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REVIEW

Health outcomes of children born after IVF/ICSI: a review of current expert opinion and literature

BCJM Fauser [a,*], P Devroey [b], K Diedrich [c], B Balaban [d], M Bonduelle [e], HA Delemarre-van de Waal [f], C Estella [g,h], D Ezcurra [i], JPM Geraedts [j], CM Howles [i], L Lerner-Geva [k], J Serna [l], D Wells [m], Evian Annual Reproduction (EVAR) Workshop Group 2011

[a] *Department of Reproductive Medicine and Gynaecology, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands;* [b] *Center for Reproductive Medicine, Vrije Universiteit Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium;* [c] *Department of Obstetrics and Gynecology, University Clinic of Schleswig-Holstein, Campus Luebeck, 23538 Luebeck, Germany;* [d] *Assisted Reproduction Unit, American Hospital of Istanbul, Guzelbahce Sokak No 20, Nisantasi, Istanbul 34365, Turkey;* [e] *Centre for Medical Genetics, Vrije Universiteit Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium;* [f] *Department of Pediatrics, Leiden University Medical Center, 2300 RC Leiden, The Netherlands;* [g] *Fundación Instituto Valenciano de Infertilidad (FIVI), Valencia University, and Instituto Universitario IVI/INCLIVA, Parc Científic Universitat de València C/Catedrático Agustín Escardino nº 9, Edificio 3, 46980 Paterna, Spain;* [h] *Departamento de Biología Molecular and Centro de Biología Molecular 'Severo Ochoa' (CSIC-UAM), Universidad Autónoma de Madrid, Madrid, Spain;* [i] *Global Development and Medical Unit, Merck Serono SA Geneva, Chemin des Mines 9, 1202 Geneva, Switzerland;* [j] *Department of Genetics and Cell Biology, Research Institute GROW, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands;* [k] *Woman and Child Health Research Unit, Gertner Institute for Epidemiology and Health Policy Research, Tel Hashomer 52621, Israel;* [l] *Instituto Valenciano de Infertilidad (IVI) Zaragoza, C/María Zambrano, 31, 50018 Zaragoza, Spain;* [m] *University of Oxford, Nuffield Department of Obstetrics and Gynaecology, Women's Centre, John Radcliffe Hospital, Oxford OX3 9DU, UK*

* Corresponding author. *E-mail address:* b.c.fauser@umcutrecht.nl (BCJM. Fauser).

Professor BCJM Fauser works at the Department of Reproductive Medicine and Gynaecology, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands. He is currently chair of the Division Woman & Baby. His past positions involved Professor of Reproductive Medicine at the Erasmus University in Rotterdam, Saal van Zwaanenberg professorship at the Free University Brussels Belgium, Visiting Professor at Stanford University California USA, and a Fullbright postdoctoral fellowship at The University of California San Diego USA. His scientific contributions mainly involve ovarian (dys)function and ovarian stimulation (around 330 international scientific publications and a total of over 12.000 citations). he is internationally active in many organisations such as ESHRE, WHO and COGI.

Abstract The Sixth Evian Annual Reproduction (EVAR) Workshop Group Meeting was held to evaluate the impact of IVF/intracytoplasmic sperm injection on the health of assisted-conception children. Epidemiologists, reproductive endocrinologists, embryologists and geneticists presented data from published literature and ongoing research on the incidence of genetic and epigenetic

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abnormalities and congenital malformations in assisted-conception versus naturally conceived children to reach a consensus on the reasons for potential differences in outcomes between these two groups. IVF-conceived children have lower birthweights and higher peripheral fat, blood pressure and fasting glucose concentrations than controls. Growth, development and cognitive function in assisted-conception children are similar to controls. The absolute risk of imprinting disorders after assisted reproduction is less than 1%. A direct link between assisted reproduction and health-related outcomes in assisted-conception children could not be established. Women undergoing assisted reproduction are often older, increasing the chances of obtaining abnormal gametes that may cause deviations in outcomes between assisted-conception and naturally conceived children. However, after taking into account these factors, it is not clear to what extent poorer outcomes are due to the assisted reproduction procedures themselves. Large-scale, multicentre, prospective epidemiological studies are needed to investigate this further and to confirm long-term health consequences in assisted-conception children.

KEYWORDS: assisted reproduction, imprinting disorders, intracytoplasmic sperm injection, infertility, IVF, children outcome

Introduction

Assisted reproduction treatment has advanced significantly since the first IVF infant born over 30 years ago (Steptoe and Edwards, 1978). An estimated 3.75 million births have resulted from assisted conceptions (ESHRE, 2010), incorporating techniques such as intracytoplasmic sperm injection (ICSI; Palermo et al., 1992) and testicular sperm extraction (TESE; Devroey et al., 1994). Within Europe and countries such as Turkey and Russia, 493,184 treatment cycles were reported in 2007, with a 7.6% increase compared with 2006 (de Mouzon et al., 2012). Most treatment cycles (55.7%) were performed in France, Germany, Spain, the UK and Italy (de Mouzon et al., 2010, 2012). A trend towards increased use of ICSI has been observed worldwide (Nyboe Andersen et al., 2008, 2009).

Considering this increase, it is important to assess the potential health risks in assisted-conception children. Debate continues about whether identified risks are attributable to assisted reproduction techniques or to intrinsic parental characteristics affecting gamete quality and fertility.

The age-related diminution of the quality and fertility of oocytes is key in assisted reproduction: a high proportion of human oocytes have chromosome abnormalities and this increases with maternal age (Balasch and Gratacós, 2012; Cheung et al., 2011; Fragouli et al., 2011b; Johnson and Tough, 2012; Pellestor et al., 2003). Despite this, childbearing at advanced reproductive age is increasingly common in developed countries. Reasons include extended time at university, advancing professional careers, contraception, late meeting of the partner, incorrect information concerning progress in assisted reproduction technologies and desire for a second child after a late first pregnancy or second marriage (Lamarche et al., 2007). In the Netherlands, mean maternal age at fertility clinic intake increased by 3.7 years over two decades, from 27.7 years in 1985 to 31.4 years in 2008 (de Graaff et al., 2011). This demographic shift toward later conception has resulted in the proportion of women over 35 years at intake almost quadrupling from 7.9% to 31.2%. As more women delay childbearing until later in life, the quantity as well as the quality of oocytes obtained is reduced. In addition, unfavourable health outcomes to both mother and child are increased (Glasser et al., 2011).

Assisted reproduction treatment involves manipulating several steps in human reproduction, including hormones to down-regulate pituitary function and stimulate the ovary for supernumerary oocyte production, in-vitro maturation of oocytes, direct injection of immature spermatozoa into oocytes, in-vitro culture of preimplantation embryos before transfer to the uterus and cryopreservation of either gametes or embryos (Zegers-Hochschild et al., 2009a,b). A large body of literature has investigated whether these procedures have the potential to alter normal gamete and embryo development and affect the health of assisted-conception children.

Of particular concern is the possibility of genomic imprinting disorders in assisted-conception children due to disturbances in the establishment and maintenance of imprinting during gametogenesis, fertilization and embryonic development (Amor and Halliday, 2008; Owen and Segars, 2009). According to the 'developmental origins of adult disease' hypothesis, prenatal conditions may change organ development and function in developing organisms (Barker, 1995). The resulting physiological, metabolic and endocrine changes can be persistent and may predispose children to increased susceptibility to disease in later life. It remains to be seen whether assisted reproduction procedures affect the epigenetic processes that occur during critical points in early embryonic development and result in long-term health consequences.

This paper provides an overview of the published literature on the incidence of genetic abnormalities and congenital malformations in assisted-conception children versus naturally conceived children and gives insights on potential differences. Collecting and interpreting data from epidemiological studies of assisted-conception children is challenging, due to a lack of uniformity of clinical definitions and differences in data collection methods. These are also highlighted in this review. The Sixth Evian Annual Reproduction (EVAR) Workshop Group Meeting was held in Evian, France in April 2011 to discuss prenatal, neonatal and long-term outcomes in assisted-conception children. It provided a unique opportunity for the faculty to present and discuss the latest scientific information on these issues. The outcomes of previous EVAR meetings have been published (Devroey et al., 2009; Diedrich et al., 2007, 2011; Fauser et al., 2008, 2011). Here are reported the opinions of the EVAR Workshop Group 2011 on the safety and impact of fertility techniques in assisted-conception children and the potential causes of deviations in genetic abnormalities.

