REVIEW

Comparison Between Children Born After Natural Conception and Those Born After Assisted Reproduction Technology: A Review of the Literature and Results from our Survey

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Abstract

Many studies have concluded that IVF/ICSI pregnancies carry an increased risk for multiplicity, perinatal mortality, preterm birth and low birth weight in comparison with pregnancies after spontaneous conception. Others have investigated the incidence of congenital malformations in IVF/ICSI conceptions and reported a possible increase in the incidence of central nervous system defects, chromosomal abnormalities, urogenital abnormalities and limb malformations. Concerns have been raised that assisted reproduction technology (ART) might increase the malformation rate in offspring, particularly after intracytoplasmic sperm injection (ICSI). However, other studies have concluded that there is no evidence of an increase in the occurrence of malformations.

The aim of this study is to summarize the present knowledge concerning malformations after ART in comparison to the spontaneously conceived children and whether this increased risk could be related to ART procedures or whether it is mainly related to parental characteristics. For this study, the literature and results from a survey as well as our data were analyzed. A total of 151 infants were born after ART at our department, among which one case of trisomy (0.66%) and one case of Potter syndrome (0.66%) were observed.

In this paper, no increase in the malformation risk of infants born after ART treatment is defended.

Keywords: malformation, IVF/ICSI, choromosome abnormalities, natural conception

Özet

Normal ve Yardımla Üreme Teknolojileri Sonrası Doğan Çocukların Karşılaştırılması: Literatür Taraması ve Kendi Sonuçlarımız

Spontan gebeliklerle karşılaştırıldığında, IVF/ICSI gebeliklerinde çoğul gebelik, perinatal mortalite, erken doğum ve düşük doğum ağırlığı riskinin arttığı birçok araştırmanın vardığı ortak sonuçtur. Bazı çalışmalarda IVF/ICSI gebeliklerinde konjenital bozukluklar incelenmiş ve merkezi sinir sistemi, kromozomal, ürogenital ve ekstremite gelişim anomalilerinde olası bir artış rapor edilmiştir. Yardımla üreme teknolojilerinden (YÜT) özellikle intrasitoplazmik sperm enjeksiyonu (ICSI) sonrasında, yenidoğanda malformasyon oranının arttığına dair tereddütler bulunmaktadır. Ancak, farklı çalışmalar malformasyonların artışı ile ilgili kesin kanıtlar olmadığı sonucuna varmışlardır.

Bu çalışmanın amacı, YÜT'ten sonra oluşabilecek malformasyonların spontan gebelikler ile karşılaştırılması amacıyla güncel bilgileri özetlemek; bu risk artışının YÜT uygulamalarına mı yoksa öncelikle ebeveyn özelliklere mi bağlı olduğunu araştırmaktır. Bu çalışma için literatür taraması yapılmış ve kendi verilerimizle birlikte başka bir araştırmanın sonuçları da analiz edilmiştir. Bölümümüzde YÜT sonrası, içlerinde bir trizomi (%0.66) ve bir Potter sendromu (%0.66) vakası da olmak üzere 151 çocuk dünyaya gelmiştir.

Bu makalede, YÜT sonucunda doğan çocuklarda malformasyon riskinde artış olmadığı savunulmaktadır.

Anahtar sözcükler: malformasyon, IVF/ICSI, kromozom anomalileri, doğal konsepsiyon

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Introduction

Assisted reproduction techniques (ARTs) have become widely accepted treatment methods of infertile couple with more than 125 000 *in vitro* fertilization (IVF) cycles in Europe per year (1).

In the Nordic countries 2.2-3.9% of children (1.7-3.1% of births) were born with help of IVF in 2001. In many industrialized countries, IVF children constitute 2-4% of all newborn babies (2). Approximately 1 million children world-wide have been born through assisted reproduction technology (ART) (3) and the number of children born worldwide after IVF has greatly increased with time (4).

Since ART is increasingly used to overcome infertility other aspects have attracted attention (5). Much concern has been expressed about the health and development of children born after ART, as many adverse effects associated with these procedures can influence the outcome.

The first studies comparing rates of congenital abnormalities in children conceived as a result of ARTs mainly IVF with those in children conceived spontaneously were published in the late 1980s and early 1990s (6-9). Besides, Hansen et al. carried out the first study and examined children born between 1993 and 1997, in an attempt to determine whether and to what extent birth defects were more common in children conceived by two methods of assisted reproduction (IVF/ICSI) than in children conceived naturally (10).

Hansen et al. took a random sample of 4000 naturally conceived births and compared it with the ART births in the same period and area (n=1138) and concluded that infants conceived with ART were more likely to be delivered by caesarean section, to have a low birth weight and to be born before term. The major birth defect for ICSI, IVF and naturally conceived infants was 8.9%; 9.0% and 4.2% respectively (10). Most studies of ART results (including ICSI) have also shown an increase of congenital abnormality cases (1.3 fold) in comparison to normal pregnancies (11).

Another study examined the prevalence of low and very low birth weight infants conceived with ART (12). When the data were controlled for maternal age, parity, and plurality no significant difference could be found between children born after IVF treatment and those born without ART treatment (13,14). Others have shown that infants born after IVF have a poorer perinatal outcome than naturally conceived infants (12,15,16). Therefore, studies assessing the relationship between ARTs and birth defects have been less consistent (10,14,17,18).

The purpose of this study was on one hand to compare the neonatal outcome in singleton and twin pregnancies after assisted conception, focusing on the most recent data available, with those of spontaneously conceived singleton and twin pregnancies and on the other hand to present the knowledge concerning malformation after assisted reproduction and particularly to discuss whether ART children have an increased risk of malformations and other diseases in comparison with spontaneously conceived children. Most studies have shown that infants born after IVF have a poorer perinatal outcome than naturally conceived infants.

Antenatal complication of assisted reproduction

Premature birth

It is well established that infants conceived following IVF and ICSI are more likely to be born preterm, of low birth weight and to be a twin or higher order multiple than spontaneously conceived infants (19-21). Many well-designed studies have consistently documented association between ART and low and very low birth weight and preterm delivery (20-22).

Doyle et al. investigated possible risk factors for prematurity, low birth weight and small-for gestation-age (SGA) in children born from singleton pregnancies after IVF (23). Analysis by multiple regression indicated that hypertension during pregnancy was an independent risk for preterm delivery, low birth weight and SGA; bleeding during pregnancy was a risk factor for preterm delivery; whereas the number of embryos transferred and the type of infertility was a risk factor for low birth weight (23).

The incidence of low birth weight and very low birth weight in infants conceived with ART was disproportionately increased when compared with spontaneous conceptions. In addition, singleton infants conceived after ART and born at 37 weeks of gestation were 2.6 times more likely to be of low birth weight (24).

Recently, Bower and Hansen showed that even in singleton ART infants, there is around two-fold increase in risk of

Table 1. Perinatal outcome in singletons after *in vitro* fertilization and natural conception: results of a meta analysis of clinical trials

	Approximately absolute risk (%)		
Outcome	**IVF**	**Spontaneous**	**Odds ratio (95% CI)**
Perinatal mortality	2	0.7	2.9 (1.61-2.98)
Preterm delivery	11.5	5.3	1.95 (1.73-2.20)
Birth weight <2500 g	9.5	3.8	1.77 (1.40-2.22)
Birth weight <1500 g	2.5	1.0	2.70 (2.31-3.14)
Small for gestation age	14.6	8.9	1.60 (1.25-2.04)

Adopted from Jackson et al. (21).

