

Endocrine and Metabolic Diseases

- Osteoporosis
- DM2
- Obesity
- Metabolic Syndrome
- Hypothyroidism

Etiology of Associated Disease

- Not known
- Misbalance of ratio of Testosterone: estradiol proposed
- Part of genetic syndrome

Summary

- Incidence of KS is underestimated and under diagnosed
- KS is a syndrome with many associated medical issues
- Early diagnosis improves quality of life
- Etiology for decline in germ cells with age is unclear
- Testosterone therapy is indicated at puberty
- Fertility can be achieved with TESE/ICSI
- TESE/BX for preservation of sperm at puberty is experimental

Slide 44

1 Rebecca Sokol; 21/04/2014

SOKOL REFS for KS Talk

1. Aksglaede L, et al. Therapy of endocrine disease: Testicular function and fertility in men with Klinefelter syndrome: a review. Human Reprod Update 2006;12:39-48.
2. Bojesen A, Juul S, Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. J Clin Endocrinol Metab 2003; 88:622-6.
3. Bojesen A, et al. The metabolic syndrome is frequent in Klinefelter's syndrome and is associated with abdominal obesity and hypogonadism. Diabetes Care 2006;29:1591-8.
4. Gies et al. Spermatogonaial stem cell preservation in boys with KS: To bank or not to bank, that is the question. Fertil Steril 2012; 98:284-9.
5. Lue Y, et al. XXY male mice: an experimental model for Klinefelter syndrome. Endocrinology 2001;142:1461-70.
6. Maiburg M et al. The genetic origin Klinefelter Syndrome and its effect on spermatogenesis. Fertil Steril 2012; 98:253-60.
7. Mehta A and Paduch DA. Klinefelter syndrome:an argument for early aggressive hormonal and fertility management. Fertil Steril 2012; 98:274-83.
8. Mehta A et al Successful testicular sperm retrieval in adolescents with KS. Fertil Steril 2013; 100:970-76.
9. Oates RD: The natural history of endocrine function and spermatogenesis in KS: what the data show. Fertil Steril 2012; 98: 266-73
10. Rives N et al. The feasibility of fertility preservation in adolescents with KS. Human Reprod 2013; 28:1468-79.
11. Schiff JD. Et al. Success of testicular sperm extraction and ICSI in men with KS. JCEM 2005; 90:6263-7.
12. Simpson JL, et al. Klinefelter syndrome: expanding the phenotype and identifying new research directions. Genet Med 2003; 5:460-8.

13. Sokol RZ. It's not all about the testes: medical issues in Klinefelter patients. Fertil Steril 2012; 98:261-5.
14. Tartaglia N, et al. A new look at XXYY syndrome: medical and psychological features. Am J Med Genet 2008;15:146A:1509-22.
15. Zitzmann M, et al. . X-chromosome inactivation patterns and androgen receptor functionality influence phenotype and social charactersitcs as well as pharmacogenetics of testosterone therapy in Klinefelter patients. J Clin Endocrinol Metab 2004; 89:6208-17.

Sperm Aneuploidy and ART

Dolores J. Lamb, Ph.D., HCLD

ASRM President (2011-2012)

Director, Center for Reproductive Medicine

Professor and Vice-Chairman for Research

Scott Department of Urology

Professor of Molecular and Cellular Biology

Baylor College of Medicine

Disclosures

- **Reproductive biology and cancer research in the Lamb laboratory is supported by US National Institutes of Health grants R01 DK078121, P01 HD36289, K12 DK083014, and T32 DK00763 and by the US Department Of Defense, US Army Materiel Command PC061154**

Learning Objectives

- **At the conclusion of this presentation, the attendee should be able to:**
  - **Discuss the relationship of sperm aneuploidy, recurrent pregnancy loss and ART**
  - **Understand the known causes of sperm aneuploidy**
  - **Know the tests used to diagnose sperm aneuploidy and the test interpretation**

The Problem of Recurrent Miscarriage

- Three or more consecutive pregnancy losses
- Know
  - I
  - Idiopathic

A Majority of First Trimester Abortuses Are Chromosomally Abnormal

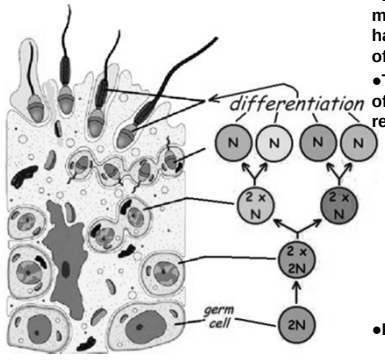
BCM Baylor College of Medicine Center for Reproductive Medicine

When Do Chromosome Abnormalities Arise?

