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ORIGINAL ARTICLE *Infertility*

The risk for four specific congenital heart defects associated with assisted reproductive techniques: a population-based evaluation

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STUDY QUESTION: Are the risks of hypoplastic left heart syndrome, transposition of great arteries, tetralogy of Fallot (TOF) and coarctation of the aorta increased in infants conceived by different assisted reproductive techniques (ARTs)?

STUDY ANSWER: ARTs, and particularly intracytoplasmic sperm injection (ICSI), are specifically associated with a higher risk of TOF.

WHAT IS ALREADY KNOWN: ARTs are associated with an increase in the overall risk of birth defects. The risk for congenital heart defects (CHDs) associated with ARTs has been evaluated as a whole but there is limited information on the risks for specific CHDs.

STUDY DESIGN, MATERIAL AND METHODS: We conducted a case–control study using population-based data from the Paris registry of congenital malformations for the period 1987–2009 and a cohort study of CHD (EPICARD) on 1583 cases of CHDs and 4104 malformed controls with no known associations with ARTs. ARTs included ovulation induction only, IVF and ICSI.

RESULTS: Exposure to ARTs was significantly higher for TOF than controls (6.6 versus 3.5%, $P = 0.002$); this was not the case for the other three CHDs. ARTs (all methods combined) were associated with a 2.4-fold higher odds of TOF after adjustment for maternal characteristics, paternal age and year of birth [adjusted odds ratios (OR): 2.4, 95% confidence interval (CI): 1.5–3.7] with the highest risk associated with ICSI (adjusted OR: 3.0, 95% CI: 1.0–8.9). No statistically significant associations were found for the other CHDs.

LIMITATIONS: Our study cannot disentangle to what extent the observed associations between the risk of TOF and ARTs are due to causal effects of ARTs and/or the underlying infertility problems of couples who conceive following ART.

IMPLICATIONS: The developmental basis of the specific association between the risk of TOF and ARTs need to be further investigated.

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COMPETING INTERESTS: None.

Key words: assisted reproductive techniques / intracytoplasmic sperm injection / congenital heart defects / tetralogy of Fallot / epidemiology

Introduction

Assisted reproductive techniques (ARTs) are known to be associated with a modest increase in the overall risk of congenital anomalies (Wennerholm *et al*., 2000; Hansen *et al*., 2002, 2005; Koivurova *et al*., 2002; Klemetti *et al*., 2005; Olson *et al*., 2005; Schieve *et al*., 2005). Relatively, little specific information exists on the risk of congenital heart defects (CHDs) for fetuses conceived following ARTs (Anthony *et al*., 2002; Hansen *et al*., 2002; Katalinic *et al*., 2004; Lie *et al*., 2005; Zhu *et al*., 2006; Reefhuis *et al*., 2009; Tararbit *et al*., 2011). Available evidence suggest an overall risk for CHDs in relation to ARTs that is comparable to that found for all congenital anomalies combined [odds ratios (OR) ~1.4–1.5] (Hansen *et al*., 2005; Tararbit *et al*., 2011). Specific associations between different methods of ARTs and categories of CHDs have also been reported by our group (Tararbit *et al*., 2011) using a subset of the data used in the present study.

However, previous studies have mostly examined the risk of CHDs in relation to ARTs for all CHDs combined or for broad categories of CHDs rather than for specific CHDs. Moreover, the associations between different methods of ARTs and specific CHDs have not been examined. Assessment of such specific associations is important as known teratogens are generally associated with the risk of one or a few specific malformations. Furthermore, specific associations between types of CHDs and ARTs may provide clues about the underlying mechanism of the higher risk of congenital malformations in fetuses conceived following ARTs.

Using population-based data from the Paris registry of congenital malformations and a cohort study of children with CHDs (the EPICARD study), we estimated the risks for four major specific CHDs: hypoplastic left heart syndrome (HLHS), transposition of great arteries (TGA), tetralogy of Fallot (TOF) and coarctation of the aorta (CoA) in relation to different methods of ARTs.

Materials and Methods

Data sources

Two sources of data were used for this study: (i) the Paris registry of congenital malformations and (ii) the EPICARD study (epidemiological study on the outcomes for congenital heart diseases). These two sources of data are briefly described below.

