A review of known imprinting syndromes and their association with assisted reproduction technologies

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An association between assisted reproduction technologies (ART) and abnormal genomic imprinting in humans has been recognized for several years; however, the magnitude of this risk and the spectrum of imprinting syndromes to which the risk applies remains unknown. Nine human imprinting syndromes have been identified but current evidence links ART with only three: Beckwith–Wiedemann syndrome, Angelman syndrome and the newly described maternal hypomethylation syndrome. There is currently a lack of evidence linking ART with the remaining six imprinting syndromes: Prader–Willi syndrome, Russell–Silver syndrome, maternal and paternal uniparental disomy of chromosome 14, pseudohypoparathyroidism type 1b and transient neonatal diabetes. Evidence from clinical reports suggests that the association between imprinting syndromes and ART may be restricted to syndromes where the imprinting change takes the form of hypomethylation on the maternal allele. In contrast, studies of gametes and early embryos suggest that ART can be associated with hypermethylation as well as hypomethylation, with imprinting changes occurring on paternal as well as maternal alleles. The health effects of ART-associated imprinting changes may also extend beyond the nine recognized imprinting syndromes.

Keywords: assisted reproduction; imprinting syndromes; Beckwith–Wiedemann syndrome; Angelman syndrome; maternal hypomethylation syndrome

Introduction

It is now five years since a series of clinical studies raised concern about a link between assisted reproduction technologies (ART) and two imprinting syndromes, Beckwith–Wiedemann syndrome (BWS) (DeBaun et al., 2003; Gicquel et al., 2003; Maher et al., 2003b; Halliday et al., 2004) and Angelman syndrome (AS) (Cox et al., 2002; Orstavik et al., 2003). These studies relied on case records and questionnaire data, and a control group was used in only one study (Halliday et al., 2004). For these reasons, subsequent interpretation and utilization of these data in the clinical setting has been difficult. Several subsequent clinic-based and population-based studies have attempted to strengthen the epidemiological links between imprinting syndromes and various types of ART including in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) (Lidegaard et al., 2005; Sutcliffe et al., 2006; Bowdin et al., 2007; Doornbos et al., 2007). Unfortunately, these studies have generally been underpowered or have suffered from other methodological difficulties, and there remains a lack of reliable data on the relative and overall risk of imprinting syndromes in children conceived using ART. It is also unclear whether a risk applies to all imprinting syndromes or just a subset thereof. Here we provide a review of known imprinting syndromes, and examine the evidence for each being associated with ARTs.

Diverse genetic and epigenetic changes underlie imprinting syndromes

Genomic imprinting is the modification of the genome so that genes from only one (rather than two) parental alleles are expressed. The mechanism underling imprinting is epigenetic, occurring via changes in DNA methylation and histone modifications rather than through alterations in DNA sequence. Among imprinted genes, those that are paternally expressed tend to promote growth whereas those that are maternally expressed tend to suppress growth, leading to the hypothesis that genomic imprinting may have evolved as a parental ‘battle of the sexes’ to regulate the maternal allocation of resources to the offspring (Moore and Haig, 1991). Approximately 1% of all human genes are thought to be imprinted, with ~50 imprinted genes identified to date, and an additional 150 genes predicted to be imprinted on the basis of DNA sequence characteristics (Luedi et al., 2007).

Imprinting syndromes are a group of medical conditions that result from the altered expression of genes that are usually imprinted. The mechanisms that alter the expression of
Imprinted genes are diverse and can be categorized into three ‘genetic’ mechanisms and one ‘epigenetic’ mechanism. The relative contribution of each mechanism varies for each imprinting syndrome. The three genetic mechanisms are: (i) large deletions or duplications of chromosomal regions that contain imprinted genes; (ii) DNA mutations in genes that are usually imprinted or in their imprinting control centers and (iii) uniparental disomy (UPD). In contrast, the epigenetic mechanism involves no alteration in DNA sequence, but changes in DNA methylation and modification of histones (epimutations) that can arise as a result of errors in imprint erasure, establishment or maintenance. Changes in DNA methylation can be further subdivided into four categories, comprising gain of methylation (hypermethylation) and loss of methylation (LOM) (hypomethylation) occurring on either the maternal or paternal allele.

