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Risk of congenital heart defects associated with assisted reproductive technologies: a population-based evaluation

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Aims	To estimate the risk of congenital heart defects (CHD) associated with assisted reproductive technologies (ART).
Methods and results	We used data from the Paris Registry of Congenital Malformations on 5493 cases of CHD and 3847 malformed controls for which no associations with ART were reported in the literature. Assisted reproductive technologies included inductors of ovulation only, in vitro fertilization, and intracytoplasmic sperm injection. Exposure to ART was higher for cases than controls (4.7 vs. 3.6%, $P = 0.008$) and was associated with a 40% increase in the maternal age, socioeconomic factors, and year of birth-adjusted odds of CHD without chromosomal abnormalities [adjusted odds ratio (OR) 1.4, 95% confidence interval (CI) 1.1–1.7]. Assisted reproductive technologies were specifically associated with significant increases in the odds of malformations of the outflow tracts and ventriculoarterial connections (adjusted OR 1.7, 95% CI 1.2–2.4) and of cardiac neural crest defects and double outlet right ventricle (adjusted OR 1.7, 95% CI 1.1–2.7). In general, we found specific associations between methods of ART and subcategories of CHD.
Conclusion	Cases with CHD were more likely to have been conceived following ART when compared with malformed controls. This higher risk for CHD varied specifically according to the method of ART and the type of CHD and may be due to ART per se and/or the underlying infertility of couples.
Keywords	Congenital heart defects • Assisted reproductive technologies • Risk factor • Epidemiology

Introduction

Congenital heart defects (CHD) are the most prevalent birth defects and comprise the most important cause of malformation-related infant mortality.[1,2] Despite the progress in prenatal diagnosis, medical and surgical management of infants with CHD[3,4] substantial risks of morbidity and mortality remain for severe cases of CHD. Risk factors of CHD include inherited[5] and non-inherited causes,[6] among which the role of assisted reproductive technologies (ART) remains uncertain.

Assisted reproductive technologies include various techniques used to achieve pregnancy in the case of male or female infertility and comprise inductors of ovulation, conventional in vitro fertilization (IVF), and IVF with intracytoplasmic sperm injection (ICSI). These techniques are increasingly used in many countries[7,8] in part due to trends towards delayed childbearing.[9] For example, in France, nearly 2.4% of all children born in 2006 were conceived following ART.[10]

Children conceived following ART are known to be at higher risk for adverse birth outcomes[11–15] including those related to multiple births, preterm delivery, and intrauterine growth retardation. There are more uncertainties regarding the risks for birth defects,[13,16–23] and in particular for specific defects such as CHD.[12,24–26] The meta-analysis by Hansen et al.[18] showed a

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moderate increase in the overall risk for birth defects in children born after IVF or ICSI compared with children born spontaneously [odds ratio (OR) = 1.40, 95% confidence interval (CI) 1.28–1.53]. However, insufficient data exist regarding specific risks for CHD that may be associated with ART.

Previous studies included a relatively small number of cases of CHD and showed inconsistent results for risks of CHD in relation to ART.[12,24–26] Moreover, specific associations between subcategories of CHD and different methods of ART have not been adequately assessed in the literature. This is important as most known teratogens and risk factors for birth defects are associated with one or a few specific anomalies.

Using population-based data from the Paris Registry of Congenital Malformations including more than 5000 cases of CHD, we estimated the risk for CHD in relation to different methods of ART for: all CHD, CHD without chromosomal abnormalities, and subcategories of CHD defined based on anatomo-embryological criteria.

Methods

Data

We used data from the population-based Paris Registry of Congenital Malformations,[4,27] which registers all cases of birth defects and chromosomal abnormalities among live-borns, still-borns (≥22 weeks of gestation), and pregnancy terminations. The Registry covers the population of women who live in Greater Paris area (Paris and its surrounding suburb) and deliver or have a pregnancy termination in a Parisian maternity unit. The annual number of deliveries in our population is about 38 000.

The Paris Registry is a member of the European Network of Registries of Congenital Malformations (EUROCAT) and of the International Clearinghouse for Birth Defects Surveillance and Research. The Registry follows the EUROCAT methodology and quality of data is routinely monitored by both EUROCAT[1] and the National Committee of Registries in France. The review of procedures regarding confidentiality of data is overseen by both the National Committee of Registries and the National Committee of Informatics and Freedom. Data are based on medical records and are collected from several sources including maternity units, neonatology wards, and cytogenetic and pathology services.

Data for the study population corresponded to the period 1987–2006 as the first case of a malformation with exposure to IVF occurred in 1987 and 2006 was the last year for which data were available.

Methods

A case–control study with malformed controls was conducted to estimate the risk of CHD in relation to ART. Cases were children/foetuses with a diagnosis of CHD. For the malformed controls, following Hook's recommendations,[28] we included a wide spectrum of malformations for which no association with ART was reported in the literature. The malformed controls were foetuses/children with isolated club-foot, angioma, skin abnormality, polydactyly, syndactyly, or congenital hip dislocation.

