

1 UNIVERSITÀ DEGLI STUDI DI VERONA

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5
6 *Surgery, Dentistry, Maternity and Infant*

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8 DOCTORAL PROGRAM IN

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10 *Surgical and Cardiovascular Sciences*

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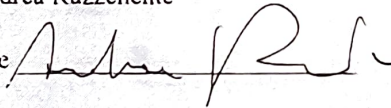
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16 TITLE OF THE DOCTORAL THESIS

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18 **Natural History and Evolution of Conotruncal Anomalies Diagnosed Before 16 Weeks'**
19 **Gestation: A Multicenter Retrospective Cohort Study**

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21 S.S.D. MED/40 GINECOLOGIA E OSTETRICIA

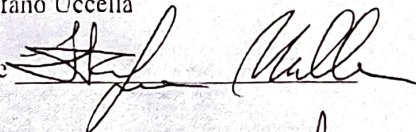
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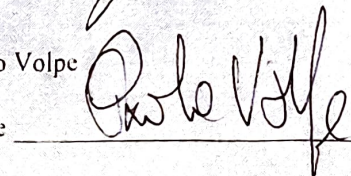
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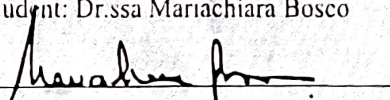
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48 **Abstract**

49 **Objective:** To assess the natural history and evolution of conotruncal anomalies (CTAs) diagnosed
50 before 16 weeks' gestation, focusing on lesion progression, genetic associations, and pregnancy
51 outcomes. A secondary aim was to establish nomograms for the fetal pulmonary artery and aorta
52 between 12+0 and 15+6 weeks' gestation in normal fetuses.

53 **Methods:** This retrospective cohort study was conducted at two Italian tertiary fetal medicine centers
54 between 2017 and 2023. Fetuses diagnosed with CTA on early fetal echocardiography (<16 weeks)
55 were identified from institutional databases. Included anomalies were anterior malalignment
56 ventricular septal defect (am-VSD), tetralogy of Fallot (TOF), TOF with pulmonary atresia (TOF-
57 PA), truncus arteriosus (TA), double outlet right ventricle (DORV), transposition of the great arteries
58 (TGA), congenitally corrected TGA (cTGA), and interrupted aortic arch type B (IAA-B). Only cases
59 with postnatal or postmortem confirmation were included. Progression was defined as morphologic
60 change requiring revision of diagnosis. Invasive genetic testing (CMA ± WES) was offered to all
61 patients. Reference measurements of the great arteries were prospectively collected in normal fetuses
62 between 12+0 and 15+6 weeks.

63 **Results:** A total of 131 fetuses were diagnosed with CTA before 16 weeks; 101 fulfilled inclusion
64 criteria. Sixty-one pregnancies (46.6%) were terminated, most commonly for abnormal genetic
65 findings (mostly 22q11.2 microdeletion). Among 70 ongoing pregnancies, intrauterine demise
66 occurred in 5 cases (7.1%) and postnatal death in 2 (2.9%). Lesion progression occurred in 19/70
67 (27.1%), mainly between the second and third trimesters. The most frequent evolutions were am-
68 VSD to TOF (n=8) and TOF to TOF-PA (n=4). Nomograms for early aortic and pulmonary valve
69 dimensions were established. Progressive TOF cases had pulmonary artery diameters below the 5th
70 centile, while stable cases were above this range.

71 **Conclusions:** CTAs diagnosed before 16 weeks are dynamic conditions, with one in four showing
72 antenatal progression. Early diagnosis warrants detailed genetic assessment, serial echocardiography,
73 and specialized prenatal counselling. Early vessel measurements may help identify fetuses at risk of
74 evolution and guide tailored surveillance.

75 **Keywords:** cardiac embryology, fetal cardiology, prenatal screening, longitudinal assessment.