The Paris registry of congenital malformations

Since 1981, the Paris registry of congenital malformations registers all cases of birth defects and chromosomal anomalies among live births, stillbirths (≥22 weeks of gestation) and pregnancy terminations. The registry covers the population of women who live in the Greater Paris area (Paris and its surrounding suburb) and deliver or have a termination of pregnancy for fetal anomaly 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 (European Surveillance of Congenital Anomalies, EUROCAT) and of the International clearinghouse for birth defects surveillance and research (Eurocat Special Report, 2009; Cocchi *et al*., 2010; Greenlees *et al*., 2011; Khoshnood *et al*., 2011). The registry follows the EUROCAT methodology and the quality of data is routinely monitored by both the EUROCAT and the French National Committee of Registries. Review of procedures regarding confidentiality of data is overseen by both the National Committee of Registries and the National Committee of Informatics and Freedom (CNIL). Data are based on medical records and are collected from several sources including maternity units, neonatology wards, cytogenetic and pathology services.

In the present study, data from the registry corresponded to the period 1 January 1987 to 31 December 2009 as the first case of a malformation with exposure to IVF occurred in 1987 and 2009 was the last year for which data were available at the time of the study.

EPICARD

The EPICARD study is an on-going prospective cohort study of all children with a CHD (Khoshnood *et al*., 2012) born to women living in the Greater Paris area (Paris and its surrounding suburbs) between 2005 and 2008 regardless of the place of delivery ($n = 317\,538$ births). The principal objectives of the study are to use population-based data from a large cohort of patients with CHDs to: (i) estimate the total and live birth prevalence, (ii) examine timing of diagnosis and assess medical and surgical management of children with CHDs, (iii) evaluate neonatal mortality and morbidity and neuro-developmental outcomes of children with CHDs and (iv) identify the factors associated with their health outcomes, especially the role of events during the neonatal period and of the initial medical and surgical management. All cases (live births, pregnancy terminations, fetal deaths) diagnosed in the prenatal period or up to 1 year of age in the birth cohorts between 1 May 2005 and 30 April 2008 were eligible for inclusion. The total number of cases of CHDs included in the study was 2867, including 2348 live births (82%), 466 pregnancy terminations (16.2%) and 53 fetal deaths (1.8%). Diagnoses were confirmed in specialized paediatric cardiology departments and for the majority of pregnancy terminations and fetal deaths by a foetopathologist examination. For others in which a pathology examination could not be done, the diagnoses were confirmed by consensus by a paediatric cardiologist and a specialist in echocardiography in the study group based on the results of prenatal echocardiography examination.

Methods

A case–control study with malformed controls was performed. Cases were fetuses/children with HLHS, TGAs, TOF and CoA. Cases included in both the Paris registry and the EPICARD study were counted once. Malformed controls were isolated congenital defects other than CHDs for which no evidence of an association with ARTs was found in the literature. As recommended by Hook (1993), we selected a wide spectrum of heterogeneous birth defects as controls in order to decrease the risk of selection bias due to shared aetiologic factors between cases and controls (Swan *et al*., 1992; Lieff *et al*., 1999). The malformations in the control group comprised cases of club-foot, angioma, skin abnormality, polydactyly, syndactyly and congenital hip dislocation in the Paris registry.

The risk (odds) of each CHDs in relation to ARTs was the main outcome measure. Data on exposure to ARTs were obtained from medical records. The same procedure for data collection and coding was used for information on ARTs in the two data sets (Paris registry and EPICARD) used in this study. Exposure to ARTs included the following categories: ovulation induction (OI) only, IVF and ICSI. Exposure to ARTs was assessed as follows: (i) a binary variable (ART yes/no), (ii) a variable in four categories (no ART, OI, IVF, ICSI) and (iii) a variable combining IVF and ICSI (IVF + ICSI) in a single category.

Potential confounding factors considered were maternal characteristics (age, occupation and geographic origin), paternal age and year of birth (or pregnancy termination). Although their exact relations to the risk for specific CHDs are not well known, these factors are associated with both exposure to ARTs and prevalence of birth defects in general (Vrijheid *et al*., 2000). Maternal occupation was coded in five categories (professional, intermediate, administrative/public service, other and none) following

the French National Institute of Statistics and Economic Studies classification. Geographic origin was coded in four categories: French, North African, Sub-Saharan African and other countries.