- **Gametogenesis**
  - Meiotic Non-Disjunction During

The Male Contribution to Recurrent Pregnancy Loss At the Sperm Chromosomal Level Is Not Always Considered During the Evaluation of the Couple With Current Pregnancy Loss Especially If the Male Is Normozoospermic

Chromosome Deletion or Gain (Aneuploid) in Offspring May Result From Defective Spermatogenesis

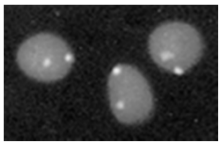


- During spermatogenesis, meiosis results in a haploid germ cell (one set of chromosomes)
- The loss of or presence of an extra chromosome results in:
  - Down's Syndrome(Trisomy 21)
  - Klinefelter (XXY)
  - Turner Syndrome (Monosomy X)
  - Edwards Syndrome (Trisomy 18)
  - Patu Syndrome (Trisomy 13)
- Fetal loss

Spermatogenic Defects Leading to Genetic Abnormalities in Sperm

- **How Do We Measure Sperm Aneuploidy?**

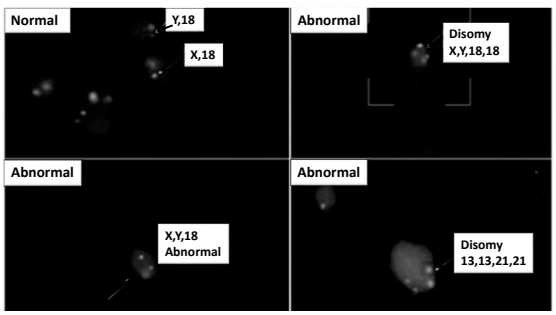
Fluorescent In Situ Hybridization (FISH)



X chromosome= green
Y chromosome= red
Chromosome 18= yellow

- Uses multicolor fluorescently labeled DNA probes specific for each chromosome
- Allows detection of number of specific chromosomes in a cell
  - Haploid = single copy of each chromosome (germ cells)
  - Diploid = double (disomic) copy of each chromosome (somatic cells)
  - Aneuploid = any deviation from haploid or diploid state

Examples of Sperm Aneuploidy

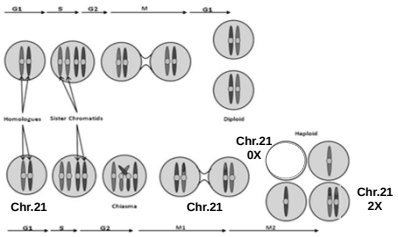


How Does Aneuploidy Occur?

- **Non-dysjunction (main mechanism in sperm)**
  - **Reduced or absent meiotic recombination**
- **Anaphase lag (nullisomic sperm)**
- **Ineffective checkpoint control**
  - **Synaptic and recombination errors cause abnormal chromosome segregation and meiotic arrest**
  - **Failure to arrest meiosis with aneuploidy**

Templado, et al, Molec Hum Repro 19:634-643, 2013

One Example of Aneuploidy



What is Normal? Average Percentages of Sperm Disomy in Healthy Individuals

Chr.#	%
1	0.08
2	0.09
3	0.20
4	0.08
6	0.04
7	0.06
8	0.03
9	0.16
12	0.14

Chr.#	%
13	0.12
15	0.10
16	0.07
18	0.06
20	0.12
21	0.17
22	0.47
X,Y	0.27

BCM
Baylor College of Medicine
Center for Reproductive Medicine

Templado, et al, Molec Hum Repro 19:634-643, 2013

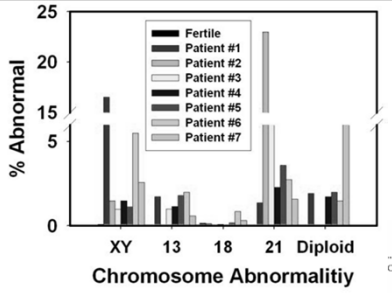
Numerical Chromosomal Abnormalities in Sperm