To date, evidence from ART-conceived patients with imprinting syndromes suggests that the increased risk of imprinting syndromes associated with ART is confined to the category of epimutations. The pathway(s) that lead to epimutations in ART-conceived children remain unresolved, but possible contributing factors include the subfertility itself (Ludwig et al., 2005; Doornbos et al., 2007; Kobayashi et al., 2007), the process of ovulation induction (Sato et al., 2007; Fortier et al., 2008), physical interference with embryos during IVF/ICSI/embryo transfer (Rivera et al., 2008) and aspects of the in vitro culture of embryos (Doherty et al., 2000; Fauque et al., 2007). There is currently no evidence that genetic mechanisms (DNA mutations or cytogenetic deletions/duplications) are associated with ART, and there is no particular reason to expect that such mutations would favour imprinted genes. Maternal UPD (matUPD) is however associated with advanced maternal age (Kotzot, 2004) and therefore matUPD is expected to occur more commonly in ART pregnancies because women using ART are typically older.

This review will focus on nine recognized imprinting syndromes: AS, BWS, Prader–Willi syndrome (PWS), Russell–Silver syndrome (RSS), maternal and paternal UPD (patUPD) of chromosome 14 (matUPD14, patUPD14), pseudo-hypoparathyroidism type 1b (PHP-1b), transient neonatal diabetes (TND) and the newly described ‘maternal hypomethylation syndrome’. These phenotypes typically result from altered expression of imprinted genes; that is, rather than being expressed from one allele, the imprinted genes are either expressed from two alleles, or not expressed at all. The proportion of cases in which the underlying mechanism is epimutation varies considerably between these syndromes (Table I).

**Angelman syndrome**

AS (OMIM 105830) affects ~1 in 16 000 children and is characterized by severe intellectual disability, speech impairment, ataxia, a happy demeanor, seizures and microcephaly (Williams and Driscoll, 2007). The most common molecular mechanism underpinning AS is a deletion of 4–6 Mb at 15q11.2–15q13, found in ~70% of AS patients. PatUPD of chromosome 15 accounts for 7% of AS patients and an additional 11% have mutations in the gene UBE3A. Approximately 3% of patients with AS have an ‘imprinting defect’, evidenced by a paternal-only pattern of methylation but biparental inheritance of 15q11.2–15q13. In a small proportion (10%) of these AS patients, the imprinting defect is actually due to a deletion of the imprinting centre (Buiting et al., 2003), but the remainder are thought to represent epimutations.

ART has been implicated in AS by reports of five ART-conceived patients with epimutation-AS (Cox et al., 2002; Orstavik et al., 2003; Ludwig et al., 2005). Of these, four were conceived using ICSI (Cox et al., 2002; Orstavik et al., 2003; Ludwig et al., 2005) and one using ovarian hyperstimulation alone (Ludwig et al., 2005). A tentative link has also been drawn between AS and subfertility by the report of two patients with epimutation-AS in whom the parents had taken >24 months to become pregnant (Ludwig et al., 2005), and by a third patient with epimutation-AS who was conceived using donor insemination after unsuccessful IVF (Sutcliffe et al., 2006). The link between AS and ART is based on the rarity of AS (1/16 000), the rarity of epimutations as a mechanism of AS (~3%) and the relatively infrequent use of ART as a method of conception (2–3%). These three events are expected to coincide by chance only once every ~20 million births.

Given the rarity of epimutations as a mechanism for AS, the relative risk of epimutation-AS associated with ART would need to be high to have any detectable impact on the incidence of AS in population-based studies; it is therefore not surprising that none has been detected. In a cohort of 63 AS patients ascertained through a Dutch AS support group, there were no AS patients conceived using IVF/ICSI, although three were born following ovulation induction, one by artificial insemination and four following a time-to-pregnancy >12 months (Doornbos et al., 2007). Importantly, none of these patients were documented as having an epimutation. Similarly in a British study of 75 AS patients, none were conceived using IVF/ICSI, although two were conceived using artificial insemination (both had deletion-AS) and one was conceived naturally following previous use of ART (Sutcliffe et al., 2006). Interestingly, the latter patient had an imprinting defect, suggesting the presence of an epimutation.

**Beckwith–Wiedemann syndrome**

BWS (OMIM 130650) is an overgrowth syndrome estimated to affect 1 in 13 700 children (Shuman et al., 2005). Clinical features are highly variable but include prenatal and post-natal overgrowth, neonatal hypoglycaemia, exomphalos, macroglossia, hemihyperplasia, an increased risk of embryonal tumours (particularly Wilms tumour), but normal intellect.

