Title: Cancer risk in children born after ART

Authors: Adam Arshad[1], Alastair G Sutcliffe[2]

Departments and Institutions:

[1]North Middlesex University Hospital, Sterling Way, London N18 1QX

[2]University College London and Great Ormond Street Institute of Child Health, London

Corresponding author contact information:

Name: Professor Alastair Sutcliffe

Policy, Practice and Populations Unit, University College London, Great Ormond Street Institute of Child Health, 30 Guildford Street, London

Email: a.sutcliffe@ucl.ac.uk

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English Abstract

The influence of Assisted Reproductive Technologies (ART) on the risk of childhood cancer is poorly understood. We sought to summarise the current literature as to the risk of cancer in children born from ART. Overall, we identified that the current evidence is divergent, with the results of several cohort studies and meta-analyses on this subject ranging from there being no risk or to there being an increased risk from being conceived by ART. Further large-scale cohort studies to determine the influence of ART on cancer risk are required. If a valid risk is present, experimental studies are necessary to determine whether this risk is associated with the use of fertility drugs or is due to the parental infertility.

German Abstract

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Introduction

Since 1978 more than 8 million children worldwide have been born via Assisted Reproductive Technology (ART), with the use of ART increasing by 2.5% per year in the UK (1). It has been well established that children conceived via ART are at risk of adverse perinatal outcomes, including an increased risk of preterm birth, low birth weight and congenital defects (2, 3). However, the association between ART and cancer risk in children is less clear. The results from the contemporaneous literature range from there being an increased cancer risk, to there being no risk or to even a protective effect from receiving ART (4, 5). This article overviews the current literature as to the risk of childhood cancer after ART.

Pathophysiology

The aetiology of childhood cancers is complex. It has been suggested that fertility treatments may increase the risk of their occurrence in children. This could be mediated by epigenetic changes caused by the fertility drugs, the ART process or both. Alternatively, these genetic changes that increase the risk of childhood cancer may be pre-existent within the gametes of the infertile partner/partners.

Epigenetic Changes Secondary to Fertility Drugs

It is considered that childhood cancer pathways may start in early foetal development, where exposure to fertility treatments leads to epigenetic changes in the gametes or embryo. Exposure to fertility medications occurs at each phase of the ART procedure, including during stimulation of the follicles, the process of oocyte retrieval, the culture of the embryo's, cryopreservation and during embryo transfer.

Evidence to support this hypothesis comes from studies that have assessed genetic imprinting. Imprinting is an epigenetic form of gene regulation that regulates the expression of certain genes depending on their parental origin (6). Aberrant functioning of these imprinting genes is associated with an increased risk of both childhood (retinoblastoma, neuroblastoma and acute myeloid leukaemia) and adult cancers (breast, bladder and cervical cancer) (7).

The development and maintenance of imprinting genes occurs during gametogenesis and embryogenesis. However, environmental and physical stressors can cause abnormalities in these genes (8). Fertility treatments may act as a chemical stressor, with the medications influencing either their formation or function.

There is a paucity of evidence exploring the influence of fertility treatments on the imprinting genes in humans. However, there is better evidence in animal studies. Observational studies have noted that children born after the use of frozen embryo transfer have a higher risk of being large for gestational age (9). It is probable that epigenetic changes secondary to the cryopreservation process result in this occurrence. Furthermore, studies have reported an altered gene expression profile in the placenta, embryo's and cord blood of singletons conceived by ART compared to naturally conceived children (10, 11). In addition,

in an experimental study of mice undergoing hormonal superovulation, mouse embryos were identified to have altered methylation of the imprinting genes (12). A similar finding has been demonstrated in ruminant animals, where they have been seen to develop a condition termed large offspring syndrome secondary to the abnormal methylation of the imprinting gene IGF2R (13).

Large offspring syndrome can also occur in humans, where it is called Beckwith-Wiedemann syndrome. This syndrome was first recognised to be associated with ART in a study of children born after embryo cryopreservation (14). Children with this condition have an increased risk of hepatoblastoma and rhabdomyosarcoma (15). A study by Eroglu et al. observed that 92% of Beckwith-Wiedemann patients who were conceived by ART had epigenetic changes. Only 55% were expected to have these changes. This adds to the link between fertility treatments and imprinting errors (6).

Subfertility

Parental subfertility may also be the underlying cause of the cancer risk associated with ART. Studies have demonstrated that the gametes of infertile couples have an increased number of epigenetic changes (16). Furthermore, males with fertility problems are noted to have DNA defects in their sperm, with loosely packed chromatin and DNA damage (17). Altogether these faults may increase the risk of childhood cancer.

Study Findings

To our knowledge, there have been 9 registry-based cohort studies that have recently assessed the risk of cancer from ART (Table 1). While smaller studies have been conducted on this subject, given the rarity of childhood cancers, we chose to only discuss studies with at least 10 cancer cases.

