Dr. Louise Wilkins-Haug

Hosted By:
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Assisted reproduction, congenital malformation and imprinted genes – Is there reason for concern?

Louise Wilkins-Haug, MD,PhD

Current knowledge of malformations following IFV is preliminary

Author	N	Major	minor
Govaerts, 1998	141	0	1.4 %
Van Golde, 1999	119	1.6%	
Loft, 1999	721	3.5%	
Wennerholm, 2000	1139	4.1%	
Palmero, 2000	2059	1.1%	0.5 %
Lancaster, 2000	2762	2.5%	
Bonduelle, 2002	2840	3.4%	6.3%

Current knowledge

- Has risk been properly assessed?
- Is there a biologic reason to be concerned?

Assessment of risk - potential pitfalls

- 1) Use of poorly delineated study populations
- 2) Inconsistencies in determination of congenital anomalies
- 3) Heterogeneity of ART - ovulation induction, IVF, ICSI, assisted hatching, etc

#1 Comparing infertility population to general population

- Etiology of infertility may be tied to congenital/genetic disease

- Infertile population may be different than naturally conceiving population by known and unknown covariants

Etiology of infertility as a factor - oligospermia

Author	Def.	N	Autosomal translocations	XX/XY anomalies	Total % abnormal
Mastuda	<20	326	11 (3.4%)	5(1.5%)	4.9%
Bourr.	<10	594	23 (3.9%)	11 (1.9%)	5.8%
Abylho.	<10	180	6 (3.3%)	5 (2.7%)	6.1%

Habitual miscarriage -balanced translocations

- Newborn surveys 0.2%
- Habitual SAB couples 3 - 4 %
- > 3 consecutive SABs 9.2 %

- infertile couples 0.6 %
- >10 IVF cycles failed 3.2 %

- Stern C., 1999

Male factor – congenital bilateral absence of the vas deferens

Author	**N**	**Compound Heterozygote**	**Heterozygote**	**% CF carriers (at least one allele)**
Mercier	67	24%	42%	66%
Chillon	102	19% - two mutations	25% - one mutation	78%
		34% - mutation/5T	7% - 5T alone	85% (incl 5T)

Female factor infertility

- X/Autosomal translocations
- X chromosome mosaics
- Fragile X Premutation Carriers
- Single gene disorders

Chromosomal anomalies associated with ICSI conceptions likely reflect paternal condition

- 1.2% risk of chromosomal rearrangements
  - All of paternal origin
  - All present on paternal peripheral blood analysis

- 1.0% risk of sex chromosome aneuploidy
  - 4/5 cases determined to be of paternal origin
  - No evidence of paternal mosaics in peripheral blood (Bonduelle, 1996; In,t Veld, 1996)

Is the infertile population potentially different ?

- Known and unknown covariates of infertility and congenital malformations
  - Maternal and paternal age differences
  - Exposures - smoking
  - Prenatal care – folate, etc
  - Pregnancy termination

Assessment of risk - potential pitfalls

- 1) Use of poorly delineated study populations
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#2 Determination of congenital anomalies - controls usually from “registry“

- Completeness of exam variable
- Increased use of ultrasound in infertility patients and follow-up of identified concerns
- Timing of neonatal exam
  - 2-3% rate is in first 48 hours

Definition of anomaly

- Major versus minor
- Coding of anomaly
- Single versus multiple

Sample sizes and power for events of low frequency

- 3% rate requires 244 cases to have 95% confidence to detect a 3 fold increase

- Requires pooling of anomalies which may dilute a potential increase in a specific system or type of anomaly

Sample sizes and power for events of low frequency

- subset analyses fall victim to “multiple testing “
- testing 20 categories of anomalies would result in 1 reaching significance just due to multiple testing (5% for 20)

Assessment of risk - potential pitfalls

- 1) Use of poorly delineated study populations
- 2) Inconsistencies in determination of congenital anomalies
- 3) Heterogeneity of ART - ovulation induction, IVF, ICSI, assisted hatching, etc

#3 Heterogeneity of treatments not addressed

- IVF - per cent ICSI varies
  - Most recently 9% in European studies, 40% by CDC in 1999
- Ovulation agents; culture media; assisted hatching
- Spematid inseminations, oocyte transfers

