Assisted reproduction technology and defects of genomic imprinting

It is estimated that approximately 1% of the newborn population of the British Isles are conceived following assisted reproduction technologies such as in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI). While the long term outcome of IVF children is mostly reassuring, some concerns remain. Specifically, recent studies have suggested a possible association between assisted conception and clinical conditions of genetic origin known as genomic imprinting defects. This has arisen from several different studies observing an excess of assisted conceptions among the rare clinical disorders of Beckwith–Wiedemann syndrome (BWS) and Angelman syndrome (AS). The numbers of such patients described in the studies to date are small but indicate a clear need for large-scale investigations to clarify the link between genomic imprinting defects and assisted conception as well as to establish the exact biological basis of any such link. In view of the strong public interest in this area of medicine, it behoves all professionals working in reproductive medicine and associated areas to be aware of these emerging data and be in a position to discuss them in as informed and responsible a manner with patients, as current data limitations permit.

Introduction

Assisted reproductive technology (ART) has enabled many infertile couples to enjoy parenthood. Since the birth of Louise Brown 27 years ago, improvements in infertility treatments have resulted in increased in success rates of in vitro fertilisation (IVF) to a level that seems to have stabilised in recent years. It is now estimated that ART accounts for greater than 1% of all births in the United Kingdom and United States, and more than 30% of all twins. Several outcome studies have highlighted the increased complication rates in IVF-conceived children compared with the general population. While many of the complications are attributable to a higher number of multiple births, it has been shown that singleton IVF infants have a greater risk of low birthweight and birth defects. One of the great fears when manipulating gametes in vitro is the introduction of defects at the laboratory stage. Some researchers have questioned the genetic implications for offspring of intracytoplasmic sperm injection (ICSI), particularly for male infertility of genetic cause. Higher incidences of de novo sex chromosomal aberrations, inheritance of CF mutations and Y microdeletions and spermatozoal aneuploidy have been reported following ICSI procedures. Nevertheless, most of the long term follow up data of children conceived by IVF are reassuring.

Since 2002, geneticists have reported an increased incidence of IVF or ICSI conceptions among children with Beckwith–Wiedemann syndrome (BWS) and Angelman syndrome (AS). These conditions may be caused by errors of genomic imprinting. As more data emerge, a tentative link has formed between ART and imprinting defects. In the UK, Maher et al. found an increased frequency of IVF conceptions in BWS cohorts (4% of BWS cases were ART conceptions, compared with 1.2% of the general population). Other researchers in the United States and France describe similarly increased association of ART among children with BWS. Combining the findings of these investigations results in a 4.2-fold increase in the risk of BWS for children conceived in vitro. These reports echo studies by Cox and Orstavik suggesting a link between AS and ICSI. Such alarming reports have reinforced the need for continuous surveillance of the long term outcome of children conceived with ARTs.

The possibility of statistical misinterpretation of these somewhat tentative data is heightened by different methodological approaches, as well as absence of age and control data. While this may be entirely understandable in the context of studies of very rare disorders, it leaves open the possibility of interpretational error. Nonetheless, the apparent consistent increase of actual to expected cases of genomic imprinting disorders from these several disparate sources and studies remains unexplained and confers significant potential for parental anxiety.

What is genomic imprinting?

Mendel first described single-gene inheritance in his studies of the characteristics of garden peas. The principles of Mendelian inheritance explain up to 5000 clinically significant diseases. Since Garrod and Bateson first applied Mendel’s laws to inborn errors of metabolism, few exceptions to Mendelian inheritance have been observed. However, increasing knowledge of molecular detail in relation to uncommon disorders has unveiled atypical patterns of single-gene inheritance. Genomic imprinting is one example where Mendel’s laws are not obeyed.
On the basis of Mendelian principles, it is assumed that genes from both parents play an equal role in development. A certain allele of an autosomal gene would be equally likely to be transmitted from a parent, of either sex, to an offspring, of either sex. However, maternal and paternal genomes are not functionally equivalent; a number of genes may have modifications, specific to the parent of origin, and are said to be imprinted. Imprinted genes show preferential expression from a specific parental allele; about 50 such genes are known and are expressed according to their sex cell lineage.

In some genetic disorders (Table 1), the expression of the disease phenotype depends on whether the mutant allele has been inherited from the father or from the mother. Perhaps the best-studied examples in human disease are Prader–Willi syndrome (PWS) and AS. In approximately 70% of PWS cases, there is a cytogenetic deletion involving 15q11–q13 occurring on the chromosome 15 inherited from the patient’s father. Thus, the genomes of these patients have genetic information on 15q11–q13 that is derived solely of maternal origin. Conversely, if there is a deletion or mutation of the maternally imprinted contribution, such that the genome contains 15q11–13 of paternal origin only, the disease will manifest as AS. Thus, the parental origin of the gene has a direct influence on the disease phenotype. This is not simply a theoretical consideration; such families exist and have been the subject of study and journal reports.