Table 2. Comparison of perinatal data of ICSI and spontaneously twin birth

Outcome	ICSI twin (n=1102)	Spontaneously twin (n=2163)	Odds ratio
Birth weight	2317±591	2304±579	NS
Birth weight <1500 g	8.8%	10.15%	0.86 (0.67-1.11)
Birth weight <2500 g	59.2%	59.6%	0.98 (0.85-1.13)
Perinatal death	3.1%	2.7%	1.73 (0.91-3.28)
Still birth	2.1%	1.4%	1.52 (0.88-2.62)
Neonatal death	0.99%	1.2%	0.77 (0.38-1.55)

perinatal mortality, low birth weight and preterm birth, about a 50% increase in small for gestation age and a 30-35% increase in birth defects, compared with singletons conceived spontaneously (25).

Perinatal complication of assisted reproduction

Apgar score

The outcome, other than live birth rate, has become an important focus of investigation. Kallen et al. studied low Apgar scores and other neonatal problem like cerebral haemorrhage, neonatal convulsions, respiratory problems and neonatal sepsis using the Swedish Medical Birth register, as well as the Hospital Discharge Register and found that low Apgar score <7 at 5min in 2.6% of IVF infants as compared with 1.3% in the general population. In IVF singleton, 1.8% had low Apgar scores, as compared with 1.3% in the general population; the odd ratio (OR) adjusted for year of birth was 1.29 (95% CI 1.11-1.50) (26).

Birth defects after assisted reproduction

Classification of the malformation into minor or major

Kurinczuk and Bower found that ICSI-conceived infants were twice as likely to have major birth defects as was the general population of Western Australian infants born during the same period (27), whereas Bonduelle et al. reported no increase in the prevalence of birth defects in the same cohort of children (28). Kurinczuk and Bower et al. analysed data published by Bonduelle et al. (28), using a different malformation classification (29).

Congenital anomalies (malformations in IVF/ICSI)

Major congenital malformations are anatomical defects or chromosomal abnormalities that are present at birth and are either fatal or significantly affect the individual's function or appearance. They occur in general population in 2-3% of all births, with about 0.5-0.6% consisting of chromosomal abnormalities (30,31).

Lancaster's study from the 1980s was the first to report a higher prevalence of neural tube defects and transposition of the great vessels among IVF children (32).

Heart malformations were detected to be four-fold higher in IVF babies and not related only to multiple pregnancies (33).
Several studies have been conducted to determine whether or not ART is associated with an increased risk of birth defects (29). These studies have demonstrated a higher birth defect rate among children after ICSI compared with natural conception (10,34,35). Rates of ART-associated birth defects are 1.4 to 2 fold higher than the overall rate of 3% to 4% of births in general (36). Olson et al. found that 6.2% of IVF-conceived children had major birth defects, compared with a rate of 4.4% in naturally conceived children (37).

Early studies did not detect an increased incidence of malformations of IVF children (6,38,39). However, a long lasting survey from 1986 to 2002 has identified a double risk of congenital malformations in the newborn conceived with general IVF techniques with respect to normal population (40) and a similar and increased risk rate was also reported by other authors (11,41).

Studies assessing the relationship between ART and birth defects have been less consistent (10,14,17,18). Hansen et al. reported a prevalence of birth defects for both ICSI and IVF infants of approximately 9% compared with 4% among infants from natural conceptions (10). The studies by Hourvitz (41) and Van Steirteghem (42) did not show differences in obstetrics and neonatal outcome of pregnancies or congenital malformations in children conceived with ICSI or IVF. Agarwal and Allamaneni (43) and Ponjaert-Kristoffersen et al. (44) showed a no adverse neurodevelopment and cognitive/motor outcome in the ICSI children. In contrast, evidence of genetic malformation (45,46), excess of malformations of urogenital male's system (34), and increased cases of hypospadias were reported (14,47).

Several more recent matched cohort studies have demonstrated an increased risk of birth defects in general (10) or cardiovascular birth defects in particular (33), whereas, others did not find an increased risk (18,48). A number of studies on IVF and ICSI birth have shown a ~2 fold increased risk of congenital anomalies (27,46,49).

Hansen et al. carried out a systematic review to identify all peer published by March 2003 with data relating to the prevalence of birth defects in infants conceived following IVF and/or ICSI, compared with spontaneously conceived infants (11). Pooled results from all suitable published studies suggest that children born following ART are at increased risk of birth defects compared with spontaneous conceptions. The pooled OR in the seven selected studies was 1.40 (95% CI 1.28-1.53). When all 25 studies were analyzed, the OR was 1.29 (95% CI. 1.2-1.37). These results were significant for major birth defects and for singleton births only. Two third of the studies analysed (n=25) found a ≥25% increased risk

of birth defects in ART children. Those data confirm the data presented by Klemetti et al. who found odd ratio (OR) of the same magnitude (50). Those studies therefore suggest that ART children could have a 25-40% higher incidence of birth defects.

Westergaard et al., compared the data from Danish registry study (n=2245 IVF) children with a matched control group from the Danish Medical Birth Registry and with all birth in the Danish Medical Birth Registry. They found that 107 (4.8%) of IVF children and 103 (4.6%) in the control group were born with malformations as compared to 2.8% in the background population (18).

The prevalence of major birth defects in children born after ICSI (n=301), conventional IVF (n=837) and spontaneous conception (n=4000) and detected by 1 year of age were 8.6%, 9.0% and 4.2%, respectively, after adjustment for co-founders such as maternal age, parity and sex, the children born after ART were still twice as likely to have a birth defect as children born after natural conception (10). The major malformation rate after ICSI was 8.7%, compared with 6.1% in the population-based control cohort (35). However, most studies have concluded that children born after ICSI may have a lightly higher risk of birth defects than naturally conceived controls and are not at significantly higher risk than children conceived by IVF with standard insemination techniques (51).

The risk for congenital birth defects seems to be slightly increased in children conceived with ART (10,11,15,33) and according to some studies, twins are at a slightly higher risk than singleton (11,33). However, when the data were controlled for maternal age, parity, and plurality, no significant difference could be found between children born after IVF treatment and those born without ART treatment (13,14). The results of controlled studies indicated that the risk of congenital malformation after ART is increased as compared with natural conception (5.4 vs. 3.8%) (14).

Chromosomal defects after ART

Chromosomal abnormalities have been reported in IVF children, including Down's syndrome, Edward's syndrome and the Klinefelter syndrome (8). Athough debated, increased chromosome anomalies in IVF-ET pregnancies were observed early in 1995 (53,54). With the introduction of ICSI technique, which bypasses the natural selection, concern about a possible increase in the rate of early miscarriage or fetal malformations owing to parental genetic anomalies has increased (55,56). A higher rate of karyotypic abnormalities have been described in pregnancies conceived after ICSI, than in pregnancies conceived by IVF or with standard insemination (57). A meta analysis compared ICSI conceived fetal karyotypes with those in the normal neonatal population showed an increased risk of chromosomal abnormalities (42). Particularly, if infertility of a man is caused by some alterations, it is feasible to transmit them to the offspring conceived with ICSI (58,59).