Prospective, well designed cohort study is needed with:

- IVF population systematically assessed for AR variables – etiology of infertility, interventions
- Both populations assessed for potential confounders
  - Information obtained concurrently not based on recall
- Large Ns needed
- Same determination of anomalies in both populations

Netherlands study, Anthony 2002

- IVF (N=4224) compared to natural conceptions (314,605)
  - Congenital malformations ascertained from registry used for all births
  - Birth registry interrogated by IVF registry and coded conception type

Netherlands study, Anthony 2002

- IVF population 3.2% major malformation
- General population 2.7%

- Odds ratio 1.20 (95% CI: 1.01-1.43)
- Corrected OR 1.03 (95% CI:0.86-1.23)
  - Maternal age, parity and ethnicity

Netherlands study, Anthony 2002

- Categories of malformation (9)
- Odds ratio below 1.0
  - Skin/abdominal wall
  - Chromosomal
- Odds ratio above 1.0
  - CNS
  - CVS OR=1.56 (95%CI: 1.10-2.22)
  - Digestive
  - Respiratory
  - Urogenital
  - Skeletal/muscular

Netherlands study, Anthony 2002

- Increase in congenital malformations attributable to difference in populations (maternal age, parity, ethnicity)
- When investigated by category, cardiovascular malformations are significantly increased
  - 7/9 categories had odds ratios greater than 1.0

Netherlands study, Anthony 2002

How reliable are the registries ?

Subset of 1716 IVF children with additional questionnaire data

* 95 with congenital malformations not recorded

* 16/95 should have been recorded

* 17% under-reporting in registry

Australian study by Hansen in NEJM March 2002

- Similar and well delineated cases and general population
  - same source of data for both populations
  - only 3 centers for over 1000 AR infants (301 ICSI, 837 IVF alone)

Australian study by Hansen in NEJM March 2002

- same definition of birth defects and same time frame (to one year)
- only majors
- essentially all pregnancies get a 16-20 week US in Western Australia

Australian study by Hansen in NEJM March 2002

	major anomaly by one year of age		
ICSI	26/301	8.6 %	(5.7-12.4%)
IVF	75/837	9.0 %	(7.1-11.1%)
General Population	168/4000	4.2 %	(3.6-4.9%)

Australian study by Hansen NEJM, March 2002

- results held
  - only singletons studied
  - only singletons at term
  - adjusted for maternal age, parity, sex of the infant
  - independent exclusion of malformations possibly diagnosed early because of increased surveillance (either population)

Australian study by Hansen, March 2002

	Natural	ICSI	IVF
Tabs included	4.5%	8.6%	9.5%
Genetic excluded	4.0	8.0	8.5
Minors excluded	4.0	8.1	8.9
Chromosomal excluded	4.0	8.1	8.9
Multiple defects	0.5	2.0	1.6

