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Imprinting disorders in children born after ART: a Nordic study from the CoNARTaS group

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STUDY QUESTION: Is the risk of imprinting disorders increased in children conceived after ART?

SUMMARY ANSWER: We found an adjusted odds ratio (AOR) of 2.84 [95% CI: 1.34–6.01] for Beckwith–Wiedemann syndrome in ART children, while the risk of Prader–Willi syndrome, Silver–Russell syndrome or Angelman syndrome was not increased in children conceived after ART.

WHAT IS KNOWN ALREADY: Earlier studies, most of them small, have suggested an association between ART and imprinting disorders.

STUDY DESIGN, SIZE, DURATION: This was a binational register-based cohort study. All children conceived by ART in Denmark ($n = 45\,393$, born between 1994 and 2014) and in Finland ($n = 29\,244$, born between 1990 and 2014) were identified. The full background populations born during the same time periods in the two countries were included as controls. Odds ratios of imprinting disorders in ART children compared with naturally conceived (NC) children were calculated. The median follow-up time was 8 years and 9 months for ART children and 11 years and 9 months for NC children.

PARTICIPANTS/MATERIALS, SETTING, METHODS: From the national health registries in Denmark and Finland, we identified all children diagnosed with Prader–Willi syndrome ($n = 143$), Silver–Russell syndrome ($n = 69$), Beckwith–Wiedemann syndrome ($n = 105$) and Angelman syndrome ($n = 72$) born between 1994/1990 and 2014, respectively.

MAIN RESULTS AND THE ROLE OF CHANCE: We identified a total of 388 children diagnosed with imprinting disorders; 16 of these were conceived after ART. The overall AOR for the four imprinting disorders in ART children compared with NC children was 1.35 [95% CI: 0.80–2.29], but since eight ART children were diagnosed with Beckwith–Wiedemann syndrome, the AOR for this specific imprinting disorder was 2.84 [95% CI: 1.34–6.01]. The absolute risk of Beckwith–Wiedemann syndrome in children conceived after ART was still low: 10.7 out of 100 000 newborns. The risks of Prader–Willi syndrome, Silver–Russell syndrome and Angelman syndrome were not increased in children conceived after ART.

LIMITATIONS, REASONS FOR CAUTION: Imprinting disorders are rare events and our results are based on few ART children with imprinting disorders. The aetiology is complex and only partly clarified, and the clinical diagnoses are challenged by a broad phenotypic spectrum.

WIDER IMPLICATIONS OF THE FINDINGS: In the existing studies, results on the risk of imprinting disorders in children conceived after ART are ambiguous. This study adds that the risk of imprinting disorders in ART children is very small and perhaps restricted to Beckwith–Wiedemann syndrome.

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Introduction

Since the early days of ART, concern has prevailed whether these techniques may influence the health of the offspring. Most published studies on child outcomes have been reassuring, although the risks of preterm birth and congenital malformations are moderately increased after ART (Pandey *et al.*, 2012; Hansen *et al.*, 2013). Conflicting results have emerged on the risk of imprinting disorders, which are characterized by molecular changes affecting imprinted chromosomal regions or genes that are expressed in a parent-of-origin specific manner (Doornbos *et al.*, 2007; Gold *et al.*, 2014; Lazaraviciute *et al.*, 2014; Eggermann *et al.*, 2015). Since methylation takes place during the preimplantation stages of embryonic development, where the embryo is handled *in vitro* and cultured for up to 6 days in culture media, ART may disturb the DNA methylation, resulting in imprinting errors. Furthermore, inherited epigenetic defects in the gametes may be more frequent in infertile men and women, causing an underlying increased risk of imprinting disorders, with or without ART (Niemitz and Feinberg, 2004). Ludwig *et al.* (2005) showed that untreated couples with a time-to-pregnancy of more than 2 years had an increased risk of imprinting disorders, although they were not able to determine whether the gametes, the embryo culture or the embryo manipulation were associated with the increased epigenetic instability.

The mechanisms behind imprinting disorders are difficult to assess; firstly, due to the rarity of these disorders, as each disorder affects only 1–10 per 100 000 newborns. Secondly, these disorders may be the result of not only imprinting mechanisms but also of more classical genetic point mutations or microdeletions, which are conditions not expected to be influenced by *in vitro* techniques. Thirdly, the phenotype for each of these conditions has a relatively wide clinical spectrum, and therefore, the specific diagnosis is often not made until several years after birth. Fourthly, the cytogenetic techniques necessary to establish the exact mechanism behind each case have developed rapidly over the past two decades, which means that an increasingly higher percentage of children with these disorders are now diagnosed. And finally, there is no consensus on which disorders should be classified as imprinting disorders. The aim of this study was to assess the prevalence of Prader–Willi syndrome, Silver–Russell syndrome, Beckwith–Wiedemann syndrome and Angelman syndrome in children born after ART compared with children born after natural conception.