The incidence of karyotypic abnormalities after ICSI can be correlated with the number of sperm in the ejaculate, showing the link between the severity of male factor infertility and subsequent chromosomal aberrations in offspring (57). Male children conceived after ICSI carry the same Y chromosome micro deletions as their fathers (60,61). Statistically 190-time fold numerical chromosome abnormalities with respect to general population (62) and increased aneuploidy rate and micro deletion of the Y chromosome in OAT patients (63-67) classify infertility patients as a high risk group. Clementini et al. analysed the prevalence of chromosomal abnormalities in 2078 infertile couple referred for ART and found that 3.95% had one partner carrying a chromosomal change, and 4.95% men showed yq11 micro deletions (59). The chromosomal rearrangements were 2.1% translocations and 0.05% duplication. Categories with greater risk were represented by men with sperm count $<20x10^6$ sperm/ml, and women with history of pregnancy loss. This Sweden registry study (26), which covers the period 1992-2001, reported that among 16 280 identified IVF children, 811 had a diagnosis of a congenital malformation in the Swedish Medical Birth Registry (SMBR) (5.0%),

Table 3. Association of birth defect and IVF

Study	Year	Infant study	IVF defects (%)	Control defects (%)	Adjusted odds ratio	Specific defects increase
Dhont	1992-1997	Singleton (3048)	2.8	2	Not stated	None
		Twins (2482)	2.9	2.9		
Wester-gaard	1994-1995	All (2245)	4.6	4.6	Not stated	None
Hansen	1993-1997	Singleton (527)	9.5	4.2	2.2	Cardiovascular
		All (837)	9.0	4.2	(1.5-3.2)	
Koivurova	1990-1995	All (306)	6.6	4.4	Not stated	Urogenital
Olsen	1989-2002	Singleton (645)	5.6	3.7		Cardiovascular
		All (1462)	6.2			
Ericson	1982-1991	All (9111)	5.6	Not stated	0.89 (0.74-1.06)	Neural tube defects
Anthony	1995-1996	All (4224)	3.2	2.7	1.03 (0.86-1.23)	Cardiovascular

Adopted from Van Voorhis BJ 2006 (52).

Table 4. Congenital malformation in children after ART

Authors	Procedure	ART		Naturally conception	*p*
		No. of children with anomalies/Total (%)			
Loft et al. 1999	ICSI	20/730	(2.7)	None	
Wennerholm et al. 2000	ICSI	87/1139	(7.6)		
Bonduelle et al. 2002	ICSI	96/2840	(3.4)	None	
	IVF	12/2955	(3.8)	None	
Hansen 2002	ICSI	26/301	(8.6)		
	IVF	75/837	(9.0)		h.s
Kallen et al. 2002	ICSI	982/12 280	(8.0)	National	
	IVF			Registry	
Anthony et al. 2002	IVF	137/4227	(3.2)	8526/314 605 (2.7)	
Hansen et al. 2005	ICSI	Meta analysis of 25 studies		None	h. s.
	IVF	Meta analysis of 7 studies			

while among all children registered in SMBR during this period (a total of 2 039 943 children), 80 881 had such a diagnosis (4%) The crud odd risk ratio thus was 1.26. Among the malformed IVF children 535 (3.3%) had a severe malformation, while the figure for a general population was 45 892 (2.2), crud odd ratio 1.46. The overall conclusion from this large study was that children born after IVF have a modest but significant increased rate of congenital malformations, similar after IVF and ICSI.

Sex chromosomal aberration after ART

Many authors suggested that sex chromosome anomalies after ICSI might be due to paternal and maternal meiotic errors particularly if associated with functionally abnormal spermatozoa. A significant increase (0.83%) in sex chromosomal aberration has been reported in pregnancies after ICSI (68).

Perinatal outcome

Many data from various working group confirm the high rates of prematurity, low birth weight, and infant mortality in ART-conceived birth (10,12,15,42,46,49,69).

Preterm delivery

Preterm birth rates in the United States are 10.4% for singleton birth, 57.4% for twins, and 92.7% for triples and higher-order births (National Center for health Statistic, NCHS 2003.www.cdc.goc.nchs). Preterm delivery and low birth weight are the main cause of the increased morbidity and mortality in the neonatal period. Besides, premature delivery is associated with risks during the immediate postpartum period (congenital anomalies, respiratory distress, intraventricular haemorrhage, gastrointestinal problems, retinopathy), but has an effect on neuro- and motor development during later in life as well (70-73). The prematurity rate in IVF pregnancies was higher than that observed in French national population, even for singleton pregnancies (12.2 vs. 5.6) (74). The rate for infant mortality was (14.3 vs. 9.7%) (75).

The IVF singletons were on average born one week earlier than the controls, weight 400 g less, and had a threefold greater chance of being born by caesarean section. The higher percentage of preterm deliveries was largely due to multiple births and they contributed to neonatal conditions in 45.0% of all IVF children (76).

An increased risk for prematurity, low birth weight have not shown after IVF either with singleton (77) or with twin pregnancies (78).

Low and very low birth weight

It is well established that infants conceived following *in vitro* fertilization (IVF) and Intracytoplasmic Sperm Injection (ICSI)

Table 5. Chromosomal abnormalities in children conceived after ICSI (abnormal caryotype)

Authors (year)	No.	Sex chromosome	Autosomal chromosome	Total
Van Opstal 1997	71	4	5	9 (12.8)
Govaert et al. 1998	101	0	4	4 (3.9)
Loft et al. 1999	209	7	0	7 (3.3)
Wennerholm et al. 2000	149	0	4	4 (2.7)
Aboulgahr et al. 2001	430	6	8	15 (3.5)
Lam et al. 2001	48	0	1	1 (2.0)
Bounduelle et al. 2002	1586	10	37	37 (3.0)
Samli 2003	142	6	0	6 (4.2)
Jozwiak et al. 2004	1136	7	10	17 (1.5)

are more likely to be born preterm, of low birth weight and to be a twin or higher order multiple than spontaneously conceived infants (19-21). Even singleton births resulting from ART are associated with an increased risk of low birth weight. In singleton, 12.3% of the newborn had a birth weight <2500 g, whereas the figure observed in the French national population was 5.2% and the rate of children with birth weight <1500 g was four time higher than that found in the general French population (79).

Schieve et al. estimated that the rate of low birth weight in term singleton conceived with ART is 2.6 times that in the general population (12), and a 606 g lower mean birth weight was reported in ICSI conceived singleton births, compared with the controls (17).

Small for gestation ages

Small for gestation age (SGA) is one of the major determinants of perinatal mortality and morbidity and may relate in adult diseases. Early prediction of SGA could be helpful for health care providers and public health workers in guiding antenatal management and prevention.

The outcome of pregnancies following assisted conception have revealed adverse results compared to natural conception such as a higher rate of preterm deliveries, low birth weight and small for gestational age (SGA) in live born singleton (9,23,80-82) and twin babies (48,80,83,84). SGA, extremely low birthweight (LBW<1000 g) survivors often remain small and/or have subnormal school performance. Besides, SGA is one of the major determinants of perinatal mortality and morbidity, and may relate in adult diseases. Koudstaal et al. (2000) compared 307 IVF pregnancies with 307 control pregnancies and found that the proportion of SGA was higher in the IVF group (16.2 vs. 7.9%; p<0.001).

