ELSEVIER

European Journal of Cancer 41 (2005) 715–724

European Journal of Cancer

www.ejconline.com

Paediatric Update

# Ovulation induction, assisted conception and childhood cancer

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Received 17 June 2004; received in revised form 28 July 2004; accepted 28 July 2004
Available online 30 September 2004

**Abstract**

Rapid advances have been made in the treatment of infertility over the last 30 years following the introduction of *in vitro* fertilisation and intracytoplasmic sperm injection. Whilst effects of assisted reproductive technology (ART) on birth outcomes are well documented little is known about effects on child health after the neonatal period. Childhood cancer is one area warranting further examination. The hypothesis that cancer in children may be initiated during early fetal development means that events leading up to and around conception may be important. Whilst the few large-scale epidemiological studies that have looked at childhood cancer incidence following ART have failed to find any significant increased risk, some case-control studies have reported an increased risk of specific cancers. However, it is important not to over interpret these findings as the reason for the infertility may be the predisposing factor, rather than the procedure itself. Recent recommendations by the UK's National Health Service to offer intra-uterine insemination and one free treatment cycle for infertile couples will result in increasing numbers of children born following ART. More detailed investigations that include larger numbers plus sufficient follow-up periods and information on the underlying causes of the infertility are needed since long term outcomes for these children, in particular the risk of developing cancer, remain largely unknown.


## 1. Introduction

Worldwide, it is estimated that each year between 35 and 70 million couples have difficulty in conceiving, although over the past three decades remarkable progress has been made in the treatment of infertility. One of the first breakthroughs came in the late 1960s with the introduction of the anti-oestrogens clomiphene citrate and tamoxifen to induce ovulation in anovulatory women. The world's first ''test tube'' baby was born in 1978 [1] and since then *in vitro* fertilisation (IVF) has progressed from an experimental technique to a routinely used treatment. Further advances have involved the cryopreservation of embryos and the successful development of intracytoplasmic sperm injection (ICSI) [2] to treat couples with andrological subfertility, as well as others in whom conventional IVF has repeatedly failed. More recent techniques include blastocyst transfer, *in vitro* manipulation of embryos by assisted hatching and preimplantation genetic diagnosis.

Whilst much has been reported on short-term outcomes of assisted reproductive technology (ART) – for example, proportions of still and live births, birthweight, multiple births – there are relatively few reports on adverse outcomes or on the health of children after the neonatal period. Childhood cancer is one possible adverse outcome of ART, yet despite the fact that 1% of all children born in developed countries are now conceived by IVF, relatively few studies have investigated cancer incidence in this rapidly growing population. Cancer in children less than 15 years of age is rare, accounting for less than 1% of all malignancies diagnosed each year in developed countries, and its causes remain largely unknown

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doi:10.1016/j.ejca.2004.07.032

[3,4]. However, it has been hypothesised that some childhood cancers may be initiated during the early stages of foetal development, as is the case with some other conditions. Accordingly, events leading up to and around conception could play an important role in determining childhood cancer and therefore warrant examination. There have been several isolated reports of tumours in children born after IVF and, more recently, after ICSI, but the number of large scale epidemiological studies has been limited. Possibly of note is that diethylstilbestrol (DES), which is structurally similar to anti-oestrogens [5], administered to the mother during pregnancy has been associated with the subsequent development of cancer in children. It was identified more than 30 years ago as a cause of a highly unusual and rare vaginal cancer in late childhood and adolescence [6], and there has also been a case report of a child with acute lymphocytic leukaemia (ALL) [7].

The purpose of this review is to provide a brief introduction to the different types of ART and present the data available on the outcomes for children conceived by ART, with a particular emphasis on the risk of development of childhood cancer.

## 2. Assisted reproductive technologies – benefits and risks

The most common ART techniques currently used include intra-uterine insemination, IVF and ICSI. Their success depends on three procedures – superovulation by pharmacological stimulation of the ovary to promote the production of more than one egg; preparation of a highly motile, morphologically normal population of sperm for injection or insemination; and assisted fertilisation using techniques to aid the union of the sperm and egg. Recent articles by Rowell [8] and Braude [9] illustrate the general principles and techniques involved in assisted reproduction and are summarised in Table 1.

