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

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1 **Title**

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

4

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29

30 Running title : Assisted reproduction and congenital heart defects

## 31 **Abstract**

32 **Study question.** Are the risks of hypoplastic left heart syndrome, transposition of great arteries,  
33 tetralogy of Fallot (TOF) and coarctation of the aorta increased in infants conceived by different  
34 assisted reproductive techniques (ART)?

35 **Study answer.** ART, and particularly intracytoplasmic sperm injection (ICSI), are specifically  
36 associated with a higher risk of TOF.

37 **What is already known.** ART are associated with an increase in the overall risk of birth defects.  
38 The risk for congenital heart defects (CHD) associated with ART has been evaluated as a whole  
39 but there is limited information on the risks for specific CHD.

40 **Study design, material and methods.** We conducted a case-control study using population-  
41 based data from the Paris registry of congenital malformations for the period 1987-2009 and a  
42 cohort study of CHD (EPICARD) on 1583 cases of CHD and 4104 malformed controls with no  
43 known associations with ART. ART included ovulation induction only, IVF and ICSI.

44 **Results.** Exposure to ART was significantly higher for TOF than controls (6.6 vs. 3.5%,  
45  $P=0.002$ ); this was not the case for the other three CHD. ART (all methods combined) were  
46 associated with a 2.4-fold higher odds of TOF after adjustment for maternal characteristics,  
47 paternal age and year of birth (Adjusted OR 2.4, 95% CI 1.5–3.7) with the highest risk  
48 associated with ICSI (Adjusted OR 3.0, 95%CI 1.0-8.9). No statistically significant associations  
49 were found for the other CHD.

50 **Limitations.** Our study cannot disentangle to what extent the observed associations between  
51 risk of TOF and ART are due to causal effects of ART and/or the underlying infertility problems  
52 of couples who conceive following ART.

53 **Implications.** The developmental basis of the specific association between the risk of TOF and  
54 ART and in particular the possible role of neural crest cells in this association need to be further  
55 investigated.

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58 support from INSERM (Paris, France) and the Institut de Veille Sanitaire (Saint-Maurice,  
59 France). The EPICARD study was supported by three grants from the Ministry of Health  
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61 AREMCAR Association (Association pour la Recherche et l'Etude des Maladies  
62 Cardiovasculaires).

63 **Competing interests.** None.

64 **Key Words**

65 Reproductive techniques, assisted

66 Intracytoplasmic sperm injection

67 Heart defects, congenital

68 Tetralogy of Fallot

69 Epidemiology

## 70 **Introduction**

71 Assisted reproductive techniques (ART) are known to be associated with a modest increase in  
72 the overall risk of congenital anomalies (Wennerholm et al., 2000; Hansen et al., 2002;  
73 Koivurova et al., 2002; Hansen et al., 2005; Klemetti et al., 2005; Olson et al., 2005; Schieve et  
74 al., 2005). Relatively little specific information exist on the risk of congenital heart defects  
75 (CHD) for foetuses conceived following ART (Anthony et al., 2002; Hansen et al., 2002;  
76 Katalinic et al., 2004; Lie et al., 2005; Zhu et al., 2006; Reefhuis et al., 2009; Tararbit et al.,  
77 2011). Available evidence suggest an overall risk for CHD in relation to ART that is comparable  
78 to that found for all congenital anomalies combined (OR~1.4-1.5) (Hansen et al., 2005; Tararbit  
79 et al., 2011). Specific associations between different methods of ART and categories of CHD  
80 have also been reported by our group (Tararbit et al., 2011) using a subset of the data used in the  
81 present study.

