Human Reproduction Vol.22, No.12 pp. 3237–3240, 2007 doi:10.1093/humrep/dem268
Advance Access publication on October 5, 2007

A survey of assisted reproductive technology births and imprinting disorders

Sarah Bowdin[1,7], Cathy Allen[2], Gail Kirby[3], Louise Brueton[1], Masoud Afnan[4], Christopher Barratt[5], Jackson Kirkman-Brown[4], Robert Harrison[2], Eamonn R. Maher[3] and William Reardon[6]

[1]Clinical Genetics Unit, Birmingham Women's Hospital, Edgbaston, Birmingham, UK; [2]Human Assisted Reproduction Ireland, Rotunda Hospital, Dublin, Ireland; [3]Division of Paediatrics and Child Health, University of Birmingham, Edgbaston, Birmingham, UK; [4]Assisted Conception Unit, Birmingham Women's Hospital, Birmingham, UK; [5]Division of Reproductive Biology and Genetics, University of Birmingham, Birmingham, UK; [6]National Centre for Medical Genetics, Our Lady's Hospital for Sick Children, Dublin, Ireland

[7]Correspondence address: E-mail: sarah.bowdin@bwhct.nhs.uk

BACKGROUND: Genomic imprinting is an epigenetic process in which allele-specific gene expression is dependent on the parental inheritance. Although only a minority of human genes are imprinted, those that have been identified to date have been preferentially implicated in prenatal growth and neurodevelopment. Mutations or epimutations in imprinted genes or imprinting control centres are associated with imprinting disorders such as Angelman syndrome (AS) and Beckwith–Wiedemann syndrome (BWS). Recently, an increased frequency of assisted reproductive technology (ART) conceptions has been reported in children with BWS and AS. However, the risk of imprinting disorders in ART children is unknown. METHODS: We undertook a survey of 2492 children born after ART in the Republic of Ireland and Central England with the aim of detecting cases (both clinically diagnosed and previously unrecognized) of BWS and AS in this cohort. The response rate to an initial questionnaire was 61%, corresponding to data for 1524 children. After evaluation of the questionnaire, 70 children were invited for a detailed clinical assessment, and 47 accepted (response rate of 67%). RESULTS: In this entire cohort, we detected one case of BWS and no cases of AS. We did not find evidence that there exists a significant group of ART children with unrecognized milder forms of AS or BWS. CONCLUSIONS: Although previous studies have suggested an increased relative risk of BWS and AS after ART, our findings suggest that the absolute risk of imprinting disorders in children conceived by ART is small (<1%). Precise risk estimates of risk are difficult to define because of the rarity of the conditions and incomplete response rates to the questionnaire and clinical examination invitations. Hence further investigations are indicated to (i) refine the absolute and relative risks of imprinting disorders after ART and (ii) ensure that changes in ART protocols are not associated with increased frequencies of epigenetic changes and imprinting disorders in children born after ART.

Keywords: ART; IVF; ICSI; imprinting disorders; child follow-up

Introduction

Assisted reproductive technology (ART) births accounts for >1% of all births in the UK and USA, and more than 30% of all twin births (ESHRE, 2005). Several outcome studies have highlighted increased complication rates in IVF-conceived children compared with the general population (MRC Working Party on Children Conceived by In Vitro Fertilisation, 1990; FIVNAT (French In Vitro National), 1995; Dhont *et al.*, 1999). Some of the complications have been attributed to a higher frequency of multiple births, but studies have also shown that singleton IVF infants have a greater risk of low birthweight (Schieve *et al.*, 2002) and birth defects (Hansen *et al.*, 2002; Belva *et al.*, 2006). In addition, some researchers have questioned the genetic implications for offspring of intracytoplasmic sperm injection (ICSI), particularly for male infertility of genetic cause. However, although higher incidences of de-novo sex chromosomal aberrations (Bonduelle *et al.*, 1999), inheritance of CF mutations (Van der Ven *et al.*, 1996) and Y microdeletions (Pryor *et al.*, 1997), have been reported following ICSI procedures, most of the long-term follow-up data of children conceived by IVF are reassuring (Sutcliffe *et al.*, 2001; Leunens *et al.*, 2006).