76

77

78 **INTRODUCTION**

79 Advances in ultrasound resolution and operator expertise now allow reliable fetal echocardiography
80 as early as 12–14 weeks' gestation, with most authors defining “early fetal echocardiography” as an
81 examination performed before 16 weeks.^{1–3} Although the majority of structural heart defects are
82 already established by the end of the 8th week of gestation, when fetal heart embryogenesis is largely
83 complete, certain lesions including conotruncal anomalies (CTAs),^{4–6} may evolve during gestation
84 and their potential for progression complicates prenatal counselling. While mid-gestation
85 echocardiography remains the cornerstone of prenatal CHD diagnosis,^{7,8} the increasing use of early
86 fetal echocardiography has raised questions regarding the diagnostic reliability of early findings,
87 specifically for evolutive lesions such as CTAs. Indeed, available data are limited in the literature
88 elucidating the natural history of these conditions particularly when diagnosed in the first trimester
89 or early second trimester.^{4,5} The primary aim of this study was therefore to evaluate the antenatal
90 evolution of CTAs diagnosed before 16 weeks' gestation, with attention to lesion progression, genetic
91 abnormalities, and pregnancy outcomes. A secondary aim was to establish early-gestation
92 nomograms for aortic and pulmonary artery diameters (12+0–15+6 weeks), to assess whether great-
93 artery size at first evaluation may help identify fetuses at risk of CTA progression.

94

95 **METHODS**

96 This multicentre retrospective cohort study was conducted at two Italian tertiary fetal medicine
97 units—IRCCS Ca' Granda Ospedale Policlinico in Milan and Ospedale di Venere in Bari—between
98 January 2017 and December 2023. Institutional databases (Viewpoint™ and Astraia™, software
99 GmbH, Germany) were queried to identify all fetuses who underwent early fetal echocardiography
100 before 16 weeks' gestation and received a diagnosis of a conotruncal anomaly (CTA). Conotruncal
101 anomalies included anterior malalignment ventricular septal defect (am-VSD), tetralogy of Fallot
102 (TOF), TOF with pulmonary atresia (TOF-PA), TOF with absent pulmonary valve (TOF-APV),
103 truncus arteriosus (TA), double outlet right ventricle (DORV), transposition of the great arteries
104 (TGA), congenitally corrected TGA (cTGA), and interrupted aortic arch type B (IAA-B). Only cases
105 with available prenatal follow-up and postnatal confirmation—via postnatal echocardiography,
106 surgical findings, or postmortem examination—were eligible. First-trimester terminations of
107 pregnancy (TOP) without postmortem confirmation were excluded. All examinations were performed
108 using Voluson E8 or E10 ultrasound systems (GE Healthcare, Zipf, Austria) equipped with high-
109 frequency probes (C4-8-D, RIC6-12-D, C2-9-D). Stored 2D/3D images and videoclips were reviewed

110 retrospectively by two experienced fetal cardiologists (PV, VDR). The primary objective was to
111 identify antenatal progression of CTAs. Progression was evaluated at two timepoints: mid-gestation
112 (19+0–20+6 weeks) and third trimester (28+0–34+0 weeks). Progression was defined as significant
113 morphological changes across serial evaluations that required modification of the initial diagnosis.
114 To interpret early biometric findings in CTA fetuses, we prospectively collected aortic and pulmonary
115 valve measurements between 12+0 and 15+6 weeks' gestation in a separate cohort of structurally
116 normal fetuses⁹, given the absence of published reference nomograms for great artery dimensions in
117 this gestational age window, and the limited data available before 16 weeks.^{9–11} Pregnancies were
118 eligible for the normative dataset if dating was confirmed by first-trimester crown-rump length (CRL)
119 and the pregnancy resulted in a liveborn, appropriate-for-gestational-age infant without structural or
120 genetic abnormalities. For each gestational week (12, 13, 14, and 15 weeks) we calculated centiles
121 (1st, 3rd, 5th, 10th, 25th, 50th, 75th, 90th, 95th, 97th, 99th) for both the aortic and pulmonary valves.
122 All patients with a prenatal diagnosis of CTA were offered invasive diagnostic testing (chorionic
123 villus sampling or amniocentesis). Chromosomal microarray analysis (CMA) was performed in all
124 tested cases. Whole-exome sequencing (WES) was offered when CMA returned normal results. Data
125 extracted from institutional database included: gestational age at diagnosis, indication for early fetal
126 echocardiography, serial echocardiographic finding, pregnancy outcomes and genetic test results. All
127 patients provided informed in accordance with institutional protocols, including consent for image
128 storage and the use of anonymized clinical data for research and quality-control purposes

