## BRIEF COMMUNICATION

# Anthropometric, metabolic and endocrine evaluation of children born following assisted conception and methylation analysis of imprinted genes

H.L. MILES, J. PEEK, P. HOFMAN, M. HARRIS, N. BILLETT, FERTILITY ASSOCIATES[1] AND W. CUTFIELD

Liggins Institute, 2-4 Park Avenue, Grafton, Auckland

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Assisted reproduction has become widely practiced over the last 25 years and demand is growing .One to three percent of births in Western countries are the result of assisted reproductive technology (ART) and so far more than a million babies have been born world wide (Gosden, Trasler, Lucifero, & Faddy, 2003).

In vitro fertilisation (IVF) brings together oocytes obtained from ovarian follicles in suprerovulated cycles with prepared sperm in the laboratory. Intracytoplasmic sperm injection (ICSI) is a specialised form of IVF, developed for male factor infertility, in which fertilisation is achieved by the injection of a single sperm directly into the cytoplasm of the egg. Cleavage stage embryos ( day 2-3) derived from fertilised oocytes are replaced in the uterus (embryo transfer) for pregnancy to occur. The further on in embryo cleavage that transfer occurs the more opportunity there is for selection of competent embryos and a recent approach has been to culture embryos to the blastocyst stage (day 5-6), however the safety of extended in vitro culture is unknown (Braude & Rowell, 2003; Schultz & Williams, 2002).

The births of small for gestational age infants, anatomical anomalies and imprinted gene disorders have been reported inconsistently in follow-up series. Population based data suggests that singleton babies born following ART have a risk of low birth weight 2.6 times that of the general population and an increased risk of preterm delivery (Schieve et al., 2002; Winston & Hardy, 2002). A retrospective study of IVF and ICSI pregnancies in Western Australia found an increased risk of major birth defect by one year of age with an adjusted odds ratio of 2.0 (Hansen, Kurinczuck, Bower, & Webb, 2002). There have also been reports of increased risk of neural tube defect and oesophageal atresia (Winston & Hardy, 2002). There is a slightly higher incidence of sex chromosome aneuploidy in ICSI which may be related to the underlying cause of the male infertility (Koulischer, Verloes, Lesenfants, Jamar, & Herens, 1997). There is also an unexpectedly high incidence of the imprinted gene disorders Beckwith Wiedemann syndrome and Angelman syndrome following assisted reproduction (Gicquel et al., 2003; Gosden et al., 2003; Maher et al., 2003). There is a lack of long term follow up data for children born following ART. The main focus of published follow up studies for children born following ART have been psychomotor and neurological assessments performed in early childhood (Retzloff & Hornstein, 2003). No formal assessment of anthropometric, metabolic and endocrine parameters has been published.

Although maternal and paternal genomes are nearly equivalent in their genetic contribution to the embryo they carry different epigenetic information, which results in sex specific expression of certain imprinted genes. Over 50 such genes have been identified in the human genome, many related to placental development embryogenesis and growth (Ferguson-Smith, Lin, Tsai, Youngson, & Tevendale, 2003; Geuns, De Rycke, Van Steirteghem, & Liebaers, 2003; Meehan, 2003).

Differential DNA methylation results in expression or repression of imprinted genes (Bird & Jaenisch, 2003).There is genome wide demethylation followed by remethylation in the preimplantation embryo (Reik, Dean, & Walter, 2001). Gamete and embryo manipulation and *in vitro* culture may disturb the process of genomic imprinting and lead to abnormal imprinting and related diseases (Geuns et al., 2003). *In vitro* culture systems cause imprinting defects in animal models; decreased methylation of the imprinted sheep gene IGF II receptor following *in vitro* culture leads to fetal overgrowth and large offspring syndrome (Young et al., 2001) and culture of mouse embryos in Whittens medium results in loss of methylation and abnormal expression of the normally silent paternal H19 allele (Doherty, Mann, Tremblay, Bartolomei, & Schultz, 2000).

Beckwith Wiederman Syndrome is associated with chromosome 11 p15 abnormalities. Biallelic paternal expression of 11p15 is associated with increased birth weight, organ overgrowth, abdominal wall defects, an increased risk of Wilms and other embryonic tumours (Gosden et al., 2003). Changes in methylation of two important control regions have been shown to result in the Beckwith Wiedemann phenotype. H19 DMR (demethylated region) controls the imprinting of the telomeric IGF II and H19 genes. This region is normally hypomethylated on the maternal allele resulting in H19 expression and silencing of the IGF II gene and hypermethylated on the paternal allele. Abnormal hypermethylation of the maternal H19 DMR results in biallelic expression of IGF2. Kv DMR (demethylated region) is thought to control the centromeric cluster of genes including the KCNQ1 gene and its antisense

[1] F. Graham, M. Birdsall, R. Fisher

transcript KCN10T1 and the cell cycle regulatory protein (p57) encoded by the CDKN1C gene. Kv DMR is normally hypermethylated on the maternal allele and hypomethylated on the paternal allele. Loss of maternal methylation in this region results in silencing of CDN1C and biallelic expression of KCNQ10T1 (Du et al., 2003).A recent study from the United States performed molecular studies on 6 children with Beckwith Wiederman Syndrome born following ART, they found that 5 of the six had specific epigenetic alterations associated with BWS, four with hypomethylation of Kv DMR and one with hypermethylation of H19 DMR (DeBaun, Niemitz, & Feinberg, 2003).

We hypothesise that peri-conceptual manipulation of gametes and embryos in vitro leads to alterations in programming, via epigenetic processes, resulting in metabolic and endocrine changes in childhood. These epigenetic changes caused by differential methylation of imprinted genes may cause subtle changes undetected at birth but becoming manifest in childhood. We propose to recruit a cohort of 50 children born following assisted reproductive techniques and 50 naturally conceived controls. The study group will consist of children aged between 4 and 10 years born following replacement of fresh embryos produced using both conventional IVF and ICSI techniques. The control group will consist of naturally conceived siblings and friends of our subjects matched as closely as possible for age and sex. Detailed anthropometric data will be collected for each child and a DEXA scan will be performed to assess total body composition. A bone age will be performed to assess skeletal maturity which will aid in our analysis of the anthropometric data. A fasting blood sample will be drawn for glucose, insulin, IGF 1, IGFBP3, grehlin, adiponectin and leptin. DNA samples will be harvested from white blood cells and stored for later DNA methylation analysis. We propose to look at several candidate genes including the methylation status of H19 DMR and Kv DMR on Chromosome 11p15.

We hope to make a significant contribution to the body of knowledge regarding childhood outcome of ART pregnancies and to investigate the possible longer-term implications for these children. ART animal models may be valuable tools to determine whether specific aspects of the evolving ART process may lead to long term programmed changes in humans.

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