Seven of these studies demonstrated no overall increase in the risk of cancer from ART (18-24). However, several studies demonstrated an increased risk in the cancer subtypes in the ART cohort. Spaan et al. showed a non-significant increase in the risk of lymphoblastic leukaemia (HR=2.44, 95% CI: 0.81–7.37) and melanoma (HR=1.86, 95% CI: 0.66-5.27) (20). Furthermore, Williams et al. reported no overall increase in the risk of cancer, but in exploratory analysis, an increased risk of Hepatoblastoma (SIR: 3.64, 95% CI: 1.34–7.93, p=0.02) and Rhabdomyosarcoma (SIR: 2.62, 95% CI: 1.26–4.82, p=0.02) amongst ART children (18). This study assessed 106,013 ART patients in the United Kingdom and compared their risk of cancer development against the incidence rates in the general population. The investigators attributed the increased risk of hepatic tumours to birth weight, with children < 1000 grams (g) being at the greatest risk. A repeated study by the same team published in 2018 assessed the risk of cancer for children born after donor ART only (19). Reassuringly this study also reported no overall increased risk of cancer, but an elevated incidence of hepatoblastoma amongst children with a birth weight <2500g.

Similar findings were reported in the largest study on this topic to date (25). Spector et al. conducted an American cohort study assessing the risk of childhood cancer among 275,686 IVF conceived and 2,266,847 naturally conceived children. No association was identified between IVF and cancer risk. This non-significant result was maintained when comparing children conceived by donor eggs vs. autologous eggs, frozen embryos vs. fresh embryos, ICSI vs. no ICSI, assisted hatching vs. no assisted hatching and by day of embryo transfer. However, a significantly elevated rate of hepatic tumours (HR: 2.46, 95% CI: 1.29 –4.70) and Rhabdomyosarcoma (HR: 1.50, 95% CI: 0.81–2.84) was reported. Not dissimilar to the UK study, these findings had large confidence intervals reflecting the rarity of the incident cancers

A Scandinavian study assessed 91,796 children conceived by IVF and 358,419 children conceived naturally in Denmark, Finland, Norway and Sweden from 1982 to 2007. The average follow-up was 9.5 years. The investigators determined that IVF was associated with a non-significant increased risk of overall cancers (HR: 1.08, 95% CI: 0.91 – 1.27). However, a significant increased risk of central nervous system tumours (HR: 1.44, 95% CI: 1.01–2.05) and combined carcinomas (HR: 2.03, 95% CI: 1.06–3.89) was noted (22).

This increased risk of neurological tumours was observed by Hargreaves et al, who assessed the Danish population registry. The investigators identified an increased risk of cancer amongst recipients of frozen embryo transfer ART (HR: 2.43, 95% CI: 1.44–4.11). In subgroup analysis, this increased risk was primarily due to an enhanced risk of leukaemia (HR: 2.87, 95% CI: 1.19–6.93) and sympathetic nervous system tumours (HR: 7.82, 95% CI: 2.47–24.70). Interestingly, this finding was restricted to only frozen embryo transfer ART. Use of IVF or ICSI was not associated with an increased cancer risk (26). Overall, the increased risk of leukaemia after ART has also been reported in other cohort studies (20, 23)

The results of these cohort studies differ from three of the four meta-analyses conducted on this subject area. A recent meta-analysis by Wang et al. reviewed 48 articles, combining a total 327,884 children and 578 cancer diagnoses. The investigators stated that children conceived by ART had a higher risk of developing cancer (RR: 1.16, 95% CI: 1.01–1.32), haematological malignancies (RR: 1.39, 95% CI: 1.21–1.60) and other solid tumours (RR: 1.57, 95% CI: 1.14–2.16). With regards to the specific cancers, fertility treatments were associated with a significant increased risk of leukaemia (RR: 1.31, 95% CI: 1.09–1.57) and hepatic tumours (RR: 2.26, 95% CI: 1.32–3.85) (5).

These findings were corroborated by two other meta-analyses. Hargreaves et al demonstrated in their meta-analysis of 25 cohort and case-control studies an increased risk of all cancers, haematological malignancies, leukaemia, neuroblastoma and retinoblastoma from ART (all $p < 0.05$) (27). A differing analysis of 11 cohort studies on this topic identified an increased risk of cancer from ART (RR: 1.33, 95% CI: 0.62-2.85). While this increased risk was not statistically significant, this was considered to be due to the small sample size (as only 11 studies were included in the analysis). Furthermore, in this meta-analysis all types of cancer were assessed together, which could have disguised specific increases in the risk of the cancer subtypes (28). Only one of the four

meta-analyses identified no association between ART and childhood cancer. (21). However, a significant proportion of the included studies in this analysis were of a poor design (specifically case-control studies). Cohort studies that use registry population data from the UK, USA and Nordic countries are considered to be the most methodologically robust.