Nevertheless, the rate of preterm labour, low birth weight and SGA were comparable according to a study conducted by Reubinoff et al. who compared 260 singleton IVF pregnancies and 260 naturally conceived singleton controls, matched 1:1 for maternal age, parity, ethnic origin, location and date of delivery (77). Besides, higher pre-gravid BMI and parity seem to reduce the occurrence of SGA triplets. However, lean mothers, especially nulliparous, may be the most important target population for nutritional intervention in triplet pregnancies (85).

Neonatal morbidity

Studies that demonstrated that IVF children are at increased risk of various adverse neonatal outcomes (16,26,89) have not clearly addressed whether these risks were independent of LBW, preterm birth or multiple birth or were an effect of one of these. Pinborg et al. also found an increased risk for IVF twins being admitted to the NICU as compared with both spontaneous twins and IVF/ICSI singleton (90). Meta-analyses have shown increased neonatal intensive care units (NICUs) admissions in both IVF singleton (20,21) and IVF twins (20,91).

In addition to the morbidity, maternal mortality is increased as well (72,90,92,93). Reubinoff et al. studied 260 consecutive singleton IVF pregnancies and 260 naturally conceived singleton controls matched 1:1 for maternal age, parity, ethnic origin, and location of delivery (77). The rate of most antenatal complications was similar in both groups. The Caesarean section rate was significantly higher among IVF patients (41.9 versus 15.5%). The rates of preterm labour, low birth weight, small and very SGA, neonatal intensive care unit admissions and perinatal mortality were comparable. The authors concluded that when controlling for maternal age, parity, ethnic origin, and location and date of delivery, singleton IVF pregnancies do not carry an increased risk for prematurity, low birth weight, or maternal or fetal complications.

A group of 307 IVF pregnancies were compared with 307 control pregnancies by Koudstaal et al. after elaborate matching for an extensive number of maternal characteristics, as well as for the hospital that provided the obstetrics care (94). Four Dutch university hospitals contributed to the study. In case with spontaneous onset of labour, gestational age at delivery was 3 days shorter in the IVF group (275 versus 278 days, p=0.05). The proportion of SGA was higher in the IVF group (16.2 versus 7.9, p<0.001).

Perinatal mortality

An increased perinatal mortality has been shown by Tallo et al. (95). Higher rate of prematurity, low birth weight and perinatal

Table 6. Summary of the results of various clinical trial comparing children born after IVF and those after naturel conception (control)

References	IVF (n)	Pre-maturity <37 WA		<2500		SGA	
	n	IVF	Control	IVF	Control	IVF	Control
Rufat et al. 1994	916	12.2	0.0	12.3	0.0	15.0	0.0
Tan et al. 1992	494	14.0	8.0	14.0	7.0	16.0	10.0
Friedler et al. 1992	863	19.3	0.0	14.6	6.4	0.0	0.0
Wang et al. 1994	465	16.0	6.2	0.0	0.0	16.3	10.0
Tanbo et al. 1995	355	14.9	9.5	11.5	6.7	4.3	3.6
FINAT 1997	9432	9.1	0.0	10.5	0.0	14.4	0.0
Koudstaal et al. 2000	307	15.0	5.9	13.8	6.9	16.2	7.9

FINAT: all French centres.
Adopted from Olivennes et al. 2002 (86).

Table 7. Perinatal outcome in singleton after IVF and natural conception: results of meta analysis of clinical trial

Outcome	IVF (%)	Spontaneous (%)	Odds ratio (95% CI)
Perinatal mortality	2	0.7	2.9 (1.61-2.98)
Preterm delivery	11.5	5.3	1.95 (1.73-2.20)
Birth weight <1500 g	9.5	3.8	1.77 (1.40-2.22)
Birth weight <2500 g	2.5	1.0	2.70 (2.31-3.14)
Small for gestation age	14.6	8.9	1.6 (1.25-2.04)

Adopted from Jackson et al. 2004 (21).

Table 8. Comparison of perinatal data of ICSI and spontaneously singleton birth

General data	ICSI (n=1655)	Control (n=3278)	*p* value-Odds ratio
Birth weight	3227±585	3268±574	*p*<0.001
Birth weight <1500 g	1.9%	1.5%	1.25 (0.78-1.99)
Birth weight <2500 g	7.9%	7.0%	1.14(0.91-1.44)
Perinatal death	1.2%	0.7%	1.73 (0.91-3.28)
Still birth	0.8%	0.4%	1.99 (0.92-4.30)
Neonatal death	0.4%	0.3%	1.39 (0.53-3.65)

Adopted from Ombelet et al. 2005 (87).

Table 9. Comparison of perinatal data of ICSI and spontaneously twin birth

General data	ICSI (n=1102)	Control (n=2163)	*p* value-Odds ratio
Birth weight	2317±591	2304±579	NS
Birth weight <1500 g	8.8%	10.15%	0.86 (0.67-1.11)
Birth weight <2500 g	59.2%	59.6%	0.98(0.85-1.13)
Perinatal death	3.1%	2.7%	1.73 (0.91-3.28)
Still birth	2.1%	1.4%	1.52 (0.88-2.62)
Neonatal death	0.99%	1.29%	1.39 (0.53-3.65)

Adopted from Ombelet et al. 2005 (87).

mortality were found in specific group of singleton pregnancies by the majority of different studies of large numbers of IVF pregnancies. (Australian IVF collaborative group, MRC working party on children conceived by IVF, FIVNAT) (9,15,48,82,96,97).

Cerebral palsy and ART children

Children born after IVF have been found to have an increased risk of cerebral palsy (98). Register-based cohort studies reported an increased risk of cerebral palsy 1.7-2.8 fold in IVF children (98-100). The risk for cerebral palsy and risk for epilepsy increased significantly by 1.69 and by 1.54 respectively.

Increased risk for cerebral palsy and for other neurological impairment is associated with multiple birth (98,101-103). The highest rate of cerebral palsy is in surviving children whose co-twins or triplet died *in utero* (102,104). Lidegaard et al. carried out a study involving 442 349 singleton non-IVF and 6052 IVF children, derived from the national registry of patients and the central register of psychiatric disease in Denmark and found a significant increased risk of cerebral palsy with a rate ratio of 1.8 and of sleeping disturbances with a rate ratio of 2.0 (100).

Abnormal imprinting in Birth Defects Syndromes

Genomic imprinting is a mechanism of genetic regulation in which only one of the parental allele is selectively expressed and the other one is repressed (105,106). Differential DNA methylation leading to expression of only 1 of 2 parental alleles is a mechanism of gene regulation known as genomic imprinting. Several syndromes caused by imprinting defects, including Beckwith-Wiedemann syndrome and Angelman syndrome, have been reported to be more prevalent in children born after IVF as reviewed in Niemitz and Feinberg (107). Marques et al. showed an increase of these syndromes in babies conceived with ART and found a correlation between anomalous imprinting and hypo-spermatogenesis (108). Besides, an association between AS, BWS and ICSI children has been found (109-111).