- **Infertile males exhibit a 10-fold increase in the incidence of sperm specific chromosomal abnormalities, even when a normal somatic karyotype is present**

BCM
Baylor College of Medicine
Center for Reproductive Medicine

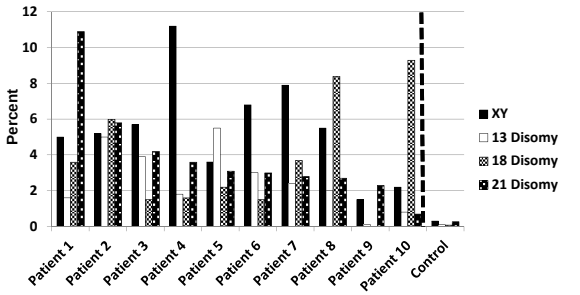
Selected Infertile Men Exhibit High Levels of Sperm Aneuploidy

Nearly 25% of Patient #2's Sperm Have an Extra Chromosome 21
With ICSI, Predict 25% of Embryos with Down's Syndrome



BCM
Baylor College of Medicine
Center for Reproductive Medicine

High Sperm Aneuploidy In a Subset of Recurrent Pregnancy Loss Male Partners



Which Categories of Spermatogenic Deficiency Exhibit Elevated Sperm Aneuploidy?

- **Oligoasthenoteratozoospermia (OAT)**
- **Oligoteratozoospermia**
- **Oligozoospermia**
- **Non-obstructive azoospermia**
- **Normozoospermic men with Recurrent Pregnancy Loss**

Other Types of Patients With Increased Sperm Aneuploidy

- **Unilateral or bilateral cryptorchidism after orchidopexy**
  - **Moretti, et al, J.Androl 28:194-199, 2007**
- **Varicoceles**
  - **Baccetti, et al.,J. Androl 27:94-101, 2006**
- **Chemotherapy (Beiomycin, doxorubicin, vincristine, dacarbazine, novantrone, oncovin, velban, prednisone**
  - **Tempest, et al, Hum Reprod 23:251-258, 2008**
  - **Choy and Brannigan, Fert Steril 100: 1187-1190, 2013**
- **Germline *TP53* Mutations (Li-Fraumeni syndrome (p53))**
  - **Paulasova, et al., Cancer Genetics 204: 278-281, 2011**

Lifestyle Issues May Affect Sperm Aneuploidy Levels

- **Cigarette smoke**
- **Alcohol consumption**
- **Diazepam (>0.3 mg/kg/day > 6 mo)**
  - Baumgartner, et al, 2001
- **Finasteride**
  - Collodel, et al., Arch Androl 53:229-233, 2007
- **Benzene**
  - Liu,et al., Yi Chuan Xue Bao 30:117-1182, 2003
  - Li, et al, Yi Chuan Xue Bao 28:589-5942, 2001
- **Pesticides/Fenvalerate/Carbaryl/Polychlorinated biphenyl, *p,p'*-DDE/Organophosphate pesticides/Pyrithroids**
  - Perry Hum Reprod Update 14:233-242, 2008
  - McAuliffe, et al., Env Health Perspec 120: 535-540, 2012

Reviewed in Hwang, et al., Therapeutic Advances in Urology, 2010

What Level of Sperm Aneuploidy Can Result in Aneuploidy in the Offspring?

- There is an association between fathering aneuploid offspring or recurrent abortions and moderately increase levels of sperm aneuploidy
  - Moderate increases in the rates of aneuploidy are clinically significant (2-3x)
    - Templado, et al, Molec Hum Repro 19:634-643, 2013
    - Down syndrome, Turner
      - Blanco, et al., Am J Hum Genet 63:1067-1072, 1998
      - Carrell, J Androl 29:124-133, 2008
      - Nagvenkar, et al., Fert Steril 84:925-931, 2005
  - Controversial
    - Hixon, et al, 1998, Eskenazi, et al, ,2002

BCM
Baylor College of Medicine
Center for Reproductive Medicine

Who Should Be Tested for Sperm Aneuploidy?