We estimated the risk (odds) of CHD when compared with malformed controls in relation to different methods of ART. This risk was estimated for the following outcomes: (i) all CHD combined, (ii) CHD without chromosomal abnormalities, (iii) CHD without chromosomal abnormalities and excluding isolated ventricular septal defects

(VSD), and (iv) 10 mutually exclusive subcategories of CHD (Table 1, subcategories 1–10) defined based on anatomo-embryological criteria and classified by consensus by two paediatric cardiologists (L.H. and D.B.). Three additional subcategories (Table 1, subcategories 11–13) were defined by re-grouping, based on their common developmental and genetic origins,[29] certain types of CHD included in the 10 preceding subcategories.

The main predictor variable was exposure to ART, which included the following: inductors of ovulation only (IO), IVF, and ICSI. Exposure to ART was assessed: (i) as a binary (ART yes/no) variable and (ii) as a variable in four categories (no ART, IO, IVF, and ICSI), and for IVF and ICSI combined (IVF + ICSI).

Variables considered as potentially confounding factors included year of birth, maternal age, occupation, and geographic origin. These factors are known to be related to both exposure to ART and prevalence of birth defects[22,30] even if their specific associations with CHD are not well documented. Maternal occupation was coded according to the French National Institute of Statistics and Economic studies (INSEE) classification: professional, intermediate, administrative/public service, other, and none. These categories represent a gradient from the highest to the lowest occupational group in France. The 'other' group comprised: artisan/small business owner, shop keeper/shop assistant, service worker, skilled worker, and unskilled worker, for which each category represented a small number of deliveries in our population. Maternal geographic origin was coded as: French, North African, other African, and other countries.

Analyses for the largest three groups of cases (all CHD, CHD without chromosomal abnormalities, and CHD without chromosomal abnormalities and excluding isolated VSD) were also done separately for singletons.

Power

Assuming a type-I error of 0.05, we had a power of 80% to detect an OR of 1.5 for the overall risk of CHD in relation to IVF (exposure ≈2%). Assuming a case–control ratio of 1:4, we had a power of 80% to detect an OR of 2.0 associated with IVF exposure for subcategories of 1000 cases. For subcategories of 100 cases, we had an 80% power to detect an OR of 3.0 or more. For exposure to ICSI (≈0.6%), we had a power of 80% to detect an OR of 2.0 for the overall risk of CHD and 3.0 for subcategories of 1000 cases.

Statistical analyses

The odds of CHD in relation to ART was estimated using logistic regression models for each outcome, after taking into account year of birth, maternal age, occupation, and geographic origin. The adjustment for maternal age was made using fractional polynomials.[31]

Separate logistic regression models were estimated for each of the following outcomes: (i) all CHD, (ii) CHD without chromosomal abnormalities, (iii) CHD without chromosomal abnormalities and excluding VSD, and (iv) each of the 13 CHD subcategories that included at least 100 cases. The following CHD subcategories were not analysed separately as they included less than 100 cases: anomalies of heart position, anomalies of coronary arteries, anomalies of venous connections, discordant atrioventricular connections, and isolated atrioventricular septal defects. However, these cases were included in the analyses for all CHD/CHD without chromosomal abnormalities/excluding VSD.

We tested whether the effects associated with ART (specifically IO and IVF for which data were available for the entire study period) changed over time; i.e. we tested for interaction effects between IO/IVF and time using nested models with likelihood ratio tests. None

Table 1 Subcategories of congenital heart defects defined according to anatomo-embryological criteria[a]

Subcategories of CHD	Malformations included	ICD 10
Anomalies of heart position	Heterotaxy syndrome[b]/isomerism of atrial appendages	Q206
	Mirror-image arrangement	Q893
Malformations of the outflow tracts and ventriculoarterial connections	Transposition of great arteries (complete)	Q203
	Vascular malpositions	
	Double outlet right ventricle	Q201
	Double outlet left ventricle	Q202
	Cardiac neural crest defects	
	Common arterial trunk	Q200
	Tetralogy of Fallot	Q213
	Aortopulmonary septal defect	Q214
	Pulmonary valve atresia + VSD	Q220 + Q210
	Atresia of pulmonary artery	Q255
	Interrupted aortic arch/atresia of aorta	Q252
	Overriding aorta	Q2542
	Defects of aortic valves/left outflow tract	
	Stenosis of aortic valve	Q230
	Insufficiency of aortic valve	Q231
	Defects of pulmonary valves/right outflow tract	
	Pulmonary valve atresia	Q220
	Pulmonary valve stenosis	Q221
	Pulmonary valve insufficiency	Q222
	Other defects of pulmonary valve	Q223
	Pulmonary infundibular stenosis	Q243
	Other defects of great vessels	Q258/Q259
Malformations of the atrioventricular valves and atrioventricular connections	Defects of tricuspid valve	
	Tricuspid valve stenosis/atresia	Q224
	Ebstein's anomaly	Q225
	Other defects of tricuspid valve	Q228
	Unspecified tricuspid valve defect	Q229
	Defects of mitral valve	
	Mitral stenosis	Q232
	Mitral insufficiency	Q233
	Unspecified mitral defects	Q239
	Common atrium/cor triloculare biventriculare	Q2115
	Atrioventricular septal defects	Q212
Functionally univentricular CHD	Double inlet ventricle	Q204
	Hypoplastic left heart syndrome	Q234
	Hypoplastic right ventricle	
	Hypoplastic right heart syndrome	Q226
	Cor biloculare	Q208
Anomalies of the great arteries	Anomalies of ascending aorta	
	Hypoplasia of ascending aorta	Q2540
	Supravalvular aortic stenosis	Q253
	Aneurysm or dilatation of aorta	Q2545
	Aneurysm of sinus of Valsalva	Q2543
	Coarctation of aorta	Q251
	Anomalies of aortic arch	Q2541/Q2544
	Anomalies of pulmonary artery	
	Stenosis of pulmonary artery	Q256
	Other defects of pulmonary artery	Q257
Anomalies of coronary vessels	Defect of coronary vessels	Q245