The majority of BWS patients have an epimutation affecting the maternal allele of one of two differentially methylated regions (DMRs) at chromosome 11p15, DMR1 (regulating the genes H19 and IGF2) and DMR2 (regulating the genes CDKN1C and KCNQ1). In over half of all BWS patients, the epimutation is hypomethylation at DMR2, whereas 2–7% of patients have hypermethylation at DMR1 (Shuman et al., 2005). The remaining BWS patients have patUPD of chromosome 11p, a cytogenetically visible chromosome abnormality, or a DNA mutation in the gene, CDKN1C.
Table I. Molecular mechanisms underlying known imprinting syndromes.

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<tr>
<td>Prevalence</td>
<td>1 in 16 000</td>
<td>1 in 17 500</td>
<td>1 in 13 700</td>
<td>1 in 100 000</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>1 in 500 000</td>
<td>Unknown</td>
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<td>Molecular mechanism</td>
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<td>Cytogenetic deletion/ rearrangement, %</td>
<td>70</td>
<td>70</td>
<td>1–2</td>
<td>&lt;1</td>
<td>Rare</td>
<td>0</td>
<td>0</td>
<td>40</td>
<td>0</td>
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<td>Uniparental Disomy, % (UPD)</td>
<td>7 (Pat UPD15)</td>
<td>25 (Mat UPD15)</td>
<td>20 (Pat UPD11p)</td>
<td>5 (Mat UPD7)</td>
<td>&gt;95 (Mat UPD14)</td>
<td>100 (Pat UPD14)</td>
<td>1 case (Pat UPD 20q)</td>
<td>40</td>
<td>0</td>
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<td>DNA mutation in gene/ imprinting centre, %</td>
<td>5–10</td>
<td>&lt;1</td>
<td>10</td>
<td>Unknown</td>
<td>0</td>
<td>0</td>
<td>Most familial cases + some sporadic cases</td>
<td>0</td>
<td>0</td>
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<td>Epimutations (total), %</td>
<td>2.5</td>
<td>&lt;1</td>
<td>65</td>
<td>64</td>
<td>1 report</td>
<td>Unrecorded</td>
<td>Unknown</td>
<td>Possibly some sporadic cases</td>
<td>20</td>
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<tr>
<td>Maternal hypomethylation, %</td>
<td>2.5, 5 ART patients</td>
<td></td>
<td>50–60, &gt;60</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Paternal hypomethylation, %</td>
<td>0</td>
<td>0</td>
<td>2–7</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Paternal hypermethylation, %</td>
<td>0</td>
<td>&lt;1</td>
<td>0</td>
<td>64 (11p)</td>
<td>1 report</td>
<td>0</td>
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ART-conceived patients are shown in bold type.
To date, more than 60 BWS patients who were conceived using IVF/ICSI have been reported (DeBaun et al., 2003; Gicquel et al., 2003; Maher et al., 2003b; Halliday et al., 2004; Chang et al., 2005; Lidegaard et al., 2005; Rossignol et al., 2006; Sutcliffe et al., 2006; Bowdin et al., 2007; Doornbos et al., 2007; Gomes et al., 2007). In the great majority of these patients the underlying molecular mechanism has been hypomethylation on the paternal allele of DMR2. BWS patients have also been reported where conception has been assisted by ovulation induction (Chang et al., 2005; Sutcliffe et al., 2006; Doornbos et al., 2007), and others have been conceived without ART, but following >12 months subfertility (Doornbos et al., 2007); the significance of these observations remains uncertain.

BWS remains the only imprinting syndrome for which data exist to allow an estimation of the relative risk of imprinting syndromes in ART pregnancies. Halliday et al. (2004) calculated that BWS patients were approximately nine times more likely to have been conceived by ART than patients without BWS. If we assume that epigenetic mechanisms account for ~65% of BWS cases (Table I), and that the increased risk from ART applies only to epimutations and not to genetic mutations, then it can be estimated that BWS patients with an epimutation are approximately 14 times more likely to have been conceived by ART than patients without epimutation BWS.