Beckwith-Weidemann and Angelman Syndromes are complex disorders of growth and development associated with aberrant imprinting at chromosome 11q15.5 (112) and the UBEA3 gene locus on chromosome 15q11-13 (109), respectively. DeBaun et al. (110) and Maher et al. (113) demonstrated a high prevalence of Beckwith-Wiedeman in children conceived by ICSI. A relationship could be found between IVF and Beck-

Table 10. Results of meta analysis of perinatal outcome in *in vitro* fertilization (IVF) singletons

Inclusion criteria	Helmerhorst et al. 2004 (20) 14 case-controlled studies	Jackson et al. 2004 (21) 15 case-controlled studies	McDonald et al. 2005 (88) 14 case-controlled studies
Sample size	5361 IVF 7038 control	12 283 IVF and 1.9 million controls	6728 IVF and 8454 controls
Outcomes			
Perinatal mortality	1.68 (1.11-2.55)*	2.2 (1.6-3.0)	2.40 (1.59-3.63)
Preterm birth <37 weeks	2.04 (1.80-2.32)*	2.0 (1.7-2.2)	1.93 (1.36-2.74)
Very preterm birth <32-33 weeks	3.27 (2.03-5.28)*	3.10 (2.00-4.80)	2.99 (1.54-5.80)
Low birth weight <2500 g	1.70 (1.50-1.92)*	1.8 (1.4-2.2)	1.40 (1.01-1.95)
Very low birth weight <1500 g	3.00 (2.07-4.36)*	2.7 (2.3-3.1)	3.78 (4.29-5.75)
Small for gestation age	1.40 (1.15-1.71)*	1.6 (1.3-2.0)	1.59 (1.20-2.11)
NICU	1.27 (1.16-1.40)*	1.60 (1.30-1.96)	1.60 (1.30-1.96)

* Summary relative risk with 95% CI NICU=neonatal intensive care unit

Table 11. Results of meta analysis of perinatal outcome in *in vitro* fertilization (IVF) twins

Inclusion criteria	Helmerhorst et al. 2004 (20) 10 case-controlled studies	McDonald et al. 2005 (88) 8 case-controlled studies
Sample size	3437 IVF twins 3429 SC-twins	2308 IVF twins 2326 SC-twins
Outcomes		
Perinatal mortality	0.58 (0.44-0.77)*	1.40 (0.22-9.11)†
Preterm birth, <37 weeks	1.07 (1.02-1.13)*	1.57 (1.01-2.44) ††
Very preterm birth, <32-33 weeks	0.95 (0.78-1.15)*	1.03 (0.4-2.9)
	1.03 (0.99-1.08)*	1.13 (0.85-1.51)
Low birth weight <2500 g	0.89 (0.74-1.07)*	1.22 (0.5-2.9)
Very low birth weight <1500 g	1.27 (0.097-1.65)*	0.92 (0.62-1.38)
Small for gestation age	1.05 (1.01-1.09)*	2.22 (1.64-3.02)
NICU		

†=matched at least for maternal age; NICU=neonatal intensive care unit
††=matched for maternal age and parity; Summary relative risk and 95% CI.

with-Wiedeman syndrome (110,113,114), retinoblastoma (115) and Angelman Syndrome (109,111), which caused by defects in genomic imprinting.

Another 3 children who conceived after ICSI were shown to be having non-inherited imprinting abnormalities at the Angelman gene locus (3,109,111). However, Lidegaard et al. found no indication of an increased risk of imprinting diseases after IVF, but an 80% increased risk of cerebral palsy by comparing a 442 349 singleton non-IVF and 6052 IVF born children (100). The number of children with specific imprinting disease in the non-IVF group was 54: fortyfour kidney cancers, five retinoblastoma, three Prader-Willi Syndrome and two Russel-Silver Syndrome. However, a significantly increased risk of cerebral palsy in IVF group, with a rate ratio (R:R) (IVF:non IVF) of 1.8 [95% confidence interval (CI) 1.2-2.8; $p<0.01$], and of sleeping disturbances, with an RR 2.0 (95% CI 1.2-3.3) could be found.

In contrast, study of DNA-methylation patterns at chromosome 1q11 in children born after ICSI reveals no imprinting defects (116). Several molecular studies reported by Manning et al. (116) did not reveal an increase in imprinting defects among chromosomes from a small sample of children born after ICSI (117) or spermatozoa collected for ICSI procedure. Maher (118) showed that an absolute risk of imprinting disorders after IVF is very small and even absent (100). Other authors claim that, given the rarity of these congenital syndromes (1/300 000 birth for Angelmann syndrome and 1/15 000 births for Beckwith-Weidemann syndrome), also a small frequency increase in IVF children must be seriously considered (112,119).

Table 12. Perinatal mortality in twin pregnancies after ART compared with the natural conception

Study	ART (%)		Natural (%)		Relative risk (95% CI)	
Dhont et al.	61/2482	(24.6)	82/2482	(33.0)	0.74	(0.54-1.03)
Dhont et al.	0/230		6/230	(2.61)	0.08	(0.0-1.36)
Fitzimmons et al.	4/112	(35.7)	48/216	(22.2)	0.16	(0.06-0.43)
Isakkson et al.	0/40		6/200	(30.0)	0.38	(0.02-6.56)
Koudstaal et al.	3/192	(15.6)	1/192	(0.52)	3.00	(0.31-28.6)
Tallo et al.	2/260	(7.7)	4/72	(55.6)	1.00	(0.26-3.85)
Total	72/3128	(23.0)	147/3392	(43.3)	0.58	(0.44-0.77)

Helmerhorst FM et al. 2004 (20)

Cancer risk in IVF/ICSI

No general cancer risk has been seen for the women undergoing ovarian hyper stimulation (121). Besides, no general cancer risk has been seen for children except for unexpected increase of histiocytosis cases (26). A few case-controlled studies have suggested an increased risk of neuroblastoma (122,123) and leukaemia (124,125). Another cohort studies among IVF children have not shown an increased risk of neuroblastoma or leukaemia compared with the general population to date (15,126,127). The incidence of childhood cancer was also not increased in children conceived spontaneously (n=9478) of women with history of subfertility. Many authors could not find an increase in childhood cancer compared to all children born in the same time interval (90,99,128-130).

Physical examination, health, chronic illness and growth

IVF and spontaneously conceived children did not differ regarding weight, height and head circumference (34,90,131, 132). The incidence of vision or hearing impairment was not increased in IVF children in most studies (34,90,132). Saunders et al. conducted a detailed review of IVF children in comparison to naturally conceived children (133); the study did not show an independent effect of IVF on the growth and physical outcome of the children. However, Bonduelle et al. reported more childhood illness in children born after IVF or ICSI than after spontaneous conception (34).

Motor and intellectual development

Many years ago, Yovich et al. reported the development of their 20 IVF children. Development test were made at 1 year old and no abnormalities were observed (134).

The group of Norfolk reported the follow-up of 110 IVF children (aged 13-30 months) compared with a control group of spontaneously conceived children. No difference was found for neurological and psychomotor evaluation (135).

Brandes et al. (128) had studied and compared 116 IVF children with 116 non-IVF control children, they found that the developmental indices of the IVF infants were within the normal range, and did not differ from those of their matched controls, but a correlation was found between multiple births and a lower developmental index (Bayley and Stanford- Binet). Kuivurova et al. studied a cohort of 229 IVF children with a cohort of 558 controls representing the general population matched appropriately for sex, year of birth, area of residence, parity, maternal age and social class and concluded that the growth of IVF children was behind that of control children, but psychomotor development was normal (136).

