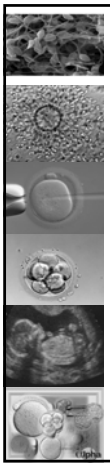
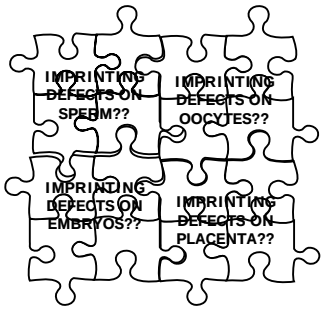
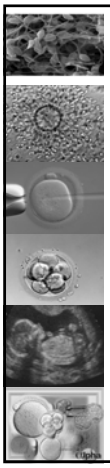




What are the consequences of all that? What happens to the ART conceived children??



Children Conceived after different intervention protocols

Anastasiou Olga, Mandelbaum Jacqueline

IVF Lab, Department of Reproductive Medicine, Hopital Tenon , Paris

ART & Epigenetics

Recently data collected from various animal species reported that the new reproductive technologies could be the origin of epigenetic disorders in animals conceived by these reproductive techniques.

In the human species, it was in 2001 that the alert was given by several publications correlating imprinting diseases with ART.

Insight from animals…

- Studies from ruminants born after ART have documented the frequent appearance of an overgrowth syndrome, known as LOS: Large Offspring Syndrome. (Sinclair et al, 2000)

LOS occurs mainly after embryo culture and nuclear transfer cloning..

Studies by Young et al, 2001 showed a correlation between LOS and loss of methylation of the maternal Insulin-like Growth Factor II Receptor-IGF2R gene.

- Cloned Bovine Preimplantation embryos are characterised by an aberrant methylation reprogramming correlated with a reduced developmental potential (Santos et al, 2003; Beaujean et al, 2004; Bourc'his et al, 2001).

Follow up of Children conceived after ART

While the new reproductive technologies have become now a routine treatment of infertility and more than > 3 million children worldwide are currently born (2% of all births in France), the fate of these children remains a topic of debate... Is there or not an increase in anomalies after ART?

- If we consider only singletons, it is largely admitted that ART babies present higher perinatal risks and low birth weight. Concerning however the congenital defects..there's a controversial debate, since some authors do not find any increase (Sutcliffe 95, Verlaenen 95, Isaksson 02 , Zadori 03 , Ponjaert Kristoffersen 05), others they do (Hansen 2005, Buckett Tan and 05, Schieve 05), and still some believe that they are linked to infertility problems of the couple (Zhu 2007)

- Some singletons from couples with a TTP> 2 years without treatment and without ART, present an increase of congenital malformations (genital), compared to children conceived naturally (Buckett & Tan, 2005; Ludwig, 2005)

ART & Beckwith Wiedemann syndrome (BWS)

What is the BWS?

BWS is a congenital overgrowth syndrome, occurring approximately 1:15,000 births.

MAIN CHARACTERISTICS:

- Macrosomia – Macroglossia
- Prematurity
- Hemihypertrophy
- Exophalos
- Abdominal Wall Defects
- Tumours: Wilms (kidney), Hepatoblastomas, Rhabdomyosarcomas, Neuroblastomas

BWS is associated with multiple distinct genetic and epigenetic alterations of the chromosomal band 11p15:

Genetic & Epigenetic causes of BWS:

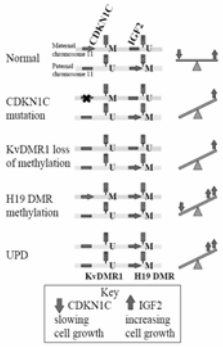
The four most common causes BWS:

5% : Mutation in $p57^{kip2}$ / CDKN1C
CDKN1C growth suppressing gene.

40-50%: Loss of methylation in the imprinted region KvDMR1, leading to reduced expression of the maternally inherited gene CDKN1C.

5-10%: Methylation of maternal H19 DMR, leading to overexpression of IGF2.