Prader–Willi syndrome
PWS (OMIM 176270) affects approximately 1 in 17 500 children and is characterized by neonatal hypotonia, childhood onset obesity, cognitive impairment, distinctive behavioural characteristics, hypogonadism and a characteristic facial appearance (Cassidy and Schwartz, 2006). There is not a single gene responsible for PWS, but most aspects of the PWS phenotype result from absence of paternal expression of a cluster of non-coding RNAs known as ‘HBII-85’ (Sahoo et al., 2008). In the great majority of PWS patients, the underlying molecular mechanism is either a 4–6 Mb chromosome deletion at 15q11.2–15q13 (70%) or matUPD of chromosome 15 (25%). Epimutations causing PWS are very rare, accounting for <1% of PWS patients, and take the form of hypomethylation on the paternally-inherited allele (Buiting et al., 2003). To date, there have been no reports of patients conceived using ART who have PWS as a result of an epimutation.

Several studies have attempted to find an association between PWS and ART. One study surveyed 163 patients with sporadic PWS and found 9 of the 163 that were conceived using ART (2 using ICSI and 7 using fertility drugs), however, molecular data were available for only 2 patients, both of whom had a chromosome deletion (Sutcliffe et al., 2006). Another study surveyed 86 patients with PWS and found 4 of the 86 were conceived using various forms of ART (2 using IVF/ICSI, 1 using artificial insemination and 1 using ovulation induction); an additional 5 of the 86 reported prior subfertility, however, a 15q deletion was found in all ART-conceived patients for whom molecular data were available (Doornbos et al., 2007). Lidegaard et al. (2005) undertook a registry study of 6052 children conceived using ART and did not find any patients with PWS; however, this result must be interpreted with caution because only 3 PWS patients were detected out of 442 349 controls, suggesting that the study methodology failed to ascertain most PWS diagnoses.

The example of PWS illustrates the epidemiological difficulties in detecting possible ART-associated risks for imprinting syndromes that are themselves very rare. If there were no increase in the risk of epimutation-PWS associated with ART, then even in countries where ART use is common, an ART-conceived baby with epimutation-PWS would be expected to be born only once every ~70 million births. An increase in the risk of epimutations of similar magnitude to that observed for BWS (×14 increase) would lead to the birth of a baby with epimutations-PWS only once every 5 million births, a frequency that would be virtually impossible to detect.

Russell–Silver syndrome
RSS (OMIM 180860) is a disorder of decreased growth that is estimated to affect 1 in 100 000 children. RSS is characterized by intrauterine and post-natal growth retardation plus variable additional features including fifth finger clinodactyly, limb length asymmetry, a typical facial phenotype and variable learning disabilities (Saal, 2007).

RSS differs from other imprinting syndromes in that three distinct imprinted loci on two different chromosomes have so far been implicated. The most important mechanism underlying RSS is hypomethylation on the paternal allele of DMR1 at 11p15 (Gicquel et al., 2005; Netchine et al., 2007), the same locus for which hypomethylation on the maternal allele causes BWS. This epimutation is responsible for over half of all patients with RSS (Gicquel et al., 2005; Netchine et al., 2007). Maternally inherited chromosome duplications involving the 11p15 DMR1 can also cause an RSS-like phenotype (Fisher et al., 2002; Eggermann et al., 2005), and a maternally inherited duplication at the 11p15 DMR2 has been reported in one RSS patient (Schonherr et al., 2007). Approximately 5% of patients with RSS have matUPD of chromosome 7 (Netchine et al., 2007). Two imprinted loci have been implicated in the matUPD7 phenotype, a DMR on 7p12.2 (including the gene GRB10) and a DMR at 7q32.2 (including the gene MEST), however, to date, no patients have been reported with RSS due to epimutations at these loci. Maternally inherited duplications that include the 7p DMR have been associated with an RSS-like phenotype (Joyce et al., 1999; Monk et al., 2000). In ~30% of patients with RSS the underlying mechanism is unknown.

There is currently little evidence linking RSS with ART. To date, there have been only five patients reported with RSS and who were conceived using IVF/ICSI (Svensson et al., 2005; Bliek et al., 2006; Kagami et al., 2007; Galli-Tsinopoulou et al., 2008), and molecular data are available for only two. One ICSI-conceived girl with an RSS-like phenotype was found to have hypomethylation at the paternal allele of DMR1 on 11p15 (Bliek et al., 2006) and an IVF-conceived girl with RSS was found to have hypermethylation on the paternal allele of the 7q DMR (MEST) (Kagami et al., 2007). The significance of the latter result is uncertain because MEST hypermethylation is not a recognized cause of RSS, and partial hypermethylation of MEST was also detected in the girl’s father.
MatUPD14 syndrome
Approximately 50 patients with maternal UPD14 (matUPD14) have now been reported (Kotzot and Utermann, 2005; Mitter et al., 2006; Kagami et al., 2008), however, matUPD14 may be significantly more common because the phenotype is relatively non-specific and molecular testing is not routinely undertaken. Some matUPD14 patients are detected because of the presence of a Robertsonian translocation involving chromosome 14 (Kotzot and Utermann, 2005).