Continued

Table 1 *Continued*

Subcategories of CHD	Malformations included	ICD 10
Ventricular septal defects	Ventricular septal defects	Q210
Anomalies of the atria and interatrial communications	Cor triatrium Interatrial communications	Q242 Q2110/Q2112/Q2113/Q2114/Q2118

Anomalies of venous connections	Anomalous systemic venous connection
	    Persistent left superior vena cava
	    Other defects of great veins
	Anomalous pulmonary venous
	    connection
	    Total anomalous pulmonary venous
	        connection
	    Partial anomalous pulmonary venous
	        connection
	    Unspecified anomalous pulmonary
	        venous connection
		
		Q261
		Q260/Q265/Q266/Q268/Q269
		
		Q262
		
		Q263
		
		Q264

Discordant atrioventricular connections	Discordant atrioventricular connection Unspecified defects of cardiac chamber and connections	Q205 Q209
TGA, heterotaxy syndrome[b] and discordant atrioventricular connections		Q206/Q203/Q205/Q209

Cardiac neural crest defects and double outlet right
    ventricle without ventricular hypoplasia
		Q200/Q213/Q214/Q220 + Q210/Q252/Q255/
		    Q2542/Q201 without Q226 and Q234

Isolated atrioventricular septal defects		Q212

[a]See the Methods section for details.
[b]Definition of heterotaxy syndrome was based on the article by Jacobs *et al.*[44]

of the interaction effects between ART and time were statistically significant (detailed results not shown; available from authors). We also tested for any interaction effects between ART and singleton/multiple births.

The statistical significance level was set at $\alpha = 0.05$ and all tests were two-sided.

As recommended by Rothman[32] and Savitz and Olshan[33] in the case of observational studies, such as ours, aimed at detecting patterns of (specific) associations, no adjustment was made for multiple comparisons in analysing the associations between the a priori chosen subcategories of CHD and ART.

Analyses were done with Stata 9 software (Statacorp, TX, USA).

Results

Study population

After excluding data with missing information on ART (2% of cases), the study population included 5493 cases of CHD, 4459 cases of CHD without chromosomal abnormalities, and 3104 without chromosomal abnormalities and excluding VSD (*Table 2*).

The subcategories of CHD comprised from 124 to 2248 cases (*Table 2*; subcategories of CHD with less than 100 cases are not shown—detailed data available from authors). The largest subcategory was the VSD (*n* = 2248) representing nearly 40% of all cases. The subcategory malformations of the outflow tracts and ventriculoarterial connections was the second largest subcategory and comprised 1088 cases.

After excluding controls with missing data for ART (3.5% of controls), the study population included a total of 3847 malformed controls, comprising isolated congenital hip dislocation (*n* = 1299), polydactyly (*n* = 769), club foot (*n* = 733), angioma (*n* = 515), skin abnormality (*n* = 367), or syndactyly (*n* = 164).

Maternal age was missing for 35 (0.6%) cases and 23 (0.6%) controls. Data on maternal geographic origin were missing for 1.7% of the cases and 2.1% of the controls, and information on maternal occupation was missing for 7.9% of the cases and 3.1% of the controls.

Most socio-demographic characteristics were different between cases and controls. Mothers of cases were older, less often of French origin, and were more likely to be in the occupational category 'none' than mothers of controls. On the other hand, mothers who had conceived following ART were more likely to be of French origin and to be in the occupational category 'professional' (highest category) than mothers who had conceived spontaneously (detailed results not shown; available from authors).

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Table 2 shows the proportions of cases and controls that were exposed to ART. Overall, CHD cases were more likely to have been conceived following ART when compared with controls (4.7 vs. 3.6%, respectively, *P* = 0.008). Exposure to the different

Table 2 Number of cases and controls and proportions of children/foetuses conceived after assisted reproductive technologies

Category[a]	Subjects with complete data on ART (n)	Exposure to ART (%)	P-value[b]
Controls	3847	3.6	
All CHD	5493	4.7	0.008
CHD without chromosomal abnormalities	4459	4.9	0.003
CHD without chromosomal abnormalities and excluding VSD	3104	5.0	0.005
Malformations of the outflow tracts and ventriculoarterial connections	1088	5.6	0.003
Malformations of the atrioventricular valves and atrioventricular connections	608	2.6	0.231
Functionally univentricular CHD	402	2.5	0.253
Anomalies of the great arteries	371	5.6	0.331
Ventricular septal defects	2248	5.0	0.006
Anomalies of the atria and interatrial communications	124	4.8	0.463
TGA, heterotaxy syndrome, and discordant atrioventricular connections	475	1.3	0.362
Cardiac neural crest defects and double outlet right ventricle without ventricular hypoplasia	537	1.8	0.014

[a]Subcategories of CHD with less than 100 cases are not shown.
[b]Comparison of the proportion of children/foetuses conceived after ART between the subcategory of CHD and the controls.