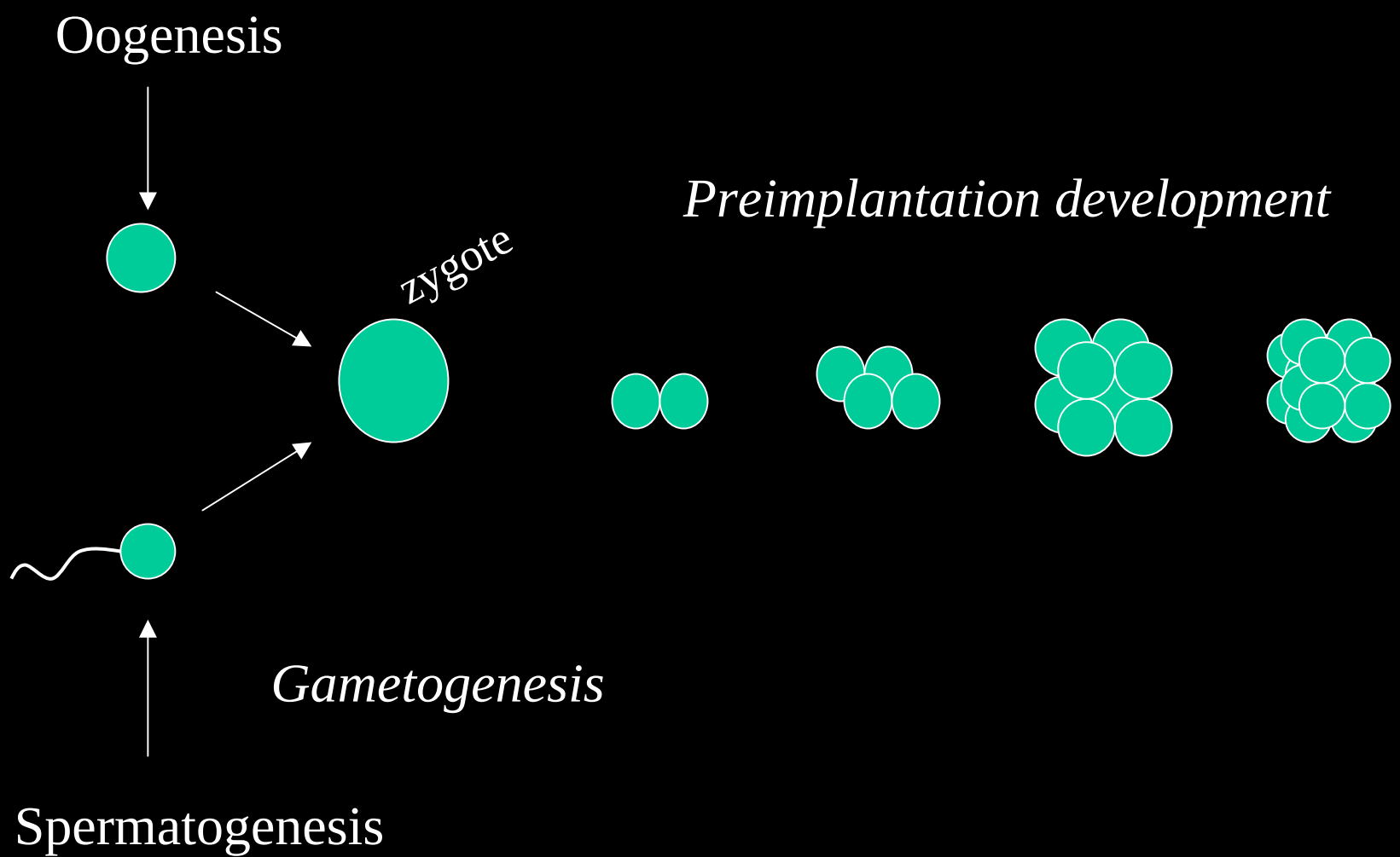
Australian study

- More study is needed
- A small increase, perhaps doubling is probably present
- Issue of multiple anomalies raised
- Due to IVF/ICS or some also to intrinsic to characteristics of infertile population