20%: Uniparental disomy – UPD (both chromosomes inherited by the father) – overexpression of IGF2 & no CDKN1C expression)



BWS & ART Techniques

Publication	No of Cases	ART Technique	Epigenetic Defect
Sutcliffe *et al*, 1995	1	IVF	-
Koudstaal *et al*, 2000	1	IVF	-
Olivennes *et al*, 2001	1	ICSI	-
Boerrigter *et al*, 2002	1	ICSI	-
Bonduelle *et al*, 2002	1	ICSI	-
Maher *et al*, 2003	6	IVF & ICSI	Kcnq1ot1
Debaun *et al, 2003*	7	IVF & ICSI	Kcnq1ot1 & H19
Gicquel *et* al, 2003	6	IVF & ICSI	Kcnq1ot1
Halliday *et al*, 2004	4	IVF & ICSI	Kcnq1ot1
Sutcliffe *et al*, 2005	11	ICSI, IVF,IUI,stimulation	Kcnq1ot1
Chang *et al*, 2005	19	ICSI, IVF,IUI,stimulation	Kcnq1ot1
Gomes *et al*, 2007	1	ICSI	Kcnq1ot1

BWS & ART Techniques

It is worth to note that....

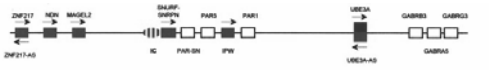
- All these studies concerning BWS have described an increased frequency in children born with the aid of assisted reproductive technology (ART) in patients with the BWS imprinting disorder(4-5% compared with 0.9-1.3% in the general population).
- These studies hqve identified patients who were conceived by disparate ART methods, including IVF, ICSI, embryo cryopreservation, ovarian stimulation so there does not appear to be an association with a specific ART technique.
- Although BWS can have a wide aetiology, in case of ART-born children, the defect found is predominantly epigenetic, and most specifically it involves the maternal allele.

ART & Angelman Syndrome (AS)

What is the Angelman syndrome (AS)?

- The prevalence of Angelman Syndrome among children and young adults is between 1:10,000-20,000.
- The consistent clinical characteristics include ataxia, hypotonia, severe mental and motor retardation, epilepsy and speech impairment.
- AS can have a wide aetiology: 70% of AS cases result from de novo maternal deletions involving chromosome 15q11.2-q13; 2% result from paternal uniparental disomy of 15q11.2-q13; and 2 to 3% result from imprinting defects:

The epigenetic defect associated with AS involves loss of methylation on the SNRPN imprinting centre(IC) which is normally methylated/silenced on the maternal allele.



AS & ART Techniques

Publication	No.of Cases	ART Technique	Epigenetic Defect
Sutcliffe, 1995	1	IVF	-
Koudstaal, 2000	1	IVF	-
Olivennes, 2001	1	ICSI	-
Cox, 2002	2	ICSI	SNRPN
Orstavic, 2003	1	ICSI	SNRPN

Although epigenetics account only for ~3% of AS , all 3 ART conceived children had sporadic imprinting defects manifested as hypomethylation

Moreover

Ludwig *et al,* 2005 have suggested an increased prevalence of imprinting defects in patients with AS born to subfertile couples: Couples with TTP>2 years and ART therapy present a double RR of conceiving a child with ID.

ART & Retinoblastoma (Rb)

•Retinoblastoma (RB) is an embryonic malignant neoplasm of retinal origin. It almost always presents in early childhood.

40% of RB cases are hereditary and both eyes are usually affected. The rest 60% are sporadic 'unilateral' cases.

Incidence of RB worldwide is 1:17,000 – 20,000 births

•The RB1 gene is a tumour suppressor, acting in the repression of E2F-regulated genes and plays a role in DNA damage induced G1/S cell cycle arrest.

In some unilateral RB cases, hypermethylation of its 5'end promoter region can lead to loss of function.

ART & Retinoblastoma (Rb)

In 2001 the first IVF-born child with an unilateral retinoblastoma was published (Anteby, 2001). Since then another 7 cases have been reported (Cruysberg, 2002; Moll, 2003; Lee, 2004).

All these cases are uni or bilateral and the children were conceived with either ICSI (1) or IVF(7).

BUT... Not one single case of retinoblastoma was identified in any of the large cohort studies designed to look at the incidence of malignancies in ART children (Kip, 2001; Bruinsma, 2000; Lemer-Geva, 2000)... WHY??

- ART associated cases have been overlooked & were not documented??
- Mothers of ART- born babies do not easily reveal that they underwent this procedure??
- Publication of first case triggered attention and a more careful questioning is carried out??
- New ovulation drugs or combination of them can influence the retinoblastoma gene??

What about recent cohort studies?