In the United Kingdom (UK), one in six couples seeks medical help in order to conceive. Clinics in the

Table 1
Summary of techniques involved in assisted reproductive technology (ART)

| Technique | Summary | Indications |
|---|---|---|
| Superovulation | Multifollicular development is induced by the use of oral anti-oestrogens (clomifene citrate or tamoxifen) or injection of follicle stimulating hormone (FSH) or gonadotrophin-releasing hormone (GnRH) analogues or antagonists with gonadotrophins | |
| Sperm Preparation | Semen samples are prepared such that highly motile and morphologically normal sperm are selected for and the seminal plasma, leucocytes and bacteria are removed to promote capacitation | |
| Intrauterine insemination | Prepared motile sperm sample is deposited in the uterus just prior to the release of an egg or eggs in a natural or stimulated cycle. Gonadotrophins can also be used to induce mild superovulation | Unexplained infertility, problems associated with ejaculation and following the failure to conceive after ovulation induction |
| Gamete Intrafallopian Transfer | Both the eggs and sperm are placed directly in the ampullary portion of the fallopian tube allowing IVF to occur at the natural site. Treatment can either be carried out transvaginally or laparoscopically and involves superovulation. (Can only be used in women who have at least one patent fallopian tube. Rarely used due to the simplicity and success of IVF) | Idiopathic infertility, failed intrauterine insemination, endometriosis and mild male infertility |
| *In vitro* fertilisation | Gonadotrophin-releasing hormone analogues and gonadotrophins are used to induce ovulation. Eggs are collected transvaginally, oocytes retrieved and cultured. Each oocyte is inseminated with 50 000–100 000 motile morphologically normal sperm and transferred to the uterus 2 or 3 days later. Concurrently progesterone or human chronic gonadotrophin is given. (Recent approaches have been to extend the embryo culture time to 5 or 6 days) | Severe tubal damage, bilateral salpingectomy, a history of endometriosis, mild male infertility, idiopathic and immunological infertility |
| Intracytoplasmic sperm injection | The selected sperm is directly injected into the cytoplasm of the egg. Although it requires micro-manipulation, it is a relatively simple and straightforward robust procedure | Ejaculatory disorders, failure of conventional IVF, presence of antisperm antibodies. Allows use of epididymal or testicular sperm if the ejaculatory ducts are blocked, if there is a congenital bilateral absence of the vas deferens, undeveloped sperm and failure of vasovasostomy or epididymovasostomy |

UK using ART are required, as a condition of their license, to report all treatments and the outcome of all births to the Human Fertilisation and Embryology Authority (HFEA). Data for 2000–2001 from HFEA on success rates in each of its approved clinics in the UK are shown in Table 2. Whilst approximately one in five treatment cycles are successful, adverse outcomes including ectopic pregnancy, miscarriage, stillbirth and neonatal death are associated with one in every 20 cycles (Fig. 1). The live birth rate is influenced by duration of infertility and a woman's first cycle of IVF carries, on average, a 17.4% success rate, decreasing to 14.4% by the fifth attempt (www.hfea.gov.uk). The success of IVF is strongly influenced by the mother's age and it has been reported that women over the age of 35 have a significantly smaller ovarian volume and around a third of the number of follicles compared with women under the age of 35 years [10]. Furthermore, an ovarian volume of less than 3 ml has been linked to poor response to ovulation induction during treatment for IVF, implying that ovarian reserve is markedly reduced [11]. Whilst at present there are no accurate markers that estimate ovarian reserve in pre-menopausal women with a normal pattern of menstruation, Wallace and Kelsey [12] recently reported that ovarian volume accurately predicts the number of follicles that remain in these women assuming that they are not taking hormonal contraceptives. Moreover, they went on to describe how measurement of ovarian volume by transvaginal sonography may determine reproductive age and ovarian reserve which is of obvious benefit for women being evaluated for ART.