Since 2002, there have been reports of an increased incidence of IVF or ICSI conceptions amongst children with Beckwith–Wiedemann syndrome (BWS) and Angelman syndrome (AS) (reviewed in Allen and Reardon, 2005). In a

retrospective study, we found an increased frequency of ART conceptions in BWS cohorts (4% of BWS cases were ART conceptions, compared with 1.2% of the general population) (Maher *et al.*, 2003). Similar results have been reported from USA and France (DeBaun *et al.*, 2003; Gicquel *et al.*, 2003). Combining the findings of these investigations suggested a 4.2-fold increase in the risk of BWS for children conceived *in vitro* (Gosden *et al.*, 2003). However, further interpretation of these studies was limited because of a reliance on case records and questionnaire data to determine the method of conception, and a lack of control groups. A case–control study in an Australian population estimated the absolute risk of BWS when ART is used as the means of conception to be 4/14 894 ($\approx$1/4000, or nine times greater than the general population) (Halliday *et al.*, 2004).

In addition, two studies suggested a link between AS and ICSI (Cox *et al.*, 2002; Orstavik *et al.*, 2003).

BWS and AS are model imprinting disorders which result from altered expression or mutations in imprinted genes that are critical for normal growth and development. Thus BWS is characterized by pre- and/or post-natal overgrowth, anterior abdominal wall defects, macroglossia, neonatal hypoglycaemia, hemihypertrophy, ear pits and creases, renal anomalies and facial naevus flammeus (Elliott *et al.*, 1994). BWS children are at increased risk of developing embryonal tumours, especially Wilm's tumour for which screening is recommended in this group. The genetics of BWS are complex but it seems that overexpression of the paternally expressed growth promoter IGF2 and/or loss of the maternally expressed candidate growth suppressor CDKN1C function can cause BWS (Maher and Reik 2000; Weksberg *et al.*, 2003; Maher 2005). In about 40–50% of children with BWS, there is loss of maternal allele methylation at an imprinting control centre (KvDMR1/IC2), associated with silencing of CDKN1C expression (Cooper *et al.*, 2005; Diaz-Meyer *et al.*, 2005). This epigenetic change has been detected in almost all children with BWS who were born after ART suggesting that the ART procedure may increase the likelihood of loss of maternal KvDMR1 methylation.

AS is characterized by severe mental retardation, delayed motor development, poor balance, jerky movements, absence of speech and a happy disposition. AS is caused by loss of expression or mutation in the maternally expressed UBE3A gene on chromosome 15. In a small number of naturally conceived AS patients, $\sim$2%, there is loss of methylation at an imprinting centre (similar to that seen in BWS ART cases at KvDMR1) (Clayton-Smith and Laan, 2003). However, the frequency of these rare epimutations appears to be increased in AS children conceived by ART (Cox *et al.*, 2002; Orstavik *et al.*, 2003). These observations suggest that the ART procedure may predispose to loss of methylation at key imprinting control centres implicated in the pathogenesis of BWS and AS. BWS and AS are rare disorders, $\sim$1 in 14 500 and 1 in 15 000 live births, respectively, and the incidences of BWS and AS caused by imprinting defects are estimated to be 1 in 30 000 and 1 in 750 000, respectively. However, in a small follow-up study of children conceived by ART, the frequency of BWS was 1 in 91 (Sutcliffe *et al.*, 1995). Furthermore, it could be postulated that milder incomplete forms of BWS and AS might be overlooked unless they were sought specifically. In order to investigate (i) the frequency of imprinting disorders in children with ART and (ii) whether some ART children might have incomplete, clinically undiagnosed, forms of BWS and AS, we undertook a questionnaire survey designed to identify children with phenotypes consistent with BWS and AS.