129 **RESULTS**

130 During the study period, 131 fetuses were diagnosed with a conotruncal anomaly (CTA) before 16
131 weeks' gestation (**Figure 1**). Of these, 61 pregnancies (46.6%) underwent termination of pregnancy
132 (TOP), including 30 in the first trimester and 31 in the second trimester. Because postmortem
133 confirmation was unavailable for first-trimester TOP cases, they were excluded from the final
134 analysis, leaving 101 fetuses in the study cohort. Among the 31 second-trimester TOPs, genetic
135 abnormalities were identified in 14 fetuses, with 22q11.2 microdeletion representing the most
136 common abnormality. Extracardiac anomalies were present in 15 cases, and in 12 cases the TOP was
137 requested in isolated CTA. Of the 70 pregnancies that continued beyond the mid-trimester anomaly
138 scan intrauterine demise (IUD) occurred in 5 cases (7.1%), and postnatal death occurred in 2
139 additional cases (2.9%). The most frequent prenatal diagnoses were anterior malalignment VSD (am-
140 VSD) and tetralogy of Fallot (TOF) (**Figure 2**). Among the 70 ongoing pregnancies, 19 fetuses
141 (27.1%) demonstrated antenatal progression of the cardiac lesion. Seven (7/19, 36.8%) showed
142 progression before 22 weeks' gestation while 12 (12/19, 63.2%) exhibited progression during the

143 third trimester. The most common patterns of evolution were am-VSD to TOF (n=8), and TOF to
144 TOF with pulmonary atresia (TOF-PA) (n=4). Additional phenotypic changes were documented in
145 fetuses initially diagnosed with DORV (n=2) and fetuses with TGA (n=4). One fetus with
146 congenitally corrected TGA (cTGA) developed complete atrioventricular block with hydrops in the
147 third trimester, resulting in intrauterine death (**Figures 3–4**). Among the 66 ongoing cases with
148 available first-trimester combined screening data, 48.5% (32/66) showed at least one abnormal
149 indirect marker suggestive of congenital heart disease (CHD) such as increased nuchal translucency
150 (NT), tricuspid regurgitation, or abnormal ductus venosus flow. Normative biometric data for the
151 aortic and pulmonary valves between 12+0 and 15+6 weeks' gestation were used to generate centile
152 charts (**Tables 1–3**). The distribution of aortic and pulmonary valve measurements throughout
153 gestational age window is shown in **Figures 5** and **Figure 6**. Distinct biometric patterns were
154 observed between progressing and non-progressing CTA cases. In TOF cases that progressed to TOF-
155 PA, the pulmonary artery diameter was consistently below the 5th centile for gestational age, whereas
156 in non-progressing TOF the hypoplastic pulmonary artery was above the 5th centile at diagnosis.
157 Among am-VSD cases, all but one case (31st centile) that progressed had pulmonary artery diameters
158 between the 10th and 25th centiles, whereas all non-progressing case showed values above the 25th
159 centile.

160 **DISCUSSION**

161 Understanding the natural history of conotruncal anomalies (CTAs) diagnosed in the first trimester is
162 essential to refine prenatal counselling, optimize surveillance, and anticipate perinatal needs. In this
163 multicentre study, we evaluated 70 ongoing pregnancies with CTA diagnosed before 16 weeks'
164 gestation and observed that approximately one in four fetuses (27.1%) exhibited antenatal progression
165 of the cardiac lesion. This confirms that CTAs represent dynamic and evolving conditions rather than
166 fixed anatomical defects, particularly when detected early in gestation. Most prior studies have
167 examined CTA evolution from the mid-gestation diagnosis onward, with limited attention to earlier
168 gestational ages^{5,12} Only a small number of reports have followed fetuses longitudinally from the
169 early gestational ages.¹³ Our findings therefore provide a unique perspective on the earliest stages of
170 CTA development and their subsequent morphological evolution. Notably, 63.2% of all progression
171 events occurred between the mid-trimester and the third trimester, highlighting the importance of
172 continued surveillance through late gestation. The most frequent progression was from anterior
173 malalignment VSD (am-VSD) to tetralogy of Fallot (TOF) (in 8 out of the 17 total number of ongoing
174 am-VSD), reflecting the embryological contribution of outlet septum deviation to progressive
175 subpulmonary obstruction. Anterocephalad malalignment of the outlet/conal septum can occur even