Table 3 Logistic regression analyses of the associations between assisted reproductive technologies (all methods combined) and congenital heart defects

	Cases	Crude OR[a]	95% CI	Adjusted[b] OR[a]	95% CI
All	All CHD	1.0	Ref.	1.0	Ref.
		1.3	1.1−1.6	1.3	1.0−1.6
	CHD without chromosomal abnormalities	1.0	Ref.	1.0	Ref.
		1.4	1.1−1.7	1.4	1.1−1.7
	CHD without chromosomal abnormalities and excluding VSD	1.0	Ref.	1.0	Ref.
		1.4	1.1−1.8	1.5	1.1−1.9
Singletons only	All CHD	1.0	Ref.	1.0	Ref.
		1.1	0.8−1.5	1.1	0.8−1.5
	CHD without chromosomal abnormalities	1.0	Ref.	1.0	Ref.
		1.1	0.8−1.5	1.2	0.9−1.6
	CHD without chromosomal abnormalities and excluding VSD	1.0	Ref.	1.0	Ref.
		1.1	0.8−1.5	1.2	0.8−1.6

[a]Odds ratios represent the odds of a birth (including live births, stillbirths, and pregnancy terminations) with congenital heart disease (cases) relative to the odds of a birth with one of the malformed controls (see the Methods section for details).
[b]Adjusted for maternal age, geographic origin, occupation, and year of birth.

methods of ART was also significantly different between cases and controls ($P = 0.013$); in particular, 1.9% of the cases were born following IVF vs. 1.3% of the controls and 0.6% of the cases were born following ICSI vs. 0.3% of the controls.

Table 3 shows the crude and adjusted associations between the overall risk of CHD and ART (all methods combined). Exposure to ART was associated with a 1.3-fold increase in the maternal age, socioeconomic factors, and year of birth-adjusted odds of all CHD (adjusted OR 1.3, 95% CI 1.0−1.6). For CHD without chromosomal abnormalities and CHD without chromosomal abnormalities and excluding isolated VSD, estimates were comparable to (slightly

higher than) those observed for all CHD (adjusted OR 1.4, 95% CI 1.1−1.7 and adjusted OR 1.5, 95% CI 1.1−1.9, respectively).

Table 4 shows the crude and adjusted ORs between the risk of CHD and the different methods of ART. The adjusted ORs for IVF and ICSI were similar and the combined IVF + ICSI exposure was associated with a 1.4-fold increase in the odds of CHD (adjusted OR 1.4, 95% CI 1.0−2.9). The combined IVF + ICSI exposure was also associated with a 1.5-fold increase in the odds of CHD without chromosomal abnormalities (adjusted OR 1.5 95% CI 1.1−2.1) and a 1.7-fold increase in the odds of CHD without chromosomal abnormalities and excluding VSD (adjusted OR 1.7 95%

Table 4 Logistic regression analyses of the associations between different methods of assisted reproductive technologies and congenital heart defects

Cases	method of ART	crude OR[a]	95% CI	adjusted[b] OR[a]	95% CI
All					
All CHD	None	1.0	Ref.	1.0	Ref.
	Inductors of ovulation only	1.1	0.8–1.5	1.2	0.9–1.6
	IVF	1.4	1.0–2.0	1.4	1.0–2.0
	ICSI	2.3	1.1–4.4	1.4	0.7–2.7
	IVF + ICSI	1.6	1.2–2.1	1.4	1.0–1.9
CHD without chromosomal abnormalities	None	1.0	ref.	1.0	ref.
	Inductors of ovulation only	1.2	0.9–1.6	1.2	0.9–1.7
	IVF	1.5	1.1–2.1	1.6	1.1–2.3
	ICSI	2.4	1.2–4.8	1.4	0.7–2.9
	IVF + ICSI	1.7	1.2–2.3	1.5	1.1–2.1
CHD without chromosomal abnormalities and excluding VSD	None	1.0	ref.	1.0	ref.
	Inductors of ovulation only	1.1	0.8–1.6	1.3	0.9–1.8
	IVF	1.6	1.1–2.3	1.7	1.2–2.6
	ICSI	2.5	1.2–5.2	1.5	0.7–3.2
	IVF + ICSI	1.7	1.3–2.4	1.7	1.2–2.4
Singletons only					
All CHD	None	1.0	Ref.	1.0	Ref.
	Inductors of ovulation only	0.9	0.6–1.4	1.0	0.7–1.5
	IVF	1.4	0.8–2.4	1.2	0.7–2.1
	ICSI	1.4	0.6–3.2	1.2	0.5–2.7
	IVF+ICSI	1.4	0.9–2.2	1.2	0.8–1.9
CHD without chromosomal abnormalities	None	1.0	ref.	1.0	ref.
	Inductors of ovulation only	1.0	0.7–1.4	1.1	0.7–1.6
	IVF	1.4	0.8–2.4	1.3	0.7–2.3
	ICSI	1.5	0.6–3.4	1.3	0.5–3.0
	IVF + ICSI	1.4	0.9–2.2	1.3	0.8–2.1
CHD without chromosomal abnormalities and excluding VSD	None	1.0	ref.	1.0	ref.
	Inductors of ovulation only	0.8	0.6–1.3	1.0	0.7–1.6
	IVF	1.4	0.8–2.5	1.5	0.8–2.7
	ICSI	1.4	0.6–3.4	1.2	0.5–3.1
	IVF + ICSI	1.4	0.9–2.3	1.4	0.8–2.3

[a]Odds ratios represent the odds of a birth (including live births, stillbirths, and pregnancy terminations) with congenital heart disease (cases) relative to the odds of a birth with one of the malformed controls (see the Methods section for details).
[b]Adjusted for maternal age, geographic origin, occupation, and year of birth.