The receptive language development, behaviour and temperaments of singleton infants (1 year post partum) who were conceived after IVF (n=65) and those matched control (n=63) were eveluated by using Bayley behaviour rating scales (137). Sutcliffe et al. compared 221 ICSI children at a mean of 18

Table 13. Reported cases of ART children with genomic imprinting disease

Disease	Reference	No. of Cases	Technology performed	Loss of imprinting	Country
Beckwith-	Sutcliffe et al. 1995	1	IVF	ND	UK
Wiedeman	Koudstaal et al. 2000	1	IVF	ND	Netherlands
syndrome	Olivennes et al. 2001	1	IVF/ICSI	ND	Belgium
	Bonduelle et al. 2002	1	ICSI	ND	Belgium
	Boerringter et al. 2002	1	IVF/ICSI	ND	Belgium
	DeBauan et al. 2003	7	IVF/ICSI	KCNQ1 H19	USA
	Gicquel et al. 2003	6	IVF/ICSI	KCNQ 10T1	France
	Maher et al. 2003	6	IVF/ICSI	KCNQ 10T1	UK
Angelman	Cox et al. 2002	2	ICSI	SNRPN	Norway
syndrome	Orstavik et al. 2003	1	ICSI	SNRPN	Germay
Silver-Russel syndrome	MRC Working Party 1990		IVF	ND	UK

Adopted from Lucifero D et al. 2004 (120)

months with 208 naturally conceived children, using Griffiths scales of mental development and found no difference between the two groups (17,138).

Ponjaert-Kristoffersen et al. investigated the development of 5-year old ICSI conceived children using the Weschler preschool and primary scales of intelligence and the Peabody development motor scaler (n=300) children each group they found no significant difference between the groups (139). Many small studies that based on interviews or neurological examinations, at up to five years old did not found differences regarding neurological abnormalities (133,139,140).

Pinborg et al. assessed the prevalence rate of neurological sequelae in Denmark in a nation wide cohort of singleton (n=5130) and twin after IVF/ICSI (n=3393) techniques and in population based control group (n=10 239) of naturally conceived children (90). They found that twin of ART have a similar risk of neurological sequelae as singleton after ART and their naturally conceived peers. Place et al. analysed a group of 66 ICSI conceived children, 3 and 5 years of age, concerning the formal development and intellectual assessment showed that there were striking similarities between the groups (131). Sutcliffe and Bonduelle et al. assess the neurodevelopment of a 5-year old children conceived naturally (n=488) versus IVF (n=424) and ICSI (n=511) using the Weschler preschool and primary scales of intelligence and the McCarthy motor scales and they found no difference between the three groups in terms of physical and audiometry screening and eye checks (34,141).

Psychological studies
Long term psycho-social effects

A trend was shown towards more behavioural problem in IVF children (n=27) in comparison to spontaneously conceived children (n=23) at the age of 8-9-years reported by their teacher (142). Bonduelle et al. reported that physiotherapy and speech therapy were significantly more common in ICSI children compared to normally conceived children (132). However, there was no difference regarding psychological therapy. Wennerholm et al. found no difference regarding psychological therapy or therapy with a social welfare officer (143).

Discussion

The cause of an increased rate of birth defects in children born after IVF is unknown. One may speculate that pre-existing metabolic-vascular changes or the hormonal therapy in early pregnancy in women undergoing ART may be a predisposing factor for the lower birth weight of singleton and twin infants (144). Other factors should also be taken into account such as the great heterogeneity in the population of parents who undergo ART, the diversity of problems inherited with the infertile couple the presence, duration, and type of infertility as well as the age of the mother and father. All of these factors could contribute to the increase rate of birth defects after ART. It has long been recognized that ART, as currently performed, poses a substantial risk for multiple birth and accompanying sequelae (145).

Previous studies have demonstrated that placentas from ART pregnancies had increased pathological features compared with naturally conceived pregnancies (146,147). The elevated birth defects risks could be a consequence from the multitude of protocols used for ovarian stimulation, and procedural factors involving manipulations of oocytes, sperm and embryos (10). Some authors do not attribute elevated birth defect risks to treatments (13,49,55,148), but rather to factors associated with parental selection. ICSI pregnancies have revealed increase in *de novo* autosomal rearrangements and sex chromosomal abnormalities (57). However, it is not established whether these chromosomal abnormalities are a direct results of ICSI procedure or are related to genetic or cytogenetic abnormalities that may occur in association with male factor infertility. Nevertheless, couples with infertility might have higher rates of congenital abnormality, whether they conceive after ART or spontaneously. Many studies showed an increased risk of spontaneous abortion of patients conceived after clomiphene citrate treatment both with and without ART (149,150).

The factors of the adverse outcome
1- Multiple pregnancies

The increase in multiple births is known to be largely due to the widespread use in the treatment of sub fertility of inadequately monitored ovulation induction and multiple embryo transfer (151,152). In a case control study of all ART pregnancies in the Dutch-speaking part of Belgium (48), perinatal outcome of singleton pregnancies after IVF was significantly worse than that of pregnancies after spontaneous conception, mainly because of the increased rate of preterm births.

Many authors have found that ART twins to be at a disadvantages at terms of prematurity (84,153,154), low birth weight (84,153), higher caesarean section rate, length of stay in the neonatal intensive care unit (154), and perinatal mortality (83,84). Population studies have shown a three to seven fold higher incidence of cerebral palsy in twins compared to singleton and one over ten fold higher in triple (102,155). About half of twins, over 90% of triplets and nearly all quadruplets are born preterm (<37 weeks) compared to 10% of singleton (156). The average weight of a newborn twins is about 800 g less than that of a singleton (157). As many as 10% of twins, 32% of triplets and over half the quadruplets weight less than 1500 g (158).

2- Parent-related factors

Some authors do not attribute elevated birth defect risks to

Table 14. Birth weight (g) in live born children after IVF/ICSI

Number of children	IVF/ICSI twin (n=3393)	IVF/ICSI singleton (n=5130)
Gestational age (weeks)	35.9±2.96	39.3±2.24
Birth weight (M±SD)	2508±615	3457±629
<1500 g	7.5%	1.5%
1500-2499 g	34.9%	4.4%
>2500 g	57.0%	94.1%

Adopted from Pinborg A et al. 2004 (90)

Table 15. Comparison of neonatal data of singletons and twins after ICSI

General data	ICSI singleton (n=1655)	ICSI twin (n=1102)	Odds ratio
Birth weight <1500 g	1.9	8.8	$p<0.001$
Birth weight <2500 g	7.9	59.2	$p<0.001$
Perinatal death	1.2	3.1	$p<0.001$
Still birth	0.8	2.1	$p<0.003$
Neonatal death	0.4	0.9	NS

Adopted from Ombelet W. 2005 (87)

treatments (13,49,57,148), but rather to factors associated with parental selection.

Maternal age

Failure to consider maternal age might bias toward a positive association for defects that have a strong association with advanced maternal age (159). Several groups documented increasing aneuploidy rates with advanced maternal age [160,161]. Besides, paternal risk factors associated with male factor infertility were found not to play a role in development outcome (148).

