Assisted reproductive therapies and imprinting disorders—a preliminary British survey


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BACKGROUND: Recent reports have suggested a higher risk of Beckwith–Wiedemann syndrome (BWS) and Angelman syndrome (AS) after assisted reproductive technologies (ARTs), but it is unclear whether this might also apply to other disorders of genomic imprinting. METHODS: We contacted families of children with BWS, AS, Prader–Willi syndrome (PWS) and transient neonatal diabetes mellitus (TNDM) to determine use of ART. RESULTS: A statistically significant increased frequency of ART in children with BWS was confirmed [2.9%, 95% confidence interval (CI) 1.4–6.3% vs 0.8% expected] but there was no significant association with PWS or TNDM. Consideration of the molecular subgroup of BWS and AS suggested the feasibility of association with ART. CONCLUSIONS: These differences may relate to variations in (i) the molecular mechanisms for disordered imprinting in the different disorders and (ii) the susceptibility of specific imprinting control regions to ART-associated methylation alterations (epimutations).

Key words: ART/BWS/imprinting/IVF/PWS

Introduction
Following reports of children conceived by ICSI (Cox et al., 2002; Ludwig et al., 2005) who developed a very rare form of Angelman syndrome (AS), three independent studies have described an increased frequency of assisted reproductive technology (ART) births (both ICSI and IVF) among children with Beckwith–Wiedemann syndrome (BWS) imprinting disorder (Debaun et al., 2003; Maher et al., 2003; Gicquel et al., 2003).

Patients and methods
In order to evaluate further the relationship between ART and disordered imprinting, we obtained a ‘conception history’ from the families of children with four model imprinting disorders. We included fertility drugs (clomiphene and other drugs inducing ovulation) in our enquiry as one report inferred (Ludwig et al., 2005) that a history of infertility rather than ART itself may be implicated in causation. With relevant ethical approval, we sent out a questionnaire as used in Ludwig et al. (2005) to (i) families belonging to the AS, Prader–Willi syndrome (PWS) and BWS patient support groups and (ii) families with a diagnosis of AS, BWS, PWS and transient neonatal diabetes mellitus (TNDM) known to the Regional Genetic Centres in Birmingham, London and Manchester and to the National Genetics Centre (Dublin) or national research studies (BWS, AS and TNDM). Ethical approval did not allow us to re-contact non-responders in view of the sensitive nature of our enquiry.

Results
Questionnaires were sent to 213 families who had a child with BWS and a total of 83 replies were received (response rate of 39%). Four of the 83 families (5%) had familial BWS and these were excluded from further analysis (none of these was conceived by ART). Of the remaining 79 sporadic cases, 11 (14%) were conceived following ART or treatment to induce ovulation. Methods of assisted conception included ICSI (n = 5), IVF (n = 1) and fertility drugs (n = 5). To allow for a potentially
Syndromic imprinting defects detected in ART-conceived children

Ireland confirmed that rates of ART are similar to those in other parts of the British Isles. The number of children conceived with TNDM was 1993 (1987–2000) and for the parents, 1960 (1954–1966) for the mothers and 1962 (1954–1966) for the fathers.

A total of 384 families with a child with AS were contacted and 81 replies were received (response rate 21%). Six (7%) had a family history of AS (none of whom had ART) and, out of the remaining 75 children, three (4%) had a history of assisted conception. One family used artificial insemination by donor (AID), another used intrauterine insemination by donor and one had previously used IVF. Assuming there were no more cases of assisted conception in the non-responders, this gives a minimum of 0.8% ART births in this group. The median years of birth for the AS children was 1993, IQR 1988–1998, 1964 (1956–1968) for the mothers and 1962 (1954–1966) for the fathers.

A total of 522 families with a child with PWS were contacted. There were 169 replies (response rate 32%) of which six (4%) had a family history of PWS (none of whom had ART). Nine (6%) of the remaining 163 were conceived using ART or assisted conception techniques (two used ICSI and seven used fertility drugs). Allowing for a biased response rate, the minimum frequency of assisted conception births is 2%. Median year of birth was 1994, IQR (1987–1999). Parents’ median years of birth were 1960 (1954–1966) and 1959 (1951–1965) for mothers and fathers, respectively.

Finally, all known UK cases of TNDM were contacted. Out of the 38 questionnaires sent out, 23 replied (60%) and no families had a history of TNDM. Only one (4%) was conceived with a history of subfertility (previous use of IVF on the cycle prior to conception) and, taking the total 38 as the denominator, the minimum frequency was 3%. Median date of birth for the TNDM children was 1993, IQR 1991–1998, 1964 (1956–1968) for the mothers and 1962 (1954–1966) for the fathers.