Materials and Methods

The two study centres were the Birmingham Women's Hospital Assisted Conception Unit, Birmingham, England (HFEA centre #0119), and Human Assisted Reproduction Ireland at the Rotunda Hospital, Dublin, Republic of Ireland. Ethics approval for the study was obtained from the local research ethics committees. Couples who had delivered a live born child or children following ART between 1989 and 2002 (Dublin) and 1997 and 2003 (Birmingham) were identified from the units' records. Those who had a stillbirth were excluded for sensitivity reasons. A questionnaire was sent to all families who agreed to participate (Appendix 1 in Supplementary data). Addresses were cross-checked with hospital records and, in the event of 'return to sender' (8% of invitation letters), a newer address was identified where possible. The questionnaire was divided into five main headings—history of the conception and pregnancy, birth defects, chromosome analysis, specific queries relating to phenotypic signs of an imprinting disorder and history of other children. This questionnaire was developed by experts in BWS and AS (ERM and WR), based on the suggested diagnostic criteria for these specific conditions (Elliott *et al.*, 1994; Williams *et al.*, 2005).

The data were examined and interpreted by a clinical geneticist. Phenotypic signs consistent with BWS or AS were evaluated in the context of gestations and birthweights to highlight significant features. Where possible in each case reported to have only a single phenotypic sign known to be associated with an imprinting disorder, the hospital notes were reviewed to confirm the parental report. In those cases where the only positive sign was a facial birthmark, a photograph was obtained initially. Following the review of the questionnaires, photographs and notes, all children with clinical signs consistent with a possible imprinting disorder were invited to attend a genetics clinic. Children were examined by a clinical geneticist and blood tests were taken for molecular analysis when clinically indicated. Data from completed questionnaires were entered into a customized database.

Letters of reassurance were sent to all other participants whose information provided no clues to a genomic imprinting defect.

Results

1559 families were identified as having successfully delivered 2492 children following ART. 1017 families completed and returned the questionnaires, providing information on 1524 children (61% of those eligible). Table I provides details of the respondents.

174 of the 1524 children (11%) were reported to have one or more phenotypic features of an imprinting disorder as listed on the questionnaire (Table II).

70 children were identified as possibly warranting clinical assessment. 47 accepted an appointment to be examined in the genetics clinic (an acceptance rate of 67%). The main reason given by non-attenders was 'my child is fine'. Four of the children examined had clinical features that might be consistent with BWS (Table III). One of these children was already

Table I. Pregnancies and children of responders.

Pregnancies	Total	1182	
	Singleton	875	74%
	Multiple	307	26%
	IVF	815	64%
	IVF/ICSI	457	36%
Children	Total	1524	
	Singleton	875	57%
	Multiple	649	43%
	Males	782	51%
	Females	742	49%

Table II. Number of children with one or more positive questionnaire answers to individual phenotypic features of BWS or AS.

Phenotypic feature recorded	Number of positive answers on questionnaire	Percentage of all respondents ($n = 1524$)
Neonatal hypoglycaemia	93	6.1
Facial birth mark	75	4.9
Abdominal hernia	21	1.3
Umbilical hernia	8	0.5
Limb asymmetry	9	0.6
Macroglossia	11	0.7
Epilepsy	17	1.1
Failure to acquire speech	2	0.1
Movement abnormalities	8	0.5

Table III. Phenotypes of children tested for BWS.

Child number	Phenotype
1	Neonatal hypoglycaemia, facial birthmark, macroglossia
2	Macrosomia, umbilical hernia
3	Facial birthmark, inguinal hernia, co-triplet of child 2
4	Macroglossia, facial birthmark, umbilical hernia, limb asymmetry, developmental delay

known to have BWS (child 4) and the underlying molecular mechanism was shown to be loss of methylation at KvDMR1. Blood samples were taken for molecular analysis from the other three children, and all were negative for paternal mosaic disomy and loss of methylation at KvDMR1. One of the children examined (not listed above) had features consistent with AS, however molecular analysis failed to detect a deletion, disomy or imprinting centre mutation at the AS region of chromosome 15.

