DOES ASSISTED REPRODUCTIVE TECHNOLOGY (ART) INCREASE THE RISK OF BIRTH DEFECTS?

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Teratology Society, 2012

Disclosures

Current Advisory Boards and Consultation

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MULTIPLE GESTATIONS AND ASSISTED REPRODUCTIVE TECHNOLOGIES (ART)

- In U.S. 30% pregnancy rate with 3% triplets and 30% twins. In U.K. 20% pregnancy rate, < 10% twins and 1% triplets
- Multiple gestations lead to complications due to neonatal sequelae of prematurity.
- Half of all ART babies in the U.S. are a twin or triplet

MULTIPLE GESTATIONS AND ART: Birth Weight

- Lower birth weight in twin gestations, but similar whether natural or assisted reproduction.
- That little to no change occurs in birth weight in ART twins suggests underlying maternal predisposition (related to difficulty conceiving).
- In singletons, however, odds ratio = 3 for very low birth weight or = 2.2 for premature birth and for perinatal morality.

MULTIPLE GESTATIONS AND ART: Birth Defects

- Birth defects increased in both monozygotic (MZ) and dizygotic twins in general population and would be expected in ART twins for this reason alone.
- Certain ART methods (blastocyst transfer) increase MZ twinning, independent of ovulation stimulation.

BIRTH DEFECTS AND ART

- Are structural malformations increased?
- Are chromosomal abnormalities increased?

REGISTRIES ASSESSING ANOMALIES IN ART / IVF

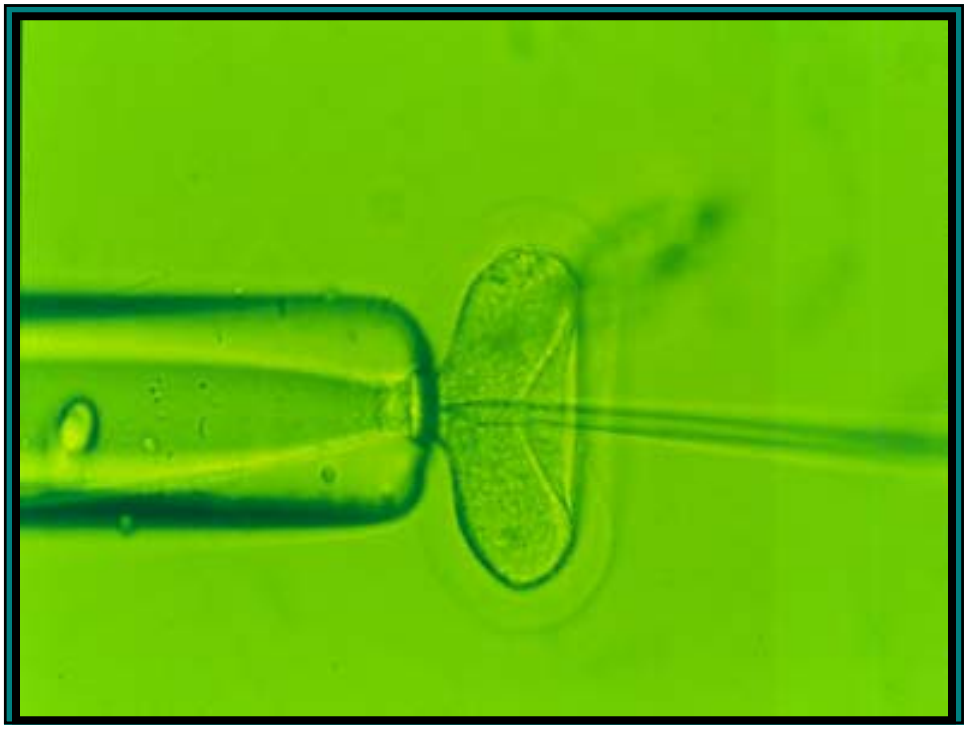
- IVF alone: (prior to 1993)
- IntraCytoplasmic Sperm Injection (ICSI): 1993 onward. Approximately half ART cycles.