Materials and methods

Before the meeting, epidemiologists, reproductive endocrinologists, embryologists and geneticists prepared presentations based on published literature and ongoing research on the incidence of genetic abnormalities and congenital malformations in assisted-conception children versus naturally conceived children, in order to arrive at a consensus on the potential reasons for any differences in outcomes between these groups.

The meeting was sponsored by Merck Serono Geneva, planned, organized and executed by DE and Olga Salvidio. (Merck Serono Geneva) with CMH. in attendance. It was chaired by PD (Free University Brussels, Brussels, Belgium), BCJMF (University Medical Centre, Utrecht, The Netherlands) and KD (University Clinic of Schleswig-Holstein, Luebeck, Germany). The speakers included BB (American Hospital of Istanbul, Istanbul, Turkey), MB (Vrije Universiteit Brussel, Brussels, Belgium), HAD-vdW (Leiden University Medical Centre, Leiden, The Netherlands), CE (Fundación Instituto Valenciano de Infertilidad, Valencia, Spain; Universidad Autónoma de Madrid, Madrid, Spain), JPMG (Maastricht University, Maastricht, The Netherlands), LL-G (Gertner Institute for Epidemiology and Health Policy Research, Tel Hashomer, Israel), JS (Instituto Valenciano de Infertilidad Zaragoza, Spain) and DW (University of Oxford, Oxford, UK).

EMBASE and Medline databases searches for English language publications were conducted separately for each section of the review. Searches were run following the meeting and limited to from 2006 to January 2013 to ensure pertinence of the data. The search strategies and results are presented in Appendices 1–4 (Supplementary material available online). This report includes the key publications identified and publications known to the authors and discussed during the Sixth EVAR Workshop Group Meeting. Discussions providing information on techniques to identify oocyte chromosome abnormalities, the potential mechanisms of injury in cryopreservation and epigenetic/imprinting processes relied more heavily on individual knowledge and expertise of the authors. Likewise, the pitfalls in conducting and assessing epidemiological studies in assisted reproduction were primarily based on discussions at the meeting. Evidence from observational studies and meta-analyses on chromosomal abnormalities, developmental changes and imprinting errors in children born after assisted reproduction was supported by in-depth literature searches.

The role of the oocyte in embryogenesis

Oocyte chromosome abnormalities

Oocyte quality affects embryo development and its potential for implantation and healthy pregnancy. Intrinsic factors affecting oocyte quality include chromosomal abnormalities and genetic defects. A role for maternal nutrition and metabolism has been postulated (Adamiak et al., 2005; Cardozo et al., 2011) and the effect of obesity on oocyte quality and reproductive outcome has been studied (Singh et al., 2012; Zander-Fox et al., 2012). Extrinsic factors may include the use of ovarian stimulation protocols (Baart et al., 2007, 2009; Seggers et al., 2012) and assisted reproduction treatment-specific procedures (Ebner et al., 2005; Mäkinen et al., 2013). Ovarian stimulation may suppress the meticulous natural selection occurring in oocyte maturation, potentially allowing oocytes with compromised quality to develop.

Standard criteria for classifying oocyte morphology are needed to ensure selection of oocytes with maximal chances of pregnancy (Rienzi et al., 2011). Reports regarding the relationships between oocyte morphological characteristics and embryo quality are conflicting. Good-quality metaphase-II oocytes are defined as being spherical and having a translucent cytoplasm with homogeneous fine granularity and no inclusions, a round or ovoid first polar body with a smooth surface, a perivitelline space of normal size and a colourless zona pellucida with regular shape. However, only about a third of all oocytes retrieved have these ideal characteristics (Veeck, 1988). A consensus defining the minimum criteria for oocyte and embryo morphology assessment was developed in Istanbul, Turkey (Alpha Scientists In Reproductive Medicine and ESHRE Special Interest Group, 2011a,b).

Extracytoplasmic and cytoplasmic oocyte dysmorphisms

Severe cytoplasmic deviations such as a centrally located granular cytoplasm or translucent cytoplasmic vacuoles (referred to as smooth endoplasmic reticulum clusters, SERC) appear to indicate genetic, epigenetic or metabolic defects that may lead to morphologically and/or genetically abnormal embryos (Serhal et al., 1997; Yakin et al., 2007).

A large, dark, spongy granulated area in the cytoplasm — previously defined as centrally located granular cytoplasm or as organelle clustering — has been implicated in decreased survival and impaired in-vitro development after cryopreservation (Balaban et al., 2008). A high aneuploidy rate (52.2%), low implantation rates and low ongoing pregnancy rates were also reported in embryos from oocytes with centrally located granular cytoplasm (Kahraman et al., 2000; Meriano et al., 2001).

SERC may have a detrimental effect on cryosurvival and developmental competence of embryos (Balaban et al., 2008). Increased biochemical pregnancy rates followed by decreased clinical pregnancy rates have been noted after embryo transfer (Otsuki et al., 2004). SERC have been related to compromised fertilization and blastula formation rates versus unaffected sibling oocytes (Ebner et al., 2008a,b). Patients with one or more gametes with SERC had significantly higher spontaneous miscarriage rates and, once pregnancy was achieved, more obstetric problems, neonatal deaths and lower birthweights in SERC-positive cycles. In one case, SERC aggregations in all retrieved oocytes in three consecutive ICSI cycles were associated with repeated multiple fetal anomalies (Akarsu et al., 2009).

Giant oocytes

Giant oocytes — approximately twice the cytoplasmic volume of regularly sized oocytes — occur sporadically (Balakier et al., 2002). Embryos from giant oocytes were initially excluded as a possible source of digynic triploidy (Balakier

et al., 2002; Rosenbusch et al., 2002). Giant oocytes were associated with abnormal cleavage in cohort embryos, but not with abnormal symmetry, implantation or pregnancy rates in a cohort of 97,556 oocytes (0.12% giant oocytes; Machtinger et al., 2011). They do not appear to reflect the quality of remaining oocytes in the ovary. Additional studies are needed to confirm a direct correlation between giant oocyte presence and increased risk of chromosomal abnormalities in sibling oocytes.

Oocyte quality and age

Chromosome abnormalities in oocytes from older women

Suboptimal assisted reproduction in older women results from a decline in oocyte quantity and quality, the latter characterized primarily by chromosome abnormalities. Oocytes with chromosome abnormalities are common in older women undergoing IVF treatment (Anahory et al., 2003; Angell et al., 1993; Battaglia et al., 1996; Pellestor et al., 2003; Zhivkova et al., 2007). Older women failing to conceive with their own oocytes are able to conceive using donor oocytes from younger women, further supporting the link between age, poor oocyte quality and reduced IVF success (Borini et al., 1995; Navot et al., 1991).

Aneuploidy, the most clinically significant chromosome abnormality, arises mostly due to segregation errors during female meiosis and is closely associated with advancing age. The relationship between increasing maternal age and trisomy is well recognized (Hassold and Hunt, 2009; Morton et al., 1988; Risch et al., 1986), rising from 2% to 3% for women in their twenties to >30% for women in their forties (Hassold and Hunt, 2009).

Reports of full karyotype analysis on the first polar body, confirmed by metaphase-II oocyte analysis, indicate a low aneuploidy rate (3% in one study) in oocytes of young fertile women (Fragouli et al., 2009). Comparative genomic hybridization (CGH) was used to provide detailed cytogenetic analysis of 308 first and second polar bodies from the fertilized oocytes of 70 women (average age of 40.8 years; Fragouli et al., 2011a). The total oocyte abnormality rate was 70%, and metaphase-II anomalies predominated over metaphase-I anomalies (50% aneuploidy rate versus 40.3%).

In a large-scale series of over 20,000 oocytes, aneuploidies were detected in polar bodies using fluorescence in-situ hybridization for chromosomes 13, 16, 18, 21 and 22. Despite assessing only five chromosomes, almost every second oocyte (46.8%) was abnormal: most errors involved chromatid loss, predicting predominance of trisomies (53%) over monosomies (26%) in the resulting embryos (2:1; Kuliev et al., 2011). Of the detected anomalies in oocytes, 40% were complex.