Limitations to the existing literature

Given the limited and conflicting existing literature, we require further studies to investigate the relationship between childhood cancer and ART. These studies must provide an adequate length of follow-up to determine the childhood cancer risk. While most childhood cancers occur before the age of 5, a few develop in adolescence (e.g. Hodgkin's lymphoma and bone tumours). A longer follow-up period would allow for the detection of these malignancies. Furthermore, these studies must have a large enough cohort to allow for the detection of the cancers and adequately analyse cancer risk. Paediatric cancers are rare, and a limited sample size reduces the statistical accuracy. Within this large sample size, the differing forms of ART treatment must be sub-analysed. This is with interest to children conceived with frozen embryo transfer. Freezing and thawing has been implicated to cause epigenetic changes in animal studies (29), with the analysis by Hargreaves et al. identifying an increased risk of cancer after frozen embryo transfer only (26). Finally, within these future studies cancer risk must be adjusted for confounding factors that could influence the relationship between ART and cancer. These include maternal age, maternal smoking status and previous foetal loss (which is associated with an increased risk of childhood leukaemia in subsequent children).

Based on the current literature, we cannot infer that fertility treatments increase the risk of childhood cancer. In particular, the increased risk that some of the studies reported could be due to the underlying parental infertility. Further research is required to detail the relationship between fertility treatment, parental infertility and cancer risk. If future studies identify epigenetic defects in couples with abnormalities in spermatogenesis or ovarian failure, but not in those whose infertility is secondary to mechanical problems (e.g. tubal blockage), then we can be more inclined to consider whether fertility treatments result in epigenetic changes of the embryo.

Several studies have reported an association between ART and the risk of hepatoblastoma in long-term follow-up. We have discussed how ART is associated with an increased risk of Beckwith-Wiedemann syndrome, which is known to increase the risk of hepatoblastoma and rhabdomyosarcoma. In the study by Williams et al, none of the 16 children who developed hepatoblastoma had an imprinting disorder (18). The authors stated that if imprinting disorders were present, they were either subclinical or had not been reported by

physicians. In the study by Spector et al., no information was available as to the risk of Beckwith-Wiedemann syndrome in the cohort.

Conclusion

The current literature indicates that there may be a potential association between ART and an increased risk of childhood cancer. However, this risk, if at all present, appears to be low.

While this should provide reassurance to individuals seeking ART, greater clarity as to the risk of tumorigenesis is required. To do this, further follow-up of children conceived by ART for cancer occurrence is required. Future studies must have an adequate number of participants, with the potential pooling of studies to help determine the true risk that ART provides.

Furthermore, an attempt to understand the potential mechanism for this putative increased cancer risk should be undertaken. Overall the senior author of this study is participating in several international collaborations (involving American, Nordic, Dutch and Australian groups) which will help improve understanding on whether any putative risks are merely random highs, or there is a pattern with even greater numbers. Watch this space.

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Table 1: Recent cohort studies that have assessed the cancer risk amongst children conceived by ART

Study	Number of ART children	Number of Cancers in ART cohort	Overall Cancer Risk	Specific Cancers increased risk
Williams et al. 2013	106,013	108	SIR: 0.98 (95% CI: 0.81 – 1.19)	Hepatoblastoma, Rhabdomyosarcoma
Sundh et al. 2014	91,796	181	HR: 1.08 (95% CI: 0.91 – 1.27)	CNS, Epithelial
Reigstad et al. 2016	25,782	51	HR: 1.21 (95% CI: 0.90 – 1.63)	-
Lerner-Greva et al. 2017	9,042	21	RR: 1.18 (95% CI: 0.80 – 1.75)	Retinoblastoma, Renal
Williams et al. 2018	12,137	12	SIR: 0.83 (95% CI: 0.43 – 1.45)	Hepatoblastoma
Spector et al. 2019	275,686	321	HR 1.17 (95% CI: 1.00 – 1.36)	Hepatic
Spaan et al. 2019	24,269	93	HR 1.00 (95% CI 0.72 – 1.38)	ALL (NS), Melanoma (NS)
Gliboa et al. 2019	64,317	85	OR: 0.95 (95% CI: 0.76 – 1.19)	-
Hargreave et al. 2019	36,221	90	HR: 1.20 (95% CI: 0.96 – 1.49)	Leukaemia, Sympathetic nervous system cancers

* ART: Assisted Reproductive Technologies; ALL: Acute lymphoblastic leukaemia; CNS: Central Nervous System; HR: Hazard Ratio; OR: Odds Ratio; SIR: Standardised Incidence Ratio; NS: Non-significant