Imprinting disorders after assisted reproductive technologies

Øjvind Lidegaard[a], Anja Pinborg[b] and Anders Nyboe Andersen[b]

Purpose of review

To assess the evidence of an increased risk of imprinting diseases in children born after use of assisted reproductive technologies.

Recent findings

Imprinting disorders occur when the epigenetic programming during gametogenesis is disturbed, or when this programming is not sufficiently sustained during the process of fertilization and early embryonic development. Ten case or case-reference reports have been published suggesting that compared with reference populations, a higher proportion of children with imprinting diseases were conceived by assisted reproductive technologies. These reports are inconsistent in linking the risk to a specific assisted reproductive technology, and a cytogenetic examination assessing the exact genetic imprinting mechanism was not always provided. Two national systematic follow-up studies on 6052 Danish and 16,280 Swedish in-vitro fertilization children found none and two children with imprinting diseases, respectively. These figures correspond approximately to the expected number of children with imprinting disease from the general population.

Summary

The evidence of an increased risk of imprinting diseases in children conceived by assisted reproductive technologies is limited. The published case reports, however, call for a systematic multinational long-term follow-up of children born after assisted reproductive technologies.

Keywords

assisted reproductive technologies, epigenetics, imprinting disorder, intracytoplasmic sperm injection, in-vitro fertilization

Curr Opin Obstet Gynecol 18:293–296.

[a]Gynaecologic Clinic Juliane, Marie Centre, Copenhagen, Denmark and [b]Fertility Clinic, Juliane Marie Centre, Copenhagen, Denmark

Correspondence to: Professor Øjvind Lidegaard MD, Dr. Med. Sci., Gynaecologic Clinic, Juliane Marie Centre, Rigshospitalet, DK-2100 Copenhagen, Denmark
Tel: +45 45 65 12 48; fax: +45 45 65 12 45; e-mail: lidegaard@dadlnet.dk

Current Opinion in Obstetrics and Gynecology 2006, 18:293–296

Abbreviations

ART	assisted reproductive technologies
ICSI	intracytoplasmic sperm injection
IVF	in-vitro fertilization

1040-872X

Introduction

During the latest few decades, several rare diseases in humans have been thought to be caused by errors in epigenetic programming which occur during the maturation of germ cells. These so-called imprinting disorders have been known for many years, but their pathogenetic background was unknown until recently.

The diseases are all rare with an incidence rate of about 1–10 in 100 000 children, and are characterized by growth abnormalities, different kinds of malformations, mental retardation and/or childhood cancers.

Epigenetic programming

When primordial germ cells migrate along the genital ridge, the blocked genes, which are found in differentiated somatic cells, in general become 'unblocked' by a demethylation process, in order to ensure in the first instance the differential potential of these gametes [1].

When the germ cells are recruited and begin their maturation process, they differentiate to become capable of all the steps in the fertilization process, including, for sperm cells, a highly developed motile capability. This differentiation is characterized by a blocking of the majority of genes in the genome, chemically ensured by methylation of the unblocked genes. While the majority of genes are silenced and a minority are active, few genes are differentially blocked, so that the gene in one allele is blocked while the other remains active. This differential blocking of the same gene in two alleles is called imprinting.

Currently, about 75 imprinted human genes have been identified. The imprinting process is controlled by other genes, the *imprinting control centres*, typically located near the imprinted genes on the same chromosome. The proportion of imprinted genes may, however, be much larger if the results from a recent animal study also apply to the human genome [2].

The differentiating and imprinting process occurs earlier during maturation in the developing spermatogonia than in the recruited oogonia, but for both, the process is accomplished before fertilization [1].

Soon after fertilization, the paternal genome and subsequently the maternal genome are again demethylated – erased or unblocked – in order to ensure the full omni-

potent differential potential of the zygote. The exception to this erasing process is the imprinted genes, which typically sustain their programming from their germ cell era, in order to give the developing embryo specific messages from the mother and father, respectively.

Some reprogramming may, however, take place – intended or not – in the imprinted genes during the general erasing process soon after fertilization. Hence, theoretically, the physical environment of the early embryo could influence this imprinting reprogramming, and thereby leave its stamp on the coming child.

Ten case or case-reference reports have been published suggesting that assisted reproductive technologies (ART) imply an increased risk of imprinting diseases. The present survey explores the evidence that children born after in-vitro fertilization have an increased risk of imprinting diseases compared with spontaneously conceived children.