- Oligospermic men
- Oligoasthenozoospermic men
- Recurrent pregnancy loss

BCM
Baylor College of Medicine
Center for Reproductive Medicine

Take Home Message

- Sperm aneuploidy is important to evaluate in male partners of women with recurrent pregnancy loss
- Sperm fluorescent *In situ* hybridization is the method commonly used to detect sperm aneuploidy
  - Chr. 13, 18, 21, X, Y
  - Consistent with a viable but affected offspring
- Normal fertile men display low levels of sperm aneuploidy
- Men with increased levels of sperm aneuploidy should undergo genetic counseling
- Options
  - PGD with ICSI, natural conception, sperm donor, adoption, remain childless


Thank You
Texas Medical Center, Houston

IMPRINTING DISORDERS AND ART

JOE LEIGH SIMPSON, M.D.

Senior Vice President for Research and Global Programs, March of Dimes Foundation

President, International Federation Fertility Societies (IFFS)

ESHRE 2014

OBJECTIVES

1. **State the relative increase (odds ratio) in birth defects associated with ART**
2. **Be able to state at least two potential explanations for the increased frequency of birth defects in ART**
3. **Be able to state how you would inform couples embarking upon ART concerning risk for birth defects in their offspring**

META-ANALYSES: ART and Birth Defects

	Year	Studies Accepted	OR	95% C.I.
Rimm	(2004)	19	1.29	(1.01-1.67)
Hansen	(2005)	25 *	1.29	(1.21-1.37)
Wen	(2012)	46	1.37	(1.26-1.48)
Hansen	(2013)	45 *	1.32	(1.24-1.42)

**** 14 reports accepted in 2005 report excluded in 2013***

ART AND BIRTH DEFECTS (Reports 2005-2013)

Significant Differences

	ART Years	Odds Ratio	95% C.I.	Country
Kallen (2005)	1982-2001	1.44	(1.32-1.57)	Sweden
Hansen (2005)	1994-1998			Australia
Kallen (2010)	2001-2007	1.25	(1.15-1.37)	Sweden
Klemmetti (2005)	1996-1999	1.31	(1.10-1.57)	Finland
Fuji (2010)	2006	1.17	(0.81-1.69)	Japan

ART AND BIRTH DEFECTS (Reports 2005-2013)

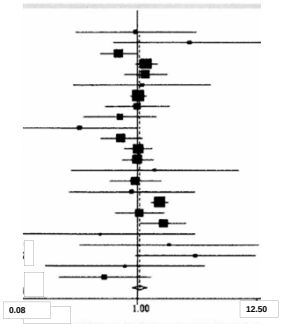
Significant Differences

	ART Years	Odds Ratio	95% C.I.	Country
Davies (2012)	1986-2002	1.24	(1.09-1.41)	Adelaide, Australia
Halliday (2010)	1991-2004	1.36	(1.19-1.55)	Australia
Kelly-Quon (2013)	1995-2000	1.25	(1.12-1.39)	California, USA
Hansen (2012)	1984-2002	1.53	(1.30-1.79)	Perth, Australia
No Significant Difference				
Moses (2014)	2007-2011	1.01	(0.67-1.52)	Colorado, USA

PITFALLS AND ANALYSIS

- Current ART methods and embryo culture methods earlier era and lack generalizability
- Multiple ovulation stimulation and embryo culture techniques
- In population-based studies lack of consistent anomaly surveillance, leading to differing anomaly rates reflecting inclusion minor anomalies

BIRTH DEFECTS: IVF VERSUS ICSI (Wen, 2012)



ASSISTED CONCEPTIONS AND BIRTH DEFECTS STUDY DESIGN (DAVIES, 2012)

- South Australia Registry (1986-2002)
- Birth defects sought before 5th birthday
- Included terminations for anomalies <20 weeks, within 28 days birth, or reported from multiple other sources
- 6163 Assisted conceptions/ 308,974 Births
- Multiple ($p<0.001$) differences between assisted and spontaneous conceptions – age, socioeconomic status, race, nulliparity, paternal occupation, smoking, multiple gestation, diabetes, anemia.