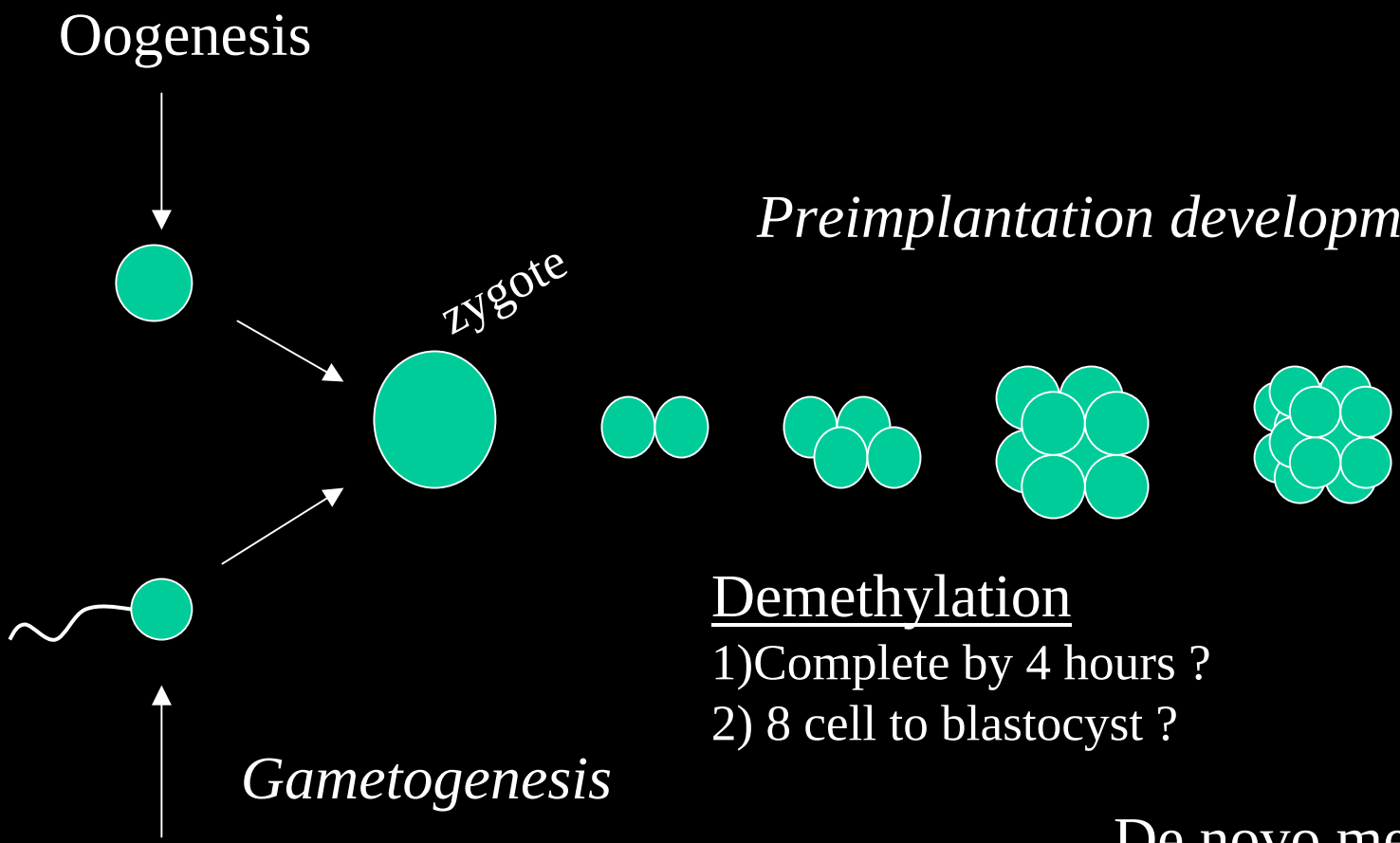
Is there a biologically plausible concern regarding genetic damage?

- Both gametogenesis and preimplantation are times of epigenetic mechanisms
  - Alterations in DNA structure rather than sequence
  - Turning on/off of embryonic genes

Potential sites of imprinting



Assisted reproduction and imprinting



Demethylation

1)Complete by 4 hours ?

2) 8 cell to blastocyst ?

De novo methylation

1) Implantation ?(Monk, 95)

2) Inner cell mass only (Santos, 2002)

Controls of imprinting

- Germline
  - Cis regulatory elements direct allele specific imprinting
  - DMRs –differential methylation regions
- Postfertilization
  - DMRs retained despite genome wide demethylation and de novo methylation
  - Demethylation in morula stage
  - De novo methylation in pregastrula (Kafri,1993)

Control of methylation

- Dnmt1 methyltransferase
  - Syntehsized in oocyte cytoplasm
  - Maintains DNA methylation of imprinted alleles
  - Traffics to nucleus at 8 cell stage only
  - Implies other methyltrasnferases needed

Timing of imprinting (Ohno, 2001)

- Mouse imprinted genes – conflicting evidence for monoallelic transcription in preimplantation embryos
- ASO-RNA-FISH
- Igf2
  - First detected at 2 cell stage
  - Biallelic up to morula
  - Maternal allelle silenced during blastocyst
  - In vitro cultured showed bias toward maternal transcription up to morula

Timing of imprinting (Monk, 2001; Salpekar, 2001)