Evidence from animal models

Doherty *et al.* [3] demonstrated in mice that in-vitro culture of the embryo causes the normally silenced imprinting paternal gene H19 to be hypomethylated and consequently aberrantly expressed. Khosla *et al.* [4] showed that changes in culture medium for in-vitro culture of mice embryos altered the expression and methylation of imprinted genes, and also affected their birth weight.

The so-called 'large offspring syndrome' is seen in in-vitro cultured cattle or sheep embryos which are transferred back into asynchronous uterine environments [5,6]. Large offspring syndrome is also seen after nuclear transfer cloning, in which cloned embryos are cultured *in vitro* and then transferred into an asynchronous recipient.

These findings indicate that, in fact, epigenetic reprogramming may take place during in-vitro culture of animal embryos, and that the timing of transfer could be crucial for the risk of imprinting disturbances.

Human studies

Before evaluation of the published reports, four methodological circumstances should be stressed.

First, imprinting diseases may be caused by classical gene mutations, microdeletions as well as by disturbances in the epigenetic process in patients with an intact genome, that is with an intact DNA sequence. The proportion of patients with specific imprinting syndromes attributable to these quite different aetiologies differs substantially between the various imprinting diseases. For example, more than 50% of patients with Beckwith-Wiedemann syndrome have different kinds of methylation disorders without any detectable gene mutation or deletion, whereas in patients with Angelman syndrome, less than 3% have methylation disorders, the majority being caused by classical genetic disorders [7•]. It should be emphasized that only imprinting diseases where the pathogenetic background is caused by methylation disturbances and the classical genetic mutations or deletions have been ruled out, could be influenced by the culture media.

Second, the knowledge of imprinting diseases is new. Many children have been and still are coded with less specific diagnosis codes, including children with nonimprinting diseases. Furthermore, in many countries, specific diagnosis codes do not exist for several imprinting diseases. In addition, the clinical manifestations of a certain imprinting disease differ depending on the degree of inactivation or hyperactivity of a certain gene. Therefore epidemiological studies are far from easy to realise.

Third, some imprinting diseases are not diagnosed as such until the child has reached a certain age. In order to assess the true incidence of imprinting diseases, children have to be followed for several years after birth whether conceived with or without ART. If the incidence of imprinting diseases in a population of ART children is compared with the incidence in a control population, it is therefore crucial that the follow-up period is similar for both populations.

Finally, some unexplained causes of infertility in women and men may be due to so far unrecognized epigenetic disorders affecting their eggs or spermatozoa, increasing the risk of epigenetic disorders in their offspring with or without the assistance of ART. Thus epigenetic disorders may be the cause of infertility rather than a consequence of the techniques used to treat infertility [7•]. A recent German report on Angelman syndrome supports this view [8].

Case reports and case-reference studies

In 10 case-only or case-reference studies published, it has been suggested that among children with epigenetic diseases there may be a higher proportion of ART children compared with a more or less well matched control population [8–17].

These reports are summarized in Table 1. The majority of studies are on Beckwith-Wiedemann syndrome, with four case reports on Angelman syndrome, one on Prader-Willi syndrome and one concerning retinoblastoma. In total, 57 children born after ART have been reported suffering an imprinting disease. With more than 1 mil-

Table 1 Case-only or case-reference reports on imprinting diseases in children conceived by in-vitro fertilization with or without ICSI

Study, year, country [Ref.]	*N*	*n*	Disease	ART	Reference population
Case series					
DeBaum 2003, USA [9]	65	3	Beckwith-Wiedemann	IVF/ICSI	0.76% of all births
Gicquel 2003, Fr [10]	149	6	Beckwith-Wiedemann	IVF: 4, ICSI: 2	1.3% of all births
Maher 2003, UK [11]	149	6	Beckwith-Wiedemann	IVF: 3, ICSI: 3	1.2% of all births
Halliday 2004, Aus [12]	37	4	Beckwith-Wiedemann	IVF: 3, ICSI: 1	1 of 148 matched controls
Chang 2005, USA [13]	341	19	Beckwith-Wiedemann	IVF: 5, ICSI: 5	None
Sutcliffe 2006, UK [14]	213	6	Beckwith-Wiedemann	IVF: 1, ICSI: 5	0.8% of all births
Cox 2002, USA [15]	2	2	Angelman syndrome	IVF: 0, ICSI: 2	None
Ørstavik 2003, N [16]	1	1	Angelman syndrome	IVF: 0, ICSI: 1	None
Ludwig 2005, D [8]	79	3	Angelman syndrome	IVF: 0, ICSI: 3	None
Sutcliffe 2006, UK [14]	384	0	Angelman syndrome	IVF: 0, ICSI: 0	0.8% of all births
Sutcliffe 2006, UK [14]	522	2	Prader-Willi syndrome	IVF: 0, ICSI: 2	0.8% of all births
Moll 2003, NL [17]	NA	5	Retinoblastoma	IVF: 4, ICSI: 1	1–1.5% of all births