Discussion

This study is the first, to our knowledge, to examine a cohort of children conceived after ART specifically for signs and symptoms of two imprinting disorders, BWS and AS. Previously, ART procedures have been associated with a relative risk of 4.2 for BWS, which would correspond to an incidence of 1 in 3372 (compared with the rate of 1 in 14 500 seen in the general population). However, Sutcliffe *et al.* (1995) reported one child with BWS out of a cohort of 91 children born after embryo cryopreservation. In addition, Olivennes *et al.* (2001) reported one case of BWS in a cohort of 73 children born after ART. These observations suggested that the frequency of BWS among ART children might be markedly higher than in the general population. The phenotype of BWS is very variable, and some children with isolated hemihyperplasia have been shown to have loss of methylation at KvDMR1 (Shuman *et al.*, 2006). We hypothesized that BWS, particularly milder forms, might be underrecognized in ART cohorts. Hence we designed a questionnaire survey to detect both ART children diagnosed with BWS/AS and to identify children with features suggestive of BWS/AS but without a formal diagnosis. However, only one child with molecularly proven BWS was identified in a cohort of 1523, suggesting that although the relative risk of BWS may be increased, the absolute risk of BWS post-ART is small ($<1\%$) and is not as high as suggested by the reports of Sutcliffe and Olivennes. We did not detect a single case of hemihyperplasia and although three further children had some stigmata of BWS, none satisfied clinical criteria for the diagnosis of BWS and molecular genetic testing was negative. Our questionnaire response rate of 61% may have resulted in some children with an imprinting disorder not being detected. Ethical considerations prevented us from tracing the children of non-responders, however, both research groups are major referral centres for imprinting disorders and we are not aware of any additional children with BWS or AS that might have been included in the cohorts.

Animal models of imprinting disorders occurring after ART suggest that the risk of BWS or AS may relate to the precise ART protocol (Young *et al.*, 1998), and therefore the risk might vary between assisted conception units. Children with post-ART AS have rare epimutations with loss of maternal allele methylation. Although it could be postulated that ART might increase the risk of somatic mosaic imprinting defects at the AS locus resulting in a "mild-AS phenotype", we could not find evidence for this (despite a low threshold for inviting a child for clinical examination). However, as the incidence of AS caused by imprinting defects is estimated to be in the region of 1 in 750 000, there is an 85% chance that our study would not detect a 50-fold increased risk given the small numbers in our cohort.

Our study should serve as a template for further, preferably prospective, clinical studies to further assess the risk of ART-related imprinting disorders. Such studies should also include children born after ovarian stimulation, since AS resulting from an imprinting defect has been reported in children conceived with the use of ovulation induction alone (Ludwig *et al.*, 2005). In addition to specifically seeking evidence of known, albeit mild, imprinting disorders, careful note should be made of congenital anomalies and growth disorders (as in our study). Recently, it has been demonstrated that children with BWS or transient neonatal diabetes mellitus may display loss of methylation at additional loci (i.e. hypomethylation is not restricted to 11p15.5 and 6q24, respectively) (Mackay *et al.*, 2006; Rossignol *et al.*, 2006). Although such hypomethylation is not exclusively seen in those conceived using ART, it is reasonable to hypothesize that the excess of growth retardation and congenital malformations in ART neonates might be caused by epigenetic alterations related to *in vitro* embryo culture and/or infertility (Horsthemke *et al.*, 2004; Zhu *et al.*, 2006). Hence

further investigation comparing epigenetic status of natural and ART-conceived children with congenital anomalies or intrauterine growth disorders may provide further insights into the relationship between ART and genome methylation.

Supplementary Data

Supplementary data are available at http://humrep.oxfordjournals.org

Funding

We are grateful for funding from the following: Children's Medical and Research Foundation, Our Lady's Hospital for Sick Children, Dublin, Ireland. Birmingham Children's Hospital Research Foundation, Birmingham, UK and WellChild.

References

Allan C, Reardon W. Assisted reproduction technology and defects of genomic imprinting. *BJOG* 2005;**112**:1589–1594.

Belva F, Henriet S, Liebaers I, Van Steirteghem A, Celestin-Westreich S, Bonduelle M. Medical outcome of 8-year-old singleton ICSI children (born≥32 weeks' gestation) and a spontaneously conceived comparison group. *Hum Reprod* 2007;**22**(2):506–515.

Bonduelle M, Camus M, DeVos A, Staessen C, Tournaye H, Van Assche E. Seven years of intracytoplasmic sperm injection and follow-up of 1987 subsequent children. *Hum Reprod* 1999;**14**(Suppl 1):243–264.