MatUPD14 is characterized by pre- and post-natal growth retardation, hypotonia, facial dysmorphism, obesity, early onset puberty and variable intellectual outcome (Kotzot and Utermann, 2005). The phenotype is thought to result from altered gene expression at a 14q32 DMR that is usually paternally methylated (Geuns et al., 2007). To date, only one patient has been reported with matUPD14 occurring as a result of an epimutation, paternal hypomethylation at the 14q32 DMR in the presence of biparental inheritance of chromosome 14 (Temple et al., 2007). Three patients have also been described with matUPD14 syndrome resulting from a submicroscopic deletion at 14q32 on the paternal allele (Kagami et al., 2008). There have been no reported ART-conceived patients with matUPD14 syndrome.

PatUPD syndrome
PatUPD14 (OMIM 608149) is very rare, with ~30 patients described in the literature to date (Kotzot and Utermann, 2005; Kagami et al., 2008). PatUPD14 is characterized by polyhydramnios, premature labour, skeletal abnormalities including small chest and frequently early death (Kotzot and Utermann, 2005). Like matUPD14, the phenotype is thought to result from altered gene expression at the 14q32 DMR. Although originally defined by the presence of UPD14, more recently some patients with a patUPD14 syndrome-like phenotype have been found to have either sub-microscopic chromosome deletions at 14q32 or epimutations resulting in hypermethylation at 14q32, both involving the maternal allele (Kagami et al., 2008). There has been no report of patUPD14 syndrome resulting from an epimutation, nor has there been report of any patient with patUPD14 syndrome having been conceived using ART.

Pseudohyoparathyroidism 1b
PHP-1b (OMIM 603233) manifests as hypocalcaemia and hyperphosphataemia due to resistance to parathyroid hormone. PHP-1b is caused by mutations or epimutations in regulatory regions of the gene GNAS1. GNAS1 encodes the \(\alpha\)-subunit of the stimulatory G protein, and in the proximal renal tubule, transcripts of the \(\alpha\)-subunit are derived only from the maternal allele.

PHP-1b occurs in familial and sporadic forms. The great majority of familial cases result from a genetic mutation, a 3-kb microdeletion located 220 kb upstream of GNAS1 that results in hypomethylation on the maternal allele (Bastepe et al., 2003; Bastepe et al., 2005). Patients with sporadic PHP-1b also show hypomethylation on the maternal allele of GNAS1, but in sporadic PHP-1b the hypomethylation is more extensive and there is usually no microdeletion or detectable alteration in DNA sequence (Bastepe et al., 2005; Liu et al., 2005). It is likely that some sporadic cases of PHP-1b result from an epimutation involving hypomethylation of the maternal allele of GNAS1 (Liu et al., 2005). To date, only one patient has been described with PHP-1b caused by patUPD of chromosome 20q (Bastepe et al., 2001). There have been no patients with PHP-1b reported to have been conceived using ART.

Transient neonatal diabetes mellitus
TND (OMIM 601410) presents in the neonatal period with growth retardation and hypoglycaemia. At 6q24 there exists a DMR that is usually methylated on the maternal allele and unmethylated on the paternal allele and regulates two imprinted genes, ZAC and HYMAI (Gardner et al., 2000; Arima et al., 2001). TND results from a ‘double dose’ of the paternal epigenotype, with the underlying molecular mechanism being paternal chromosome duplication in 40% of patients, patUPD6 in 40% and in 20% hypomethylation at the maternal allele of 6q24 DMR (Temple, 2007).

To date, there have been no documented instances of patients with TND born following IVF/ICSI. Sutcliffe et al., (2006) sought conception data from 23 registry-based TND patients and found one patient in whom the parents had previously used IVF; however, this patient was shown to have patUPD6. Mackay et al. (2006) also reported two patients with TND who were conceived naturally, but whose parents had previously experienced infertility. Both TND patients had maternal hypomethylation at 6q24, with one also having maternal hypomethylation at other loci (see Section maternal hypomethylation syndrome below).