AUSTRALIA – NEW ZEALAND ART *(1979-1993)*

	Singleton Births	Malformations
IVF	6388	185 *(2.9%)*
GIFT	3409	99 *(2.9%)*

IVF alone without ICSI: Risk not considered increased for many years

Lancaster, 1995



ICSI AND DE NOVO CHROMOSOMAL ABERRATIONS

	ICSI Offspring	General Population
Sex chromosomal	9 *(0.83%)*	~ 0.2%
Numerical		
Autosomal	9 *(0.83%)*	~ 0.2-0.6%
Numerical	5	
Structural	4	
TOTAL	18 *(1.66%)*	0.45%

Data: Bonduelle et al., 1998

HYPOSPADIAS IN ICSI OFFSPRING

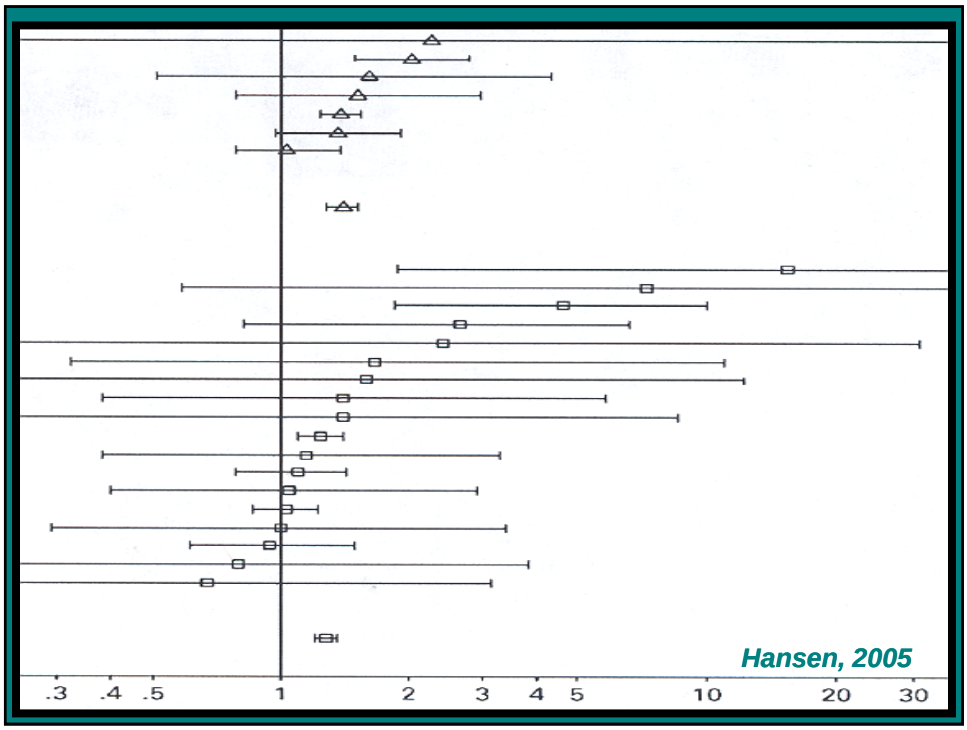
- Wennerholm et al. *(2000):* RR 3.0 *(1.09-6.50)* compared to Swedish Medical Birth Registry and Registry of Congenital Malformation
- Ericson and Kallen *(2001):* RR 1.5 *(1.0-2.1)*
- Klemetti et al (2005)- 76/10,000 v 29/10,000

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

POPULATION-BASED STUDIES (IVF and ICSI)

- Western Australia *(Perth):* 30-40% increase in birth defects *(Hansen et al., 2005)*
- Finland (Registry-based): Odds ratio 1.3 (95% confidence limits 1.1→1.6) comparing 4,559 IVF, 4,467 other ART and 27,078 controls.



META-ANALYSIS

- 19 studies IVF or ICSI
- Odds ratio for birth defects 1.29 (95% confidence limits 1.01-1.67).
- Insufficient power to evaluate individual anomalies. Hypospadias greatest risk; others arguable (cardiovascular, musculoskeletal, gastrointestinal, neural tube defects).
- Maternal age higher in IVF/ICSI

Rimm et al.