Statistical analysis

The odds of each of the four specific CHDs versus controls in relation to ARTs was estimated using logistic regression models, after taking into account year of birth, maternal characteristics (age, occupation and geographic origin) and paternal age. Paternal age was missing for 20.6% of the study population. We used multiple imputation (Little and Rubin, 2002) for missing data on paternal age. Paternal age was imputed in 20 sets of data for each CHD separately using the case/control status, exposure to ARTs, maternal age and year of birth/termination. The pooled (over the 20 data sets) adjusted ORs for the association between ARTs and risk of each specific CHD were estimated using the method described by Little and Rubin (2002). In order to explore the possible role of multiple pregnancies in the association between ARTs and CHDs, we also conducted analyses with further adjustment for multiple pregnancies and tested for any interaction effect between multiple pregnancies and ARTs.

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

Analyses were done with Stata 11 software (Statacorp, Texas, USA).

Ethics approval

No specific ethical approval was needed for this particular analysis. The French CNIL has authorized the surveillance and research activities of the registry using anonymous data and has approved the EPICARD study.

Results

Study population

After excluding cases with missing data on ARTs (3% of cases), the study population comprised 353 cases of HLHS, 444 cases of TGA, 395 cases of TOF and 391 cases of CoA. Approximately 14% of cases of HLHS, 3% of TGA, 20% of TOF and 10% of CoA were associated with chromosomal anomalies. The study population included 4104 malformed controls with complete information on ARTs, which comprised 1436 with congenital hip dislocation, 824 with clubfoot, 782 with polydactyly, 517 with angioma, 381 with skin abnormality and 164 with syndactyly with complete information on ARTs; 3% of controls had missing data on ARTs.

Table I summarizes the results of the comparison of the maternal, paternal and pregnancy characteristics of cases of CHDs (all four specific CHDs combined) and controls. Overall, mothers of cases of CHDs were older, more likely to be from North Africa and in the occupational category 'none' when compared with mothers of controls. Stillbirths and terminations of pregnancy for fetal anomaly were more frequent for cases of CHDs than controls.

When comparisons of the characteristics of cases and controls were done for the four defects separately (detailed results not shown—available from authors), for CHDs other than TOF, the characteristics of cases and controls were for the most part comparable, except that mothers of cases of CoA were more likely to be from North Africa than controls. Most sociodemographic characteristics were different between cases of TOF and controls. Mothers of cases of TOF were significantly older and more likely to be from North Africa than controls. Mothers of cases of TOF were also

Table I Associations between predictor variables and case/control status.

Characteristics	Controls		Cases		P
	n	%[a]	*n*	%[a]	
Mother					
Age (years)					
Mean (SD)	30.4 (5.2)		30.9 (5.5)		
Median (p25–p75)	30 (27–34)		31 (27–35)		
<20	59	1.4	22	1.4	0.011
20–29	1809	42.8	654	40.1	
30–34	1434	33.9	531	32.6	
35–39	722	17.1	316	19.4	
≥40	203	4.8	107	6.6	
Missing[b]	23	0.5	12	0.7	
Geographic origin					
France	2412	57.9	882	54.5	<0.001
North Africa	433	10.4	247	15.3	
Sub-Saharan Africa	550	13.2	163	10.1	
Other	770	18.5	327	20.2	
Missing[b]	85	2.0	23	1.4	
Occupation					
None	1083	26.3	440	29.7	<0.001
Professional	997	24.2	343	23.2	
Intermediate	856	20.8	263	17.8	
Administrative/public service	852	20.7	249	16.8	
Other	330	8.0	185	12.5	
Missing[b]	132	3.1	162	9.9	
Father					
Age (years)					
Mean (SD)	33.9 (6.6)		34.4 (6.7)		
Median (p25–p75)	33 (29–38)		33 (30–38)		
<20	5	0.1	3	0.2	0.133
20–29	890	25.8	277	22.6	
30–34	1198	34.7	422	34.4	
35–39	734	21.3	281	22.9	
≥40	623	18.1	244	19.9	
Missing[b]	800	18.8	415	25.3	
Pregnancy					
Multiplicity					
Singletons	2768	96.1	1382	95.8	0.756
Twins	103	3.6	57	4.0	
Triplets	8	0.3	3	0.2	
Outcome					
Stillbirths	7	0.2	46	2.8	<0.001
Live births	4231	99.6	1074	65.4	
Pregnancy terminations	12	0.3	522	31.8	

[a]% calculated with the total number of cases or controls without missing data as a denominator.
[b]% of missing data calculated with the total number of cases or controls as a denominator.

more likely to be in the occupational category 'none' than controls (data not shown).