- Lidegaard 2005 presented the Danish National IVF cohort study....During the 7-year study period, 442349 non-IVF and 6052 IVF children were examined..and they don't find an increased incidence of any imprinting disease in the IVF-children group.
- Sutcliffe 2006 explored the registries of BWS,AS,PWS and transient neonatal diabetes mellitus children...They confirm a statistically significant increased frequency of ART in children with BWS (2.9% vs 0.8%)..but ..babies arose from different ART techniques IUI, stimulation etc. (questionnaire response rate:30%)
- Bowdin 2007 in a cohort of 1524 ART born children(2 IVF centers in Central England & Republic of Ireland), they detected only 1 case of BWS and no cases of AS or other syndrome.
- Doornbos 2007 in the Dutch registry of BWS, AS, PWS children, 6.4% were born after ART: ICSI,IVF,IUI,stimulation (x 3 than in the Dutch population). Moreover.. 6.8% of them were born after a fertility problem (TTP.12 months) but with no form of ART.

Is there a correlation with specific techniques?

- ✓ Children affected by epigenetic syndromes are born after different ART techniques: IVF, ICSI, blastocyst transfer, frozen embryo transfer etc
- ✓ It is remarkable that in the different imprinting disorders in animals & humans (LOS-BWS-AS) described following ART, the epimutation involves a loss of maternal allele methylation.

(Cases of Prader Willi syndrome, due to hypermethylation of paternal allele, have not been reported after ART even though the same SNRPN IC is involved as in AS)

Epigenetic disturbances arise in oocytes & embryos and spermatozoon is not implicated??

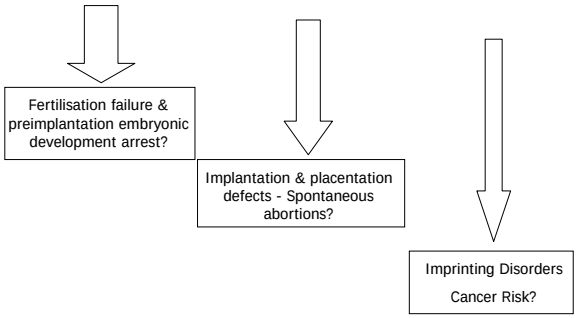
- Superovulation?
- Different fertility drugs & doses?
- *In Vitro Maturation* technique?

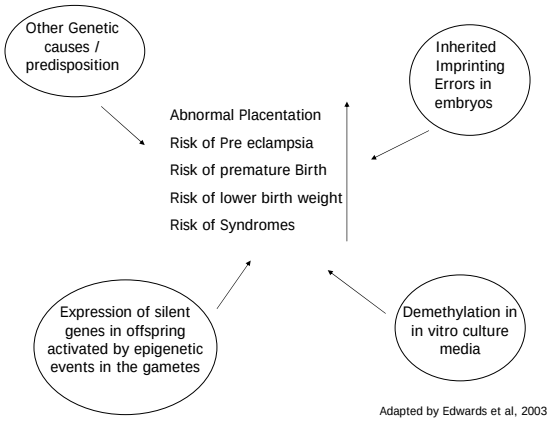
HOWEVER: Rossignol *et al* , 2006 observed that some BWS patients conceived after ART displayed abnormal methylation at loci other than 11p15: This loss of methylation was only partial, suggesting mosaicism of epimutation, occuring after fertilisation during early embryonic development rather than during oocyte maturation...

OR

Infertility per se is associated with an increased risk and superovulation increases this risk even further??

What can be the consequences in ART of these epigenetic defects?





Adapted by Edwards et al, 2003

Conclusions….

- **The most significant consequence of the recent reports suggesting a link between ART and imprinting disorders is the possibility that they might indicate « an iceberg scenario »**
- **AS and BWS would not occur because their specific loci are particularly sensitive to epimutations but rather because epimutations are equally likely to occur at other loci and result in phenotypes such as low birth weight or even have influence on the long term health of ART children**
- **There is therefore an obvious need to define the absolute risk of imprinting disorders after ART by prospectively following large cohorts of ART children while keeping on with fundamental research programs**

Epilogue…

Whatever the 'true' incidence of Epigenetic defects is after ART, there is little doubt that a heightened awareness with a closer follow-up of ART conceived children is needed.

The 'bells of worry & caution' are ringing...Clinical practitioners of all medical disciplines should listen carefully to their sounds and act wisely until strong evidence (for or against) an association between ART and Imprinting disorders is made available

Finally … a 'grand merci'….