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

- South Australia Registry
- 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
- 1986-2002
- 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

Conceptions	Percentage*	Odds Ratio Unadjusted	Odds Ratio 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, livebirths 1-28 days and any reported anomaly <5 years

Davies et al., 2012 NEJM

ADJUSTED BIRTH DEFECTS BY PROCEDURE (SINGLETONS)

PROCEDURE

- Fresh IVF 1.05 (0.82-1.35)
- Frozen IVF 1.08 (0.76-1.53)
- Fresh ICSI 1.73 (1.35-2.21)
- Frozen ICSI 1.70 (0.65-1.85)
- Donor Insemination 1.37 (0.98-1.92)
- Intrauterine Insemination 1.46 (1.09-1.95)
- "Clomiphene at home" 3.19 (1.32-7.69)
- Spontaneous after prior ART 1.26 (1.01-1.57)
- Subfertile without ART 1.37 (1.02-1.83)

Davies et al., 2012

Or,

- Are increased risks related to ART per se?
- Do increased risks relate to underlying reason why ART needed?

TECHNICAL VARIABLES IN ART

- Ovulation stimulation regimes
- Obtaining and handling gametes
- Embryo culture
- Cryopreservation

HANDLING SPERM

- Polyvinylpyrolidone *(PPV)* to slow sperm
- Hyperosmotic swelling *(of sperm tail)* with sodium citrate, fructose
- Media *(Hepes)*
- Light source
- Air

OOCYTE ASPIRATION AND HANDLING

- Type media: Earle's; heparin
- Air: 80% N, 10% O, 10 % room air,

  or 90% N, 5% O, 5% 4oom air
- Hepafiltration?

EMBRYO CULTURE

- Media composition not also disclosed (Proprietary)
- Supplementation:
  - ± human or maternal serum albumin
  - ± synthetic serum substitute
  - ± bovine serum albumin *(no longer used)*
- Traditional culture medias developed for first three days, but now cultures must extend to five days

BLASTOMERE (DAY 3-4)

BLASTOCYST (DAY 5-6)

LENGTH OF TIME IN CULTURE

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

- Selection against aneuploidy?
- What is the appropriate culture media for days 3-5/6?

CONCLUSION: TECHNICAL VARIABLES

- Multiple at each step
- Plausible that certain variables differentially deletinously compared to in vivo conception, either through ovulation stimulation or embryo culture

CRYOPRESERVATION

- Cleavage stage embryos having fewer cells upon thawing show lower pregnancy rates
- Do epigenetic changes arise during cyropreservation?

- Are increased risks related to ART per se?

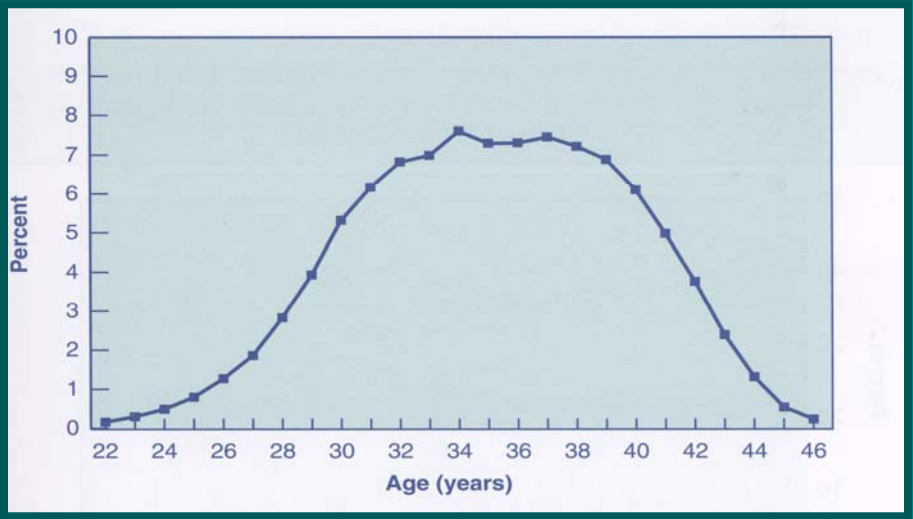
Or,

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

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.