176 in the presence of a formed subpulmonary infundibulum (am-VSD). However, the anterior deviation
177 of the outlet septum might contribute to progressive outflow obstruction with evolution in TOF both
178 during fetal life and after birth.^{14–16} Similarly, 4 out of the 15 TOF cases progressed to TOF with
179 pulmonary atresia (TOF-PA), reflecting advancing obstruction of the right ventricular outflow tract,
180 which has been associated with poorer postnatal prognosis.^{17–19} Evolution was also documented in
181 fetuses with an initial diagnosis of DORV or TGA, emphasizing that even lesions traditionally
182 considered structurally stable may undergo functional or anatomical changes as gestation advances.
183 These changes included the emergence of pulmonary outflow obstruction and evolving coarctation—
184 findings consistent with the concept that morphofunctional remodeling of the great arteries continues
185 beyond early cardiac looping stages.^{4,20} The case of cTGA complicated by late-onset complete
186 atrioventricular block is also consistent with reported risks of rhythm disturbances emerging in the
187 late second or early third trimester.^{21,22} A major contribution of this study is the establishment of
188 normative data for aortic and pulmonary artery diameters between 12 and 15+6 weeks' gestation.
189 Existing reference charts for these structures before 16 weeks are scarce,^{9–11} which has limited the
190 interpretation of great-artery findings in first-trimester CTA assessments. Our data offer new insight
191 into early great vessel development and support early risk stratification. Importantly, we observed
192 that TOF cases progressing to TOF-PA consistently demonstrated pulmonary artery diameters below
193 the 5th centile, while non-progressing TOF cases presented above this threshold. Likewise, evolving
194 am-VSDs predominantly clustered between the 10th and 25th centiles, whereas non-progressing
195 cases were above the 25th centile. These findings suggest that early great-artery biometry may have
196 predictive value for identifying fetuses at risk of progression, warranting further validation in
197 prospective studies. Our findings also underscore the strong genetic burden associated with CTAs.
198 Consistent with previous literature, 22q11.2 microdeletion emerged as the most frequent cytogenetic
199 abnormality.^{13,23–26} Lastly, nearly half of the fetuses exhibited abnormal first-trimester cardiac
200 markers—including increased nuchal translucency, tricuspid regurgitation, or abnormal ductus
201 venosus flow—supporting the integration of cardiac evaluation into first-trimester risk assessment.
202 Overall, this study highlights the need for structured management pathways for CTAs diagnosed early
203 in gestation. This includes high-resolution ultrasound, serial fetal echocardiography, comprehensive
204 genetic testing, and planned delivery in specialized centres. Early recognition and monitoring of these
205 lesions can facilitate timely intervention and minimize unexpected perinatal complications.

206 **Strengths and limitations**

207 This study has several notable strengths. First, it represents one of the largest cohorts of CTAs
208 diagnosed before 16 weeks' gestation with longitudinal follow-up through pregnancy. Second, all

209 included cases had definitive confirmation of the diagnosis through postnatal evaluation or
210 postmortem examination, ensuring diagnostic accuracy. Third, the prospective collection of aortic
211 and pulmonary artery biometry in the normal population provides valuable reference data for early
212 gestational assessment of great-artery size—an area where validated nomograms have been lacking.
213 However, some limitations must be acknowledged. The retrospective design introduces potential
214 selection and information bias, despite the comprehensive image review by experienced fetal
215 cardiologists. First-trimester TOP cases without postmortem confirmation could not be included,
216 potentially underestimating the true prevalence of severe CTA phenotypes. Additionally, although
217 the study identifies promising early predictors of lesion evolution, prospective studies with larger
218 sample sizes are needed to validate their clinical utility, refine surveillance intervals, and optimize
219 prenatal counselling strategies.