### Disomy

In classical inheritance, the offspring receives an equal chromosomal contribution from each parent. Disomy refers to the unusual and rare occurrence whereby an individual receives both autosomal genes at a given locus from the same parent. Hence, that region of the chromosome is disomic—both contributions from one parent. Such a situation may have no clinical sequelae, but consider the situation of a baby disomic for a gene for which the parent was an asymptomatic carrier of an autosomal recessive mutation. Now, although only one parent is a mutation carrier, the child is affected, having homozygosity of the carrier state. This phenomenon is well reported.

What if the child is disomic for a gene that is imprinted? Not surprisingly, this exact situation has been described and paternal disomy of chromosome 15q11–13 will result in no maternal contribution and a presentation of AS. Conversely, maternal disomy at this same region causes PWS; as in the deletional form, there is no paternal contribution.

### How are genes imprinted?

At an imprinted locus, only one allele is active and the inactive one is marked epigenetically, that is, there is a stable alteration in DNA other than the sequence itself. Epigenetic modifications include histone acetylation, cytosine methylation or both and essentially alter chromatin organisation. Methylation is one of the best-studied epigenetic modifications of DNA and all imprinted genes show differences in methylation patterns between maternal and paternal alleles. Loss of imprinting can involve hypomethylation or hypermethylation, depending on the gene.

Imprinting occurs at two stages; gametogenesis and embryonic development. Imprints are established during the development of the germ cells. The imprinted genes initially undergo demethylation as primordial germ cells migrate along the genital ridge to the fetal gonad. Subsequently during gamete maturation, methylation is reestablished by DNA methyltransferases that specifically target one of the two parental alleles for silencing. Further changes occur after fertilisation. Firstly there is genomewide demethylation, which is then followed by passive remethylation in the zygote genome. However, methylation marks on imprinted genes are protected from demethylation so that parental imprints are preserved in the developing embryo. Normal embryogenesis cannot proceed without this dynamic reprogramming of the epigenome at different stages.

### Imprinted genes in development

Genomic imprinting represents a form of gene regulation. Many imprinted genes are known to play important roles in fetal growth and development, and also in tumour suppression. Moll et al. reported an increase in retinoblastoma in IVF children and this is reminiscent of other reports of cancer in children born after assisted reproductive technologies. Animal studies have shown that loss of imprinting at the maternal Igf2 gene is associated with overgrowth and large offspring syndrome, a condition some researchers have compared with BWS in humans where organ overgrowth also occurs.

**Table 1. Conditions associated with genomic imprinting defects.**

<table>
<thead>
<tr>
<th>Condition</th>
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<tbody>
<tr>
<td>PWS</td>
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<tr>
<td>AS</td>
</tr>
<tr>
<td>BWS</td>
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<tr>
<td>Russell–Silver syndrome</td>
</tr>
<tr>
<td>Wilm’s tumour</td>
</tr>
<tr>
<td>Osteosarcoma</td>
</tr>
<tr>
<td>Bilateral retinoblastoma</td>
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<tr>
<td>Rhabdomyosarcoma</td>
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</table>

macroglossia (Fig. 1). In utero there may be a large and thickened placenta, polyhydramnios, a long umbilical cord and a fetus measuring large for gestational age. Additional, more variable features include hemihypertrophy, ear pits and creases, renal anomalies and facial nevus flammeus. BWS children are at increased risk of developing embryonal tumours, especially Wilms’tumour for which condition screening is recommended in this group.

BWS can occur by a variety of mechanisms (Table 2), but in the majority of cases imprinted genes from chromosome 11p15.5 have been implicated. About 60% of sporadic cases have epigenetic changes of methylation causing alterations in the expression of the paternally expressed alleles IGF2 and KCNQ1OT, or maternally expressed genes such as H19 and CDKN1C. Twenty percent of cases are attributable to uniparental disomy where the inheritance of two alleles from the same parent causes loss of imprinting of a normally imprinted gene. These data from sporadic cases of BWS contrast with the results of molecular analysis of the ART-associated cases; of 19 cases found, 14 cases were tested; all were positive for hypomethylation of KCNQ1OT, and all were negative for UPD13–15 (Table 3).