Clayton-Smith J, Laan L. Angelman syndrome: a review of the clinical and genetic aspects. *J Med Genet* 2003;**40**:87–95.

Cooper WN, Luharia A, Evans GA, Raza H, Haire AC, Grundy R, Bowdin SC, Riccio A, Sebastio G, Bliek J *et al.* Molecular subtypes and phenotypic expression of Beckwith-Wiedemann syndrome. *Eur J Hum Genet* 2005;**13**:1025–1032.

Cox GF, Burger J, Lip V, Mau UA, Sperling K, Wu BL, Hosthemke B. Intracytoplasmic sperm injection may increase the risk of imprinting defects. *Am J Hum Genet* 2002;**71**:162–164.

DeBaun M, Niemitz E, Feinberg A. Association of in vitro fertilisation with Beckwith-Wiedemann syndrome and epigenetic alterations of LIT1 and H19. *Am J Hum Genet* 2003;**72**:156–160.

Diaz-Meyer N, Yang Y, Sait SN, Maher ER, Higgins MJ. Alternative mechanisms associated with silencing of CDKN1C in Beckwith-Weidemann syndrome. *J Med Genet* 2005;**42**:648–655.

Dhont M, De Sutter P, Ruyssinck G, Martens G, Bekaert A. Perinatal outcomes of pregnancies after assisted reproduction: case-control study. *Am J Obstet Gynecol* 1999;**181**:688–695.

Elliott M, Bayly R, Cole T, Temple IK, Maher ER. Clinical features and natural history of Beckwith-Wiedemann syndrome: Presentation of 74 new cases. *Clin Genet* 1994;**46**:168–174.

ESHRE. Assisted reproductive technology in Europe, 2001. Results generated from European registers by ESHRE. *Hum Reprod* 2005;**20**:1158–1176.

FIVNAT (French In Vitro National). Pregnancies and births resulting from in vitro fertilization: French national registry, analysis of data 1986 to 1990. *Fertil Steril* 1995;**64**:746–756.

Gicquel C, Gaston V, Mandelbaum J, Siffroi J, Flahault A, Le Bouc Y. In vitro fertilization may increase the risk of Beckwith-Wiedeman syndrome related to the abnormal imprinting of the KCNQ1OT gene. *Am J Hum Genet* 2003;**72**:1338–1341.

Gosden R, Trasler J, Lucifero D, Faddy M. Rare congenital disorders, imprinted genes, and assisted reproductive technology. *Lancet* 2003;**36**:1975–1977.

Halliday J, Oke K, Breheny S, Algar E, Amor D. Beckwith-Wiedemann syndrome and IVF: a case-control study. *Am J Hum Genet* 2004;**75**:526–528.

Hansen M, Kurinczuk JJ, Bower C, Webb S. The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. *N Engl J Med* 2002;**346**:725–730.

Horsthemke B, Gross S, Katalivic A, Sutcliffe A, Varon R, Ludwig M. Subfertility is associated with an increased risk of conceiving a child with an imprinting defect. *Am J Hum Genet* 2004;**113**:40 (Abstr).

Leunens L, Celestin-Westreich S, Bonduelle M, Liebaers I, Ponjaert-Kristoffersen I. Cognitive and motor development of 8-year-old children born after ICSI compared to spontaneously conceived children. *Hum Reprod* 2006 (Epub ahead of print).

Ludwig M, Katalinic A, Groβ S, Sutcliffe A, Varon R, Horsthemke B. Increased prevalence of imprinting defects in patients with Angelman syndrome born to subfertile couples. *J Med Genet* 2005;**42**:289–229.

Mackay D, HahnemannJ, Boonen S, Poerksen S, Bunyan D, White H, Durston V, Thomas N, Robinson D, Shield J *et al.* Epimutation of the TNDM locus and the Beckwith-wiedemann syndrome centromeric locus in individuals with transient neonatal diabetes mellitus. *Hum Genet* 2006;**119**:179–184.

Maher ER. Imprinting and assisted reproductive technology. *Hum Mol Genet* 2005;**14**(Suppl 1):R133–R138.