Materials and Methods

This cohort comprised all live-born children born in Denmark during the period 1994–2014 and in Finland between 1990 and 2014. In total, 74 637 ART children (singletons, $n = 53\,045$; twins, $n = 21\,592$) and 2 775 542 NC children (singletons, $n = 2\,701\,302$; twins, $n = 74\,240$) were included in the study. All triplets and quadruplets were excluded, but among them, no children were diagnosed with imprinting disorders. Children conceived after medically assisted reproduction without *in vitro* techniques, herein, ovarian stimulation or ovulation induction and inseminations, were included in the control group of NC children. All data were collected from the relevant national health registries in Denmark and Finland. In Denmark, the 10th version of International Statistical Classification of Diseases and Related Health Problems (ICD-10) was used throughout the study period. In Finland, the ICD-9 version was used until 1995 and the ICD-10 version was used since 1996. The Finnish Register on Congenital Malformations have an additional text following the diagnosis code specifying the syndrome. In Denmark, detailed information on the ART procedures was available from the national ART registry, whereas in Finland, information on the use of ART or not was the only available in the Finnish Medical Birth Registry, and therefore, differentiation between the different ART methods was not possible.

Statistical analyses

We described the characteristics of the mothers of children with and without imprinting disorders, born after ART and NC, respectively. Normally distributed quantitative data were summarized by means and SDs and compared using the two-sample *t*-test. Non-normally distributed quantitative data were summarized in median and quantiles and compared using the Mann–Whitney *U*-test. Categorial data were summarized in numbers and percentages and compared using Fisher's exact test. The odds ratios (ORs) of having one of the four imprinting disorders were calculated with 95% CIs. Multiple logistic regression analyses were used to adjust for maternal age, parity (nulliparous versus multiparous), year of birth, child's sex, plurality, BMI, smoking and country.

Ethical approval

In Denmark and Finland, ethical approval is not required for scientific projects solely based on registry data.

Results

Demographics

The mothers of children diagnosed with an imprinting disorder were slightly older than the mothers of children without imprinting disorders, both in the ART and NC group, but it was only statistically significant in the NC group (Table I). The prevalence of imprinting disorders did not differ significantly between boys and girls, although the figures were higher for boys among both ART and NC children (Table I). The median follow-up time was 8 years and 9 months for ART children and 11 years and 9 months for NC children. The median age at time of

Table I Descriptive data of children, with and without imprinting disorders, and their mothers.

	Assisted reproductive technology (ART)			Natural conception (NC)			ART vs NC
	Imprinting disorder	No imprinting disorder		Imprinting disorder	No imprinting disorder		
	n = 16	n = 74 621	P	n = 372	n = 2 775 239	p	p
Maternal age (mean ± SD)	35.8 ± 4.2	33.7 ± 4.3	0.06	31.1 ± 5.4	30.0 ± 5.1	<0.001	
Smokers (%)	0.0	8.0	0.25	17.7	16.1	0.43	
BMI > 30 (%)	0.0	5.7	0.32	6.7	5.3	0.23	
Nulliparous (%)	62.5	67.5	0.67	41.2	41.6	0.87	
Boys (%)	68.8	50.9	0.15	53.9	51.2	0.30	
Age at diagnosis (median, interquartile range, months)	11 [2–23]			30 [6–69]			0.20
Follow-up (median, interquartile range, years)	7.0 [3.2–10.9][1]	8.9 [4.1–14.0][2]		10.7 [6.0–16.9][1]	11.8 [5.9–17.6][2]		0.02[1]; <0.001[2]
Follow-up (median, interquartile range, years)	8.8 [4.1–14.0]			11.8 [5.9–17.7]			<0.001

diagnosis was 11 months (interquartile range, 2–23 months) for ART children and 30 months (interquartile range, 6–69 months) for NC children; $P = 0.20$.