AGE DISTRIBUTION OF WOMEN WHO HAD ART CYCLES USING FRESH NONDONOR EGGS OR EMBRYOS, 2001



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

- Do increased risks relate to why ART needed?
  - Difficulty in conceiving confers increased risk irrespective of therapy
- No true comparison group *(infertile women not undergoing ART)*

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

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Davies et al 2012

SUBFERTILITY AND BIRTH DEFECTS (SINGLETON PREGNANCIES)

	Adjusted Hazard Ratio	
	Subfertile but natural	Infertile and treated
Total	1.20 (1.07 to 1.35)	1.39 (1.23 to 1.57)
Circulatory	1.25 (0.97 to 2.15)	1.21 (0.91 to 1.62)
Genital	0.81 (0.48 to 1.38)	2.03 (1.37 to 3.01)
Urinary system	1.07 (0.68 to 1.69)	1.45 (0.94 to 2.24)
Chromosomal	0.68 (0.33 to 1.41)	0.98 (0.50 to 1.89)
Digestive	1.51 (1.04 to 2.14)	1.44 (9.94 to 2.22)
Nervous	2.01 (1.21 to 3.34)	1.39 (1.23 to 1.57)

Davies et al 2012

CLOMIPHENE AND BIRTH DEFECTS

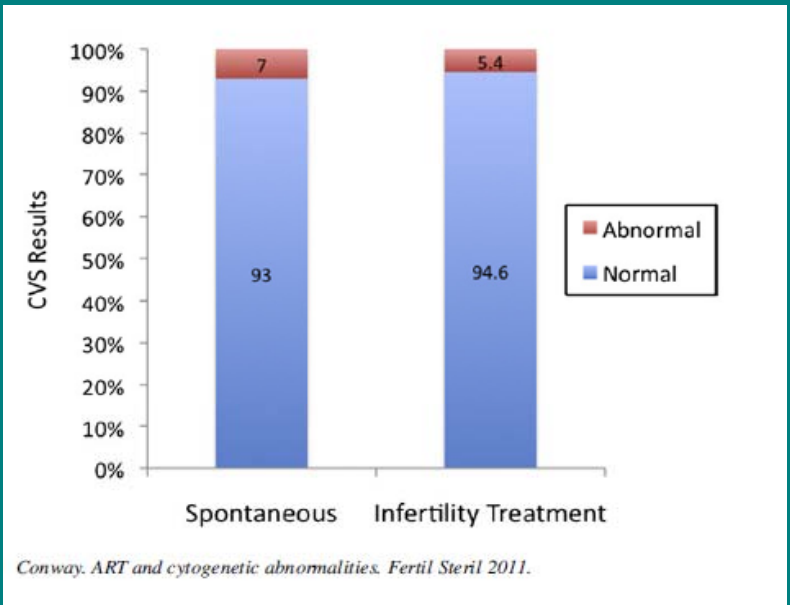
	Adjusted Odds Ratio/95% conf. interval
Anencephaly	2.3 (1.1-4.7)
Dandy-Walker	4.4 (1.7-11.6)
Cardiac-Septal	1.6 (1.1-2.2)
- Muscular	4.9 (1.4-16.8)
Coarction Aorta	1.8 (1.1-3.0)
Esophageal atresia	2.3 (1.3-4.0)
Exstrophy cloaca	5.4 (1.6-19.3)
Omphalocele	2.2 (1.1-4.5)
Craniosynostosis	1.9 (1.2-3.0)

Reefhuis, Human Reprod 26:451-457,2001.

What mechanism might be disturbed in both infertility and birth defects?