82 However, previous studies have mostly examined the risk of CHD in relation to ART for all  
83 CHD combined or for broad categories of CHD rather than for specific CHD. Moreover, the  
84 associations between different methods of ART and specific CHD have not been examined.  
85 Assessment of such specific associations is important as known teratogens are generally  
86 associated with the risk of one or a few specific malformations. Furthermore, specific  
87 associations between types of CHD and ART may provide clues about the underlying  
88 mechanism of the higher risk of congenital malformations in foetuses conceived following ART.  
89 Using population-based data from the Paris registry of congenital malformations and a cohort  
90 study of children with CHD (the EPICARD study), we estimated the risks for four major  
91 specific CHD: hypoplastic left heart syndrome (HLHS), transposition of great arteries (TGA),  
92 tetralogy of Fallot (TOF), and coarctation of the aorta (CoA) in relation to different methods of  
93 ART.

## 94 **Material and methods**

### 95 Data sources

96 Two sources of data were used for this study: 1) the Paris registry of congenital malformations  
97 and 2) the EPICARD study (Epidemiological study on the outcomes for congenital heart  
98 diseases). These two sources of data are briefly described below.

99

#### 100 *The Paris registry of congenital malformations*

101 Since 1981, the Paris registry of congenital malformations registers all cases of birth defects and  
102 chromosomal anomalies among live-births, still-births ( $\geq 22$  weeks of gestation), and pregnancy  
103 terminations. The registry covers the population of women who live in the Greater Paris area  
104 (Paris and its surrounding suburb) and deliver or have a termination of pregnancy for foetal  
105 anomaly in a Parisian maternity unit. The annual number of deliveries in our population is about  
106 38,000.

107 The Paris registry is a member of the European network of registries of congenital  
108 malformations (European Surveillance of Congenital Anomalies, EUROCAT) and of the  
109 International clearinghouse for birth defects surveillance and research (Eurocat special report,  
110 2009; Cocchi et al., 2010; Greenlees et al., 2011; Khoshnood et al., 2011). The registry follows  
111 the EUROCAT methodology and quality of data is routinely monitored by both EUROCAT and  
112 the French National Committee of Registries. Review of procedures regarding confidentiality of  
113 data is overseen by both the National Committee of Registries and the National Committee of  
114 Informatics and Freedom (CNIL). Data are based on medical records and are collected from  
115 several sources including maternity units, neonatology wards, cytogenetic, and pathology  
116 services.

117 In the present study, data from the registry corresponded to the period January 1st 1987 to  
118 December 31st 2009 as the first case of a malformation with exposure to *in vitro* fertilization

119 occurred in 1987 and 2009 was the last year for which data were available at the time of the  
120 study.

121

## 122 *EPICARD*

123 The EPICARD study is an on-going prospective cohort study of all children with a CHD  
124 (Khoshnood et al., 2012) born to women living in the Greater Paris area (Paris and its  
125 surrounding suburbs) between 2005 and 2008 regardless of the place of delivery (N = 317,538  
126 births). The principal objectives of the study are to use population-based data from a large  
127 cohort of patients with CHD to: i) estimate the total and live birth prevalence, ii) examine timing  
128 of diagnosis and assess medical and surgical management of children with CHD, iii) evaluate  
129 neonatal mortality and morbidity and neuro-developmental outcomes of children with CHD;  
130 and iv) identify the factors associated with their health outcomes, especially the role of events  
131 during the neonatal period and of the initial medical and surgical management. All cases (live  
132 births, pregnancy terminations, foetal deaths) diagnosed in the prenatal period or up to one year  
133 of age in the birth cohorts between May 1<sup>st</sup> 2005 and April 30<sup>th</sup> 2008 were eligible for inclusion.  
134 The total number of cases of CHD included in the study was 2867, including 2348 live births  
135 (82%), 466 pregnancy terminations (16.2%) and 53 foetal deaths (1.8%). Diagnoses were  
136 confirmed in specialized paediatric cardiology departments and for the majority of pregnancy  
137 terminations and foetal deaths by a foetopathologist examination. For others in which a  
138 pathology exam could not be done, the diagnoses were confirmed by consensus by a paediatric  
139 cardiologist and a specialist in echocardiography in the study group based on results of prenatal  
140 echocardiography examination.