PGD and polar body screening in women of advanced maternal age

The multiple causes of human, age-related nondisjunction are not well characterized. This complicates efforts to devise preventive measures to avoid chromosomal abnormalities associated with older maternal age. A high incidence of aneuploid preimplantation embryos suggests that if only euploid embryos are transferred, the chance of successfully having a healthy child could be increased (Munné et al., 1993). A recent study examined the outcomes of children born after preimplantation genetic diagnosis (PGD) compared with children born after ICSI and embryo transfer on day 5 (Desmyttere et al., 2012). Authors concluded that embryo biopsy for PGD does not introduce extra risk to the overall medical condition of newborn children. Multiples born following embryo biopsy appear to be at lower risk for low birthweight compared with multiples born following ICSI. Research is now focusing on PGD to improve the IVF success rate in older women.

At least 10 randomized, controlled trials on blastomeres from patients with either good (Jansen et al., 2008; Mersereau et al., 2008; Meyer et al., 2009; Staessen et al., 2008) or poor IVF prognosis (Debrock et al., 2010; Hardarson et al., 2008; Mastenbroek et al., 2007; Schoolcraft et al., 2009; Staessen et al., 2004; Stevens et al., 2004) have shown no improvement in delivery rate with PGD. Possible explanations may be that not all chromosomes were tested and that the biopsied blastomere may not always be a true representation of the embryo at the 8-cell stage because of mosaicism. The procedure itself may also negatively impact the developmental potential of the biopsied embryo, particularly if the embryologist undertaking the biopsy is inexperienced. Oocyte aneuploidy testing by polar body analysis may help improve aneuploid embryo detection before transfer, since chromosomal mosaicism should not be an issue for gamete analysis.

A proof-of-principle study confirmed polar body screening as a reliable method to analyse oocyte chromosomal status (Geraedts et al., 2011). Two centres (University of Bonn, Bonn, Germany and Società Italiana Studi di Medicina della Reproduzione, Bologna, Italy) were chosen for the study based on their experience in polar body biopsy, as legislation in their countries restricted the possibility of undertaking embryo biopsy at a later stage of development. Chromosomal analysis of first and second polar bodies was undertaken by two independent observers in each centre using 24sure (BlueGnome, UK), a comparative genomic hybridization micro-array technique that screens all chromosomes in one cell within 12 h and thus allows for fresh embryo transfer. If either or both of the polar bodies were aneuploid, the corresponding zygote was then also processed by array CGH for an independent analysis by the other centre. Both polar bodies were biopsied from 226 zygotes (42 cycles in 41 couples, average maternal age 40.0 years). The ploidy status was predicted in 195 (86%) zygotes, 55 were euploid (28%) and 140 were aneuploid (72%). At least one aneuploid zygote was predicted in each cycle with one exception, and all zygotes were predicted to be aneuploid in 19 out of 42 cycles (45%). Fresh embryos were transferred in the remaining 23 cycles (55%); one frozen–thawed embryo transfer was performed. Eight patients had a clinical pregnancy of which seven are ongoing (ongoing pregnancy rates: 17% per cycle and 30% per transfer). Array CGH was used to determine the ploidy status of 156 zygotes: 38 (24%) were euploid and 118 (76%) aneuploid. Complete information was available on both polar bodies and the corresponding zygote in 138 zygotes. In 130 (94%) zygotes, the overall euploid versus aneuploid status was

concordant with results from the polar bodies, and in 8 (6%), the results were discordant. In all the discordant cases, aneuploid results in one or both polar bodies predicted aneuploidy in the zygote but array CGH indicated that the corresponding zygote was euploid. This concordance analysis of both polar bodies and the corresponding zygote indicates that the maternal chromosomal contribution to the zygote can be predicted with acceptable accuracy (Geraedts et al., 2011).

Pitfalls in conducting and assessing epidemiological studies in assisted reproduction

Accumulation of data from fertility centres worldwide will help to determine the incidence and nature of health risks in children born after assisted reproduction and to compare various developmental outcomes with those in children conceived naturally. Several potential sources of bias must be considered in evaluating these data.

Taking the rate of congenital anomalies as an example, assessment of epidemiological data may be influenced by selection bias when identifying an appropriate control population, observation bias (including incomplete reporting and misclassification from inconsistent criteria for defining congenital abnormalities) and other confounding factors. Assisted reproduction pregnancies and children may be monitored more closely than those naturally conceived, contributing to surveillance bias. The challenges and confounding observations encountered when studying congenital anomalies in assisted-conception children are listed in Table 1. Methodological strategies are required to minimize these limitations and

Table 1 Pitfalls and observations in collecting and analysing data on congenital anomalies.

Observation bias	
Collection of registry data	Neonatal units may have a higher number of assisted-conception children, who will be under close medical surveillance
Collection of interview data	Written questionnaires, as opposed to telephone interviews, may elicit a lower malformation rate
Interobserver variation and expertise	There may be different paediatricians during the same time period, resulting in interobserver variation
Observer blinding for study group	Observer must be blinded
Classification system differences	
Definitions for congenital anomalies	Several classification systems (e.g. ICD 9—10, BPA, EuroCat) exist
Definitions for major or minor anomalies	Cut offs for major and minor anomalies vary across classification systems
Additional protocol guidelines	Certain conditions may be excluded from reporting
Coding of examination results	Data input may be via a checklist or free text (major anomalies may be coded blindly (dependent on guideline used); minor anomalies via a checklist)
Other factors	
Pregnancy outcomes	Various definitions used for live births, stillbirths and pregnancy terminations, with potential for exclusion of prenatal anomalies
Duration and point of observation	Observations may be made at different time points (e.g. birth, 6 months) and may be carried out over a period of time (e.g. from birth to 6 months)
Loss to follow up	Loss to follow-up rates differ among families with successful deliveries and those who have lost a child or have a child with a pathology
Sample size	Sample size should be adequately powered to detect minimal difference
Matching of control groups or adjustment	Maternal variables (age, parity) and chronic disease (e.g. diabetes, hypertension)
	Environmental exposure (e.g. smoking, alcohol, drugs)
	Ethnic and social background
	Educational level
	Infertility history
	Genetic background
	Plurality

BPA = British Paediatric Association Classification of Diseases; EuroCat = European Surveillance of Congenital Anomalies; ICD = International Classification of Diseases.

Table 2 Birth defect rates in infants (singletons and multiples) after transfer of frozen and fresh IVF and ICSI early cleavage-stage embryos.

Group	*Cryopreserved cycles*	*Fresh cycles*
Belva et al. (2008)		
IVF	12/390 (3.1)	112/2955 (3.8)
ICSI	35/547 (6.4)	96/2840 (3.4)
Källén et al. (2005)[a]		
IVF	81/1055 (7.7)	832/10,228 (8.1)
ICSI	36/419 (8.6)	392/4530 (8.7)

Values are *n*/total (%).
ICSI = intracytoplasmic sperm injection.
[a]Fresh IVF = 1.00, adjusted for year of birth, maternal age and number of infants in birth.

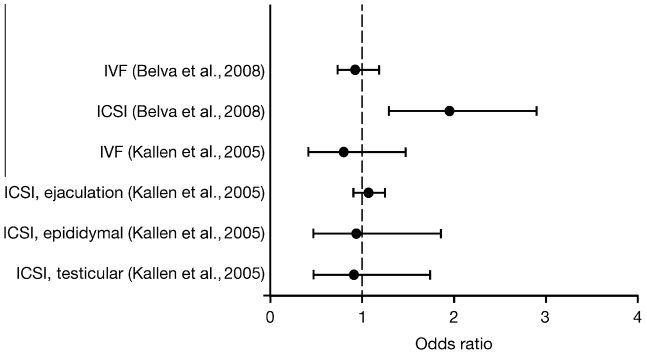

Figure 1 Odds ratios for birth defects after transfer of frozen and fresh IVF and ICSI early cleavage-stage embryos. ICSI = intracytoplasmic sperm injection (Belva et al., 2008; Källén et al., 2005).

must be considered when interpreting studies examining other outcomes.

The importance of adjusting for maternal and neonatal factors is highlighted by results from two studies comparing congenital malformation rates in IVF and ICSI children born following cryopreservation versus fresh assisted reproduction (**Table 2** and **Figure 1**). A higher risk of congenital anomalies for cryopreserved compared with fresh IVF cycles was reported in ICSI children (Belva et al., 2008). In contrast, Källén et al. (2005) adjusted the results of their study for year of birth, maternal age and number of infants born and found no differences.