CI 1.2–2.4). In contrast, we did not find any statistically significant association between IO and CHD with or without chromosomal abnormalities.

When analyses were restricted to singletons, ORs for IVF and ICSI remained >1, but the magnitude of associations decreased and CIs included the null value (*Tables 3* and *4*). However, test of the interaction effect between ART and singleton/multiple births was not statistically significant (*P* = 0.67).

Analyses for subcategories of congenital heart defects defined according to anatomo-embryological criteria

Table 5 shows the results of the analyses for the subcategories of CHD. Assisted reproductive technologies were associated with significant increases in the odds of malformations of the outflow tracts and ventriculoarterial connections (adjusted OR 1.7, 95% CI 1.2–2.4) and of cardiac neural crest defects and double outlet right ventricle (adjusted OR 1.7, 95% CI 1.1–2.7).

The adjusted ORs for ICSI were in general similar to those for IVF except for the subcategories malformations of atrioventricular valves and atrioventricular connections, functionally univentricular CHD and transposition of the great arteries (TGA), heterotaxy syndrome, and discordant atrioventricular connection. The combined IVF + ICSI category was associated with a 1.8-fold increase in the odds of malformations of the outflow tracts and ventriculoarterial connections (adjusted OR 1.7 95% CI 1.1–2.8) and a 1.8-fold increase in the odds of cardiac neural crest defects and double outlet right ventricle without ventricular hypoplasia (adjusted OR 1.8 95% CI 1.0–3.3). IO were associated with a 2.5-fold higher odds of anomalies of atria and interatrial communications (adjusted OR 2.5, 95% CI 0.7–8.7). In general, the estimates of the associations between the different methods of ART and CHD subcategories varied across the different subcategories. However, CIs for smaller subcategories were wide, reflecting the relative imprecision of the estimates (detailed results of the associations between different methods of ART and

Table 5 Logistic regression analyses of the associations between assisted reproductive technologies (all methods combined) and subcategories of congenital heart defects

Subcategories	Crude OR[a]	95% CI	Adjusted[b] OR[a]	95% CI
Malformations of the outflow tracts and ventriculoarterial connections	1.0	Ref.	1.0	Ref.
	1.6	1.2–2.2	1.7	1.2–2.4
Malformations of the atrioventricular valves and atrioventricular connections	1.0	Ref.	1.0	Ref.
	0.7	0.4–1.2	0.6	0.4–1.2
Functionally univentricular CHD	1.0	Ref.	1.0	Ref.
	0.7	0.4–1.3	0.6	0.3–1.3
Anomalies of the great arteries	1.0	Ref.	1.0	Ref.
	1.3	0.8–2.2	1.3	0.8–2.3
Ventricular septal defects	1.0	Ref.	1.0	Ref.
	1.4	1.1–1.8	1.3	1.0–1.6
Anomalies of the atria and interatrial communications	1.0	Ref.	1.0	Ref.
	1.4	0.6–3.2	2.0	0.8–5.0
TGA, heterotaxy syndrome, and discordant atrioventricular connections	1.0	Ref.	1.0	Ref.
	1.2	0.8–2.0	1.3	0.8–2.3
Cardiac neural crest defects and double outlet right ventricle without ventricular hypoplasia	1.0	Ref.	1.0	Ref.
	1.6	1.1–2.5	1.7	1.1–2.7

[a]Odds ratios represent the odds of a birth (including live births, stillbirths, and pregnancy terminations) with congenital heart disease (cases) relative to the odds of a birth with one of the malformed controls (see the Methods section for details).
[b]Adjusted for maternal age, geographic origin, occupation, and year of birth.

subcategories of CHD are not shown in the printed version; these results are available from authors/or as Supplementary material online, *Table S3*).

Discussion

On the basis of data from the Paris Registry of Congenital Malformations including more than 5000 cases of CHD, we found a 40% increase in the overall risk of CHD without chromosomal abnormalities in children conceived following ART after taking into account maternal age, socioeconomic factors, and year of birth. Our results also suggest that specific associations exist between ART and subcategories of CHD. Moreover, although IVF and ICSI were associated with significant increases in the risk of CHD, we did not find a significant association between IO and the overall risk of CHD.

When analyses were restricted to singletons only, the ORs decreased and the CIs included the null value. This suggests that any effect of ART on CHD may be in part due to multiple births. On the other hand, test of the interaction effect between ART and singletons/multiple was not statistically significant, although this may have been due to limited power of our study for detecting any interaction effects that may have existed. In any case, it is certainly possible that multiple births may be on the causal pathway between ART and CHD. This hypothesis is consistent with Reefhuis's[26] finding that 'multiple births were more likely to have birth defects, regardless of conception mode'. It should be noted however that the public health impact of ART on the risk for birth defects includes all (singleton and multiple) births.