Imprinting disorders

We identified 388 children diagnosed with the four imprinting disorders: Prader–Willi syndrome, Silver–Russell syndrome, Beckwith–Wiedemann syndrome and Angelman syndrome in Denmark and Finland during the study period. Of these children, 16 were conceived after ART (Table II). The prevalence of imprinting disorders was 21.4 per 100 000 born in the ART group and 13.4 per 100 000 in the NC group. The overall OR of imprinting disorders after ART was 1.60 [95% CI: 0.97–2.65] (Table II). After adjusting for maternal age, parity (nulliparous versus multiparous), year of birth, child's sex, smoking, BMI and country, the adjusted odds ratio (AOR) for imprinting disorders among ART children was 1.35 [95% CI: 0.80–2.29]. When investigating the four imprinting disorders separately, we found an increased OR in children conceived after ART only for Beckwith–Wiedemann syndrome: OR, 3.07 [95% CI: 1.49–6.31]. This increased risk persisted after adjustment for potential confounders: AOR, 2.84 [95% CI: 1.34–6.01]. We found no significant differences between children conceived after ART and NC for Prader–Willi syndrome, Silver–Russell syndrome and Angelman syndrome. Among the nine Danish ART children diagnosed with an imprinting disorder, three children were conceived after IVF ($n = 24\,760$) and six children after ICSI ($n = 15\,517$). None of the children were conceived after transfer of a frozen-thawed embryo.

Discussion

In this cohort study of all children born in Denmark and Finland over 25 years, the overall risk of the four imprinting disorders together was not increased. However, we found a significantly higher risk of Beckwith–Wiedemann syndrome, also in the adjusted analysis, AOR 2.84 [95% CI: 1.34–6.01]. The increased risk of Beckwith–Wiedemann syndrome after ART is in line with several other studies demonstrating a potential association between ART and Beckwith–Wiedemann syndrome (Halliday *et al.*, 2004; Lim *et al.*, 2009; Mussa *et al.*, 2017; Johnson *et al.*, 2018). However, in our cohort, we were not able to demonstrate a potential association between ART and Prader–Willi syndrome, Silver–Russell syndrome and Angelman syndrome, as suggested by others (Cortessis *et al.*, 2018; Hattori *et al.*, 2019).

Imprinting is an epigenetic phenomenon restricting gene expression to one parental allele, while the other allele is inactivated (White *et al.*, 2015). Epigenetic modifications are an important way of controlling gene activity, without altering the DNA sequence, and it is recognized that epigenetic alterations may increase the risk of various diseases later in life, such as diabetes, hypertension and cardiovascular diseases, as well as cancer (Niemitz and Feinberg, 2004; Bateson *et al.*, 2004). Throughout the genome, there are several phases of epigenetic programming during gametogenesis and early embryo development, coinciding with the period of *in vitro* manipulation in ART (Butler, 2009). A common process for controlling gene activity is methylation, often causing inactivation of the gene, and a high frequency of imprinted methylation errors in human preimplantation embryos has been demonstrated (White *et al.*, 2015). Imprinting disorders are a group of congenital disorders with common underlying epigenetic aetiologies, where alterations affecting imprinting genes or chromosomal regions result in clinical features affecting growth, development and metabolism. Imprinting disorders related to ART might take place just after fertilization at a time, where the epigenome could be most vulnerable, and a recent meta-analysis has suggested a positive association between ART and Prader–Willi syndrome, Silver–Russell syndrome, Beckwith–Wiedemann syndrome and Angelman syndrome (Cortessis

Table II Risk of Prader–Willi syndrome, Silver-Russel syndrome, Beckwith–Wiedemann syndrome and Angelman syndrome in Finnish and Danish children born from 1990/1994 to 2014.

	Prader-Willi syndrome	Silver-Russell syndrome	Beckwith-Wiedemann syndrome	Angelman syndrome	All four imprinting disorders
Assisted reproductive technology (ART)					
Children born	74 621	74 621	74 621	74 621	74 621
Imprinting	5	<3[#]	8	<3[#]	16
Rate/10000	0.67	0.27	1.07	0.27	2.14
Natural conception (NC)					
Children born	2 775 239	2 775 239	2 775 239	2 775 239	2 775 239
Imprinting	138	67	97	70	372
Rate/10000	0.50	0.24	0.35	0.25	1.34
ART versus NC					
Crude odds ratio	1.35	1.11	3.07	0.53	1.60
[95%CI]	[0.55–3.29]	[0.27–4.53]	[1.49–6.31]	[0.07–3.82]	[0.97–2.65]
Adj.[*] odds ratio	1.03	0.82	2.84	0.51	1.35
[95%CI]	[0.37–2.84]	[0.20–3.43]	[1.34–6.01]	[0.07–3.74]	[0.80–2.29]

[#]Due to Danish law on health data, we are not allowed to show data on groups of less than three individuals.
[*]Adjustments were made for maternal age, parity (nulliparous versus multiparous), year of birth, child's sex, BMI, smoking and country.