Congenital malformations in assisted-conception children

Overview of published literature

Table 3 lists relevant papers investigating congenital malformations in IVF/ICSI children and reported imprinting defects. Several cohort studies show a higher risk of congenital defects in assisted-conception children (Anthony et al., 2002; Bonduelle et al., 2005; Ericson and Kallen, 2001; Hansen et al., 2002; Hiura et al., 2012; Klemetti et al., 2005; Merlob et al., 2005; Olivennes, 2005; Olson et al., 2005; Schimmel et al., 2006; Zwink et al., 2012); others suggest a slight or no increased risk (Daubeney et al., 2012; Fujii et al., 2010; Jäderberg et al., 2012; Källén et al., 2010; Oliver et al., 2012; Picaud et al., 2012; Sagot et al., 2012; Yan et al., 2011; Zheng et al., 2013; Zhu et al., 2006). Prospective studies likewise have conflicting conclusions (Belva et al., 2007; Bonduelle et al., 2002a; Katalinic et al., 2004) and a case–control study by Reefhuis et al. (2009) based on telephone interviews conducted 6 weeks to 24 months after birth found a 2–4-fold increase in septal heart defects, cleft lip (with or without palate) and oesophageal atresia.

Overall, two large meta-analyses show an increased risk (odds ratios, OR, between 1.29 and 1.40) for congenital malformations in children following IVF and ICSI compared with those naturally conceived (Hansen et al., 2005; Rimm et al., 2004). This finding was confirmed in a recent meta-analysis of 46 studies assessing the effect of IVF and ICSI on birth defects compared with naturally conceived children (Wen et al., 2012). However, in a meta-analysis taking into account the contribution of subfertility as a risk factor for congenital malformations, the risk diminished substantially (OR 1.01; Rimm et al., 2011). ICSI children do not appear to have a significantly increased risk of developing major malformations compared with those born using conventional IVF procedures. More malformations, primarily urogenital, occur using testicular versus ejaculated spermatozoa.

Similarly, an epidemiological study of a single Australian population registry examined birth defects diagnosed before the child's fifth birthday ($n = 308,974$). The study showed an increased risk of birth defects associated with IVF (OR 1.47, 95% confidence interval, CI, 1.33–1.62) that was not significant after adjustment for parental factors, such as age of gametes and infertility status of patients (Davies et al., 2012). In the same study the risk of birth defects associated with ICSI increased after multivariate analysis, but this difference was not apparent if cryopreserved oocytes were used. Further data showing the effects of cryopreservation on the risk of birth defects are described below.

A decrease in the number of birth defects over time in assisted-conception children in Western Australia has been recently reported, although in this study the number of assisted reproduction-related major birth defects remained higher compared with those observed in naturally conceived children (Hansen et al., 2012).

A study examining the association between congenital heart defects and assisted reproduction treatment using a case–control study design showed that exposure to assisted reproduction was higher in children with tetralogy of Fallot compared with controls (6.6% versus 3.5%; $P = 0.002$). The authors conceded that they were not able to determine whether this effect was due to assisted reproduction or to the underlying fertility problems of the parents (Tararbit et al., 2013).

Interpretation of study data is confounded by differences in methodology, definitions used to classify the birth defects, and evaluation and reporting of congenital

Table 3 Key published papers cited.

Congenital malformations in IVF children
Ericson and Kallen (2001)
Anthony et al. (2002)
Bonduelle et al. (2002a)
Hansen et al. (2002)
Katalinic et al. (2004)
Rimm et al. (2004)[a]
Bonduelle et al. (2005)
Hansen et al. (2005)[a]
Klemetti et al. (2005)
Merlob et al. (2005)
Olivennes (2005)
Olson et al. (2005)
Schimmel et al. (2006)
Zhu et al. (2006)
Belva et al. (2007)
Reefhuis et al. (2009)
Fujii et al. (2010)
Källén et al. (2010)
Yan et al. (2011)
Rimm et al. (2011)[a]
Davies et al. (2012)
Daubeney et al. (2012)
Picaud et al. (2012)
Jäderberg et al. (2012)
Zwink et al. (2012)
Sagot et al. (2012)
Tararbit et al. (2013)

Development in IVF and ICSI children
Bowen et al. (1998)
Sutcliffe et al. (2001)
Bonduelle et al. (2003)
Leslie et al. (2003)
Place and Englert (2003)
Ponjaert-Kristoffersen et al. (2004)
Ponjaert-Kristoffersen et al. (2005)
Leunens et al. (2006)
Belva et al. (2007)
Belva et al. (2008)
Knoester et al. (2008)
Wennerholm et al. (2009)
Pinborg et al. (2010)
Belva et al. (2011)
Cooper et al. (2011)

Imprinting in IVF and ICSI children
Cox et al. (2002)
Debaun et al. (2003)
Gicquel et al. (2003)
Maher et al. (2003a)
Maher et al. (2003b)
Ørstavik et al. (2003)
Halliday et al. (2004)
Chang et al. (2005)
Lidegaard et al. (2005)
Ludwig et al. (2005)
Maher (2005)
Svensson et al. (2005)
Bliek et al. (2006)
Rossignol et al. (2006)
Sutcliffe et al. (2006)
Bowdin et al. (2007)
Doornbos et al. (2007)
Gomes et al. (2007)
Kagami et al. (2007)
Galli-Tsinopoulou et al. (2008)
Gomes et al. (2009)
Katari et al. (2009)
Lim et al. (2009)
Manipalviratn et al. (2009)
Santos et al. (2010)
Strawn et al. (2010)
Tierling et al. (2010)
Turan et al. (2010)
Zheng et al. (2011)
Oliver et al. (2012)
Hiura et al. (2012)
Oliver et al. (2012)
Zheng et al. (2013)

[a]Meta-analysis.

abnormalities (e.g. evaluation at birth versus later time points and evaluation by parents, family physician, paediatrician or geneticist). Telephone interviews such as those used by Reefhuis et al. (2009) are less likely to elicit objective information compared with reviews of hospital records, detailed written questionnaires or clinical examinations. It is also difficult to determine whether the increased risk of birth defects seen is related to the procedures themselves or to factors such as the infertility *per se*.

Large, prospective studies of nonselected populations are required to establish the risks for congenital anomalies in assisted-conception children and determine whether these are related to the techniques or to parental genetic defects. Such studies will also need to investigate fertility in ICSI-conceived children, plus the occurrence of cancer and other long-term health risks.

Prospective cohort studies in Israel

Israel presents a unique opportunity to study a nonselective assisted reproduction treatment population, as the national insurance policy covers all IVF procedures for the first and second child, for all women under the age of 45. In a 2002 survey, 1657 IVF/ICSI cycles per million population per annum were documented in the country (Collins, 2002).

A prospective cohort study evaluating congenital malformation risk diagnosed in newborn infants conceived by assisted reproduction treatment in eight IVF units in Israel from 1997 to 2004 (9042 live births), compared with naturally conceived infants (213,737 live births), found an increased crude risk for all congenital malformations after assisted reproduction treatment (OR 1.73, 95% CI 1.58–1.89; Farhi et al., 2013a,b). The risk remained

significant (OR 1.5, 95% CI 1.35—1.66) after controlling for maternal age, religion and education, gender of the child, week of birth and year of treatment. No differences in risk for congenital malformations were observed between conventional IVF and ICSI and there was no difference between singleton and multiple births. Study data were obtained using an established computerized database of the study cohort linked to the national live birth registry to determine the results of pregnancy and birth outcomes. The study's limitations, such as small sample size, incomplete ascertainment of congenital malformations and inadequate information regarding antenatal care and termination of pregnancies, must be noted and highlight the challenges in assessing this type of data. It is also possible that pregnancy complications following IVF/ICSI, such as spontaneous miscarriage, could account for fewer congenital malformations in live births. In a telephone survey of 1161 Israeli women with singleton pregnancies (561 conceived using IVF/ICSI, 600 conceived naturally) from 2006 to 2008, no differences in the rate of abnormal test results (genetic tests, ultrasound, amniocentesis and chorionic villus sampling) and congenital malformations were observed (Farhi et al., 2013a,b).