220 **Conclusions**

221 Conotruncal anomalies diagnosed before 16 weeks' gestation exhibit considerable antenatal
222 variability, with approximately 27% demonstrating progression—most commonly during the third
223 trimester. Early diagnosis should prompt comprehensive prenatal counselling and structured follow-
224 up, particularly in settings where choices regarding pregnancy continuation are constrained by
225 gestational age limits. Serial fetal echocardiography remains essential for monitoring anatomical and
226 functional changes, and comprehensive genetic testing, including microarray and potentially WES,
227 should be offered given the high prevalence of associated abnormalities. Early great-artery
228 dimensions, particularly pulmonary artery calibre, may help differentiate stable from potentially
229 progressive lesions within each CTA subgroup and thus support individualized risk stratification. By
230 providing the largest dataset to date with longitudinal assessment of first-trimester CTA evolution
231 and newly established early gestational nomograms, this study contributes substantially to the
232 understanding of early cardiac development, improving prenatal management and perinatal planning
233 for affected families.

234

235 **Conflicts of interest:** none.

236 **Funding:** none.

237

238 **References**

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327

328 **Tables Legend**

329 **Table 1.** Nomograms of pulmonary artery diameter (mm) in the normal population between 12+0-
 330 and 15+6-weeks gestations (median and 1st, 3rd, 5th, 10th, 25th, 75th, 90th, 95th, 97th, 99th centiles
 331 per gestational age).

332 **Table 2.** Nomograms of aorta diameter (mm) in the normal population between 12+0- and 15+6-
 333 weeks gestations (median and 1st, 3rd, 5th, 10th, 25th, 75th, 90th, 95th, 97th, 90th centiles per
 334 gestational age).

335 **Table 3.** Median for AO/AP ratio and AP/AO ratio according to gestational age.

336

337 **Figures Legend**

338 **Figure 1.** Flow chart displaying the 131 CTAs cases and outcomes. *Abbreviations: TOP, termination*
 339 *of pregnancy; CTA: conotruncal anomaly; amVSD, ventricular septal defect with anterior*
 340 *malalignment; TOF: tetralogy of Fallot; DORV: double outlet right ventricle; TGA: transposition of*
 341 *great arteries; cTGA: congenitally corrected TGA.*

342 **Figure 2.** Bar chart showing the distribution of the 70 ongoing CTA cases according to CTA subtype.
 343 *Abbreviation: am-VSD: anterior malalignment ventricular septal defect; TOF: tetralogy of Fallot;*
 344 *PA: pulmonary atresia; TA: truncus arteriosus; DORV: double outlet right ventricle; TGA:*
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347 **Figure 3.** Fetus with an outlet VSD with anterior malalignment (am) and right-sided aortic and ductal
 348 arches detected at 14 weeks of gestation, which progressed to TOF in the second trimester. A. Early
 349 echocardiography showed an isolated outlet VSD with am. B-C. Second-trimester echocardiography
 350 documented progression from outlet VSD with am to TOF. B. An outlet VSD with am. C. The main
 351 pulmonary artery was diminutive (arrow), smaller than the ascending aorta (*), with aortic and ductal
 352 arches positioned to the right of the trachea (T). Antegrade pulmonary artery flow is present (arrow).
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 354 *of Fallot; L, left; R, right.*

355 **Figure 4.** Fetus with TOF diagnosed at 13 weeks of gestation, which progressed to TOF with
 356 pulmonary atresia (PA) by the third trimester. A. Early fetal echocardiography demonstrated an outlet

357 VSD with anterior malalignment (am) and a main pulmonary artery (arrow) smaller than the
 358 ascending aorta. B. Mid-trimester echocardiography showed the same findings: an outlet VSD with
 359 am and a hypoplastic pulmonary artery (arrow) relative to the ascending aorta. C. Third-trimester
 360 echocardiography documented progression to TOF with PA, characterized by absent antegrade right
 361 ventricular outflow and retrograde flow via the ductus arteriosus (arrow). *Abbreviations: TOF,*
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 363 *malalignment.*

364 **Figure 5.** Distribution of pulmonary artery diameters according to gestational age at measurement.

365 **Figure 6.** Distribution of aortic diameters according to gestational age at measurement.

366

367

368 Tables

369 **Table 1.** Nomograms of pulmonary artery diameter (mm) in the normal population between 12+0
 370 and 15+6 weeks gestations (median and 1st, 3rd, 5th, 10th, 25th, 75th, 90th, 95th, 97th, 99th
 371 centiles per gestational age).