Adverse outcomes associated with ART may be dependent upon many factors including the underlying causes of infertility, drugs used to induce superovulation and to maintain early stages of pregnancy, and the processes involved in the IVF/ICSI techniques, such as sperm preparation, freeze-thawing of embryos, culture media and conditions used in growing embryos and delayed fertilisation [13]. However, the range of subfertility conditions treated by ART has widened over the past 20–30 years, potentially confounding the evaluation of

Table 2
*In vitro* fertilisation (IVF) National Data statistics adapted from Human Fertilisation and Embryology Authority (HFEA) website www.hfea.gov.uk

| | Number of treatment cycles | | Subsequent number of live births | | Live birth rates (%) | |
|---|---|---|---|---|---|---|
| | <38 years old | All ages | <38 years old | All ages | <38 years old | All ages |
| Total number started | 19006 | 25273 | 4777 | 5513 | 25.9 | 21.8 |
| At egg collection | 18163 | 23957 | 4863 | 5625 | 26.8 | 23.5 |
| At embryo transfer (IVF/ICSI) | 16828 | 22116 | 4855 | 5615 | 28.9 | 25.4 |
| (IVF only) per embryo transfer | 9214 | 12350 | 2651 | 3002 | 28.8 | 24.3 |
| (ICSI only) per embryo transfer | 7628 | 9813 | 2186 | 2519 | 28.7 | 25.7 |
| Frozen embryo replacements (per embryo transfer) | 4176 | 5707 | 705 | 838 | 16.9 | 14.7 |

Data were obtained from all HFEA-approved clinics in the UK. The rates are presented according to the type of treatment, the overall rate, the rate when the female is under 38 years old and the number of live births per cycle started, per egg retrieval and per embryo transferred, where twins and triplets are counted as one live birth event. Embryo transfer data are sub-divided into combined IVF and intracytoplasmic sperm injection (ICSI), IVF only, ICSI only and frozen embryo replacement which combines both IVF and ICSI and does not include those cycles that were abandoned prior to embryo transfer.

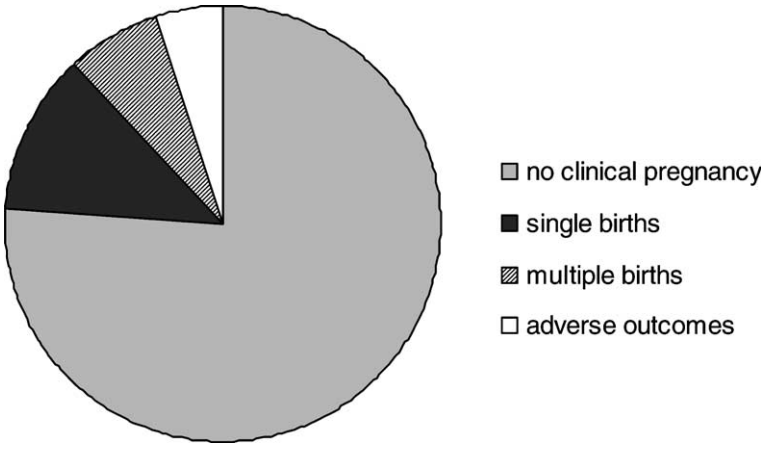


Fig. 1. Results of assisted reproductive treatment cycles reproduced from the Health Fertilisation and Embryology (HFEA) website www.hfea.gov.uk.

treatment outcomes [14]. Following IVF, there is a higher risk of multiple births (www.hfea.gov.uk) (Fig. 1), preterm delivery [15,16] and low birthweight [17–21]. Additionally, an increased risk of congenital anomalies has been suggested [19,22–25], although others have reported no difference in outcome between children conceived by IVF and those conceived naturally [26–29].

Ovulation induction has been practised for many years, but is not subject to the same regulatory processes as IVF and ICSI, so it is more difficult to assess any long-term effects of treatment. However, it has been reported that treatment with human menopausal gonadotrophin may account for the increase in low birthweight babies after ART, as it can cause an increase in insulin-like growth factor binding protein 1 which has been linked to intrauterine growth restriction [30].