AS is a neurogenetic disorder characterised by severe mental retardation, delayed motor development, poor balance, jerky movements, absence of speech and happy disposition (Fig. 2). Sporadic cases of AS are linked with a loss of function of the maternal allele of \textit{UBE3A} on chromosome 15 resulting from a deletion (70%), a point mutation, uniparental disomy (2–3%) or an imprinting defect (7–9%) (Table 4). This is in contrast to findings by Cox et al. and Orstavik et al. where, in all three cases conceived by ICSI, AS was due imprinting defects caused by aberrant methylation (Table 3); a mechanism which, in the whole of the AS population, is thought to account for only 5% of cases.

More recently, Horstemke et al. reported their study among 79 patients in the German AS Support Group. Sixteen were born to subfertile couples and 4 of these 16 were due to a sporadic imprinting defect. The relative risk of AS was significantly increased among patients conceived by ICSI and among those treated by hormonal measures. This observation extends the possible risk of imprinting defects to other modalities of treatment for infertility beyond ICSI. The authors concluded that genetic predisposition, combined with superovulation, rather than ICSI, increases the risk of conceiving a child with an imprinting defect.

From these sets of observations in patients with BWS and AS, it is clear that IVF conceptions are statistically over-represented. That the molecular-specific subgroups of AS and also of BWS represent so uncommon a molecular mechanism adds up to serious concerns on the possible effects of assisted reproductive techniques on epigenetic mechanisms and imprinting. Such concerns are compounded by animal observations that overgrowth in the IVF-conceived offspring correlates with loss of methylation in the maternally imprinted IGF2 receptor gene. In a similar vein, studies of pre-implantation mouse embryos have shown that embryo culture conditions such as presence or absence of calf serum can influence the expression and methylation status of imprinted genes.

**Mechanism of imprinting defect in ART**

It is unknown at which step, or steps, the association of ART with imprinting defects occurs. Because imprinted genes are functionally haploid, they may be vulnerable to mutations or epimutations when placed in an abnormal \textit{in vitro} environment. Curiously, so far only maternal loss of imprinting has been described in the ART-associated cases although data is limited to small numbers.

Possibilities for the introduction of a genetic error include the following:

1. Elimination of natural selection. ICSI bypasses natural selection and may overcome intrinsic barriers to the fertilisation of abnormal gametes (e.g. those with defective imprinting) of either sex. High frequencies of cytogenetic abnormalities are detected in oocytes generated from IVF. It should also be acknowledged that epigenetic
errors could be a significant cause of the underlying infertility rather than a consequence of the treatment.

2. Abnormal oocyte activation. ICSI introduces the sperm acrosome and digestive enzymes into the ooplasm and may disturb intracellular homeostatic mechanisms.

3. Removal of cumulus cells. Oocytes are denuded of their surrounding cumulus cells prior to micromanipulation. The cumulus complex may have a role in maintaining the oocyte in meiotic arrest, and its removal may be associated with the oocyte’s irreversible commitment to undergo germinal vesicle breakdown. By altering the meiotic competence of the oocyte, such manipulation may leave open the possibility of defective genomic imprinting.

4. Mechanical disruption of the oocyte. The injecting needle may cause mechanical disruption of intracellular structures such as the meiotic spindle.

Imprinting defects have been described following IVF with and without ICSI suggesting that the error occurs in a step or steps common to both procedures. Indeed, to date there is no convincing data that ICSI entails more dangers than IVF. Common and potentially causative steps include the following:

5. Culture conditions of the ovum. Factors such as the length of exposure to specific media and growth factors therein may alter oocyte maturation. Studies of mouse embryos have demonstrated that H19 methylation and expression can be altered by the culture medium used. Niemitz and Feinberg hypothesise that the methionone content of media may be a critical factor influencing methylation changes.

It seems that in vitro effects are greater on the oocyte, consistent with the relatively late completion of imprinting in oogenesis. Testicular sperm extraction (TESE) procedures collect immature spermatozoa, but the paternal imprinting process seems to be complete at this stage. This would point to potential extra risks with in vitro maturation (IVM) of oocytes, but so far there have been no reports of AS or BWS in the approximately 300 infants born to date.

6. Embryo culture. Data from animal studies have demonstrated aberrant maternal allele methylation occurring in embryos cultured in vitro, so it remains possible that prolonged embryonal cell culture per se might predispose to abnormal methylation. This may have implications for the practice of extending culture to blastocyst stage. In vitro blastocyst culture, being an abnormal environment for this stage, may potentially represent an embryonic imprinting insult.

7. Subfertility and superovulation. It is possible that subfertility itself and imprinting defects share a common aetiology, and that superovulation rather than in vitro gamete manipulation may increase the risk of conceiving a child with defect of genomic imprinting.