On the basis of our findings, we calculated attributable risk fractions, which would represent the proportion of cases that may be caused by ART, or equivalently, the proportion of cases that would be avoided were the exposure to ART removed ceteris paribus, 'if' the associations we found between the risk of CHD and ART can be assumed to represent causal relations (this may of course not be the case for reasons that are discussed further below). The attributable risk fraction estimates suggest in particular that around 2% of the CHD without chromosomal abnormalities may be caused by ART and 1.2% by IVF + ICSI. These proportions are similar to those calculated for certain other major risk factors of CHD[34] and may increase as exposure to ART is likely to increase over time.

To our knowledge, there are no embryological or physiopathological hypotheses to explain the specific associations between ART and the subcategories of CHD that we found in our study. In particular, genomic imprinting disorders found in children born after ART[35] seem unlikely to explain all of the observed associations. Moreover, our results for CHD without chromosomal abnormalities suggest that associations between CHD and ART are not due to the association of the latter with chromosomal abnormalities. Our results may be helpful for generating hypotheses regarding underlying mechanisms for the association between the risk of CHD and different methods of ART.

Our study has certain limitations. Although our study included a large number of cases of CHD, exposure to ART, and particularly to ICSI, remains infrequent in the general population and in our Registry data. Consequently, the CIs for the estimates of the associations between subcategories of CHD and IVF and more so ICSI were wide, indicating the limited precision of some of our estimates.

Moreover, we made no adjustment for multiple comparisons in analysing the associations between the a priori chosen

subcategories of CHD and ART. Although this is consistent with the recommendations by Rothman[32] and Savitz and Olshan[33] in the case of observational studies, such as ours, aimed at detecting patterns of specific associations, controversies remain among experts as to the right statistical approach to multiple comparisons. In any case, the specific associations we found can only be considered exploratory and need to be further investigated.

The choice of malformed controls may have been a source of selection bias[36,37] due to associations that may exist between exposure to ART and malformations included as controls. In order to minimize such bias, following Hook's recommendations,[28] we selected a wide spectrum of malformations for which no associations with ART were described in the literature. This methodology is often used in the field of birth defects[38] and in particular in assessing the teratogenic effects of medications.[39,40]

Although this approach of choosing a heterogeneous group of malformations as controls can lower the risk of selection bias by diluting any effects due to unknown (i.e. hitherto unreported in the literature) associations that may exist between ART and one or more of the malformations selected as controls, the possibility of residual bias cannot be excluded. This bias may result in an underestimation of the true association (i.e. what one would observe with an ideal set of non-malformed controls) between ART and CHD if ART is associated with an increase in the risk of the malformations included as controls. Conversely, the overestimation of the associations between ART and CHD can occur if ART is associated with a decrease in the risk of malformations included as controls, although such an association seems unlikely. Moreover, a differential misclassification bias for exposure assessment cannot be excluded if exposure to ART is ascertained in a different way in the case of CHD compared with the malformations included as controls. However, we are not aware of any a priori reason or empirical evidence to suggest that such a bias may exist for ART.

The frequency of missing data on exposure was different between cases and controls. This may have resulted in biased (over or under) estimates of the associations between CHD and ART if the distribution of exposure was different between subjects with complete data and those with missing data. Nevertheless, the overall frequency of missing data in this study was low and we have no reason to believe that the distribution of exposure was indeed different for cases and controls with missing data.

We explored specific associations that may exist between different types of CHD and ART by conducting separate analyses for subcategories of CHD defined a priori based on anatomic and/or embryological criteria. An important caveat that needs to be considered is that our criteria for defining these subcategories can be arguable; alternative and equally, if not more, valid subcategories may be envisaged. Notwithstanding these considerations, our results suggest that indeed specific associations may exist between ART and risk of specific CHD subcategories, without necessarily implying that the subcategories investigated in our study are the most appropriate ones to use in this setting.

Another potential limit of our study is related to the effects of possible confounding factors that could not be taken into account.[41] In particular, we did not have adequate data for folic acid and/or multivitamins intake for women in our population.

Lack of adjustment for multivitamins use could have resulted in an underestimation of the risks associated with ART as: (i) multivitamins/folic acid intake has been shown to be associated with a lower risk of CHD[42] and (ii) a higher proportion of women who conceived after ART may have had an adequate multivitamins/folic acid intake.[26]

Another potentially important confounding variable that we could not adjust for was paternal age. As paternal age is correlated with maternal age, we partially adjusted for paternal age by taking maternal age into account. Nevertheless, lack of full adjustment for paternal age may have resulted in an overestimation of risks, particularly for ICSI that is used more often, although not exclusively, in the case of male infertility.[8]

More broadly, in the association between ART and CHD, the question of the role of underlying infertility vs. that of any treatment effects of ART *per se* remains an open one.[14,16,17,43] Some studies have attempted to separate the effects of underlying infertility from any treatment effects due to ART by adjusting for the duration of infertility/involuntary childlessness.[17] This strategy has been criticized by Hansen *et al.*[19] as this variable may be 'synonymous with exposure'. This is consistent with the data from our Registry in which 99% of the cases/controls with exposure to ART had a duration of infertility of 2 years or more.

Although for most subcategories of CHD the associations between IVF and ICSI were similar, our findings suggest that ICSI may be associated with a higher risk for some subcategories of CHD when compared with conventional IVF. However, CIs for the estimates of the risks associated with ICSI for subcategories of CHD were wide. Hence, while suggestive, our results cannot lead to a definitive conclusion regarding higher risks due to ICSI vs. IVF for certain subcategories of CHD.