Davies et al., 2012

BIRTH DEFECTS ADJUSTED AND UNADJUSTED

		Odds Ratio	
Conceptions	Percentage*	Unadjusted	Adjusted
All Assisted	8.3%	1.47	1.28
All Spontaneous	5.8%		
IVF alone	7.2%	1.26	1.07 (0.90-1.26)
ICSI alone	9.9%	1.77	1.57 (1.30-1.90)

Includes terminations <20 weeks, live births 1-28 days and any reported anomaly <5 years

Davies et al., 2012 NEJM

BIRTH DEFECTS: ICSI / IVF versus Traditional IVF

- **No consistent difference observed in overall frequency of structural birth defects**
- **Hypospadias increased in offspring of ICSI pregnancies. Could reflect transmission of pleiotropic paternal genes that contributed to male infertility**
- **Sex chromosomal abnormalities seem increased in ICSI offspring**
  - **Relationship to balanced autosomal translocation in ICSI father (2%) and mothers (1.2% - 2.0%)**

HYPOSPADIAS IN ICSI OFFSPRING

- **Wennerholm et al. *(2000):* RR 3.0 *(1.09-6.50)* compared to Swedish Medical Birth Registry and Registry of Congenital Malformation**
- **Ericson and Kallen *(2001):* RR 1.5 *(1.0-2.1)***
- **Klemetti et al (2005)- 76/10,000 v 29/10,000**

Explanations for Increased Risk Birth Defects

- **Are increased risks related to ART per se?**

Or,

- **Do increased risks relate to underlying reason why ART needed?**

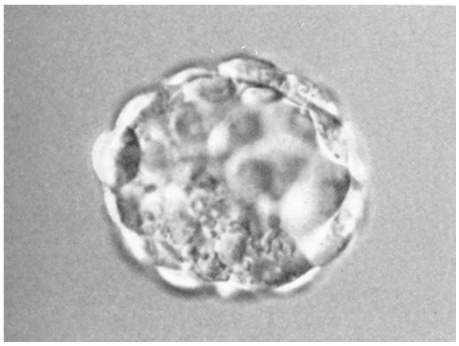
TECHNICAL VARIABLES IN ART

- Ovulation stimulation regimes
- Obtaining and handling gametes
- Embryo culture
- Cryopreservation
- Many potential confounders previously employed in IVF/ICSI no longer utilized (2013)

EMBRYO CULTURE

- Media composition not also disclosed (Proprietary)
- Supplementation has varied:
  - ± human or maternal serum albumin
  - ± synthetic serum substitute
- Considerable changes decade by decade

BLASTOCYST (DAY 5-6)



INCREASED LENGTH OF TIME IN CULTURE FOR BLASTOCYSTS

One third of cleavage stage embryos do not survive in vitro to day 5.

- **Selection against aneuploidy?**
- **Traditional culture medias developed for first three days, but now cultures must extend to five days. Appropriate culture media for day 6?**

DIFFERENTIAL GENE EXPRESSIONS WITH DIFFERENT EMBRYO CULTURE MEDIA

- **Mouse embryos in Whitten's media misexpressed 114 genes *(Affymetrix microarray chip)* compared to in vivo embryos**
- **Incubation in KSOM / AA medium misexpressed 29 genes**

Rinaudo and Schultz, Reproduction 128:301, 2004

CONCLUSION: TECHNICAL VARIABLES

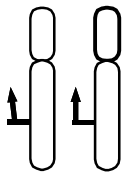
- **Potential disturbances at each step**
- **Plausible that certain genes differentially expressed compared to in vivo conception, either through ovulation stimulation or embryo culture**
- **Few contemparous population-based reports and these show lower OR (*Fuji 2006 OR 1.17; Moses 2007-11 OR 1.01)***

IS IMPRINTING THE EXPLANATION FOR INCREASED BIRTH DEFECTS?