As part of the IVF process, oocytes are collected and transferred to a culture medium containing the essential nutrients and electrolytes required for fertilisation and maintenance of embryo growth. Leese et al. [31] have reported that *in vitro* culture and manipulation can alter oocyte and embryo cell physiology leading to changes in early embryo gene expression patterns. Others have reported that *in vitro* culture can alter the gene expression considered central to embryo development and thus specifically influence the methylation status of parentally imprinted genes [32–35]. Formulation of the media used in human embryo culture is not always divulged to IVF clinics and, whilst embryos will adapt to imperfect media, the long-term consequences and effects on gene expression are not known [36]. There is clearly a need to develop standard embryo culture protocols and to examine whether extended culture time might result in an increased number of imprinting disorders.

Concerns have arisen over genetic and congenital anomalies reported in children born following ICSI, especially where epididymal or testicular sperm and sperm from men whose infertility is of genetic origin have been used. How or why these anomalies occur is not known. It is possible that the procedure may influence the natural maturation process of sperm, damage gametes, cause mechanical injury to the spindle leading to genetic defects or they may occur as a consequence of the underlying genetic causes of infertility

One possible explanation is that direct injection of a single sperm into the oocyte as part of the ICSI process bypasses the natural selection mechanisms sperm encounter in the course of natural conception allowing fertilisation to occur using sperm with impaired motility and morphology. It has also been suggested that patients with male factor infertility may have DNA defects in their sperm, as abnormalities such as DNA damage and loosely packed chromatin have already been reported in poor quality semen samples [37–39]. Furthermore, increased levels of DNA damage have been reported in sperm samples with abnormal motility and morphology from patients undergoing ICSI with a negative association between the number of sperm with damaged DNA and success of fertilisation [40]. Whilst it appears that there is an inherent mechanism to prevent the transmission of defective DNA, techniques are needed to screen sperm for DNA damage prior to use for ICSI to reduce the possibility of the incorporation of defective genetic material and to improve the efficacy of the procedure.

In't Veld et al. [41] proposed an increased risk of sex anomalies in children born following ICSI which, given reports of excess chromosomal abnormalities in male and female partners of ICSI candidate couples, would not be entirely unexpected [42,43]. Karyotype analyses of children conceived by ICSI have shown an increased incidence of paternally transmitted structural aberrations and sex-chromosome abnormalities [44–46]. Bowen et al. [47] have reported developmental differences in children conceived by ICSI and, although no cytogenetic tests were carried out on the children, the observation that differences were greater for boys than girls provides some evidence for possible transmission of chromosomal anomalies from father to son. One possible explanation for the reported increase in sex chromosomal aberrations following ICSI is that autosomal aberrations often lead to miscarriage, whereas sex chromosomal aberrations are compatible with full-term pregnancies [48]. Whilst it is possible to predict some chromosomal abnormalities by parental karyotyping, it is by no means definitive and there is an argument to carry out prenatal diagnosis in all ICSI treatment cycles [46].

The recent studies of DeBaun et al. [49], Gicquel et al. [50] and Maher and colleagues [51] have added to growing concerns about the relationship between ART and epigenetic defects in the offspring. Epigenetic phenomena include those involving genomic imprinting where differences in gene expression are controlled by the parental origin of the allele and can entail either rigid monoallelic expression or in some cases preferential or even total expression of the specific parental allele. Changes in gene expression are transmitted during cell division and whilst they do not cause mutations in the DNA code they do involve altered control of DNA expression, e.g. by hypomethylation (activating oncogenes and chromosomal rearrangement) or hypermethylation (affecting tumour suppressor genes) and chromatin modifications. Imprinted genes are often located near CpG islands or GC-rich sequences and show differences in methylation between maternal and paternal alleles. The modification of chromatin structure due to altered methylation along with the methylation status of CpG islands are important elements in the transcriptional regulation of genes. Human embryo studies suggest that reprogramming of the parental imprint occurs during early embryonic development.