Growth and development in IVF/ICSI-conceived children

Outcomes in children born after IVF

IVF pregnancies have been associated with a higher risk of complications and adverse perinatal outcomes compared with naturally conceived pregnancies (Helmerhorst et al., 2004; Jackson et al., 2004; McDonald et al., 2005). Several factors that may influence prenatal growth and development are shown in **Figure 2**.

In a large, retrospective cohort study examining the long-term health effects of hormone stimulation in mothers and their children (Ceelen et al., 2008b), birthweight, birthweight standard deviation scores and gestational age were significantly lower in 225 8—18-year-old IVF-conceived children compared with naturally conceived singleton children (Ceelen et al., 2008a). The study involved 26,428 women with subfertility problems in one of 12 IVF clinics in the Netherlands between 1980 and 1995 (19,840 women received IVF treatment, 6588 women did not; Ceelen et al., 2008b). A more recent retrospective cohort study, comparing 1246 fertile and 461 infertile healthy women, showed that the infants of infertile women were smaller and had a lower birthweight. Authors concluded, however, that the pathology of the infertility had a greater impact on fetal growth rather than the infertility therapies themselves (Cooper et al., 2011).

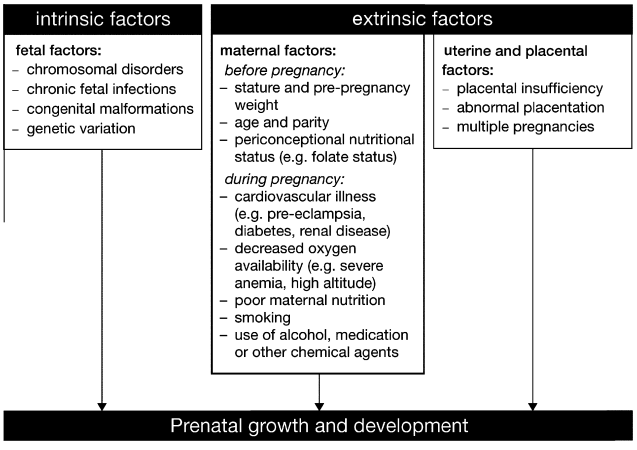

Figure 2 Biological factors influencing growth and development. Adapted from: Ceelen et al., 2008b.

Analysis of several cardiometabolic measures in the same study found small but significant increases in blood pressure (BP) and fasting glucose concentrations in IVF children compared with controls (Ceelen et al., 2008a). Both systolic and diastolic BP were higher in IVF children (109 ± 11 versus 105 ± 10 mmHg in controls; $P < 0.001$; and 61 ± 7 versus 59 ± 7 mmHg in controls; $P < 0.001$, respectively). IVF children had higher fasting glucose concentrations (5.0 ± 0.4 versus 4.8 ± 0.4 mmol/l in controls; $P = 0.005$) and were 2.5 times more likely to be in the highest fasting glucose quartile (≥5.2 mmol/l) than controls (highest quartile versus lowest quartile: 95% CI 1.2—5.2). However, other indicators of insulin resistance, such as fasting insulin concentrations and insulin resistance measures, were similar between IVF and naturally conceived children. No differences in height, weight and body mass index were observed between groups. Although a 3—4-mmHg higher systolic BP and 1—2-mmHg higher diastolic BP in IVF children appear to be insignificant, a major impact on public health cannot be ruled out.

A possible mechanism for the reported rise in arterial BP has been proposed (Scherrer et al., 2012). Scherrer's group documented a significant increase in vascular dysfunction in a group of 65 12-year-old assisted-conception children conceived after hormonal stimulation and ICSI or IVF, but not in children conceived *in vivo* from mothers who underwent hormonal stimulation. The accompanying editorial (Celermajer, 2012) highlighted that the prognostic significance of the arterial abnormalities reported in this small study remained unknown and further research is required to identify their possible cause.

Body composition, assessed by anthropometry and dual-energy X-ray absorptiometry, was studied in another subset of 233 IVF children (139 pubertal) and 233 age- and gender-matched control children (143 pubertal) at the Vu University Medical Centre, The Netherlands (Ceelen et al., 2007). IVF children had a lower subscapular-triceps skinfold ratio and a higher sum of peripheral skinfolds, peripheral body mass and percentage of peripheral body fat compared with controls. A nonsignificant tendency towards higher total body fat was also seen in IVF children. No differences in bone mineral composition between IVF children and controls were found.

In the same group of 233 IVF children (115 boys and 118 girls) there were no differences in timing and progression of puberty between IVF and age-matched control adolescents (Ceelen et al., 2008c). Bone age appeared more advanced in pubertal IVF-conceived girls, but not in boys, compared with controls. Additionally, dehydroepiandrosterone sulphate and LH concentrations were higher in IVF-conceived girls than in controls. No functional limitations in terms of general cognitive ability, school performance (need for extra help, repeating a grade, special education) and rates of learning and developmental disorders, were found in IVF

children at the end of primary and secondary school (Wagenaar et al., 2008). One study showed that British Ability Scale scores were higher in assisted-conception children compared with naturally conceived children, although these differences could be almost fully explained by inequalities in socioeconomic circumstances (Carson et al., 2011).

Overall, development in children born after IVF appears similar to that of age- and gender-matched controls in terms of post-natal growth and puberty, bone mineralization and cognitive function (Ceelen et al., 2007, 2008a,b,c, 2009; Wagenaar et al., 2008).

Outcomes in children born after ICSI and TESE

Cognitive and motor development studies using various assessment scales have generally found no differences between ICSI, IVF and naturally conceived children (Bonduelle et al., 2003; Leslie et al., 2003; Leunens et al., 2006; Place and Englert, 2003; Ponjaert-Kristoffersen et al., 2004, 2005; Sutcliffe et al., 2001). Lower scale scores were found for ICSI versus naturally conceived children in two studies (Bowen et al., 1998; Knoester et al., 2008). Other parameters are comparable between ICSI children and those in the general population (i.e. illness/surgical interventions, cognitive/motor development, growth, weight, height, head circumference, body mass index, pubertal staging, genital examination, inhibin B concentrations (at puberty), Sertoli cell function, salivary testosterone; Belva et al., 2007, 2008, 2011; Wennerholm et al., 2009).

Matched cohort studies from the same centre showed similar neonatal outcomes for IVF and ICSI children and for TESE and ICSI children, including singletons. Overall, the risk of major congenital malformations was slightly higher with ICSI (OR 1.3) versus the general population, and more malformations (OR 1.54, 95% CI 0.99—2.42), mostly in the urogenital system, were observed using testicular versus ejaculated spermatozoa.

Outcomes in children born after cryopreservation

A literature review suggested that pregnancies and infants conceived following slow freezing of embryos are not associated with an increased risk of adverse obstetric and perinatal outcomes (Wennerholm et al., 2009). The review included IVF/ICSI children born after cryopreservation, slow freezing and vitrification of early cleavage-stage embryos, blastocysts and oocytes. No differences in congenital malformation rates were found between children born using cryopreservation techniques and children from fresh transfer in population-based registries from Australia (Shih et al., 2008), Sweden (Källén et al., 2005) and the USA (Sassisted reproduction treatment, 1993, 1994, 1995, 1996, 1998, 1999, 2000). In three small studies including children from natural conception as controls, no differences were found in neonatal outcome or congenital malformation rate (Sutcliffe, 2000; Sutcliffe et al., 1995a; Wennerholm et al., 1998).