- Human imprinted genes – variability in onset of expression which is timing and tissue specific, also variable from embryo to embryo
- cDNA from oocytes, 4, 8, blastocyst stages
  - SNRPN, PEG1, UBE3A each expressed
  - Only SNRPN expressed from monoallele (paternal copy)
  - SNRPN monoallelic by 4 cell stage (Huntriss, 1998)

Can imprints be altered ? (Doherty, 2000)

- In mice, monoallelic versus biallelic expression of H19 dependent on media
  - Standard versus supplemented with amino acids
- Biallelic expression concordant with loss of methylation
- Not all imprinted genes affected
  - Snrpn not altered in same media system

Can imprints be altered? (Khosia, 2001)

- Effect of serum addition to media
  - Controls – in vivo conception and implantation
  - Media alone – in vitro
  - Media with fetal calf serum – in vitro
- 1/3 of blastocysts in supplemented media implanted and survived to day 14 fetuses

Timing of imprints (Khosia, 2001)

- Presence of fetal calf serum supplemented media
  - Smaller fetuses compared to both control groups
  - Decreased expression of H19 and IGF2
  - Increased methylation at imprinting center of H19

Any relationship between IVF and imprinting disorders?

- 100 children with the majority conceived by IVF with ICSI with ejaculated sperm (Manning 2000)
  - no imprinting defects of chromosome 15

Any relationship between IVF and imprinting disorders?

- Angelman syndrome and ICSI (Cox, 2001)
  - Imprinting defect occurs in < 5% of cases
  - Maternal allele is unmethylated
  - Case report of two children conceived with ICSI
    - Isolated imprinting defect in both
    - 1/300,000

Any relationship between IVF and imprinting disorders - Beckwith Wiedemann

- LGA, macroglossia, omphalocele
- overgrowth syndromes - omphalocele, CDH
  - altered closures due to organomegaly
  - increased risk of malignancy

Association of BWS with ICSI
(DeBaun, 2003)

- Registry of BWS since 1994

	IVF conceptions	ICSI
Registry	4.6 %	70%
1999 (CDC)	0.76%	42%

- At least a 6 fold increase in BWS

Association of BWS with ICSI
(DeBaun, 2003)

- 5/6 children had imprinting errors of LIT1
  - Maternal allele DMR hypomethylated
  - Generally 60% of BWS due to LIT1
  - Without LIT1 imprint (methylation), LIT1 expression increased suppressing other genes in region

Association of BWS with ICSI
(DeBaun, 2003)

- 1/6 children had abnormal imprinting of LIT1 and H19
  - Hypermethyltion of H19 DMR
  - 15% with H19 imprinting errors
- 1/6 children had no aberrant methylation

Any relationship between IVF and imprinting disorders - Large offspring syndrome (LOS) in cattle

- Cattle and sheep with LGA at birth, congenital malformations
  - Can occur in 1/3 of in vitro conceptions
- Young and Fiarburn, 2000 - theorized as due to problems in imprinted genes --- epigenetic misprogramming

Emerging information on LOS and imprinting in animals

- LOS in sheep associated with reduced expression of Igf2r (Young, 2001)
  - through loss of methylation following embryo culture
  - locus not imprinted in humans

In vitro compared to in vivo cultures (Niemann, 2000)

- Altered levels of expression
  - Heat shock protein
- Transcription of some genes lacking
  - Connexin43 gene (crucial for maintenance of compaction)
- New transcription of other genes
  - bovine leukemia inhibitory factor (bLIF) and LIF-receptor-beta (LR-beta) genes

LOS produced in sheep (Lazzarri, 2002)

- In vitro culture with supplemented media
  - Increased number of cells in blastocysts
  - Increased overall size of balstocysts
  - Increased transcripts for developmentally important genes Hsp70.1, Cu/Zn-SOD, Glut-3, Glut-4, bFGF, and IGFI
  - Correlated with LOS in offspring

Where does this leave investigations of congenital malformation?

- Risk of congenital malformation following assisted reproduction may equal a doubling of background risk
- Potential genetic causes resulting in infertility and malformations need to be addressed
  - Oligospermia/chromosomal abnormalities
  - CBAVD and cystic fibrosis
- Alterations in imprinted genes are suspected based on animal LOS studies and initial human studies of Beckwith Wiedemann