Many imprinted genes are involved in foetal growth and development and the expression of these genes appears to be influenced by chemical and physical stresses [35]. It is possible that the stressful process of ICSI may lead to the disruption of these genes. Effects of the loss and function of imprinted genes can be catastrophic, as is evident in Beckwith–Wiedemann (BWS), Prader–Willi (PWS) and Angelman (AS) syndromes, where imprinting defects in the 11p15 (BWS) and 15q11-q13 region (AS and PWS) have been reported [52–56]. AS, which is a rare congenital condition (approximately 0.67 per 10 000 live births), has been reported in children born following ICSI [57,58] and involves a deficient maternal copy of the imprinted gene. As the maternal allele *SNPRN* methylation imprint occurs at the time of fertilisation (or later), it has been suggested that ICSI may have an aetiological role in the cases of AS [59].

Frequent alterations in the imprinting and methylation of several genes on the 11p15 chromosomal segment are associated with BWS including abnormal methylation of *H19* and *LIT1*. In sporadic BWS, *KvDMR1* loss of methylation is the most common molecular abnormality observed [60–62]. In 13 of 14 ART-associated BWS cases analysed to date, loss of methylation at a differentially methylated region (*KvDMR*) in the *KCNQ1* gene has been reported [49–51]. It has been suggested that loss of *KvDMR1* methylation may cause the downregulation of the expression of *CDKN1C* and a loss of imprinting of *IGF2*, both of which are candidate BWS genes [63]. Engel et al. [62] reported that the frequency of *KvDMR1* loss of methylation was higher in children with BWS born following ART compared with those conceived naturally. This suggests that imprinting errors in *KvDMR1* may account for the relationship between ART and BWS.

An association between these types of congenital anomalies and the molecular processes which underlie them and embryonal tumours is well-established and the most frequent associations are with overgrowth syndromes such as Beckwith–Wiedemann and Simpson–Gobai–Behmel, and with congenital anomalies that are features of such syndromes [64–68]. Wilms' tumour is reported most often, occuring in 11% of children with BWS before the age of 4 years, followed by hepatoblastoma and neuroblastoma [66]. Other cancers observed, though less frequently, are rhabdomyosarcoma and adrenocortical carcinoma. A conservative estimate of the incidence of BWS in the general population is 0.13 per 10 000 live births [51]. Hence, whilst childhood cancer is rare, with 1 in 600 children being diagnosed with a malignancy before the age of 15 years, BWS is rarer still – more than a hundred times less common. However, Maher et al. [51] reviewing the notes of 149 patients on a British BWS registry found that six (4%) were born following ART. Based on the assumption that approximately 1% of births in the general population took place following ART, only 1.49 would have been expected. Three of Maher's six cases were conceived by IVF and three by ICSI. Similar findings have been reported from a French registry by Gicquel et al. [50] who also identified six children born following ART amongst 149 patients with BWS, against 1.94 expected (Odds Ratio (OR) = 3.2, 95% Confidence Interval (CI), 1.4–7.3). Two of these six children were conceived by ICSI. These kinds of observations have refuelled interest in the possible direct relationship between ART procedures and childhood cancer.

## 3. Childhood cancer

The causes of childhood cancer remain largely unknown. Epigenetic mechanisms are increasingly believed to play an important role in human carcinogenesis, independently of their role in congenital genetic syndromes such as BWS, AS or PWS [69]. Disrupted gene expression such as loss of imprinting of growth regulatory genes has been identified in a variety of childhood tumours, including loss of imprinting of the insulin-like growth factor 2 gene in Wilms' tumour [70]. It is possible that the drugs and procedures involved in ART may lead to epigenetic modification of DNA and alter imprinted gene expression, potentially resulting in the development of cancer in the offspring.