Another study evaluated the safety of cryopreservation in combination with IVF and ICSI, prenatal diagnosis and neonatal outcome in children conceived from frozen—thawed ICSI embryos (cryoICSI) and frozen—thawed IVF embryos (cryoIVF) (Belva et al., 2008). Questionnaire and physical examination data at 2 months from 547 cryoICSI children and 390 cryoIVF children were compared with each other and with infants born after fresh embryo transfer. Data were also compared with earlier results from fresh ICSI and IVF embryos. Major malformations were more frequent in cryoICSI liveborns (6.4%) than in cryoIVF liveborns (3.1%; OR 2.15, 95% CI 1.10—4.20) and fresh ICSI liveborns (3.4%; OR 1.96, 95% CI 1.31—2.91). Increased rates of de-novo chromosomal anomalies (3.2%) were found in cryoICSI fetuses/children compared with the fresh ICSI group (1.7%; OR 1.96, 95% CI 0.92—4.14; NS). Subsequently, results from the Australian registry showed a lower number of birth defects following ICSI with frozen embryo cycles (6.6%; OR 1.10, 95% CI 0.65—1.85) compared with fresh cycles (10.7%; OR 1.73, 95% CI 1.35—2.21; Davies et al., 2012). In a population-based cohort study of the Danish IVF registry, singletons born between 1995 and 2006 after cryopreserved embryo transfer were compared with singletons from fresh embryo transfer and with nonassisted reproduction singletons (Pinborg et al., 2010). A higher mean birthweight was found in the cryopreserved group (3578 ± 625 g) versus the fresh transfer group (3373 ± 648 g) and in the cryopreserved group versus the nonassisted reproduction group (3537 ± 572 g). Children in the cryopreserved group had a lower adjusted risk of low birthweight (OR 0.63, 95% CI 0.45—0.87) and preterm birth (OR 0.70, 95% CI, 0.53—0.92) compared with those in the fresh transfer group. Similar low birthweight and preterm birth rates were observed when comparing the cryopreserved group with the nonassisted reproduction treatment group. Perinatal mortality rate was doubled in cryopreserved versus nonassisted reproduction treatment (1.6% versus 0.8%, respectively) singletons and the adjusted risks of very preterm birth and neonatal admittance were also increased. No significant differences in the prevalence of birth defects, neurological sequelae, malignancies and imprinting-related diseases were observed between the cryopreserved group and two control groups. A more recent study also reported a higher birthweight after cryopreservation compared with fresh embryo transfer (Nakashima et al., 2013). Further data, from the German IVF registry, showed a significantly higher birthweight in singleton neonates after cryopreservation compared with fresh cycles from gestational weeks 34 to 42 based on 73,461 transfer cycles (Bühler et al., 2010).

Growth was similar between 255 children born after IVF and 225 children from natural pregnancies after 18 months follow up (Wennerholm et al., 1998). In another study of 343 children born after ICSI (78 born after IVF and 81 after cryopreservation), 2-year growth was similar to that in naturally conceived controls irrespective of assisted reproduction method (Nakajo et al., 2004). A UK study found small differences in mental development and clinical neurological assessment between 91 children born after cryopreservation and 83 naturally conceived controls and concluded that overall development was not cause for concern (Sutcliffe et al., 1995b).

A recent meta-analysis of five studies including data from 4282 vitrified oocytes, 3524 fresh oocytes and 361 slow-frozen oocytes showed no difference in the rates of ongoing pregnancy, embryo quality, embryo cleavage and fertilization between vitrified and fresh oocytes (Cobo and Diaz,

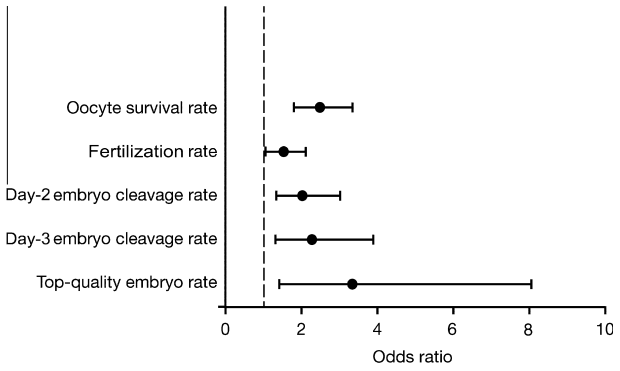

Figure 3 Results from a meta-analysis of five studies involving 4282 vitrified oocytes, 3524 fresh oocytes and 361 slow-frozen oocytes (2005—2009). Adapted from: Cobo and Diaz, 2011.

2011). However, fertilization, oocyte survival, top-quality embryo and embryo cleavage rates were higher in vitrified compared with slow-frozen oocytes (**Figure 3**). Data comparing infant outcomes after vitrification and slow freezing of blastocysts and oocytes are limited at present (Wennerholm et al., 2009). However, a recent systematic review and meta-analysis of 11 studies, comparing obstetric and perinatal complications after cryopreserved versus fresh embryo transfer, concluded that pregnancies arising from the use of cryopreserved embryos have better outcomes (Maheshwari et al., 2012).

A possible explanation for the observed reduction in the risk of birth defects and better outcomes with cryopreservation compared with fresh embryo techniques is the increased likelihood that only the healthiest embryos will survive the freeze—thaw process.

Subcellular effects of cryopreservation

Cryopreservation is used in IVF/ICSI for various reasons: to safeguard surplus embryos, reduce the number of embryos transferred (and reduce the multiple pregnancy rate), delay embryo transfer and preserve fertility in cancer patients (Varghese et al., 2008). Oocytes and embryos are cryopreserved using either a slow-freezing method or a newer, faster process of vitrification (Boldt, 2011). Vitrification requires higher concentrations of cryoprotectants and results in a solid, glass-like cell free of ice crystals. Injuries during cryopreservation may be the result of either inherent biological factors (e.g. large size, spherical shape, depolymerization of the meiotic spindle) or external mechanisms of damage (e.g. toxicity, chilling, ice formation, osmotic damage; Tucker and Liebermann, 2007). Despite this, human cells have considerable ability to repair damage and protocols have evolved to minimize cryopreservation injuries (Coticchio et al., 2006).

The meiotic spindle is highly sensitive to cryoprotectants and changes in temperature. Low temperature can cause depolymerization of tubulin, potentially raising the risk of aneuploidy — one of the main concerns with oocyte cryopreservation. Studies investigating the effects of oocyte cryopreservation on meiotic spindle and chromosome configuration are conflicting. Some indicate repolymerization and repair after cryopreservation with a return to normal configuration (Bianchi et al., 2005; Cobo et al., 2001; Gook et al., 1994; Rienzi et al., 2004; Stachecki et al., 2004); others found increased chromosome abnormalities and altered spindle structures in frozen oocytes after in-vitro maturation (Park et al., 1997).

Instituto Valenciano de Infertilidad researchers have demonstrated that the meiotic spindle returns to its normal configuration irrespective of protocol (vitrification or slow freezing; Cobo et al., 2008b) and that vitrified oocytes were equivalent to fresh in terms of ongoing pregnancy rates in a prospective, randomized, controlled clinical trial (Cobo et al., 2010). No difference in fertilization rates, day-2 or -3 cleavage and blastocyst formation and embryo quality for vitrified and fresh oocytes were observed at the Instituto Valenciano de Infertilidad (Cobo et al., 2008a).

Chromosomal abnormalities in children born after ICSI

Newer assisted reproduction techniques, such as the use of testicular spermatozoa in case of nonobstructive azoospermia for ICSI, are perceived as being less 'natural' (Devroey et al., 1995), but there is little information on children born using these methods beyond the neonatal period. Surveillance of ICSI pregnancies and children for adverse health outcomes, birth parameters, major anomaly rates and chromosomal malformations has been pursued systematically in the medical genetics centre at the Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Brussels, Belgium.

In a single-centre study at this institute, a slightly lower percentage of IVF pregnancies resulted in a live birth compared with ICSI pregnancies (69.5% versus 73.3%; $P < 0.001$), indicating no increased elimination of genetic anomalies potentially linked to the ICSI technique in early pregnancy (Bonduelle et al., 2002a). A significantly higher rate (1.6%) of de-novo, noninherited chromosomal anomalies in ICSI children was observed versus the general population (0.3—0.4%) in prenatal testing (Bonduelle et al., 2002b). This appears related to low sperm concentration ($<20 \times 10^{6}$/ml) or abnormal sperm motility. No increases in de-novo chromosomal anomalies were found in 195 children conceived following TESE compared with ICSI (1794 children; Belva et al., 2011).

Imprinting disorders and IVF/ICSI

Epigenetic mechanisms and imprinting

Recent studies suggest a link between IVF/ICSI and epigenetic modifications, which can be defined as phenotypic or gene expression changes (Berger et al., 2009). Examples of epigenetic changes include DNA methylation, histone modification and other chemical alterations that affect packaging of DNA around nucleosomes. Genomic imprinting is an epigenetic mechanism associated with DNA methylation (Li et al., 1993) that 'marks' regions of DNA in a parent-of-origin specific manner. This affects genes in the vicinity of the epigenetic modification, resulting in uniparental gene expression (Koerner and Barlow, 2010; Reik and Walter, 2001).