Maher ER, Reik W. Beckwith-Wiedemann syndrome: imprinting in clusters revisited. *J Clin Invest* 2000;**105**:247–252.

Maher ER, Brueton LA, Bowdin SC, Luharia A, Cooper W, Cole T, Macdonald F, Sampson JR, Barratt CL, Reik W *et al.* Beckwith-Wiedemann syndrome and assisted reproductive technology (ART). *J Med Genet* 2003;**40**:62–64.

MRC Working Party on Children Conceived by In Vitro Fertilisation. Births in Great Britain resulting from assisted conception, 1978 to 1987. *BMJ* 1990;**300**:1229–1233.

Olivennes F, Mannaerts B, Struijs M, Bonduelle M, Devroey P. Perinatal outcome of pregnancy after GnRH antagonist (ganirelix) treatment during ovarian stimulation for conventional IVF or ICSI: a preliminary report. *Hum Reprod* 2001;**16**:1588–91.

Orstavik KH, Eiklik K, Van Der Hagen CB, Spetalen S, Kierulf K, Skjeldal O, Buiting K. Another case of imprinting defects in a girl with Angelman syndrome who was conceived by intracytoplasmic sperm injection. *Am J Hum Genet* 2003;**72**:218–219.

Pryor JL, Kent-First M, Muallem A, Van Bergen AH, Nolten WE, Meisner L, Roberts KP. Microdeletions in the Y chromosome of infertile men. *N Eng J Med* 1997;**336**:534–539.

Rossignol S, Stenou V, Gicquel C. The epigenetic imprinting defect of Beckwith-Wiedemann syndrome patients born following ART is not restricted to the 11p15 region. *J Med Genet* 2006 (Epub ahead of print).

Schieve LA, Meikle SF, Ferre C, Peterson H, Jeng G, Wilcox L. Low and very low birthweight in infants conceived with the use of assisted reproductive technology. *N Engl J Med* 2002;**346**:731–737.

Shuman C, Smith A, Steele L, Ray P, Clericuzio C, Zackai E, Parisi M, Meadows A, Kelly T, Tichauer D *et al.* Constitutional UPD for chromosome 11p15 in individuals with isolated hemihyperplasia is associated with high tumour risk and occurs following assisted reproductive technologies. *Am J Med Genet* 2006;**140A**:1497–1503.

Sutcliffe AG, D'Souza SW, Cadman J, Richards B, Mckinlay IA, Lieberman B. Outcome in children from cryopreserved embryos. *Arch Dis Child* 1995;**72**:290–293.

Sutcliffe A, Taylor B, Saunders K, Thornton S, Lieberman B, Grudzinskas J. Outcome in the second year of life after in-vitro fertilization by intracytoplasmic sperm injection: a UK case-control study. *Lancet* 2001;**357**:2080–2084.

Van der Ven K, Messer L, van der Ven H, Jeyendran RS, Ober C. Cystic fibrosis mutation screening in healthy men with reduced sperm quality. *Hum Reprod* 1996;**11**:513–517.

Weksberg R, Smith A, Squire J, Sadowski P. Beckwith-Wiedemann syndrome demonstrates a role for epigenetic control of normal development. *Hum Mol Genet* 2003;**12**:R61–R68.

Williams C, Beaudet A, Clayton-Smith J, Knoll J, Kyllerman M, Laan LA, Magenis R, Moncla A, Schinzel A, Summers J *et al.* Angelman syndrome 2005: updated consensus for diagnostic criteria. *Am J Med Genet* 2005;**140**:413–418.

Young L, Sinclair K, Wilmut I. Large offspring syndrome in cattle and sheep. *Rev Reprod* 1998;**3**:155–163.

Young LE, Fernandes K, McEvoy TG. Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. *Nat Genet* 2001;**27**:153–154.

Zhu JL, Basso O, Obel C, Bille C, Olsen J. Infertility, infertility treatments, and congenital malformations: Danish National Birth Cohort. *BMJ* 2006;**333**:679.

Submitted on January 17, 2007; resubmitted on June 14, 2007; accepted on June 18, 2007