In contrast to the many reports of an association between ART and BWS, reports on cancer in children born after ART or fertility drug treatment are limited. There have been a number of case reports which, although interesting observations, do not provide proof of a causal relationship. Melamed et al. [71] reported hepatoblastoma in a child conceived after maternal treatment with clomiphene and perganol; and there have been two subsequent reports of hepatoblastoma occurring in children conceived by IVF [72,73]. In 2001, Anteby et al. [74] reported retinoblastoma in one of 47 children with ocular abnormalities conceived by IVF, and soon after Cruysberg et al. [75] reported a second child with retinoblastoma conceived by IVF. The latter is one of the five children later described by Moll et al. [76]. The remaining case reports of childhood cancer following IVF conception include three children with neuroectodermal tumours – one medulloblastoma, one neuroblastoma, one neuro/ganglioblastoma [77]; one child with clear cell sarcoma [72]; one case of a malignant testicular tumour [78]; and one case with a congenital brain tumour [79].

More importantly, there have been six studies directly reporting the incidence of childhood cancer in cohorts of children conceived by IVF, and/or following other assisted reproduction techniques or born to women evaluated for infertility (Table 3). They include reports from the UK [80], Sweden [19], Australia [81], Israel [82], the Netherlands [83] and Denmark [84]. Findings from

Table 3
Incidence of childhood cancer in cohorts of children born following assisted reproductive techniques

| Authors (year) | Design/participants | Incidence |
|---|---|---|
| Doyle et al. (1998) | 2507 children born after ART (UK; 1978–1991 births) | 2 cancers observed, 3.5 expected, SIR 0.57 (95% CI, 0.07–2.06) |
| Bergh et al. (1999) | 5856 children conceived by IVF (Sweden; 1982–1995 births) | 4 cancers observed, 3.6 expected |
| Bruinsma et al. (2000) | 5249 children born after ART (Australia; 1979–1995 births) | 6 cancers observed, 4.3 expected, SIR 1.39 (95% CI, 0.62–3.09) |
| Lerner-Geva et al. (2000) | 332 children conceived by IVF (Israel, 1981–1994 births) | No cancers observed, 1.7 expected |
| Klip et al. (2001) | 17 000 children born to women diagnosed with sub-fertility problems: 9479 born after ART (8080 conceived by IVF); 7521 conceived naturally (Netherlands, 1980–1995 births) | 16 cancers observed, 15.5 expected, SIR 1.0 (95% CI, 0.6–1.7), RR for ART compared with natural conceptions = 0.8 (95% CI, 0.3–2.3), RR for IVF compared with natural conceptions = 0.8 (95% CI, 0.2–2.4) |
| Brinton and colleagues (2004) | 51 063 children born to 30 364 women evaluated for infertility (Denmark, 1953–1996) | 51 cancers observed, 44.7 expected, SIR 1.14 (95% CI, 0.8–1.5) |

ART, assisted reproductive techniques; IVF, *in vitro* fertilisation; SIR, standard incidence ratio; RR, risk ratio; CI, confidence interval.

all these studies are reassuring although the authors of some concede that their cohorts are not large enough to detect an increase in incidence, even if one existed, and have not been followed up for long enough [80,82]. Although cancer risk in the Danish cohort was reported to be comparable to that in the general childhood population [84], the authors suggested a possible, but non-significant, increased risk of haematological malignancies (mainly leukaemias) following the use of the ovulation stimulating drugs clomiphene (RR = 1.78, 95% CI, 0.6–4.8) and human chorionic gonadotrophin (RR = 1.54, 95% CI, 0.5–4.7). Although this is the largest study to date, even its finding was based on relatively small numbers (51 cancers reported in total, 19 haematopoietic tumours). Pooling results from the four studies which report cancer incidence in ART children and provide sufficient information [19,80,81,83] give an SIR of 1.03 (95% CI, 0.61–1.63).