In theory, IVF/ICSI could alter the normal dynamics of the imprinting process either directly, inducing abnormal epigenetic changes, or indirectly through the propagation of imprinting defects in the gametes of the parents,

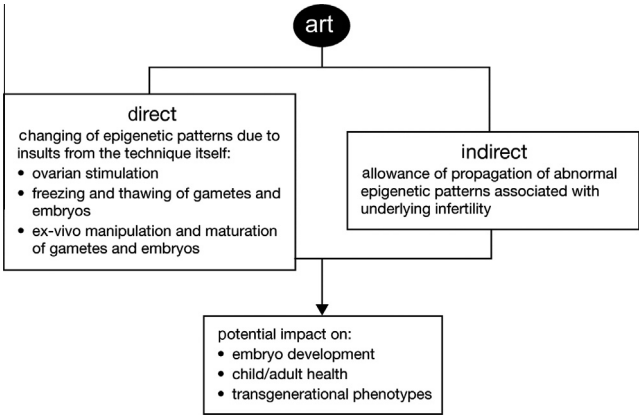

Figure 4 Assisted reproduction treatment and potential alterations of the phenotypical fetal/adult programme.

associated with their underlying subfertility (**Figure 4**). During in-vitro culture, embryos are exposed to artificial media, light and high oxygen concentrations before transfer to the uterus. IVF/ICSI may circumvent natural selection mechanisms in gamete development and subject gametes and embryos to environmental stress, potentially leading to detrimental epigenetic changes.

The advent of IVF and PGD has facilitated studies of oocytes and embryos, revealing the mechanisms that may lead to genetic abnormalities in gametes and during the preimplantation stage. Evidence of sex-specific differences in genomic imprints suggests that female gametes may be more vulnerable to the acquisition of defects than male as they do not complete the imprinting process until around the time of fertilization (**Figure 5**; Gosden et al., 2003; Reik and Walter, 2001). The male imprint is established earlier in the process of gametogenesis, prior to most of the interventions associated with fertility treatment. This sex-related difference may explain why most children with imprinting disorders born following IVF have defects associated with abnormal expression of maternally imprinted genes.

Imprinting defects and IVF/ICSI in humans

At present, data obtained in humans are inconclusive. However, some epidemiological studies indicate an increased incidence of imprinting disorders in IVF/ICSI children. Defects in the establishment or maintenance of imprinting have been studied in disorders such as Beckwith–Wiedemann syndrome (BWS; Bowdin et al., 2007; Chang et al., 2005; Debaun et al., 2003; Doornbos et al., 2007; Gicquel et al., 2003; Gomes et al., 2007; Halliday et al., 2004; Lidegaard et al., 2005; Lim et al., 2009; Maher et al., 2003b; Rossignol et al., 2006; Strawn et al., 2010; Sutcliffe et al., 2006) and Angelman's syndrome (Cox et al., 2002; Doornbos et al., 2007; Lidegaard et al., 2005; Ludwig et al., 2005; Ørstavik et al., 2003; Sutcliffe et al., 2006;). Less evidence exists linking Prader–Willi syndrome (Doornbos et al., 2007; Lidegaard et al., 2005; Sutcliffe et al., 2006) and Silver–Russell syndrome with IVF/ICSI (Bliek et al., 2006; Galli-Tsinopoulou et al., 2008; Kagami et al., 2007; Svensson et al., 2005). Since the first report in 2002 (Cox et al., 2002), there has been concern that these disorders are more prevalent in children born after IVF/ICSI (Chang et al., 2005; Maher, 2005; Manipalviratn et al., 2009) and may represent a 'tip of the iceberg' phenomenon (Maher et al., 2003a).

A variety of genetic defects can result in BWS and Angelman's syndrome, including point mutation, deletion, uniparental disomy and imprinting defects (usually involving an alteration in cytosine methylation). It is noteworthy that, in one review, 90–100% of children with BWS that were born after IVF/ICSI had imprinting defects, compared with 40–50% of children with BWS conceived without IVF/ICSI (Manipalviratn et al., 2009). In Angelman's syndrome, 71% of IVF/ICSI-conceived children with the disorder have an imprinting defect versus just 5% of affected children born after natural conception (Manipalviratn et al., 2009). Despite limitations in sample size and lack of correction for maternal age or underlying infertility diagnoses, current evidence suggests a possible association between IVF/ICSI and BWS and, to a lesser degree, for IVF/ICSI and

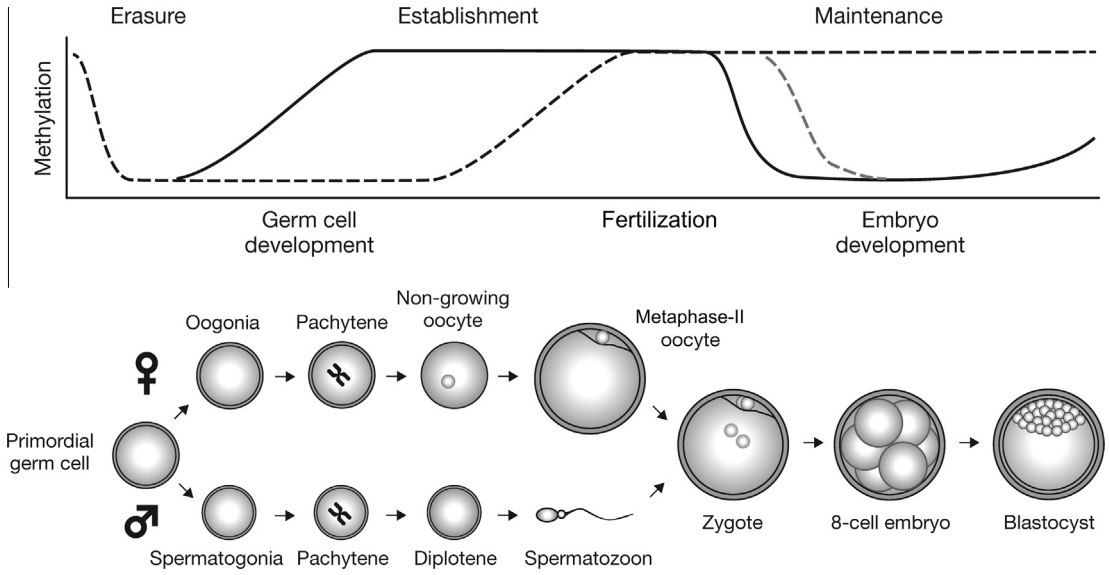

Figure 5 Stages of imprinting in early life. Adapted from: Gosden et al., 2003.

Angelman's syndrome. However, it is important to remember that absolute risk of BWS — the only imprinting syndrome for which there are data on relative risk — after IVF/ICSI is estimated to be less than 1% (Bowdin et al., 2007; Halliday et al., 2004). This suggests that routine screening for imprinting disorders in children conceived by IVF/ICSI is not necessary.

Recent reports have identified DNA methylation differences in IVF/ICSI-conceived versus naturally conceived children (Gomes et al., 2009; Katari et al., 2009; Turan et al., 2010) while others failed to see this correlation (Oliver et al., 2012; Tierling et al., 2010; Zheng et al., 2011). However, the potential clinical consequences of methylation changes are still unknown. As relatively little is known about variation in methylation within the general population, and since the biological and clinical impacts of the apparent changes are largely unknown, results must be interpreted with caution.

Imprinting and ovarian stimulation

Some authors have postulated that ovulation induction might increase the risk of imprinting defects (Ludwig et al., 2005). Oocytes that show a gain of methylation (H19) and a loss of methylation (PEG1, KvDMR1) for some imprinted genes have been detected in IVF/ICSI-treated infertile women (Khoueiry et al., 2008; Sato et al., 2007). However, age and infertility were not eliminated as potential causes of the imprinting alterations.

Imprinting and ICSI

Follow up of children born using ICSI has thus far not revealed differences in methylation patterns compared with children from standard IVF (Santos et al., 2010; Tierling et al., 2010). While ICSI appears not to alter the imprinting status of the gametes, it might facilitate the transmission of paternal imprinting abnormalities if such defects are present. It may be that spermatozoa are resistant to disruption of genomic imprinting caused by ICSI, as male imprinting is established early during spermatogenesis (**Figure 4**). This provides some reassurance for protocols involving fertilization with immature spermatozoa.