3- The underlying causes of infertility and problem inherited with the infertile couple

The greater risks for LBW and preterm delivery among singleton conceived with ART have been hypotheized to be related to the underlying infertility among women using ART (36). Couples with infertility might have higher rates of congenital abnormality, whether they conceive after ART or spontaneously. Data already exist showing that couples with infertility who conceive spontaneously have poorer perinatal outcomes than the general population (162). Couples with infertility might have higher rates of congenital abnormality, whether they conceive after ART or spontaneously. Many studies showed an increased risk of spontaneous abortion of patients conceived after clomiphene citrate treatment both with and without ART (149,150). Women who sought fertility advice or testing but not treatments were in increased risk of delivering very low birth weight infants compared with a control group with no indication of sub fertility (163).

In voluntary childless even in the absence of IVF has been an impact on prematurity rate (164,165). Singleton infants conceived with infertility treatment had a two-fold increase in LBW compared with infants conceived 3 or more months after infertility treatment was discontinued (166).

4- Chromosomal defects in the gametes of subfertile couple

ICSI pregnancies have revealed increase in *de novo* autosomal rearrangements and sex chromosomal abnormalities (55). However, it is not established whether these chromosomal abnormalities are a direct results of ICSI procedure or are related to genetic or cytogenetic abnormalities that may occur in association with male factor infertility.

It is also already well established that chromosomal abnormalities are more frequent in men with severe male factor infertility who are referred for IVF/ICSI (167). It is also known that men with sever oligozoospermia have an increased rate of chromosomal aberrations (168,169). Abnormal karyotypes were found in 3.5% of ICSI babies (n=430), as compared to none in 430 naturally conceived babies (170).

A moderate excess of chromosomal aberrations following ICSI as compared to approximately 1% described in neonatal population (30,31). These excess risks seem to be related to parental characteristics and not to ICSI technique used (46,55,171,172).

It is also, already well established that chromosomal abnormalities are more frequent in men with severe male factor infertility who are referred for IVF/ICSI (167). Kunathikom et al. reported elevated rates of abnormal sperm karyotypes, with considerable aneuploidy in sperm from infertile males when compared with fertile controls (173). ICSI pregnancy has revealed increase in *de novo* autosomal rearrangements and sex chromosomal abnormalities (55). However, it is not established whether these chromosomal abnormalities are a direct results of ICSI procedure or are related to genetic or cytogenetic abnormalities that may occur in association with male factor infertility. Previous study pointed out that disorders of genomic imprinting may be induced by some IVF procedures especially due to the, immaturity of the gametes (54).

5- The role of ovarian stimulation (medication for ovulation)

Ovulation induction is used in various clinical circumstances. Women with impaired ovarian function or anovulation require stimulation to restore or establish regular ovulation. Women who diagnosed with unexplanained infertility, mild endometriosis or mild male factor infertility, stimulation with multi-follicular development significantly improve pregnancy rates (174).

ART pregnancies may be different from naturally conceived pregnancies in several ways. Controlled ovarian hyper stimulation and administration of hCG and/or progesterone for luteal support may result in non-physiological levels of estrogens, progesterone, and relaxin which in turn may have effects on endometrial and cervical tissues and placentation and/or may impair embryo-endometrial synchronization (175-178). Previous studies have demonstrated that placentas from ART pregnancies had increased pathological features compared with naturally conceived pregnancies (146,147).

The elevated birth defects risks could be consequences from the multitude of protocols used for ovarian stimulation, and procedural factors involving manipulations of oocytes, sperm, and embryos (10). A 1.7 fold increased risk of monozygotic twinning was observed in a study of infants born after ovarian stimulation without IVF (49).

6- The effect of the ART itself (IVF, ICSI)

Many studies suggested that infants delivered after *in vitro*

fertilization and embryo transfer IVF/ET had low birth weights. However, the study conducted by Diamond et al. showed that infants conceived through IVF-ET are not predisposed *per se* to a low birth weight or delivery at an early gestational age (179).

As reviewed by Retzloff and Hornstein, no certain differences in congenital malformation rates between infants born after standard IVF or after ICSI have been demonstrated (51). The malformation rate was similar in ICSI (n=4949) and IVF (n=11 283) children (8.6% and 8.1% respectively). Besides, there was no indication of an increased malformation rate after ICSI/MESE (microsurgery epididymal sperm extraction) (7.4%) and in ICSI/testicular sperm extraction (7.5%) (26).

Bonduelle et al. found that the total malformation rate, taking into account major malformation in stillborn, terminations and live born was 4.2% in ICSI group, which included 2889 child and 4.6% in children born after IVF children (n=2995). Besides, they found that malformation in the ICSI cohort was not related to sperm origin or sperm quality. However, their study had no control group of spontaneously conceived children (55). Rimm et al. carried out a meta analysis, which included 19 studies and found that the rate of major malformations ranged from 0 to 9.5% for IVF, 1.1 to 9.7% for ICSI and 0.0 to 6.9% for controls (180). Ericson et al. estimated an increased risk of major malformations after ICSI (7.1%) as compared to standard IVF (5.3%) (14). However, in a later publication by the same authors and including a large number of children, this difference in malformations between ICSI and IVF was no longer registered (26). Lie et al. compared malformation in ICSI versus IVF in a meta analysis, which included four prospective out of 22 studies, which contained relevant data on ICSI children. They found that the prevalence's of major malformations varied from 3.0% to 9.0% across studies (181). One study showed an increase in hypospadia with ICSI (172). However, another study could not find an association between ICSI and hypospadia or any other birth defects as compared with children born after IVF (10,37,51,55).

Bonduelle et al. assessed 439 ICSI and 207 after IVF born children (2 years age), by performing standard development test and they found no indication that ICSI children have a lower psychomotor development than IVF children (148).

7- Manipulations of oocytes, sperm, embryos

The elevated birth defects risks could be consequences from the multitude of protocols used for ovarian stimulation, and procedural factors involving manipulations of oocytes, sperm, and embryos (10). Thurin et al. by analysing 520 cycles, found that implantation rates were higher during IVF versus ICSI cycles and when the embryo reached at least a 4-cell stage (183). Ludwig et al. found no increase in malformations in the groups using epididymal (3.8%) or testicular sperm (9.2%) compared to ejaculated sperm (8.4%) (46). ICSI pregnancies have revealed increase in *de novo* autosomal rearrangements and sex chromosomal abnormalities (55). However, it is not established whether these chromosomal abnormalities are direct results of ICSI procedure or are related to genetic or cytogenetic abnormalities that may occur in association with male factor infertility. *In vitro* studies of ICSI fertilization suggest a number of cytogenetic effects, including effects on spindle apparatus, microtubules, chromatin behaviour, cell cycle checkpoint, and chromosome positioning (184).

Table 16. Neonatal factors by method of conception in twin pregnancies

	GIFT	IVF	Spontaneous	*p*
Birth weight	2019±743	1973±686	2202±647	0.01
<1500 g	23%	22%	17%	NS
1500-2500 g	35%	52%	48%	NS
> 2500 g	42%	25%	35%	NS
Perinatal mortality	0.0%	2%	3%	NS

Adopted from Ocksenkuehn R et al. 2003 (182)

8- *In vitro* development

Some have speculated that epigenetic errors, such as defects in DNA methylation and imprinting, might be caused by the embryo culture that follows IVF. Besides, the *in vitro* environment in which embryos develop can affect various embryo parameters and might also impact subsequent *in vivo* development of the embryo and fetus [185]. High concentrations of glucose have been associated with decreased embryo quality (186-188). Besides, the addition of serum to media also is linked to negative effects on cleavage rates and possibly to adverse effects on DNA methylation and imprinting (189). Whereas, addition of amino acids, antioxidants, and coculture with somatic cells has been associated with favourable effects (190,191).