In conclusion, we found that cases with CHD were more likely to have been conceived following ART when compared with malformed controls. In particular, IVF and ICSI were associated with a 1.5-fold increase in the odds of CHD without chromosomal abnormalities after adjustment for year of birth, maternal age, occupation, and geographic origin. In contrast, we did not find a significant association between IO (alone) and the overall risk of CHD. In general, estimates suggested that there may be specific associations between different methods of ART and subcategories of CHD classified based on anatomo-embryological criteria. Such specific associations may reflect causal effects due to ART and/or the underlying infertility of couples who conceive following ART.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Conflict of interest: none declared.

References

1. Eurocat. Final Activity Report to European Commission March 2004 to August 2007. http://www.eurocat.ulster.ac.uk/pdf/EUROCAT-Final-EC-Report.pdf (16 December 2009).

2. Hoffman JI, Kaplan S, Liberthson RR. Prevalence of congenital heart disease. *Am Heart J* 2004;**147**:425–439.

3. Bonnet D, Coltri A, Butera G, Fermont L, Le Bidois J, Kachaner J, Sidi D. Detection of transposition of the great arteries in fetuses reduces neonatal morbidity and mortality. *Circulation* 1999;**99**:916–918.

4. Khoshnood B, De Vigan C, Vodovar V, Goujard J, Lhomme A, Bonnet D, Goffinet F. Trends in prenatal diagnosis, pregnancy termination, and perinatal mortality of newborns with congenital heart disease in France, 1983–2000: a population-based evaluation. *Pediatrics* 2005;**115**:95–101.

5. Pierpont ME, Basson CT, Benson DW Jr., Gelb BD, Giglia TM, Goldmuntz E, McGee G, Sable CA, Srivastava D, Webb CL. Genetic basis for congenital heart defects: current knowledge: a scientific statement from the American Heart Association Congenital Cardiac Defects Committee, Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. *Circulation* 2007;**115**:3015–3038.

6. Jenkins KJ, Correa A, Feinstein JA, Botto L, Britt AE, Daniels SR, Elixson M, Warnes CA, Webb CL. Noninherited risk factors and congenital cardiovascular defects: current knowledge: a scientific statement from the American Heart Association Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. *Circulation* 2007;**115**:2995–3014.

7. de Mouzon J, Lancaster P, Nygren KG, Sullivan E, Zegers-Hochschild F, Mansour R, Ishihara O, Adamson D. World collaborative report on Assisted Reproductive Technology, 2002. *Hum Reprod* 2009;**24**:2310–2320.

8. Nyboe Andersen A, Goossens V, Bhattacharya S, Ferraretti AP, Kupka MS, de Mouzon J, Nygren KG. Assisted reproductive technology and intrauterine inseminations in Europe, 2005: results generated from European registers by ESHRE: ESHRE. The European IVF Monitoring Programme (EIM), for the European Society of Human Reproduction and Embryology (ESHRE). *Hum Reprod* 2009; **24**:1267–1287.

9. Khoshnood B, Bouvier-Colle MH, Leridon H, Blondel B. Impact of advanced maternal age on fecundity and women's and children's health. *Journal de gynecologie, obstetrique et biologie de la reproduction* 2008;**37**:733–747.

10. Agence de Biomédecine. Rapport annuel et bilan des activités 2007. http://www.agence-biomedecine.fr/annexes/bilan2008/pdf/bilan_proc.pdf (16 December, 2009).

11. Bergh T, Ericson A, Hillensjo T, Nygren KG, Wennerholm UB. Deliveries and children born after in-vitro fertilisation in Sweden 1982–95: a retrospective cohort study. *Lancet* 1999;**354**:1579–1585.

12. Katalinic A, Rosch C, Ludwig M. Pregnancy course and outcome after intracytoplasmic sperm injection: a controlled, prospective cohort study. *Fertil Steril* 2004; **81**:1604–1616.

13. Koivurova S, Hartikainen AL, Gissler M, Hemminki E, Sovio U, Jarvelin MR. Neonatal outcome and congenital malformations in children born after in-vitro fertilization. *Hum Reprod* 2002;**17**:1391–1398.

14. Schieve LA, Rasmussen SA, Buck GM, Schendel DE, Reynolds MA, Wright VC. Are children born after assisted reproductive technology at increased risk for adverse health outcomes? *Obstet Gynecol* 2004;**103**:1154–1163.

15. Sutcliffe AG, Ludwig M. Outcome of assisted reproduction. *Lancet* 2007;**370**: 351–359.

16. Buckett WM, Tan SL. Congenital abnormalities in children born after assisted reproductive techniques: how much is associated with the presence of infertility and how much with its treatment? *Fertil Steril* 2005;**84**:1318–1319; discussion 1327.

17. Ericson A, Kallen B. Congenital malformations in infants born after IVF: a population-based study. *Hum Reprod* 2001;**16**:504–509.

18. Hansen M, Bower C, Milne E, de Klerk N, Kurinczuk JJ. Assisted reproductive technologies and the risk of birth defects—a systematic review. *Hum Reprod* 2005;**20**:328–338.

19. Hansen M, Kurinczuk JJ, Bower C, Webb S. The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. *N Engl J Med* 2002;**346**: 725–730.

20. Klemetti R, Gissler M, Sevon T, Koivurova S, Ritvanen A, Hemminki E. Children born after assisted fertilization have an increased rate of major congenital anomalies. *Fertil Steril* 2005;**84**:1300–1307.