### Table 3. Summary of studies of imprinting disorders after ART.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Reference</th>
<th>Molecular defect</th>
<th>No. of ART cases</th>
<th>No. analysed molecularly</th>
<th>ART</th>
</tr>
</thead>
<tbody>
<tr>
<td>BWS</td>
<td>DeBaun et al.</td>
<td>4/6 KCNQ1OT hypomethylation, 1/6 KCNQ1OT hypomethylation, and H19 hypermethylation</td>
<td>7</td>
<td>6</td>
<td>IVF and ICSI</td>
</tr>
<tr>
<td></td>
<td>Maher et al.</td>
<td>2/2 KCNQ1OT hypomethylation</td>
<td>6</td>
<td>2</td>
<td>IVF and ICSI</td>
</tr>
<tr>
<td></td>
<td>Gicquel et al.</td>
<td>6/6 KCNQ1OT hypomethylation</td>
<td>6</td>
<td>6</td>
<td>IVF and ICSI</td>
</tr>
<tr>
<td>AS</td>
<td>Cox et al.</td>
<td>2/2 Sporadic imprinting defect at 1C</td>
<td>2</td>
<td>2</td>
<td>ICSI</td>
</tr>
<tr>
<td></td>
<td>Orstavik et al.</td>
<td>Sporadic imprinting defect at 1C</td>
<td>1</td>
<td>1</td>
<td>ICSI</td>
</tr>
<tr>
<td>RB</td>
<td>Moll et al.</td>
<td>1/5 coding mutation at RB1</td>
<td>5</td>
<td>5</td>
<td>IVF and ICSI</td>
</tr>
</tbody>
</table>

### Table 4. Molecular causes of AS (adapted from Clayton-Smith and Laan).

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Cause</th>
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<tbody>
<tr>
<td>70%</td>
<td>De novo maternal deletion</td>
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<tr>
<td>5–10%</td>
<td>UBE3A mutation</td>
</tr>
<tr>
<td>2%</td>
<td>Uniparental disomy (paternal)</td>
</tr>
<tr>
<td>2%</td>
<td>Imprinting centre deletion</td>
</tr>
<tr>
<td>2%</td>
<td>Imprinting centre defect without deletion</td>
</tr>
<tr>
<td>10–15%</td>
<td>None identified</td>
</tr>
</tbody>
</table>

The current position

There are strong circumstantial observations that suggest a cause-and-effect relationship between assisted conception and clinical conditions caused by imprinting mutations. Such data as currently exist arise from patient cohorts with two specific conditions caused by imprinting defects and show an unexpected over-representation of assisted reproduction conceptions among these patient groups. The concerns that ART may predispose to imprinting defects in offspring gain further weight with consideration of animal studies germane to this area.

Infertility specialists need to be aware of these developing issues to be well enough informed to address the legitimate concerns of their patients. It must be recognised that many patients are looking outside the profession for information nowadays and specialists need to have in mind the data available from such resources as the Human Embryology Authority Web site (www.hfea.gov.uk) and the American Academy of Paediatrics (www.aap.org) when addressing concerns raised by patients. While the patient’s autonomy in decision-making is respected, an appreciation of all risks is necessary to ensure that infertile couples receive the information sufficient to make informed choices. Physicians also bear a responsibility to the health of future children and potential health risks must always be considered. The ultimate aim of infertility treatment is not just a positive pregnancy test, rather the birth of a healthy baby to healthy parents.

The absolute numbers of imprinting defects associated with IVF and ICSI are small and unlikely to deter any would-be patients from undergoing the treatment. Nevertheless, the emerging data are of concern and highlight the need for further investigation. Foremost among immediate studies should be a widespread review of ART prevalence among AS and BWS cases tested and identified in approved laboratories nationally and internationally. Concurrent with this, studies are needed to look at ART populations of children and identify if there is evidence for increased prevalence of AS and BWS in such children. Of necessity these studies will need to be large-scale, multicentred and take account of children with ‘other’ defects not known currently to be due to imprinting problems.

Infertility specialists, obstetricians, paediatricians and geneticists commonly work in separate environments, so that in the past issues pertaining to the short and long term effects of assisted conception have been reviewed in isolation. Increased cross-specialty co-operation, which would be greatly enhanced by appropriate research funding, and the promotion of follow up studies of patients born of assisted conception are clearly the means to definitively address the current impasse. The issues raised here are not to be dismissed as ‘academic’. They are real and absolute and will impact on clinicians working in infertility and on their patients.

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References