et al., 2018). Almost all imprinting disorders are diagnosed in early childhood, although the clinical diagnosis can be delayed due to a broad phenotypic spectrum. Studies on mouse embryos have shown combined superovulation and embryo culture resulting in increased disruption of genomic imprinting (Market-Velker et al., 2010). The four imprinting disorders investigated in this study originate from different genetic modifications. If some imprinting disorders are more associated with ART than others, this suggests that some loci may be more vulnerable to external events, and the potential effect of ART procedures, than others. The heterogeneity of the four imprinting disorders might be the explanation as to why we do not necessarily find a consistent increase in risk of imprinting disorders after ART. Furthermore, not all imprinting disorders are caused by methylation errors. For Beckwith–Wiedemann syndrome, uniparental disomy might be responsible for up to 20% of cases (Henry et al., 1991). Although based on only nine cases of ART children with imprinting disorders, we found a 3-fold increased risk of imprinting disorders in children conceived after ICSI versus IVF. The numbers are too small to draw any conclusions, but future studies should investigate this further. New ARTs are continuously being developed and implemented; among others, extended embryo culture from cleavage to blastocyst stage keeps the embryos for 2–4 days more in *in vitro* culture. Concomitantly, vitrification has quickly overtaken slow freezing for cryopreservation of surplus embryos. Studies have shown that children born after cryopreservation of embryos have an altered birthweight profile with a higher proportion of children being born large for gestational age (LGA), which may be caused by epigenetic modifications (Henningsen et al., 2011; Nelissen et al., 2012; Wennerholm et al., 2013). However, none of the Danish ART children with imprinting disorders were conceived after replacement of a frozen-thawed embryo. Hence, if an epigenetic modification causes LGA babies, it could be different from those potentially associated with the four imprinting disorders investigated in this study.

Ever since IVF was introduced, continuous attention has been on the outcome and health of ART children, but due to the rarity and heterogenicity of imprinting disorders, the field has been difficult to investigate. An early national Danish register study from our group could not demonstrate an increased risk of imprinting disorders (Lidegaard et al., 2005). However, several studies have suggested an association between ART and Beckwith–Wiedemann syndrome (DeBaun et al., 2003; Gicquel et al., 2003; Maher et al., 2003; Halliday et al., 2004; Sutcliffe et al., 2006; Hiura et al., 2012; Mussa et al., 2017; Johnson et al., 2018) (Table III).

The effects of both subfertility and ART on epigenetic gene regulation are unquestionably complex. Studies examining DNA methylation in children with imprinting disorders have not been able to identify specific changes in DNA methylation in selected genes, although some studies find that the methylation error rates are significantly higher in children conceived after ART (Lim et al., 2009; Lazaraviciute et al., 2014; Hattori et al., 2019). Advanced parental age is known to predispose to genetic errors causing also imprinting disorders. When Hattori et al. (2019) stratified their analyses on maternal age older or younger than 37 years for children with Prader–Willi syndrome, they found the rate of methylation errors to be significantly increased in ART compared with NC children, in mothers younger than 37 years. This indicates that not only maternal age but also ART may affect DNA methylation and potentially the risk of imprinting disorders (Cortessis et al., 2018; Hattori et al., 2019).

The strength of this register-based investigation of imprinting disorders is two large Nordic national datasets with high coverage and validity. The main weakness is the few imprinting disorders in the ART group, limiting the power of our study. A further weakness of this study is that the ICD-10 codes are not specific for the mechanisms behind these syndromes. In our analyses, we chose to use logistic regression instead of survival analyses, as we do not consider the aspect of age at diagnosis crucial, when investigating the risk of

Table III National cohorts and case-control studies investigating the prevalence of Prader–Willi syndrome, Silver–Russell syndrome, Beckwith–Wiedemann syndrome and Angelman syndrome.