372

Gestational age (wks)	Mean GA	1st	3rd	5th	10th	25th	50th	75th	90th	95th	97th	99th	n
12	12,46	1,29	1,30	1,30	1,40	1,50	1,65	1,70	1,80	1,80	1,80	1,86	38
13	13,13	1,41	1,45	1,48	1,55	1,60	1,70	1,76	1,80	1,83	1,86	1,88	16
14	14,30	1,61	1,65	1,68	1,76	1,90	2,00	2,20	2,24	2,25	2,28	2,29	20
15	15,29	2,0	2,00	2,00	2,04	2,10	2,22	2,70	3,12	3,21	3,21	3,21	15

373

374 **Table 2.** Nomograms of aorta diameter (mm) in the normal population between 12+0 and 15+6
 375 weeks gestations (median and 1st, 3rd, 5th, 10th, 25th, 75th, 90th, 95th, 97th, 90th centiles per
 376 gestational age).

377

378

Gestational age (wks)	Mean GA	1st	3rd	5th	10th	25th	50th	75th	90th	95th	97th	99th	n
12	12,46	1,06	1,10	1,10	1,17	1,20	1,30	1,48	1,53	1,60	1,60	1,64	38
13	13,13	1,21	1,25	1,28	1,30	1,34	1,40	1,50	1,55	1,60	1,60	1,61	16
14	14,30	1,50	1,53	1,55	1,60	1,64	1,75	1,80	1,91	2,01	2,04	2,08	20
15	15,29	1,89	1,90	1,90	1,94	2,00	2,10	2,40	2,53	2,57	2,58	2,59	15

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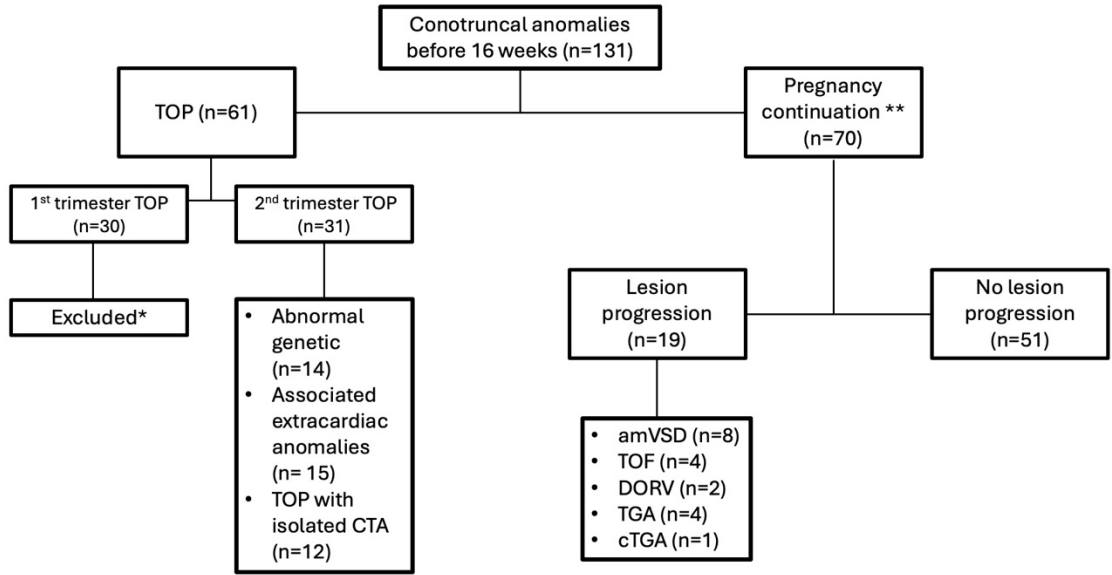
381 **Table 3.** Median for AO/AP ratio and AP/AO ratio according to gestational age.

382

Gestational age (wks)	Median AO_AP	Median AP_AO
12	0,82	1,22
13	0,87	1,15
14	0,89	1,13
15	0,95	1,05