Maternal hypomethylation syndrome
Three recent publications have indicated the existence of a novel imprinting syndrome resulting from maternal hypomethylation at multiple loci (Mackay et al., 2006; Rossignol et al., 2006; Boonen et al., 2008). Rossignol et al. (2006) studied 40 BWS patients and found that 10 of them had LOM at loci other than the 11p15 DMR2. Eleven of the 40 BWS patients in the study had been conceived using ART, and three of these exhibited LOM at multiple loci. These results indicate that the maternal hypomethylation syndrome can be associated with, but is not limited to, ART conceptions. Mackay et al. (2006) studied 12 patients with TND resulting from maternal hypomethylation at the 6q24 DMR, and found that 6 had hypomethylation at other loci. None of these patients were conceived following ART, although one was conceived following a period of subfertility. A recent report described two siblings with maternal hypomethylation syndrome, exhibiting features of TND and BWS (Boonen et al., 2008). The siblings and their mother were the product of consanguineous relationships, raising the possibility of a novel autosomal recessive defect of the imprinting mechanism being present either in the siblings or their mother.

Other imprinted loci may be associated with unknown syndromes
The nine imprinting syndromes outlined above have been defined because they present a recognizable phenotype that it usually present from early life. As noted previously, there are
an estimated 200 imprinted genes in the human genome (Luedi et al., 2007), and the majority of these are yet to be associated with a clinical syndrome or phenotype. Epimutations at some of these loci might contribute to the increased incidence of pregnancy complications and birth defects that have been observed in ART pregnancies (Horsthemke and Ludwig, 2005), while other epimutations might result in more subtle effects, such as altered predisposition to common diseases (e.g. psychiatric disorders, cancers), which could significantly influence the long-term health of ART-conceived children (Maher et al., 2003a).

Follow-up studies examining the health, growth and development of children conceived using ART have generally been reassuring (Knoester et al., 2008; Leunens et al., 2008), although one study found that IVF-conceived children were taller than their naturally conceived counterparts (Miles et al., 2007). Future studies of young adults conceived using ART may be helpful for delineating subtle phenotypes affecting health, growth or behavior that might ultimately be shown to result from aberrant imprinting.

**Summary of clinical data**

Table I shows a summary of the clinical data reviewed above, and allows two conclusions to be drawn.

First, evidence of imprinting syndromes resulting from epimutations in ART-assisted pregnancies is so far confined to three syndromes: BWS, AS and the maternal hypomethylation syndrome. It is notable that for all three syndromes the observed epigenetic defect is hypomethylation on the maternal allele of the relevant DMR. This is the same category of epimutation that was found to cause large offspring syndrome in sheep, which results from hypomethylation of the maternal IGF2 receptor gene (Young et al., 2001), and suggests that the risk of imprinting syndromes associated with ART might be confined to the subgroup of imprinting syndromes caused by hypomethylation of the maternal allele; this subgroup also includes TND and PHP-1b. The situation with RSS remains unclear; there is one report of an ART-conceived patient with hypomethylation at the paternal 11p15 DMR1, however, this patient had a ‘RSS-like’ phenotype that did not meet the diagnostic criteria for RSS (Bliek et al., 2006). A second ART-conceived RSS patient had an unusual epimutation at the 7q32 DMR that is not a recognized cause of RSS (Kagami et al., 2007).

The second conclusion is that an effect of ART is only likely to be detectable for syndromes where epimutations comprise a significant proportion of cases. It is no surprise that the best evidence for an effect of ART on imprinting comes from studies of BWS, a disorder where 65% of patients have an epimutation. For syndromes in which epimutations are infrequent, such as PWS, an effect of ART may be almost impossible to detect, particularly if the increased risk of epimutations is similar in magnitude to that observed in BWS (~14×).

**Lessons from the study of gametes and preimplantation embryos**

Additional insight into the association between ART and imprinting syndromes can be gained from studying gametes and preimplantation embryos. Such studies in humans have considerable limitations because the primary source of oocytes and embryos is infertile couples who by definition are undergoing IVF/ICSI. Although several studies have examined the patterns of gene expression or methylation in human gametes/preimplantation embryos (Huntriss et al., 1998; Monk and Salpekar, 2001; Salpekar et al., 2001; Geuns et al., 2003, 2007), the fact that these samples have been obtained from infertile couples undergoing IVF/ICSI prevents comparison with a suitable control group comprising naturally conceived embryos or naturally ovulated oocytes.