Risk of CHDs associated with ARTs

All cases

Exposure to ARTs (all methods combined, Table II) was significantly higher for cases of TOF than controls (6.6 versus 3.5%, $P = 0.002$). Exposure to the different methods of ARTs (data not shown) was also significantly different between cases of TOF and controls, in particular 2.5% of TOF were born following IVF versus 1.3% of controls and 1.3% of TOF were born following ICSI versus 0.3% of controls ($P = 0.004$). Exposure to ARTs was not associated with a significantly higher risk of other CHDs.

Exposure to ART was associated with a 2·4-fold increase in the maternal characteristics and year of birth-adjusted odds of TOF (adjusted OR = 2.4, 95% CI: 1.5–3.7) (Table III). In contrast, ARTs were not associated with statistically significant increases in the risks of HLHS, TGA or CoA and the ORs were generally close to the null value (Table III). All three methods of ARTs were associated with significantly higher odds of TOF (Table IV). In particular, ICSI was associated with a 3-fold higher odds of TOF after adjustment for maternal characteristics and year of birth (adjusted OR = 3.0, 95% CI: 1.0–8.9). There was no evidence that IVF was associated with a higher odds of TOF when compared with OI (for IVF: adjusted OR = 2.0, 95% CI: 1.0–4.2; for OI: adjusted OR = 2.5, 95% CI: 1.3–4.8). For the other three specific CHDs, no statistically significant associations were observed. Further adjustment for paternal age using the multiple imputation estimates did not modify appreciably the above estimates (data not shown).

Table II Numbers of cases and controls and proportions of fetuses conceived after ARTs.

	n	% exposed to ART	*P*[b]
Controls[a]	4104	3.5	
All cases			
HLHS	353	2.8	0.491
TGA	444	2.7	0.363
TOF	395	6.6	0.002
CoA	391	3.3	0.831
Cases without chromosomal anomalies			
HLHS	303	2.6	0.413
TGA	430	2.8	0.423
TOF	315	7.3	0.001
CoA	350	3.7	0.860

CoA, coarctation of the aorta; HLHS, hypoplastic left heart syndrome; TGA, transposition of the great arteries; TOF, tetralogy of Fallot.
[a]The following malformations were used as controls: club-foot, angioma, skin abnormality, polydactyly, syndactyly and congenital hip dislocation.
[b]Comparison of the proportion of children/fetuses conceived after ART between the specific CHD and the malformed controls.

Cases without associated chromosomal anomalies

Tables III and V show the results of the analyses for the associations between the risks of the four CHDs and ARTs (all methods combined, Table III) and separately for different methods of ARTs (Table V) for the subset of cases without associated chromosomal anomalies. All estimates were essentially the same as those found for all cases combined (i.e. when cases of each specific CHD with and without associated chromosomal anomalies were analysed together).

Results of the analyses, which included further adjustment for multiple pregnancies, were essentially the same as those found without adjustment for multiple pregnancies (data not shown). We found no statistically significant interaction effects between ARTs and multiple pregnancies for any of the four CHDs (data not shown).

Discussion

Using population-based data on nearly 1600 cases of specific CHDs, we assessed the risk of four specific CHDs in relation to ARTs. We found that ARTs (all methods combined) were associated with a 2·4-fold increased risk of TOF, after taking into account maternal age, occupation, geographic origin, paternal age and year of birth. In particular, ICSI was associated with a 3-fold higher adjusted odds of TOF. In contrast, we did not find any statistically significant increases in the risk of CHDs in relation to ARTs for the other CHDs in our study, i.e. HLHS, transposition of the great arteries (TGA) and CoA. Risk estimates were comparable when cases with chromosomal anomalies were excluded, suggesting that the associations between ARTs and TOF are not due to the association of the latter with chromosomal anomalies. Further adjustment for multiple pregnancies did not substantially modify our results.