21. Olson CK, Keppler-Noreuil KM, Romitti PA, Budelier WT, Ryan G, Sparks AE, Van Voorhis BJ. In vitro fertilization is associated with an increase in major birth defects. *Fertil Steril* 2005;**84**:1308–1315.

22. Schieve LA, Rasmussen SA, Reefhuis J. Risk of birth defects among children conceived with assisted reproductive technology: providing an epidemiologic context to the data. *Fertil Steril* 2005;**84**:1320–1324; discussion 1327.

23. Wennerholm UB, Bergh C, Hamberger L, Lundin K, Nilsson L, Wikland M, Kallen B. Incidence of congenital malformations in children born after ICSI. *Hum Reprod* 2000;**15**:944–948.

24. Anthony S, Buitendijk SE, Dorrepaal CA, Lindner K, Braat DD, den Ouden AL. Congenital malformations in 4224 children conceived after IVF. *Hum Reprod* 2002;**17**:2089–2095.

25. Lie RT, Lyngstadaas A, Orstavik KH, Bakketeig LS, Jacobsen G, Tanbo T. Birth defects in children conceived by ICSI compared with children conceived by other IVF-methods; a meta-analysis. *Int J Epidemiol* 2005;**34**:696–701.

26. Reefhuis J, Honein MA, Schieve LA, Correa A, Hobbs CA, Rasmussen SA. Assisted reproductive technology and major structural birth defects in the United States. *Hum Reprod* 2009;**24**:360–366.

27. De Vigan C, Khoshnood B, Lhomme A, Vodovar V, Goujard J, Goffinet F. Prevalence and prenatal diagnosis of congenital malformations in the Parisian population: twenty years of surveillance by the Paris Registry of congenital malformations. *Journal de gynecologie, obstetrique et biologie de la reproduction* 2005;**34**:8–16.

28. Hook EB. Normal or affected controls in case–control studies of congenital malformations and other birth defects: reporting bias issues. *Epidemiology* 1993;**4**: 182–184.

29. Houyel L. Embryologie du coeur normal. *EMC (Elsevier Masson SAS, Paris), Cardiologie* 2009; 11–001-C-10.

30. Vrijheid M, Dolk H, Stone D, Abramsky L, Alberman E, Scott JE. Socioeconomic inequalities in risk of congenital anomaly. *Arch Dis Child* 2000;**82**:349–352.

31. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. *Int J Epidemiol* 1999;**28**:964–974.

32. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology* 1990;**1**:43–46.

33. Savitz DA, Olshan AF. Multiple comparisons and related issues in the interpretation of epidemiologic data. *Am J Epidemiol* 1995;**142**:904–908.

34. Wilson PD, Loffredo CA, Correa-Villasenor A, Ferencz C. Attributable fraction for cardiac malformations. *Am J Epidemiol* 1998;**148**:414–423.

35. Manipalviratn S, DeCherney A, Segars J. Imprinting disorders and assisted reproductive technology. *Fertil Steril* 2009;**91**:305–315.

36. Lieff S, Olshan AF, Werler M, Savitz DA, Mitchell AA. Selection bias and the use of controls with malformations in case–control studies of birth defects. *Epidemiology* 1999;**10**:238–241.

37. Swan SH, Shaw GM, Schulman J. Reporting and selection bias in case–control studies of congenital malformations. *Epidemiology* 1992;**3**:356–363.

38. Khoshnood B, De Vigan C, Vodovar V, Breart G, Goffinet F, Blondel B. Advances in medical technology and creation of disparities: the case of Down syndrome. *Am J Public Health* 2006;**96**:2139–2144.

39. Arpino C, Brescianini S, Robert E, Castilla EE, Cocchi G, Cornel MC, de Vigan C, Lancaster PA, Merlob P, Sumiyoshi Y, Zampino G, Renzi C, Rosano A, Mastroiacovo P. Teratogenic effects of antiepileptic drugs: use of an International Database on Malformations and Drug Exposure (MADRE). *Epilepsia* 2000;**41**: 1436–1443.

40. Dolk H, Jentink J, Loane M, Morris J, de Jong-van den Berg LT. Does lamotrigine use in pregnancy increase orofacial cleft risk relative to other malformations? *Neurology* 2008;**71**:714–722.

41. Simpson JL. Registration of congenital anomalies in ART populations: pitfalls. *Hum Reprod* 1996;**11**(Suppl. 4):81–88.

42. Botto LD, Mulinare J, Erickson JD. Occurrence of congenital heart defects in relation to maternal mulitivitamin use. *Am J Epidemiol* 2000;**151**:878–884.

43. Zhu JL, Basso O, Obel C, Bille C, Olsen J. Infertility, infertility treatment, and congenital malformations: Danish national birth cohort. *BMJ* 2006;**333**:679.

44. Jacobs JP, Anderson RH, Weinberg PM, Walters HL 3rd, Tchervenkov CI, Del Duca D, Franklin RC, Aiello VD, Beland MJ, Colan SD, Gaynor JW, Krogmann ON, Kurosawa H, Maruszewski B, Stellin G, Elliott MJ. The nomenclature, definition and classification of cardiac structures in the setting of heterotaxy. *Cardiol Young* 2007;**17**(Suppl. 2):1–28.