Prader-Willi syndrome (PWS)						
	ART		NC		Prevalence	Ratio
	PWS	total	PWS	total	/100000	RR* [95%CI]
Källén, 2005 (S)	1	16 280	0	2 023 663	0.05	-
Lidegaard, 2005 (DK)	0	6052	3	436 297	0.7	0
Sutcliffe, 2006 (UK)	2	68 566	161	8 327 061	1.9	1.5 [0.4–6.1]
Doornbos, 2007 (NL)	2	83 818	84	3 954 461	2.1	1.1 [0.3–4.6]
Hiura, 2012 (Japan)	4	10 524	261	1 123 610	23.4	1.6 [0.6–4.4]
Gold, 2014 (US)	20	25 015	1864	3 960 909	47.3	1.7 [1.1–2.6]
Hattori, 2019 (Japan)	24	1.3%	520	98.7%	Na**	3.4 [na**]
This article (DK)	5	74 621	138	2 775 239	5.0	1.0 [0.4–2.8]
Silver-Russell syndrome (SRS)						
	ART		NC		Prevalence	Ratio
	SRS	total	SRS	total	/100 000	RR* [95%CI]
Källén, 2005 (S)	1	16 280	0	2 023 663	0.05	-
Lidegaard, 2005 (DK)	0	6052	2	436 297	0.5	0
Hiura, 2012 (Japan)	4	10 524	42	1 123 610	4.0	10.2 [3.6–28.4]
Hattori, 2019 (Japan)	8	1.3%	67	98.7%	NA**	8.9 [na**]
This article (DK)	<3#	74 621	67	2 775 239	2.4	0.8 [0.2–3.4]
Beckwith-Wiedemann syndrome (BWS)						
	ART		NC		Prevalence	Ratio
	BWS	total	BWS	total	/100000	RR* [95%CI]
DeBaun, 2003 (US)	3	30 285	62	3 920 132	1.6	6.3 [2.0–20.0]
Gicquel, 2003 (F)	6	9930	143	760 070	19.4	3.2 [1.4–7.3]
Maher, 2003 (UK)	6	43 074	143	4 277 408	3.4	4.2 [1.8–9.4]
Halliday, 2004 (Aus)	4	14 894	33	1 301 606	2.8	10.6 [3.8–29.9]
Sutcliffe, 2006 (UK)	6	68 566	73	8 327 061	0.9	10.0 [4.3–22.9]
Doornboos, 2007 (NL)	4	83 818	69	3 954 461	1.8	2.7 [1.0–7.5]
Hiura, 2012 (Japan)	6	10 524	70	1 123 610	6.7	9.2 [4.0–21.1]
Mussa, 2017 (Italy)	7	7884	31	371 988	10.0	10.7 [4.7–24.2]
Hattori, 2019 (Japan)	7	1.3%	117	98.7%	NA**	4.5 [na**]
This article (DK)	8	74 621	97	2 775 239	3.7	2.8 [1.3–6.0]
Angelman syndrome (AS)						
	ART		NC		Prevalence	Ratio
	AS	total	AS	total	/100000	RR* [95%CI]
Sutcliffe, 2006 (UK)	1	68 566	74	8 327 061	0.89	1.6 [0.2–11.8]
Doornboos, 2007 (NL)	0	83 818	63	3 954 461	1.6	0
Hiura, 2012 (Japan)	2	10 524	123	1 123 610	11.0	1.7 [0.4–7.0]
Hattori, 2019 (Japan)	4	1.3%	227	98.7%	Na**	1.3 [na**]
This article (DK)	<3#	74 621	70	2 775 239	2.5	0.5 [0.1–3.7]

RR* indicates rate ratio; Na**, not available
#Due to Danish law on health data, we are not allowed to show data on groups of less than three individuals.

disorders that are present already at birth and diagnosed during the first years of life for both ART and NC children. The difference in length of follow-up between the two groups is therefore not expected to influence our results. If anything, adjustment for length of follow-up would overestimate the risk of imprinting disorders in the ART group a little. Nevertheless, we found a lower age at diagnosis in ART children compared with NC children. A longer follow-up is needed to determine whether this results from a shorter follow-up for the ART children in this cohort or from a shift in time of diagnosis, for example, due to increased parental awareness. In the latter situation,

survival analysis might overestimate the risk of imprinting disorders after ART.

Due to lack of power, we were not able to analyse the frequency of imprinting disorders in children conceived after culturing of the embryo to the blastocysts stage. However, White *et al.* (2015), who investigated the occurrence of imprinted methylation errors in human preimplantation embryos, found evidence that methylation errors derive as early as the six- to eight-cell stage and that extended culture time to the blastocysts stage did not increase the risk of imprinting errors.

In conclusion, this large-scale cohort study demonstrated no overall increased risk of imprinting disorders in general in children conceived after ART but specifically there was an increased risk of Beckwith–Wiedemann syndrome. If only some imprinting disorders are associated with ART, this suggests that some loci may be more vulnerable to external events, and the potential effect of ART procedures, than others.

Authors' roles

All authors planned the study and discussed the results. S.R., A.L.S., A.A.H., M.G. and S.O. fitted and merged data. A.A.H. performed the analyses and drafted the manuscript. J.L.F. contributed to the data analyses and interpretation. All authors were involved in finalizing the manuscript and approved the final version. The authors agreed upon the listing of authors.

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Conflict of interest

The authors have no conflicts of interest related to this work.

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