On the basis of our findings, we calculated attributable risk fractions, which would represent the proportion of cases of TOF that may be caused by ARTs, or equivalently, the proportion of cases of TOF that would be avoided were the exposure to ARTs removed ceteris paribus, 'if' the association we found between the risk of TOF and ARTs can be assumed to represent a causal relation (this may of course not be the case in part for reasons that are discussed further below). The attributable risk fraction estimates suggested in particular that around 6.5% of the TOF may have been caused by ARTs (all methods combined) and 2% by ICSI.

Our study has certain limitations. We had limited power to detect OR lower than 2 in the association between ARTs (for all methods combined) and specific CHDs and three in the case of the different methods of ARTs. Therefore, our study may have had insufficient power to detect statistically significant associations for other CHDs.

The models used to estimate the ORs for the different defects in relation to ARTs were not nested (i.e. were separate models) and we did not formally test the statistical significance of differences in the ORs for one defect versus another. The associations were not statistically significant for any of the defects except for TOF, whereas the numbers of cases for the other CHDs were comparable to those of TOF.

A potential source of bias in our study is related to the use of malformed controls (Swan *et al.*, 1992; Lieff *et al.*, 1999). The main advantage of using malformed controls is to reduce the risk of recall or other sources of information bias. But malformed controls may also be a source of selection

Table III Logistic regression analyses of the associations between assisted reproductive technologies (ART, all methods combined) and four specific CHDs.

	CHDs	ART	Unadjusted OR[a]	95% CI	Maternal Adjusted[b] OR[a]	95% CI
All cases	HLHS	None	1.0	Ref.	1.0	Ref.
		All methods combined	0.8	0.4–1.5	0.8	0.4–1.8
	Transposition of the great arteries	None	1.0	Ref.	1.0	Ref.
		All methods combined	0.8	0.4–1.4	0.7	0.4–1.4
	TOF	None	1.0	Ref.	1.0	Ref.
		All methods combined	1.9	1.3–3.0	2.4	1.5–3.7
	CoA	None	1.0	Ref.	1.0	Ref.
		All methods combined	0.9	0.5–1.7	1.1	0.6–2.0
Cases without chromosomal anomalies	HLHS	None	1.0	Ref.	1.0	Ref.
		All methods combined	0.7	0.4–1.5	0.8	0.3–1.7
	Transposition of the great arteries	None	1.0	Ref.	1.0	Ref.
		All methods combined	0.8	0.4–1.4	0.7	0.4–1.4
	TOF	None	1.0	Ref.	1.0	Ref.
		All methods combined	2.2	1.4–3.4	2.6	1.6–4.2
	CoA	None	1.0	Ref.	1.0	Ref.
		All methods combined	1.1	0.6–1.9	1.2	0.6–2.2

[a]Odds ratios (OR) represent the odds of a birth (including live births, stillbirths and pregnancy terminations) with congenital heart defect (cases) relative to the odds of a birth with one of the malformed controls (club-foot, angioma, skin abnormality, polydactyly, syndactyly and congenital hip dislocation).
[b]Adjusted for maternal age, geographic origin, occupation and year of birth.

Table IV Logistic regression analyses of the associations between the different methods of ARTs and four specific CHDs.