Significant methylation alterations were noted in patients with male factor infertility, with differences observed between patients with azoospermia, oligozoospermia and abnormal protamine concentrations (Hammoud et al., 2010; Marques et al., 2010; Minor et al., 2011). This suggests that transmission of epigenetic alterations varies according to the cause of infertility. Assays to detect alterations in DNA methylation could reveal men at high risk of transmitting imprinting disorders (Sato et al., 2011).

Imprinting and in-vitro culture

Assisted reproduction techniques include the in-vitro culture of preimplantation embryos. No studies to date have demonstrated a direct association between the culture media and the development of adult diseases. However, different media may influence birthweight due to epigenetic modifications (Dumoulin et al., 2010). An effect of altered imprinting on birthweight would not be surprising as many imprinted genes have roles in placental development.

In some animal models, assisted reproduction treatment can induce abnormal epigenetic patterns, including alterations to genomic imprinting (Fortier et al., 2008; Mann et al., 2004; Market-Velker et al., 2010a,b; Rexhaj et al., 2011; Rivera et al., 2008; Sato et al., 2007). Although animal models are not human, it seems likely that some methods do carry a risk (albeit a relatively low one) of affecting imprinting, especially if the techniques used are suboptimal, poorly mimicking the natural environment of the oocyte/embryo. Modern media formulations may alleviate this risk compared with those used in the early years of IVF, but it is not possible to calculate an absolute risk with currently available data.

The observation that in-vitro culture, especially extended culture to the blastocyst stage, may affect embryo outcome was initially made in ruminants. A connection to imprinting was proposed, as some lambs and calves born after embryo culture exhibited overgrowth abnormalities, now collectively referred to as 'large offspring syndrome' and reminiscent of BWS (Hori et al., 2010; Horii et al., 2010; Obata et al., 2011). Studies in cows and mice are exploring the effects of IVF, in-vitro culture, somatic cell nuclear transfer and vitrification on imprinting and gene expression (Doherty et al., 2000; Khosla et al., 2001; Wang et al., 2010; Young et al., 2001).

A review of studies in animals and humans recommended caution in extrapolating data from animals, particularly mouse models (Menezo et al., 2010). It also suggested that culture media containing essential amino acids in the fertilization and early developmental stages could minimize the risk of imprinting diseases. It has been suggested that, in order to accomplish the DNA methylation, vital for the laying down and maintenance of genomic imprints, a supply of methionine may be required during the first 3 days of in-vitro culture (Menezo, 2006; Summers and Biggers, 2003). For similar reasons, it is possible that DNA methylation might be altered by ovarian stimulation, which leads to elevated concentrations of follicular homocysteine, potentially causing epigenetic disruption (Berker et al., 2009; Pacchiarotti et al., 2007; Sato et al., 2007). This highlights the importance of more open disclosure of culture methodologies among centres and manufacturers worldwide, as many culture systems remain proprietary.

Evidence of abnormal methylation in the KvDMR or IGF2/H19 imprinting control region has been reported in clinically normal individuals conceived by IVF/ICSI, supporting the hypothesis that maternal imprints are particularly vulnerable to changes induced by IVF/ICSI (Gomes et al., 2009; Shi et al., 2011), particularly as oocyte imprinting occurs during the same time period as many of the interventions used for infertility treatment (e.g. ovarian stimulation, in-vitro maturation and IVF).

Imprinting and assisted reproduction: future considerations

Parent germ cells and preimplantation embryos may be susceptible to defects in imprinted genes at different stages of development. The periconceptional period may present a

critical time during which imprinting and other key developmental functions can be altered. Current research has uncovered key events in the timing and mechanisms of imprint erasure, establishment and maintenance. However, many unanswered questions remain surrounding epigenetics, imprinting and the use of assisted reproduction, including: whether prolonged culture to blastocyst stage confers a higher risk for imprinting disorders; whether cytoplasmic and cytoskeletal disruption caused by cryopreservation increases the risk of imprinting abnormalities; what other disorders related to imprinting can be observed; and the proportion of imprinting problems potentially due to IVF/ICSI versus intrinsic parental factors such as underlying infertility. Evaluation of many more children is needed to assess the true incidence of imprinting disorders in IVF/ICSI, but the incidence, while possibly elevated relative to the general population, is likely to be low.

Conclusions

The health of children conceived following IVF/ICSI is of considerable interest. Several articles have raised concerns that children conceived by IVF/ICSI are at increased risk of poor health outcomes, ranging from congenital birth defects (Davies et al., 2012; Hansen et al., 2002; Klemetti et al., 2005; Merlob et al., 2005; Olson et al., 2005) to imprinting disorders (Odom and Segars, 2010; Strawn et al., 2010), and media reports to the lay public often exploit these. The Sixth EVAR Workshop Group Meeting was organized to obtain a detailed evaluation on whether there are differences in the rate of health outcomes such as congenital abnormalities, growth and development between IVF/ICSI-conceived children and those conceived naturally. This paper also includes an extensive review of the literature; however, the authors acknowledge that it is not a systematic review and therefore contains an element of subjectivity.

The risk for congenital malformations in children following assisted reproduction treatment (IVF and ICSI) compared with those naturally conceived was not statistically significant (Hansen et al., 2005; Rimm et al., 2004). IVF-conceived children tend to be born at lower birthweights and have higher peripheral fat mass, systolic and diastolic BP and fasting glucose concentrations (Ceelen et al., 2007, 2008a). Subsequent growth and development appear similar compared with age- and gender-matched controls (Ceelen et al., 2008b,c; Nakajo et al., 2004; Sutcliffe et al., 1995b; Wagenaar et al., 2008; Wennerholm et al., 1998, 2009). Current evidence shows no direct effect of IVF/ICSI on imprinting disorder rate (Amor and Halliday, 2008; Bowdin et al., 2007; Doornbos et al., 2007; Manipalviratn et al., 2009).

Information on the occurrence of imprinting defects in IVF/ICSI children is limited. There is a $<1\%$ absolute risk of BWS after IVF/ICSI (Bowdin et al., 2007; Halliday et al., 2004). Methylation pattern differences between ICSI and IVF children have not been found so far and there are no studies to date that have demonstrated a direct association between culture media and the development of adult diseases.

Several challenges are encountered in obtaining and evaluating data from epidemiological studies of congenital malformations, including small sample size, correction for confounding factors such as maternal age, incomplete reporting, inconsistent criteria for defining congenital abnormalities and difficulty in identifying an appropriate control population. These must be taken into account when analysing the results of past studies and in conducting future research.

Patient- and technology-related factors that could impact on outcomes in children born after IVF/ICSI were explored at the meeting. Technology-related concerns include deficiencies in culture media that could increase the risk of long-lasting epigenetic alterations, changes in oocytes following ovarian stimulation and endometrial preparation, exposure of oocytes and embryos to biochemical contaminants in IVF culture systems, bypassing of natural sperm selection during ICSI, physical damage to the ooplasma or meiotic spindle during ICSI and damage from cryopreservation and PGD. Patient-related concerns include parental age, infertility type and duration and the use of gametes from an ageing population of IVF/ICSI patients with defective genes or organelles. Technological advances in treatments cannot compensate for the natural decrease in oocyte quantity and quality in older women. The impact of oocyte quality on early embryonic survival and development was examined in detail, and techniques to improve evaluation of oocyte quality through refinement of morphological assessment were explored (Alpha Scientists In Reproductive Medicine and ESHRE Special Interest Group, 2011a,b) and via PGD methodologies such as polar body biopsy (Geraedts et al., 2011).

Well-controlled, large-scale, multicentre, prospective, long-term epidemiological studies are required to further define the most probable cause of differences in outcomes between IVF/ICSI-conceived and naturally conceived children, without ruling out the infertility factor itself from the analysis as a potential source of imprinting defects. There is a need for careful follow up of IVF/ICSI-conceived children into adulthood to determine long-term health-related consequences. In the future, the epigenetic profile of normal gametes and embryos must be explored and objectively defined. Once these processes are understood, researchers will be able to objectively test the influence of variables such as in-vitro culture systems or assisted reproduction technologies on DNA methylation and the potential effect on newborn babies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.rbmo.2013.10.013.

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