- Plausible give potential effects of culture media and incubation. However, also hypothesis of last resort
- Deleterious effect would not necessarily be embryonically lethal ("all or none" phenomenon) and could be manifested as birth defects
- But, absolute rate low given rarity of known imprinting syndrome (Beckwith-Wiedemann, Russell Silver, Prader-Willi, Angelman)

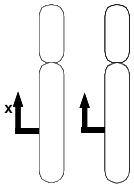
Bi-Allelic Expression

- From both chromosomes, maternally and paternally inherited alleles. Some genes designed to be expressed only by allele from a parent of given sex



Genomic Imprinting

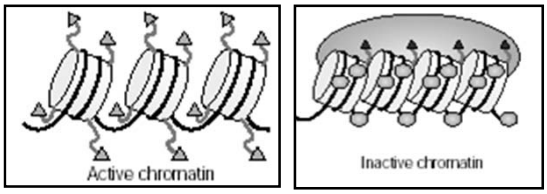
The unequal expression of maternal and paternal alleles



Maintaining Parent of Original Imprinting

- Parental mark set in germ cells
- Reversible on passage through the opposite parental germline (switch)
- Differential expression of the new parental imprint in the offspring
- Stable transmission through mitosis in somatic cells

IMPRINTING AND CHROMATIN MODIFICATIONS

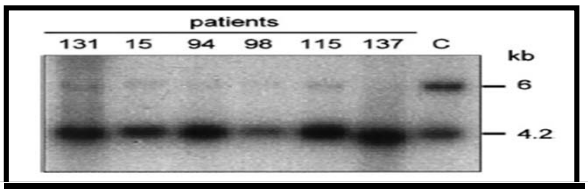


DNA methylation = Inactive

ART AND BECKWITH - WIEDEMANN SYNDROME *(BWS)*

- Overgrowth syndrome. IGF2 normally expressed only from paternal allele. Maternal H19 restrains growth by suppressing maternal IGF2. Usual mechanism in BWS: hypomethylation of maternal KCNOT1 (60%) leading to ⇧IGF2 of maternal origin.
- De Braun (2003): 7 of 65 BWS cases associated with ART, 5 requiring ICSI
  - Imprinting perturbation (*H19)* involved maternal allele, uncommon molecular basis for BWS

BECKWITH-WIEDEMANN SYNDROME



6 kb band: methylated
4.2 kb band: unmethylated

IMPRINTING DISORDERS IN ART REGISTRIES

- **Population-based *(vital statistics)* studies in Scandinavia show no increased risk overall**
- **When odds ratio for selected disorders is increased, the absolute effect is small because imprinting disorders are rare**
- **No technical feature in common with cycles resulting in birth defects.**

Are increased risks related to ART per se?

Or,

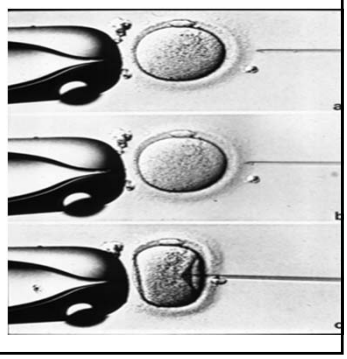
- **Do increased risks relate to underlying reason why ART needed?**
  - **Different populations: Fertile v Infertile**
  - **Populations could alter differ? re: status of imprint**

CHARACTERISTICS OF POPULATION REQUIRING INFERTILITY TREATMENT

- 10% of population (equal male and female)
- Differs from general population
  - Older age
  - May have genetic disorders with implications for offspring (e.g., Kartagener syndrome, cystic fibrosis), in both male and female partners
  - Increased balanced translocations in both male and female partners

SPERM ANEUPLOIDY AND ICSI

- 3 fold increase in sex chromosome aneuploidies after ICSI (prenatal diagnosis data)



ICSI FATHERS AND HYPOSPADIAS IN OFFSPRING

- Hypospadias is polygenic / multifactorial with 2.5% recurrence risk for first-degree relatives
- Gonadal abnormalities that necessitate ICSI for fertilization could result in decreased hormone production in father and fetus and thus hypospadias

EPIGENETIC PERTURBATION IN SPERM OF INFERTILE MALES

• MTHR, PAX 8, NTF3, SFN, HRAS	Hypermethylation
• IGF2, H19	Decreased methylation
• RASGRF1 GTL2, PLAG1, MEST, KCND1 LIT1, SNRPN	Locus – specific hypermethylation
• H3K4me, H3K27 me	Histone retention (nucleosomes)