CHD	ART	Unadjusted OR[a]	95% CI	Maternal Adjusted[b] OR[a]	95% CI
HLHS	None	1.0	Ref.	1.0	Ref.
	OI only	0.7	0.3–1.9	0.9	0.3–2.5
	IVF	0.6	0.2–2.0	0.5	0.1–2.3
	ICSI	1.8	0.4–7.9	1.6	0.3–7.2
	IVF + ICSI	0.8	0.3–2.1	0.8	0.3–2.3
Transposition of the great arteries	None	1.0	Ref.	1.0	Ref.
	OI only	0.6	0.2–1.5	0.6	0.2–1.7
	IVF	1.2	0.5–2.6	1.0	0.4–2.5
	ICSI	—	—	—	—
	IVF + ICSI	—	—	—	—
TOF	None	1.0	Ref.	1.0	Ref.
	OI only	1.5	0.8–2.9	2.5	1.3–4.8
	IVF	2.0	1.0–3.9	2.0	1.0–4.2
	ICSI	4.1	1.5–11.6	3.0	1.0–8.9
	IVF + ICSI	2.4	1.3–4.2	2.3	1.2–4.2
CoA	None	1.0	Ref.	1.0	Ref.
	OI only	0.7	0.3–1.7	1.0	0.4–2.6
	IVF	1.0	0.4–2.4	1.1	0.4–2.9
	ICSI	2.4	0.7–8.5	1.2	0.2–5.6
	IVF + ICSI	1.2	0.6–2.6	1.1	0.5–2.6

[a]Odds ratios (OR) represent the odds of a birth (including live births, stillbirths and pregnancy terminations) with congenital heart defect (cases) relative to the odds of a birth with one of the malformed controls (club-foot, angioma, skin abnormality, polydactyly, syndactyly and congenital hip dislocation).
[b]Adjusted for maternal age, geographic origin, occupation and year of birth.

bias if malformations included as controls are either directly or indirectly associated with ARTs. Risks could be under (over)-estimated if malformations included in the control group occur more (less) frequently in fetuses conceived following ARTs. By selecting a heterogeneous group of malformations with no known association with ARTs, as recommended by Hook (1993), we aimed to minimize such bias. However, the possibility

Table V Logistic regression analyses of the associations between ARTs and four specific CHDs without associated chromosomal anomalies.

CHD	ART	Unadjusted OR[a]	95% CI	Maternal Adjusted[b] OR[a]	95% CI
HLHS	None	1.0	Ref.	1.0	Ref.
	OI only	0.7	0.3–1.9	0.8	0.2–2.5
	IVF	0.5	0.1–2.0	0.3	0.0–2.4
	ICSI	2.1	0.5–9.2	1.8	0.4–8.4
	IVF + ICSI	0.8	0.3–2.2	0.7	0.2–2.3
Transposition of the great arteries	None	1.0	Ref.	1.0	Ref.
	OI only	0.6	0.2–1.5	0.6	0.2–1.8
	IVF	1.2	0.5–2.7	1.1	0.5–2.6
	ICSI	—	—	—	—
	IVF + ICSI	—	—	—	—
TOF	None	1.0	Ref.	1.0	Ref.
	OI only	1.6	0.8–3.2	2.3	1.1–4.8
	IVF	2.2	1.1–4.5	2.5	1.2–5.2
	ICSI	5.2	1.8–14.7	3.7	1.3–10.9
	IVF + ICSI	2.8	1.6–5.0	2.8	1.5–5.2
CoA	None	1.0	Ref.	1.0	Ref.
	OI only	0.8	0.3–1.9	1.1	0.4–2.8
	IVF	1.1	0.4–2.7	1.3	0.5–3.3
	ICSI	2.7	0.8–9.6	1.3	0.3–6.1
	IVF + ICSI	1.4	0.7–2.9	1.3	0.6–2.9

[a]Odds ratios (OR) represent the odds of a birth (including live births, stillbirths and pregnancy terminations) with congenital heart defect (cases) relative to the odds of a birth with one of the malformed controls (club-foot, angioma, skin abnormality, polydactyly, syndactyly and congenital hip dislocation).
[b]Adjusted for maternal age, geographic origin, occupation and year of birth.

of residual bias due to shared aetiologies between cases and malformed controls cannot be excluded.

A differential misclassification bias for exposure assessment cannot be excluded if exposure to ARTs is ascertained in a different way for cases and controls. However, we have no reason to believe that ARTs may have been ascertained differentially for cases of TOF versus the other CHDs examined in our study.

We had a relatively high proportion of missing data on paternal age. The latter is known to be associated with ARTs and more specifically with ICSI. Estimates for ICSI could therefore be biased if the distribution of paternal age was different for subjects with missing data. We used multiple imputations for imputing missing paternal age using case/control status, exposure to ARTs, maternal age and year of birth, and adjustment for paternal age did not appreciably change our results. However, residual bias due to other paternal characteristics cannot be excluded.