141

## 142 Methods

143 A case-control study with malformed controls was performed. Cases were fetuses/children with  
144 hypoplastic left heart syndrome (HLHS), transposition of great arteries (TGA), tetralogy of  
145 Fallot (TOF) and coarctation of the aorta (CoA). Cases included in both the Paris registry and  
146 the EPICARD study were counted once. Malformed controls were isolated congenital defects  
147 other than CHD for which no evidence of an association with ART was found in literature. As  
148 recommended by Hook (1993), we selected a wide spectrum of heterogeneous birth defects as  
149 controls in order to decrease the risk of selection bias due to shared etiologic factors between  
150 cases and controls (Swan et al., 1992; Lieff et al., 1999). The malformations in the control group  
151 comprised cases of club-foot, angioma, skin abnormality, polydactyly, syndactyly and  
152 congenital hip dislocation in the Paris registry.

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

160 Potential confounding factors considered were maternal characteristics (age, occupation and  
161 geographic origin), paternal age, and year of birth (or pregnancy termination). Although their  
162 exact relations to the risk for specific CHD are not well known, these factors are associated with  
163 both exposure to ART and prevalence of birth defects in general (Vrijheid et al., 2000). Maternal  
164 occupation was coded in five categories (professional, intermediate, administrative/public  
165 service, other, and none) following the French National Institute of Statistics and Economic  
166 Studies (INSEE) classification. Geographic origin was coded in four categories: French, North  
167 African, Sub-Saharan African, and other countries.

168

169

170 Statistical analysis

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

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

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

184

185 Ethics approval

186 No specific ethical approval was needed for this particular analysis. The French National  
187 Committee of Informatics and Freedom (CNIL) has authorised the surveillance and research  
188 activities of the registry using anonymous data and has approved the EPICARD study.

## 189 **Results**

### 190 Study population

191 After excluding cases with missing data on ART (3% of cases), the study population comprised  
192 353 cases of HLHS, 444 cases of TGA, 395 cases of TOF and 391 cases of CoA. Approximately  
193 14% of cases of HLHS, 3% of TGA, 20% of TOF and 10% of CoA were associated with  
194 chromosomal anomalies. The study population included 4104 malformed controls with complete  
195 information on ART, which comprised 1436 with congenital hip dislocation, 824 with club-foot,  
196 782 with polydactyly, 517 with angioma, 381 with skin abnormality, and 164 with syndactyly  
197 with complete information on ART; 3% of controls had missing data on ART.

198 Table 1 summarises the results of the comparison of the maternal, paternal and pregnancy  
199 characteristics of cases of CHD (all four specific CHD combined) and controls. Overall,  
200 mothers of cases of CHD were older, more likely to be from North Africa and in the  
201 occupational category "none" as compared with mothers of controls. Still births and  
202 terminations of pregnancy for foetal anomaly were more frequent for cases of CHD than  
203 controls.

204 When comparisons of the characteristics of cases and controls were done for the four defects  
205 separately (detailed results not shown – available from authors), for CHD other than TOF, the  
206 characteristics of cases and controls were for the most part comparable, except that mothers of  
207 cases of CoA were more likely to be from North Africa than controls. Most sociodemographic  
208 characteristics were different between cases of TOF and controls. Mothers of cases of TOF were  
209 significantly older and more likely to be from North Africa than controls. Mothers of cases of  
210 TOF were also more likely to be in the occupational category “none” than controls (data not  
211 shown).

212

### 213 Risk of CHD associated with ART

214 *All cases*

215 Exposure to ART (all methods combined, Table 2) was significantly higher for cases of TOF  
216 than controls (6.6% vs. 3.5%,  $p=0.002$ ). Exposure to the different methods of ART (data not  
217 shown) was also significantly different between cases of TOF and controls, in particular 2.5% of  
218 TOF were born following IVF vs. 1.3% of controls and 1.3% of TOF were born following ICSI  
219 vs. 0.3% of controls ( $p=0.004$ ). Exposure to ART was not associated with a significantly higher  
220 risk of other CHD.