- Chromosomal
- Imprinting

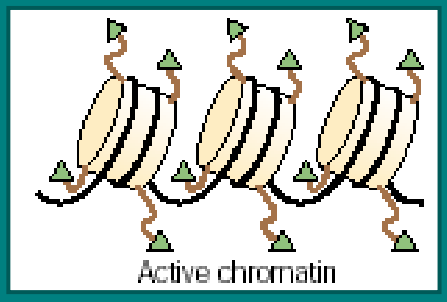
Chromosomal

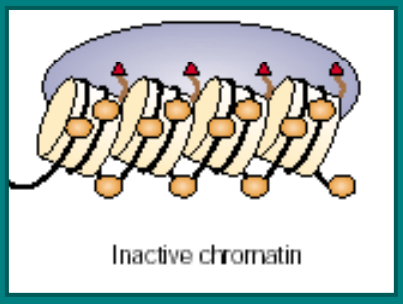


Conway. ART and cytogenetic abnormalities. Fertil Steril 2011.

IMPRINTING AS EXPLANATION FOR INCREASED BIRTH DEFECTS?

CHROMATIN MODIFICATIONS





Histone methylation
Histone deacetylation
Other

ART AND BECKWITH - WIEDEMANN SYNDROME *(BWS)*

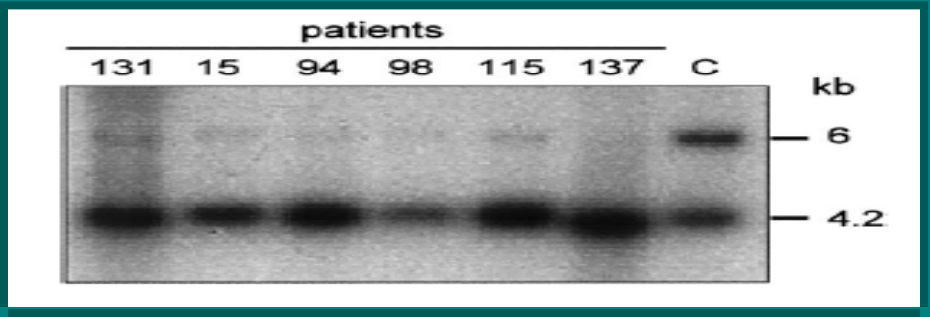
- Overgrowth syndrome (BWS) reminiscent of certain animal models.
- De Braun (2003): 7 of 65 cases associated with ART
  - Of the 7, 5 required ICSI
  - Cases studied *(N = 6)* had imprinting perturbations *(LIT1 or H19)* involving maternal allele, an uncommon molecular basis for BWS

BWS RESEARCH CENTRE
(Birmingham, U.K.)

- **149 BWS cases:**
  - ***3 IVF alone***
  - ***3 ICSI with IVF***
- **Unscheduled maternally expression 11p alleles**

Maher et al, J Med Genet, 2003

BECKWITH-WIEDEMANN SYNDROME



6 kb band: methylated
4.2 kb band: unmethylated

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

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)

IMPRINTING DISORDERS AND HUMAN ART REGISTRIES

- No technical feature common to birth defects associated with ART.
- Population-based *(vital statistics)* studies in Scandinavia show no increased risk overall or for any specific anomaly.
- Even if results were to show large relative risk the absolute effect is small because imprinting disorders are rare.

CONCLUSIONS : BIRTH DEFECTS AND ART

1. Overall malformation rate slightly increased (relative risk 1.3 – 1.4). Only specific abnormalities hypospadias and sex chromosomal abnormalities (0.6%↑) in Intracytoplasmic sperm injection (ISCI)

2. Myriad of technical variables in ART, so deleterious effect plausible.

3. ART couples not representative of the general population; thus, true control group not possible for robust comparison.

CONCLUSIONS

4. Birth defects increased in subfertile couples not requiring ART.
5. Imprinting perturbations plausible and consistent with animal studies and sperm studies, but data inconclusive and would likely confer low absolute risk even if present.
6. Trend toward single embryo transfer with 5-6 day blastocyst could further perturb imprinting and does increase frequency of monozygotic twins.