Case-control (7) and other studies constitute the remaining nine reports of cancer in children conceived by ART, or with a parental history of infertility (Table 4). Kobayashi et al. [85] identified nine children born

Table 4
Case-control and other studies of childhood cancer reporting findings for parental infertility and/or conception following assisted reproductive techniques

| Authors (years) | Participants, methods and results |
|---|---|
| van Steensel-Moll et al. (1985) | 519 cases <15 years with acute lymphocytic leukaemia; 507 controls. Postal questionnaires sent to parents. OR 6.0 (95% CI, 0.9–38.2) for history of fertility problems; OR 1.9 (95% CI, 1.0–3.5) for prenatal exposure to 'drugs to maintain pregnancy' |
| Kramer et al. (1987) | 104 children with neuroblastoma; 101 controls. Telephone interview with parents. OR 2.25 (90% CI, 1.14–4.44) for exposure to sex hormones in the 3 months before or during pregnancy |
| Kobayashi et al. (1990) | 6236 children diagnosed 1985–1989 on the Japan Children's Cancer Register. 9 children born after maternal ovulation induction; 4/887 with neuroblastoma ($P = 0.001$ *vs.* other malignancies); 2/517 with lymphoma ($P = 0.013$) |
| Schwartzbaum (1992) | 101 children <9 years with neuroblastoma (cases); 690 with other cancers (controls). Interview with mothers. OR 1.2 (95% CI, 0.6–2.3) for prenatal sex hormone exposure |
| Michalek et al. (1996) | 183 cases <15 years with neuroblastoma; 372 controls. Telephone interview with mothers. OR 3.0 (95% CI, 1.3–6.9) for any sex hormone exposure during pregnancy; OR 10.4 (95% CI, 1.2–90.0) if sex hormone treatment was for infertility |
| Roman et al. (1997) | 177 cases <30 years with leukaemia or non-Hodgkin's lymphoma; 354 controls. Medical records-based study. OR 2.1 (95% CI, 0.9–4.6) for history of infertility investigation |
| Olshan et al. (1999) | 504 cases <19 years with neuroblastoma; 504 controls. Telephone interview with mothers. OR 1.4 (95% CI, 0.9–2.1) for history of infertility; OR 1.6 (CI 0.8–3.0) for ever use of clomiphene |
| Schüz et al. (1999) | 2358 cases <15 years with cancer (1184 with acute leukaemias); 2588 controls. Postal questionnaires and telephone interviews with parents. OR 1.6 (95% CI, 1.0–2.5) for hormonal treatment for infertility for acute leukaemias |
| Moll et al. (2003)[a] | 5 children with retinoblastoma born after IVF (1 after ICSI); relative risks based on assumption that 1.0–1.5% of children in the Netherlands conceived by IVF. If 1.0% all births after IVF, RR: 7.2 (95% CI, 2.4–17.0); if 1.5% all births after IVF, RR: 4.9 (95% CI, 1.6–11.3) |

OR, odds ratio; CI, confidence intervals; IVF, *in vitro* fertilisation; ICSI, intracytoplasmic sperm injection; RR, risk ratio.
[a] Includes the case reported by Cruysberg et al. in 2002.

after maternal ovulation induction amongst 6236 children diagnosed between 1985 and 1989 and on the Japan Children's Cancer Registry. They reported a significantly increased risk for neuroblastoma (4/887; $P = 0.001$ vs. other malignancies) and also for lymphoma (2/517; $P = 0.013$ vs. other malignancies). Four of the case-control studies also concern neuroblastoma. Kramer and colleagues [86] and Michalek et al. [87] reported significantly raised odds ratios (OR) for exposure to sex hormones during or in the 3 months before pregnancy (OR = 2.25, 90% CI, 1.14–4.44; and OR = 3.0, 95% CI, 1.3–6.9, respectively). Conversely, Schwartzbaum [88] reported no significant association for prenatal sex hormone exposure (OR = 1.2, 95% CI, 0.6–2.3); and Olshan et al. [89] reported no significant associations for a maternal history of infertility (OR = 1.4, 95% CI, 0.9–2.1) or ever use of clomiphene (OR = 1.6, 95% CI, 0.8–3.0).