INCREASED TRANSLOCATIONS IN COUPLES UNDERGOING ICSI *(PER 1,000)*

	Female	Male	Newborns
Rcp	6.9	12.3	1.52
Rob	6.9	8.2	0.90
Inv	6.9	1.4	0.42
Total	20.7	21.9	2.84
	(2.07%)	*(2.19%)*	*(0.28%)*

Gekas et al., Hum. Reprod., 2001

EPIGNETIC (METHYLATION) IN SILVER – RUSSELL SYNDROME

- PEGi / MEST locus ordinarily demethylated (active gene)
- Infertile father requiring ICSI showed unscheduled methylation at four cytosine residues. During father's meiosis four additional residues became methylated = Silver-Russell syndrome transmitted to twin offspring

Eggermann et al., Amer J Med Genet C 2010

SUBFERTILITY AND BIRTH DEFECTS

- Danish national birth cohort; interviews determined infertility history
- 50,897 singleton and 1366 twins of fertile couples; Time to pregnancy (TTP) < 12 months)
- 5764 singleton and 100 twins; TTP > 12 months, but natural conception
- 4588 singleton and 1690 twins undergoing infertility treatment (singleton 398 ICSI; 1483 IVF; others "hormonal", surgery, IUI)

OR 1.2 Birth Defects

Zhu et al., BMJ 2006

BIRTH DEFECTS: SUBFERTILITY (>12 months) versus "normal" (<12 months)

	Odds Ratio	95% C.I.
Zhu, 2006	1.20	1.07-1.35
Jacques, 2010	1.30	0.98-1.72
Davies, 2012		
– Subfertile no prior pregnancy	1.29	0.99-1.68
– Spontaneous conception after prior ART	1.25	1.01-1.56

SUBFERTILITY AND BIRTH DEFECTS

- Naturally conceived pregnancies occur in couple "infertile" by traditional definitions (12 months unprotected coitus)
- Frequency of birth defects: Increased compared to controls
- Thus, increased rate in ART offspring likely reflects underlying biologic reason necessitating ART. May or may not account for all (Rimm) increase

CONCLUSIONS (1): BIRTH DEFECTS AND ART

1. ***Overall*** **malformation rate increased (relative risk 1.3). Only specific abnormalities: hypospadias and sex chromosomal abnormalities in intracytoplasmic sperm injection (ISCI)**
2. **Increase in birth defects probably similar in IVF alone versus IVF/ISI**

CONCLUSIONS (2): BIRTH DEFECTS AND ART

3. **Whether increase is due to ART or to characteristics of underlying population is unclear.**
   - **ART couples not representative of the general population; thus, true control group not possible.**
   - **Birth defects increased in sub-fertile couples not requiring ART.**

CONCLUSIONS (3): BIRTH DEFECTS AND ART

4. **Epigenetic phenomena plausible explanation but unproven. Unclear whether alterations observed are a component of subfertility or arose in ART, but former certain in selected cases**

UPCOMING ESHRE EVENTS

// ESHRE CAMPUS EVENTS

ESHRE's 30th Annual Meeting

www.eshre2014.eu

Munich, Germany
29 June - 2 July 2014

Epigenetics in reproduction

www.eshre.eu/lisbon

Lisbon, Portugal
26-27 September 2014

Endoscopy in reproductive medicine

www.eshre.eu/endoscopyoct

Leuven, Belgium
15-17 October 2014

Making OHSS a complication of the past: State-of-the-art use of GnRH agonist triggering

www.eshre.eu/thessaloniki

Thessaloniki, Greece
31 October-1 November 2014

From gametes to blastocysts – a continuous dialogue

www.eshre.eu/dundee

Dundee, United Kingdom
7-8 November 2014

Controversies in endometriosis and adenomyosis

www.eshre.eu/liege

Liège, Belgium
4-6 December 2014

Bringing evidence based early pregnancy care to your clinic

www.eshre.eu/copenhagen

Copenhagen, Denmark
11-12 December 2014

An update on preimplantation genetic screening (PGS)

www.eshre.eu/rome

Rome, Italy
12-13 March 2014

For information and registration: www.eshre.eu/calendar
or contact us at info@eshre.eu

eshre
SCIENCE MOVING
PEOPLE
MOVING SCIENCE

NOTES

NOTES

NOTES

NOTES

NOTES

NOTES

NOTES

NOTES