One study was able to study the methylation patterns in superovulated oocytes from infertile women and compare them with the methylation patterns in immature oocytes collected from fertile women undergoing laparoscopic procedures (Sato et al., 2007). Compared with oocytes from fertile women, superovulated oocytes from some infertile women were shown to have hypomethylation at the 7q32 DMR and hypermethylation at the 11p DMR1. It could not be determined whether these epimutations were the result of infertility itself, the ovulation induction process, or maternal age, however similar results were obtained from superovulated oocytes from fertile mice, implicating the ovulation induction procedure (Sato et al., 2007). Consistent with these observations, hypermethylation at the maternal 11p DMR1 has also been detected in human ES cells derived from IVF blastocysts (Li et al., 2005).

Human spermatozoa are more readily available from fertile males, providing a suitable control group, yet there is conflicting evidence regarding the effects of infertility on imprinting in spermatozoa. The 11p15 DMR1, which is normally methylated on the paternal allele, was found by one study to be normally methylated in the spermatozoa of infertile men (Hartmann et al., 2006), however, another study found hypomethylation of the 11p15 DMR1 in infertile men and this also correlated with the severity of oligospermia (Marques et al., 2004). In the same cohort there was no evidence of hypermethylation at the 7q32 DMR, which is normally unmethylated in males (Marques et al., 2004). Kobayashi et al. (2007) analysed the methylation profile of seven genes (2 paternally methylated, 5 maternally methylated) in the sperm of infertile men, and found that the sperm of oligospermic men were more likely to carry epimutations than the sperm of normospermic males. Detected epimutations fell into two groups: hypomethylation at loci that are usually methylated in sperm (11p15 DMR1; 14q32 DMR) implies a failure of imprint acquisition at these loci, whereas hypermethylation at DMRs that are usually unmethylated in sperm (7q32, 6q24, 15q DMRs) presumably results from a failure of imprint erasure.

More extensive studies have been possible in mouse embryos because embryos are available from normally fertile mice, in the absence of ovulation induction and in vitro culture. In mice, IVF and embryo culture appear to result in a higher frequency of epimutations. Extensive studies of the H19 locus in mouse embryos have yielded inconsistent results: some studies have found paternal hypomethylation (Doherty et al., 2000; Mann et al., 2004), whereas others have found maternal hypermethylation (Khosla et al., 2001; Li et al., 2005). These contrasting results might reflect the use of different mouse strains and culture media. Another
possibility is that the use of pooled embryos in these studies masked individual differences between embryos; a recent study analysing individual embryos found that some exhibited maternal hypermethylation while other had paternal hypomethylation (Fauque et al., 2007). Rivera et al. (2008) also studied individual embryos and found that IVF and embryo manipulation resulted in hypomethylation at the paternal H19 locus and hypomethylation at the maternal 11p15 DMR2 locus, the latter being the same epimutation that is associated with BWS in human ART conceptions.

The presence of epimutations affecting both maternal and paternal alleles suggests that defective maintenance of the imprint after fertilization is an important underlying mechanism. This hypothesis is supported by a recent study of methylation patterns in mouse placentae obtained following ovulation induction, which showed hypomethylation at paternal as well as maternal alleles (Fortier et al., 2008). The same epimutations were not observed in the embryos themselves, indicating a failure of imprint maintenance in the placenta, where the mechanism for imprint maintenance appears to be less robust. These findings were not observed in placentae that had not been subjected to in vitro culture or manipulation, implicating the ovulation induction process as the cause of defective imprint maintenance.

Although these studies of mouse and human gametes and embryos have yielded inconsistent results, they collectively suggest that epimutations associated with ART: (i) appear to be associated with subfertility, ovulation induction and embryo culture; (ii) can affect both maternally and paternally methylated genes and (iii) can involve either hypomethylation or hypermethylation.

Concluding comments

Despite emerging evidence of an association between ART and several imprinting syndromes, there is still limited knowledge about the cause of epimutations in ART pregnancies, the syndromes for which a risk applies and the level of risk. For syndromes in which epimutations make up only a small proportion of cases, such as PWS, it is unlikely that evidence of an increased risk associated with ART will ever by obtained. Future studies should focus on syndromes where a significant proportion of cases are caused by epimutations, such as RSS and TND. Given the rarity of all imprinting syndromes, another focus of future studies should be the long-term follow-up of the health of ART-conceived adults. Such studies may shed light on imprinting effects that extend beyond those associated with the recognized imprinting syndromes.

References


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