It seems that differential methylation of an allele during embryo culture is one of the most frequent epigenetic DNA modification due to methionine and group B vitamins present in the culture median (192,193). Several severe human genetic disorders are associated with imprinted genes including Beckwith Wiedeman (BWS), Prader-Willi and Angelman (AS) syndrome and retinoblastoma (107,194). Some have proposed that embryo cultured media used in IVF might predispose to imprinting defects in the embryo (107,195).

9- The luteal support

ART pregnancies may be different from naturally conceived pregnancies in several ways. Controlled ovarian hyperstimulation and administration of HCG and or/progesterone for luteal support may result in non-physiological level of estrogens, progesterone, and relaxin which in turn may have effects on endometrial and cervical tissues and placentation and/or may impair embryo-endometrial synchronization (175-178). Previous studies have demonstrated that placentas from ART pregnancies had increased pathological features compared with naturally conceived pregnancies (146,147). Besides, increased concentration of maternal serum human gonadotropin concentrations (MS HCG) are an independent risk factor for SGA among dichorionic twin MS HCG >2.5 MoM and associated with adverse maternal outcome among monochorionic twins (196).

Table 17. Results of a prospective study comparing infants born after ICSI with those born after conventional IVF

Outcome parameter	ICSI (n=2889)	IVF (n=2995)	*p* value
Preterm birth <37 week			
All	31.8	29.3	0.046
Singleton	8.4 (n=1499)	9.0 (n=1556)	
Twins	54.6 (n=1228)	47.6 (n=1250)	
Triplets	97.7 (n=123)	NA	
LBW <2500 g (%)			
All	26.7	26.5	0.858
Singleton	7.1 (n=1499)	7.8 (n=1556)	
Twins	48.1 (n=1228)	45.1 (n=1250)	
Triplets	54.0 (n=113)	NA	
VLBW <1500 g (%)			
All	4.4	5.6	0.031
Singleton	1.5 (n=1499)	1.8 (n=1556)	
Twins	5.2 (n=1228)	7.6 (n=1250)	
Triplets	34.5 (n=113)	NA	
Perinatal mortality ≥20 weeks			
All	1.87	2.33	0.238
Singletons	1.25 (n=1499)	0.77 (n=1556)	
Twins	2.38 (n=1228)	3.87 (n=1250)	
Triplets	4.27 (n=113)	NA	

NA: not available; LBW: low birth weight; VLBW: very low birth weight
Adopted from Bonduelle et al. 2002 (55).

10- Freezing/Thawing

After conventional IVF was established and the cryopreservation technique was developed, the first baby produced after applying this technique was born in 1984 (197).

However, there are reported associations with birth defects and ovulation induction and cryopreservation (15,198,199).

Bergh et al. found that singleton, born after cryopreservation, had a significantly lower risk of preterm birth and LBW, when compared with conventional IVF in a stimulated cycle (15). Kallen et al. compared 1000 infants born after cryopreservation with more than 10 000 infants born after conventional IVF and noticed a significant lower ORS for preterm birth (OR 0.69, 95% CI 0.58-0.83), LBW (OR 0.49, 95% CI 0.02-0.75) and low Apgar score (OR=0.26, 95% CI 0.09-0.78) (26). However, most studies show similar results for IVF cycles using fresh or cryopreserved embryos (143,200,201). Wennerholm et al. conducted a study investigating the postnatal growth and the health up to 18 months of age of children born after IVF with embryo cryopreservation and those born after standard IVF with fresh embryos and those of spontaneous pregnancies (143). There was no evidence of a difference between the three groups in term of psychomotor development. Sutcliffe et al. found that there was a little difference between children born after embryo cryopreservation and naturally conceived children (199).

11- Possible birth defect risks

Experts have yet to fully understand the underlying reasons for ART and IUI risks. However, the following factors are known or possible causes:

The difference in zygosity distribution between iatrogenic and spontaneously conceived twin, may be, counteracts the disadvantages imposed by ART pregnancies. As a higher proportion of iatrogenic twins are DZ than amongst twins who are spontaneously conceived, the iatrogenic twins in general would be expected to fare better. One study has shown iatrogenic twins to be advantaged (78).

One study that controlled for zygosity by limiting the study to DZ twins found that ART twins had a small but significant reduction in gestational age and birth weight and a higher perinatal mortality rate (153).

A monochorionic placenta carries additional risks for the fetuses due to the shared fetal circulation and consequent risk of hemodynamic imbalance. The twin-twin transfer syndrome occurs in 10-15% of monochorionic pregnancies (202) and is associated with a high perinatal mortality rate and a substantial risk of long-term neurological morbidity (203-205).

In the present study, between April 1995 and January 2006 the data for each individual infertile couple attending at the Department of Obstetrics of Gynecology and Reproductive Medicine at the University Women's Hospital of Saarland University, Germany, were entered in the University Database. Besides, details of all deliveries after natural conception and those induced by IVF/ICSI (n=139) were recorded.

All the patients included in this study took folic acid already before conception. During pregnancies, all the patients underwent routine periodic ultrasound examination and echocardiography. Regular ultrasound examination was defined as one ultrasound being performed in each trimester as suggested by the official guidelines for maternity care in Germany. Amniocentesis and karyotype study performed in women older than 35 years, in case of chromosomal abnormalities in one of the parents, in case of abnormal nuchal translucency test and on request by the patients.

In the present study at our centre, a series consisted of 151 infants (71 boys and 80 girls) born out of 139 pregnancies following IVF/ICSI, of which 126 (84.1%) were born from single pregnancies, 24 (16%) from twin pregnancies and 1 (0.66%) from triple pregnancies. There was no quadruplet pregnancies as the German legislation regulation ART specify that not more than three embryos are to be replaced in one single treatment cycle and that super-mummery zygotes are to be stored at the pronucleate stage.

Low birth weight and very low birth weight were also observed. Although, the birth weight of the infants by the birth was as follows: 108 infants (71.52%) had a mean birth weight of >2500 g, 36 (23.84%) has a birth weight of 2500 g and the remaining 7 infants (4.63%) had a birth weight of 1500 gram or less. Mature infants (115, ≥ 36 weeks were seen more often than premature 36 <36) weeks. A case of trisomy 21 was also observed in a single pregnancy (0.66%) and a case of Potter syndrome was observed in a twin pregnancy. No other clinically relevant chromosomal abnormalities were observed in the assessed pregnancies.

Conclusion

1- All of the data obtained in the different studies published on the perinatal outcome of IVF pregnancies found an increase in associated complications.

2- A large proportion of these complications can be related to the high percentage of multiple pregnancies, but they are also observed with a higher frequency in singleton pregnancies.

3- Risk factors that could explain these results are multiple.
Age and parity may be important factors. The role of IVF itself has not been demonstrated convincingly, as studies comparing infertile patients treated by IVF with patients treated by ovarian stimulation without IVF found no difference between these groups.

The population characteristics could represent an important bias in comparing the IVF population with the general population, and studies matching IVF pregnancies in spontaneous pregnancies could find different results.

However, important progress in the management of multiple pregnancies and in neonatal intensive care has considerably changed the prognosis of premature babies when the pregnancy and the babies are managed in specialized centres.

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