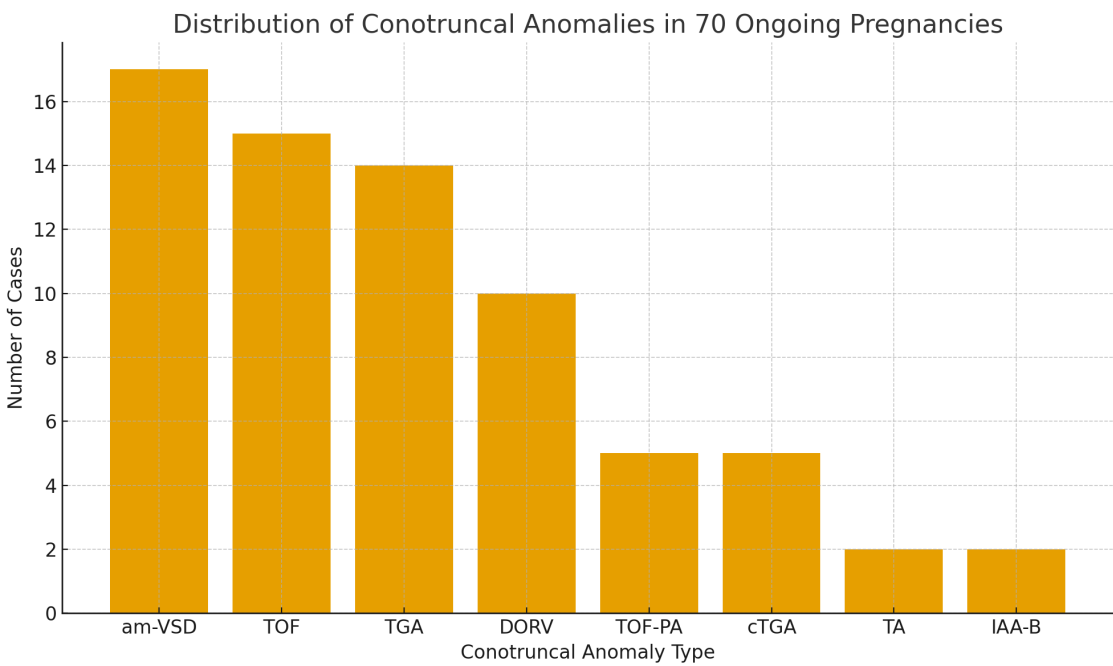
383 **Figures**

384 **Figure 1.** Flow chart displaying the 131 CTAs cases and outcomes. *Abbreviations: TOP, termination*
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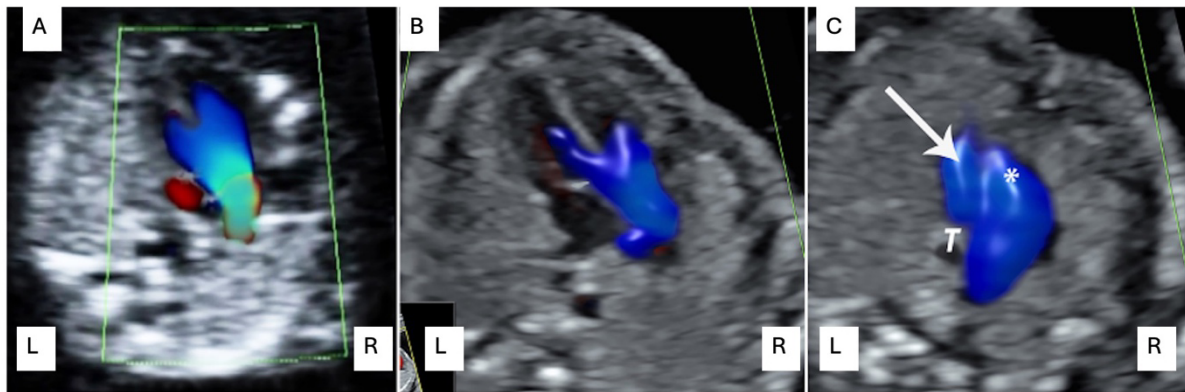


388 *postmortem confirmation of CHD not available
 ** of those, 5 fetal deaths and 2 neonatal deaths