N indicates number of children with imprinting disease, *n* the number of these conceived by ART; NA, not available.

lion ART children born, this figure which approximates 1 in 18 000 children is in itself not of much concern, as it corresponds roughly to the normal rate of imprinting diseases in nonART children.

The finding of most concern is that in ART children with imprinting diseases, the proportion with nongenetic but just methylating disturbances is in some reports higher than the expected proportion found in nonART children with these diseases.

Follow-up studies

Several follow-up studies have been published on children born after use of ART. Only two of these, however, specifically assessed the occurrence of imprinting diseases (Table 2) [18,19]. In Denmark, all 6052 in-vitro fertilization (IVF) singleton children born from 1995 through 2001 were followed to the end of year 2002, or on average 4.1 years, in order specifically to assess imprinting diseases. A group of 442 349 normally conceived singletons born through the same period made up the controls. The diseases were assessed in the National Register of Patients collecting discharge diagnoses of all hospitalized (in- and out-) patients. In the control group, 54 children were recorded with a possible imprinting disease, whereas none were found in the IVF group [18]. The expected number according to the findings among the controls was, however, only 0.74. Among the 54 in the control group, 49 had early cancers (44 kidney cancer and 5 retinoblastoma) and only five had other imprinting disorders.

From these figures, it is obvious that many children with imprinting diseases were not registered with the specific imprinting codes, but were recorded with other less specific malformation codes.

In Sweden, Källén *et al.* [19] followed 16 280 children born after IVF [30% intracytoplasmic sperm injection (ICSI)] and found two with imprinting diseases. They used a control group of more than two million normally conceived children delivered during the same period. The assessment of imprinting diseases in the ART group was done manually by going through the medical records of all the reported malformations in the ART children, among whom two appeared to have a possible imprinting disease, none of them Beckwith-Wiedemann syndrome. A similar procedure was impossible for the control group. Therefore, no assessment of imprinting diseases was done for the controls.

Although some imprinting diseases are undoubtedly recorded with nonspecific diagnosis codes, the codes for childhood cancer including neuroblastoma are more difficult to misclassify.

Both the Danish and the Swedish follow-up studies found evidence of a normal occurrence of childhood

Table 2 Systematic follow-up studies on ART children including assessment of imprinting diseases

			Imprinting disorders		
Study, year, country [Ref.]	ART	Controls	ART	Controls	Comments
Lidegaard 2005, DK [18]	6052	442 349	0	54	4.1 year follow-up
Källén, 2005, S [19]	16 280	2 039 943	2	NA	

NA, not available.

cancers among ART children. Combining the results of the two studies, only one Swedish case of retinoblastoma was found among *22 332* ART children. We have therefore relatively valid evidence to conclude that the occurrence of childhood cancers, including cancers due to imprinting disorders, is not increased, in ART children.

For the other nonmalignant imprinting diseases, currently no firm conclusions can be drawn. More systematic and controlled follow-up data are needed. The best evidence against an increased risk of nonmalignant imprinting disorders in ART children is that the risk of imprinting cancers does not appear to be increased.

The widespread use of ART calls for a systematic multinational follow-up of ART children, as recently pointed out by Niemitz and Feinberg [7•]. As a result of their complete national registers, the Nordic countries have perhaps special opportunities of achieving this goal.

Besides the possible aspect of ART, imprinting will probably be one of the main focuses in the next decade of reproductive research, as many diseases may be influenced by epigenetic programming during gametogenesis, and perhaps also in early embryonic life. It could be that the key mechanism of the so-called Barker hypothesis may be mediated through just epigenetic programming [2], as may even the later sexual orientation of the child [20].

Combined epidemiological and biomedical research will probably bring much new insight in the near future.