The question of multiple pregnancies and its association with both ARTs and the risk of congenital anomalies is an important issue to consider. There is evidence suggesting that multiple pregnancies may be associated with a higher risk of congenital anomalies (Mastroiacovo *et al.*, 1999; Glinianaia *et al.*, 2008). This may specifically be the case for CHDs, although relatively little, and at times contradictory, information exists on the associations between multiple pregnancies and CHDs (Manning and Archer, 2006; Bahtiyar *et al.*, 2007; Campbell *et al.*, 2009). Moreover, it is not clear to what extent any association between multiple pregnancies and CHDs may in fact be due to ARTs. Our results remained similar after further adjustment for multiple pregnancies and we did not find any statistically significant interaction effects between ARTs and multiple pregnancies for any of the CHDs, although this may have been due to limited power of our study for detecting interaction effects. In any case, none of the above precludes the possibility that multiple pregnancies may be on the causal pathway between ARTs and CHDs. It is worth noting, however, that the public health impact of ARTs on the risk for birth defects, including that of TOF found in our study, includes all (singleton and multiple) pregnancies.

Specific associations between ARTs and certain categories of CHDs, particularly the so-called conotruncal defects, which include TOF, have been reported (Tararbit *et al.*, 2011; Reefhuis *et al.*, 2009). In a recent study (Tararbit *et al.*, 2011), the risk of CHDs associated with ARTs was also shown to vary more generally for different methods of ARTs and categories of CHDs defined based on anatomic and clinical criteria (Houyel *et al.*, 2011). In particular, the authors found a stronger association between ICSI and the category 'Malformations of the outflow tracts and ventriculoarterial connections' that comprised, among other CHDs, the conotruncal defects.

The developmental origins of TOF are complex and not fully understood but they may involve abnormal development of neural crest cells. None of the other three CHDs studied is known to be of cardiac neural crest origin. In particular, TGA which is a defect of the outflow tract does not belong to the group of the conotruncal defects (Houyel *et al.*, 2011) and migration/proliferation of neural crest cell appear to be normal in this condition (Bajolle *et al.*, 2006). In order to further investigate, the hypothesis of the involvement of neural crest cells in the association between TOF and ARTs, we

assessed the risk for other, rarer CHDs thought to be of neural crest origin (TOF with pulmonary atresia, TOF with absent pulmonary valve and common arterial trunk). We found an increased overall risk associated with ARTs (data not shown) but the CIs were wide due to small sample sizes.

Given the uncertainties about both the developmental origins of cardiac defects and possible effects of ARTs on fetal development, the hypothesis of a potential implication of neural crest cells in the association between ARTs and TOF must be regarded as very tentative and no more than a reasonable speculation. Future observational and experimental studies using other designs (e.g. animal studies, genetic studies, fundamental research in biology of reproduction/ARTs as well as additional epidemiological studies) are needed to both further assess our observations and in order to understand the possible underlying mechanisms of the association between the risk of TOF and ARTs.

In conclusion, we found that cases of TOF were more likely to have been conceived following ARTs when compared with controls. ARTs were associated with a 2·4-fold higher risk of TOF after adjustment for maternal age, occupation, geographic origin, paternal age and year of birth; ICSI was specifically associated with a 3-fold higher risk of TOF. In contrast, we did not find statistically significant associations between ARTs and HLHS, TGA or CoA and most ORs were close to the null value. Our study cannot disentangle to what extent the observed associations between the risk of TOF and ARTs may be due to any causal effects of ARTs and/or the underlying infertility problems of couples who conceive following ARTs. Nevertheless, the developmental basis of the specific association between risk of TOF and ARTs, particularly ICSI, and the potential implication of neural crest cells in this association, need to be further investigated.

Authors' roles

B.K. conceived the study. K.T. conducted the main statistical analyses and wrote the first draft of the manuscript with B.K. N.L. and A-C.T. assisted with statistical analysis. L.H., D.B. and F.G. contributed to the conceptualization of ideas and made suggestions about the required analyses. L.H. and D.B. provided expertise as paediatric cardiologists. All of the authors contributed to the interpretation of findings and revisions of the article.

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Conflict of interest

None declared.

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