389 **Figure 2.** Bar chart showing the distribution of the 70 ongoing CTA cases according to CTA subtype.
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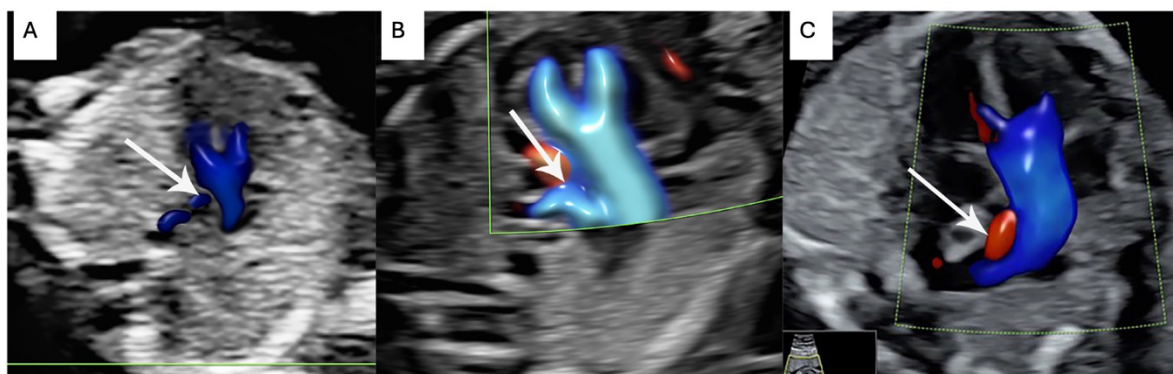


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403

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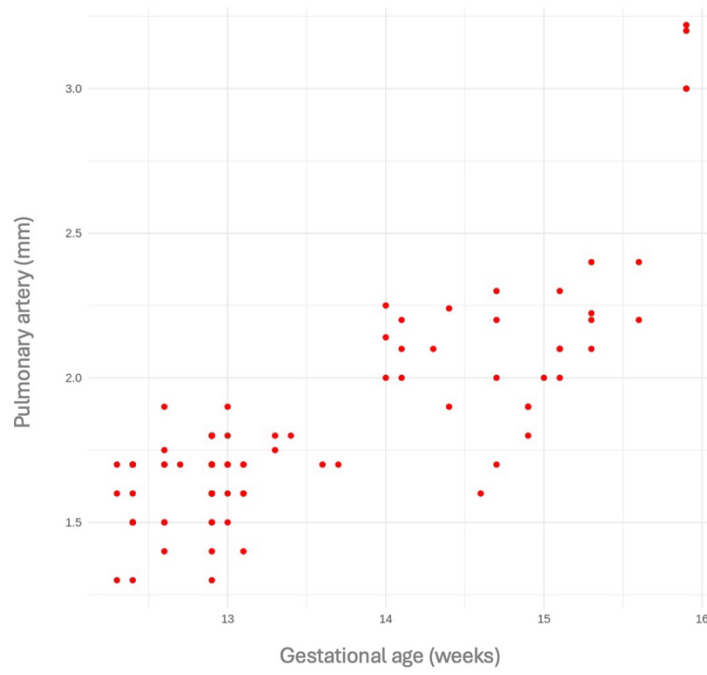


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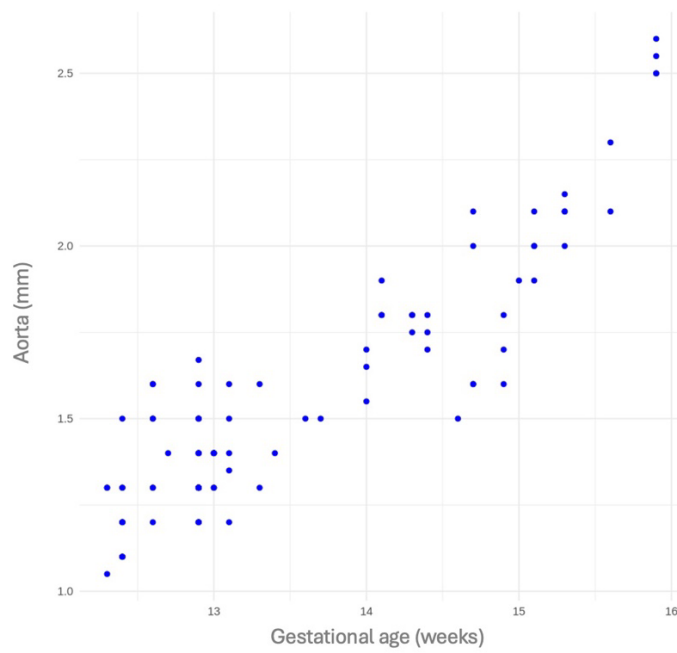
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417

418 **Figure 6.** Distribution of aortic diameters according to gestational age at measurement.



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