Conclusion

Several case reports suggest that a higher proportion of children with imprinting diseases are conceived by ART, compared with the expected incidence of ART children in the general population. Animal models have suggested that culture media may influence the imprinting process. On the other hand, two follow-up studies could not confirm such an increased risk of imprinting diseases. In both, however, a substantial misclassification of many imprinting diseases may have occurred. The fact that imprinted childhood cancers, which are less likely to be misclassified, were not increased in ART children is the strongest evidence against a generally increased risk of imprinting diseases in ART children.

A systematic, long-term multinational follow-up on ART children is warranted in order to clarify whether imprinting diseases occur more frequently in ART children, and if so, whether it is the infertility in itself, the medical treatment, or the culture media, that are responsible for an eventually increased risk.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 349–350).

1 Gosden R, Trasler J, Lucifero D, Faddy M. Rare congenital disorders, imprinted genes, and assisted reproductive technology. Lancet 2003; 361: 1975–1977.

2 Luedi P, Hartemink AJ, Jurtle RL. Genome-wide prediction of imprinted murine genes. Genome Res 2005; 6:875–884.

3 Doherty AS, Mann MR, Tremblay KD, *et al*. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. Biol Reprod 2000; 62:1526–1535.

4 Khosla S, Dean W, Brown D, *et al*. Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. Biol Reprod 2001; 64:918–926.

5 Sinclair KD, Young LE, Wilmut I, McEvoy TG. In-utero overgrowth in ruminants following embryo culture: lessons from mice and a warning to men. Hum Reprod 2000; 15 (Suppl 5):68–86.

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

7 • Niemitz EL, Feinberg AP. Epigenetics and assisted reproductive technology: A call for investigation. Am J Hum Genet 2004; 74:599–609.
This survey describes the molecular mechanism in imprinting disorders, bring up the experiences from animal models to human laboratories, and outlines the specific epigenetic changes in different imprinting diseases.

8 Ludwig M, Katalinc A, Gross S, *et al*. Increased prevalence of imprinting defects in patients with Angelman syndrome born to subfertile couples. J Med Genet 2005; 42:289–291.

9 DeBaun MR, Niemitz L, Feinberg AP. Association of in vitro fertilization with Beckwith-Wiedemann Syndrome and epigenetic alterations of LIT1 and H19. Am J Hum Genet 2003; 72:156–160.

10 Gicquel C, Gaston V, Mandelbaum J, *et al*. In vitro fertilisation may increase the risk of Beckwith-Wiedemann Syndrome related to the abnormal imprinting of the KCNQ1OT gene. Am J Hum Genet 2003; 72:1338–1341.

11 Maher ER, Brueton LA, Bowdin SC, *et al*. Beckwith-Wiedemann syndrome and assisted reproduction technology (ART). J Med Genet 2003; 40:62–64.

12 Halliday J, Oke K, Breheny S, *et al*. Beckwith-Wiedemann Syndrome and IVF: A case–control study. Am J Hum Genet 2004; 75:526–528.

13 Chang AS, Moley KH, Wangler M, *et al*. Association between Beckwith-Wiedemann syndrome and assisted reproductive technology: a case series of 19 patients. Fertil Steril 2005; 83:349–354.

14 Sutcliffe AT, Peters CJ, Bowdin S, et al. Assisted reproductive therapies and imprinting disorders – a preliminary British survey. Hum Reprod 2006 (in press) [Epub December 16, 2005].

15 Cox GF, Bürger J, Lop V, *et al*. Intracytoplasmic sperm injection may increase the risk of imprinting defects. Am J Hum Genet 2002; 71:162–164.

16 Ørstavik KH, Eiklid K, Hagen CB, *et al*. Another case of imprinting defect in a girl with Angelman syndrome who was conceived by intracytoplasmic sperm injection. Am J Hum Genet 2003; 72:218–219.

17 Moll AC, Imhof SM, Cruysberg JRM, *et al*. Incidence of retinoblastoma in children born after in-vitro fertilisation. Lancet 2003; 361:309–310.

18 Lidegaard Ø, Pinborg A, Andersen AN. Imprinting diseases and in vitro fertilisation. Danish National IVF cohort study. Hum Reprod 2005; 20:950–954.

19 Källén B, Finnström O, Nygren KG, Olausson PO. In vitro fertilization (IVF) in Sweden: Risk for congenital malformations after different IVF methods. Birth Defects Res 2005; 73:162–169 (Part A).

20 Mustanski BS, DuPree MG, Nievergelt CM, *et al*. A genomewide scan of male sexual orientation. Hum Genet 2005; 116:272–278.