The three remaining case-control studies reported findings for leukaemia and/or non-Hodgkin's lymphoma. Van Steensel-Moll et al. [90] reported a significantly increased risk for prenatal exposure to 'drugs to maintain pregnancy' (OR = 1.9, 95% CI, 1.0–3.5), but not for a history of fertility problems (OR = 6.0, 95% CI, 0.9–38.2) in children with ALL. Roman et al. [91] in a medical records-based study reported a non-significant OR of 2.1 (95% CI, 0.9–4.6) for leukaemia or non-Hodgkin's lymphoma in children and young adults diagnosed under 30 years of age who were born to mothers who had undergone infertility investigations. Schüz et al. [92] reported a significantly raised Odds Ratio for maternal hormonal treatment for infertility in children with acute leukaemias (OR 1.6, 95% CI, 1.0–2.5).

The final and most recent report of note concerns five Dutch children with retinoblastoma, four of whom were conceived by IVF and one by ICSI [76]. They reported a significantly raised RR of 7.2, assuming 1.0% of all Dutch births followed IVF (95% CI, 2.4–17.0), and 4.9, assuming 1.5% of all births arise in this way (95% CI, 1.6–11.3). However, a problem with all these studies is that they are based on small numbers, and most may also be subject to recall bias [86–90,92].

It is difficult to make direct comparisons as there are differences among the studies, for example, in relation to the timing and exact nature of treatments, but for some cancers there may possibly be an increased risk.

## 4. Summary

The findings to date suggesting that gamete and/or embryo manipulation or the use of fertility drugs are related to adverse outcomes should not be over-interpreted. However, there is some basis for concern as the findings from case-control studies suggest an increased risk of some specific cancers, although findings from cohort studies suggest essentially a null risk. Even if the effects are real, it might nonetheless be the medical reason for the infertility rather than the procedure itself that is the most important predisposing factor. Infertile couples treated with ART may already have an increased number of epigenetic defects in their gametes, which come to light during the ART process. It will be important for future investigations to ascertain the underlying causes of infertility. Epigenetic changes are unlikely to be caused by the ART process if they prove only to be found in couples where ovarian failure or sperm mutational defects are a problem, and not in those with purely mechanical problems such as blocked tubes.

There is need for more detailed investigation on the outcomes of ART in children conceived by these techniques, especially on the risk of developing cancer. Future studies need to include large enough numbers, have an adequate control population, and a long enough follow-up period. Complementary laboratory research into the effects on pregnancy outcomes of culture conditions, including the type of media used and length of embryo culture prior to transfer, and gene imprinting and subsequent birth defects is vital in gaining a better understanding of the ART processes. Further cohort studies linked with national cancer registry data are needed; and, although logistically challenging, pooling of cohort studies from different countries, or national multi-centre studies, would provide ways of increasing the numbers available.

The UK's National Health Service (NHS) has undertaken to fund one free IVF treatment cycle for infertile couples by April 2005, provided the women is under the age of 40 years. A recommendation has also been made for the NHS to offer intra-uterine insemination (IUI). These new measures will lead to increasing numbers of children born following ART, despite the fact that long-term outcomes for these children remain largely unknown. Within the UK, the HFEA has a unique population-based registry of children born following ART. However, the data remain confidential under the Human Fertilization and Embryology Act (1990). A UK Medical Research Council (MRC) working party is currently considering the need for access to this data, to enable more research on the long-term outcomes for children. Some small studies in European countries have been undertaken based on locally assembled registers (Netherlands) or on nationwide indicators of a subfertility (treatment) episode (Sweden, Denmark, UK). Australia has also contributed, but there is a notable lack of relevant studies from within the United States of America (USA), despite widespread use of these techniques.

## Conflict of interest statement

None declared.

## Acknowledgements

We gratefully acknowledge the help of Mike Bradburn and Anna Cargill (Cancer Research UK Medical Statistics Group) in producing the pooled estimate of cancer risk in ART children. The work of the Childhood Cancer Research Group is supported by the Department of Health and the Scottish Executive. The work of the Epidemiology and Genetics Unit at the University of York is supported by the Leukaemia Research Fund.

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