An estimate for the incidence of ART in the general UK population was obtained using records kept by the Human Fertilisation and Embryology Authority (HFEA). Enquiry to Ireland confirmed that rates of ART are similar to those in other parts of the British Isles. The number of children conceived after IVF/ICSI techniques during the period 1991–2002 was 68,566 (including unknown outcomes of pregnancies). Over the same time period, 8,395,627 children were born in the UK (UK Office National Statistics & Information Services Department, Scotland). This means that a maximum 0.8% of births during this period were IVF/ICSI conceptions (the HFEA excluded induction of ovulation or clomiphene in its figures and therefore these were removed from the core analysis). This period covers a large number of the births collected in this study and is a good estimate of the proportion expected in the UK.

This UK population estimate was compared with the frequency of IVF/ICSI conceptions in our data. Six children with BWS and two with PWS were conceived by IVF/ICSI, giving minimum frequencies (allowing for non-responders) of 2.9%, 95% CI 1.4–6.3%, and 0.4%, 95% CI 0.1–1.4%, respectively. As the 95% CI in the BWS group does not contain the UK estimate, this suggests a significantly higher frequency of ART conception in children with BWS that is not due to chance (see Figure 1).

For those families who returned the questionnaire, we compared the molecular status of ART cases (when known) with that reported in unselected patients (see Table I). All ART BWS children (n = 8) that had undergone molecular analysis had loss of maternal allele methylation at the KvDMR1 imprinting control region (expected 50%). Molecular data were available for the three AS cases with a history of assisted conception: one (who was conceived by AID/intrauterine insemination by donor/had previously used IVF) had loss of maternal allele methylation at the SNRPN imprinting control region (expected incidence 4%). The other two cases both had a maternal 15q deletion (expected incidence 75%). The two PWS assisted conception cases (both ICSI conceived) for whom molecular data were available had a
paternal 15q11.2 deletion and the TNDM patient who was born after previous IVF had uniparental disomy.

Discussion
We have confirmed an association between ART and BWS. Furthermore, all eight post-ART BWS cases with a molecular genetic diagnosis had loss of maternal allele methylation at the KvDMR1 imprinting control region (expected 50%). Although the absolute frequency of BWS after ART is small (<1%), this association is important as it confirms that in humans, as in some animals, ART may be associated with epigenetic changes that can lead to human disease. However, key questions are what is the cause of the increased susceptibility to epigenetic alterations and whether these post-ART epigenetic changes are restricted to BWS or may be associated with other phenotypes. Epimutations at the SNRPN imprinting control region are a rare cause of AS (population frequency ~1 in 300 000) but to date five of seven children with AS conceived by ICSI have had this subtype (including our case), so ART-related BWS and AS appear to be specifically associated with loss of maternal allele methylation at a critical imprinting control region. Two hypotheses have been proposed to explain this association (Maher, 2005). First, based on animal studies, it has been suggested that in vitro embryo culture might predispose to KvDMR1 or SNRPN demethylation. Alternatively, it may be that there is an increased risk of an imprinting disorder following ART because of an association with infertility per se rather than with in vitro embryo culture (e.g. treatment for infertility such as ovarian stimulation might be implicated and/or susceptibility to epigenetic defects might be responsible for both infertility and an increased risk of imprinting defects). In support of this latter hypothesis is the observation that there is an increased frequency of SNRPN epimutations among AS children born after ART and other assisted conception techniques including induced ovulation (Ludwig et al., 2005).

To our knowledge, this is the first investigation of PWS and TNDM and ART. We did not find evidence of an association between ART and PWS. However, paternal allele deletions and maternal uniparental disomy are the overwhelming causes of PWS and so this finding reinforces the hypothesis that the association between ART and imprinting disorders such as BWS and AS is because of a specific link to loss of methylation at a critical imprinting control region. At this stage, it is not clear whether KvDMR1 and SNRPN imprinting control regions are especially sensitive to epimutations or whether ART might be associated with demethylation at other imprinting control regions. A subset of TNDM patients (~25%) have an isolated methylation defect (loss of maternal allele methylation) of an imprinted CpG island at chromosome 6q24, and we did not find a link between TNDM epimutations and ART. However, TNDM is a rare disorder and analysis of multiple national cohorts of TNDM patients will be required to define the frequency of ART in TNDM patients reliably.

Our study illustrates the problems encountered in undertaking research on possible long-term morbidity of ART without linkage between the HFEA database and disease registers and reliable information on the frequency of different assisted conception techniques in the reference population. Careful follow-up of ART children is required to define the precise absolute risks of different imprinting disorders and confirm or refute suggestions of possible increased risks of childhood tumours such as retinoblastoma (Moll et al., 2003), or others in which aberrant imprinting is part of the aetiology. Nevertheless, our findings illustrate how specific molecular markers can be used to identify susceptible patient subgroups (e.g. BWS children with KvDMR1 loss of methylation) to increase the power of studies investigating linkage between ART and imprinting disorders. To address the risk of ART conception resulting in an imprinting disorder, we would first need to know the reliable population frequencies of all these disorders, probably impossible for BWS as the phenotype merges with normality in the milder cases. Secondly, we would have to link these cases with conception status via an improved HFEA database. We hope our report adds to the evidence that such a study is needed.

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References

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