221 Exposure to ART was associated with a 2.4-fold increase in the maternal characteristics and  
222 year of birth-adjusted odds of TOF (Adjusted OR= 2.4, 95%CI 1.5 – 3.7) (Table 3). ~~The odds of~~  
223 ~~TOF associated with ART remained similar after further adjustment for paternal age.~~ In contrast,  
224 ART were not associated with statistically significant increases in the risks of HLHS, TGA or  
225 CoA and the ORs were generally close to the null value (Table 3). All three methods of ART  
226 were associated with significantly higher odds of TOF (Table 4). In particular, ICSI was  
227 associated with a three-fold higher odds of TOF after adjustment for maternal characteristics and  
228 year of birth (Adjusted OR= 3.0, 95%CI 1.0-8.9). There was no evidence that IVF was  
229 associated with a higher odds of TOF as compared with OI (for IVF: Adjusted OR=2.0, 95%CI  
230 1.0 – 4.2; for OI: Adjusted OR= 2.5, 95%CI 1.3 – 4.8). For the other three specific CHD, no  
231 statistically significant associations were observed. Further adjustment for paternal age using the  
232 multiple imputation estimates did not modify appreciably the above estimates (data not shown).

233

234 *Cases without associated chromosomal anomalies*

235 Tables 3 and 5 show the results of the analyses for the associations between the risks of the four  
236 CHD and ART (all methods combined, Table 3) and separately for different methods of ART  
237 (Table 5) for the subset of cases without associated chromosomal anomalies. All estimates were

238 essentially the same as those found for all cases combined (i.e. when cases of each specific CHD  
239 with and without associated chromosomal anomalies were analysed together).

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

## 244 Discussion

245 Using population-based data on nearly 1600 cases of specific congenital heart defects (CHD),  
246 we assessed the risk of four specific CHD in relation to assisted reproductive techniques (ART).  
247 We found that ART (all methods combined) were associated with a 2.4-fold increased risk of  
248 tetralogy of Fallot (TOF), after taking into account maternal age, occupation, geographic origin,  
249 paternal age, and year of birth. In particular, ICSI was associated with a three-fold higher  
250 adjusted odds of TOF. In contrast, we did not find any statistically significant increases in the  
251 risk of CHD in relation to ART for the other CHD in our study, i.e., hypoplastic left heart  
252 syndrome (HLHS), transposition of the great arteries (TGA), and coarctation of the aorta (CoA).  
253 Risk estimates were comparable when cases with chromosomal anomalies were excluded,  
254 suggesting that the associations between ART and TOF are not due to the association of the  
255 latter with chromosomal anomalies. Further adjustment for multiple pregnancies did not  
256 substantially modify our results.

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

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

268 The models used to estimate the odds ratios for the different defects in relation to ART were not  
269 nested (i.e., were separate models) and we did not formally test the statistical significance of

270 differences in the odds ratios for one defect vs. another. The associations were not statistically  
271 significant for any of the defects except for TOF, whereas the numbers of cases for the other  
272 CHD were comparable to those of TOF.

273 A potential source of bias in our study is related to the use of malformed controls (Swan et al.,  
274 1992; Lieff et al., 1999). The main advantage of using malformed controls is to reduce the risk  
275 of recall or other sources of information bias. But malformed controls may also be a source of  
276 selection bias if malformations included as controls are either directly or indirectly associated  
277 with ART. Risks could be under (over)-estimated if malformations included in the control group  
278 occur more (less) frequently in foetuses conceived following ART. By selecting a heterogeneous  
279 group of malformations with no known association with ART, as recommended by Hook (1993),  
280 we aimed to minimize such bias. However, the possibility of residual bias due to shared  
281 aetiologies between cases and malformed controls cannot be excluded.

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

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

292 The question of multiple pregnancies and its association with both ART and the risk of  
293 congenital anomalies is an important issue to consider. There is evidence suggesting that  
294 multiple pregnancies may be associated with a higher risk of congenital anomalies  
295 (Mastroiacovo, et al., 1999; Glinianaia et al., 2008). This may specifically be the case for CHD,

296 although relatively little, and at times contradictory, information exist on the associations  
297 between multiple pregnancies and CHD (Manning et al., 2006; Bahtiyar et al., 2007; Campbell  
298 et al., 2009). Moreover, it is not clear to what extent any association between multiple  
299 pregnancies and CHD may in fact be due to ART. Our results remained similar after further  
300 adjustment for multiple pregnancies and we did not find any statistically significant interaction  
301 effects between ART and multiple pregnancies for any of the CHD, although this may have been  
302 due to limited power of our study for detecting interaction effects. In any case, none of the  
303 above precludes the possibility that multiple pregnancies may be on the causal pathway between  
304 ART and CHD. It is worth noting however that the public health impact of ART on the risk for  
305 birth defects, including that of TOF found in our study, includes all (singleton and multiple)  
306 pregnancies.

307 Specific associations between ART and certain categories of CHD, particularly the so-called  
308 conotruncal defects, which include TOF, have been reported (Reefhuis et al., 2009; Tararbit et  
309 al., 2001). In a recent study (Tararbit et al., 2011), the risk of CHD associated with ART was  
310 also shown to vary more generally for different methods of ART and categories of CHD defined  
311 based on anatomic and clinical criteria (Houyel et al., 2011). In particular, the authors found a  
312 stronger association between ICSI and the category “Malformations of the outflow tracts and  
313 ventriculoarterial connections” that comprised, among other CHD, the conotruncal defects.

314 The developmental origins of TOF are complex and not fully understood but they may involve  
315 abnormal development of neural crest cells. None of the other three CHD studied is known to be  
316 of cardiac neural crest origin. In particular TGA which is a defect of the outflow tract does not  
317 belong to the group of the conotruncal defects (Houyel et al., 2011) and migration/proliferation  
318 of neural crest cell appear to be normal in this condition (Bajolle et al., 2006). In order to further  
319 investigate, the hypothesis of the involvement of neural crest cells in the association between  
320 TOF and ART, we assessed the risk for other, rarer CHD thought to be of neural crest origin  
321 (TOF with pulmonary atresia, TOF with absent pulmonary valve, and common arterial trunk).

322 We found an increased overall risk associated with ART (data not shown) but the confidence  
323 intervals were wide due to small sample sizes.

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

332 In conclusion, we found that cases of TOF were more likely to have been conceived following  
333 ART when compared with controls. ART were associated with a 2.4-fold higher risk of TOF  
334 after adjustment for maternal age, occupation, geographic origin, paternal age and year of birth;  
335 ICSI was specifically associated with a three-fold higher risk of TOF. In contrast, we did not  
336 find statistically significant associations between ART and HLHS, TGA or CoA and most ORs  
337 were close to the null value. Our study cannot disentangle to what extent the observed  
338 associations between risk of TOF and ART may be due to any causal effects of ART and/or the  
339 underlying infertility problems of couples who conceive following ART. Nevertheless, the  
340 developmental basis of the specific association between risk of TOF and ART, particularly ICSI,  
341 and the potential implication of neural crest cells in this association, need to be further  
342 investigated.

**343 Role of authors**

344 B. Khoshnood conceived the study. K. Tararbit conducted the main statistical analyses and wrote  
345 the first draft of the manuscript with B. Khoshnood. N. Lelong, A-C. Thieulin assisted with  
346 statistical analysis. L. Houyel, D. Bonnet and F. Goffinet contributed to the conceptualization of  
347 ideas and made suggestions about the required analyses. L. Houyel and D. Bonnet provided  
348 expertise as paediatric cardiologists. All of the authors contributed to the interpretation of  
349 findings and revisions of the article.

350

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358

**359 Conflict of interest**

360 None declared

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**Table 1. Associations between predictor variables and case/control status.**

characteristics	controls		cases		p
	n	% <sup>§</sup>	n	% <sup>§</sup>	
<b>Mother</b>					
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*	23	0.5	12	0.7	
Geographic origin					
France	2412	57.9	882	54.5	
North Africa	433	10.4	247	15.3	
Subsaharan Africa	550	13.2	163	10.1	
Other	770	18.5	327	20.2	
missing*	85	2.0	23	1.4	
Occupation					<0.001
none	1083	26.3	440	29.7	
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*	132	3.1	162	9.9	
<b>Father</b>					
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*	800	18.8	415	25.3	
<b>Pregnancy</b>					
Multiplicity					0.756
singletons	2768	96.1	1382	95.8	
twins	103	3.6	57	4.0	
triplets	8	0.3	3	0.2	
Outcome					<0.001
still-births	7	0.2	46	2.8	
live-births	4231	99.6	1074	65.4	
pregnancy terminations	12	0.3	522	31.8	

\* % of missing data calculated with the total number of cases or controls as a denominator

§ % calculated with the total of cases or controls without missing data as a denominator



Table 2. Numbers of cases and controls and proportions of fetuses conceived after Assisted Reproductive Technologies (ART).

		N	% exposed to ART	p <sup>†</sup>
Controls *		4 104	3.5	
All cases	Hypoplastic left heart syndrome	353	2.8	0.491
	Transposition of the great arteries	444	2.7	0.363
	Tetralogy of Fallot	395	6.6	0.002
	Coarctation of the aorta	391	3.3	0.831
Cases without chromosomal anomalies	Hypoplastic left heart syndrome	303	2.6	0.413
	Transposition of the great arteries	430	2.8	0.423
	Tetralogy of Fallot	315	7.3	0.001
	Coarctation of the aorta	350	3.7	0.860

\* The following malformations were used as controls: club-foot, angioma, skin abnormality, polydactyly, syndactyly and congenital hip dislocation.

† Comparison of the proportion of children/fetuses conceived after ART between the specific CHD and the malformed controls.

Table 3. Logistic regression analyses of the associations between assisted reproductive technologies (ART, all methods combined) and four specific congenital heart defects (CHD).

	CHD	ART	Unadjusted OR*	95% CI	Maternal Adjusted† OR*	95% CI
All cases	Hypoplastic left heart syndrome	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
	Tetralogy of Fallot	None	1.0	ref.	1.0	ref.
		All methods combined	1.9	1.3 - 3.0	2.4	1.5 - 3.7
	Coarctation of the aorta	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	Hypoplastic left heart syndrome	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
	Tetralogy of Fallot	None	1.0	ref.	1.0	ref.
		All methods combined	2.2	1.4 - 3.4	2.6	1.6 - 4.2
	Coarctation of the aorta	None	1.0	ref.	1.0	ref.
		All methods combined	1.1	0.6 - 1.9	1.2	0.6 - 2.2

\* 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).

†Adjusted for maternal age, geographic origin, occupation and year of birth.

**Table 4.** Logistic regression analyses of the associations between the different methods of assisted reproductive technologies (ART) and four specific congenital heart defects (CHD).

CHD	ART	Unadjusted OR*	95% CI	Maternal Adjusted† OR*	95% CI
Hypoplastic left heart syndrome	None	1.0	ref.	1.0	ref.
	Ovulation induction 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.
	Ovulation induction 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	/	/	/	/
Tetralogy of Fallot	None	1.0	ref.	1.0	ref.
	Ovulation induction 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
Coarctation of the aorta	None	1.0	ref.	1.0	ref.
	Ovulation induction 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

\* 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).

†Adjusted for maternal age, geographic origin, occupation and year of birth.

**Table 5.** Logistic regression analyses of the associations between assisted reproductive technologies (ART) and four specific congenital heart defects (CHD) without associated chromosomal anomalies.

CHD	ART	Unadjusted OR*	95% CI	Maternal Adjusted† OR*	95% CI
Hypoplastic left heart syndrome	None	1.0	ref.	1.0	ref.
	Ovulation induction 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.
	Ovulation induction 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	/	/	/	/
Tetralogy of Fallot	None	1.0	ref.	1.0	ref.
	Ovulation induction 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
Coarctation of the aorta	None	1.0	ref.	1.0	ref.
	Ovulation induction 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

\* 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)

†Adjusted for maternal age, geographic origin, occupation and year of birth.

