Postnatal persistence of fetal cardiovascular remodeling associated with assisted reproductive technologies: a cohort study.

Running title: Children conceived by ART, cardiovascular characteristics.

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ABSTRACT

Objective: to assess the postnatal persistence of fetal cardiovascular remodeling associated to assisted reproductive technologies (ART), in children at 3 years of age.

Design: A cohort study of children conceived by ART.

Setting: Maternal-Fetal Medicine Unit, Hospital Clinic Barcelona, Spain.

Population sample: 80 singleton pregnancies conceived by ART and 80 spontaneously conceived (controls) followed from fetal life up to childhood.

Methods: Cardiovascular evaluation was performed at 3 years of corrected age, including echocardiography, carotid intima-media (cIMT) by ultrasound and blood pressure.

Main Outcome Measures: Postnatal persistence of cardiovascular changes in children conceived by ART.

Results: As compared to controls, children conceived by ART showed larger atria (right atrial area: control 4.9 cm^2 (0.9) vs. ART 5.5 cm^2 (0.9), $p<0.001$), more globular ventricles (right ventricular sphericity index: control mean 1.8 (SD 0.5) vs. ART 1.6 (0.2), $p<0.001$), and signs of systolic (tricuspid annular plane systolic excursion: control 18 mm (2) vs. ART 16 mm (3), $p<0.001$) and diastolic dysfunction (isovolumic relaxation time: control 68 ms (12) vs. ART 79 ms (12), $p<0.001$). ART children also presented increased systolic blood pressure (control 90 mmHg (6) vs. ART 94 mmHg (5), $p<0.003$) and cIMT (control 0.52 μm (0.14) vs. ART 0.60 μm (0.16), $p<0.001$) as compared to those spontaneously conceived.

Conclusions: Cardiovascular changes previously reported in ART fetuses persist postnatally at 3 years of age. These results underscore the importance of future studies for assessing the long-term cardiovascular health associated to ART.

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KEY WORDS: assisted reproductive technologies, postnatal, heart, echocardiography, blood pressure, carotid intima-media thickness.

Tweetable abstract: Cardiovascular changes described in fetuses conceived by ART, persist in children at 3 years of age.

INTRODUCTION

It is now estimated that more than 5.4 million babies have been conceived worldwide since the first *in vitro* fertilization (IVF) baby was born in 1978.(1) Thus, the potential health risks associated with these treatments are of great importance to public health. Although most of children conceived by assisted reproductive technologies (ART) are born healthy, concerns regarding long-term cardiometabolic health in ART children are increasing due to the accumulative evidence suggesting that ART may have lasting negative repercussions for the health of individuals conceived through these techniques.(2, 3)

Various studies have shown that children born after ART have poorer metabolic and vascular profiles than naturally conceived children, with elevated systolic and diastolic blood pressures, higher fasting glucose levels, increased central adiposity and vascular dysfunction with pulmonary hypertension.(4-8) According to these authors, the changes mentioned seems to be independent of prematurity, birthweight and parental characteristics; suggesting that features related to ART could directly contribute to these adverse health effects.(9)

Recently, cardiac remodeling *in utero* that persists into early postnatal life was demonstrated in ART offspring.(10) In this study, fetal echocardiography enabled to show signs of cardiac remodeling and dysfunction in ART fetuses mainly in the form of larger atria, shorter ventricles, thicker myocardial walls, reduced longitudinal motion and impaired relaxation. A postnatal follow-up of the same cohort at 6 months of age showed not only postnatal persistence of cardiac remodeling but also demonstrated increased blood pressure and vascular wall thickness.(10) However, cardiovascular outcomes in later stages of life is still controversial with studies reporting changes in left ventricular function(11, 12) and others suggesting right ventricular dysfunction only evident under stressful conditions.(13)

The aim of the present study was to evaluate the persistence, into childhood, of cardiac findings observed in ART fetuses. For this purpose, we conducted a cardiovascular follow-up study from our prenatal ART cohort into early childhood.

METHODS

Study population and protocol

A prospective cohort study including 80 children conceived by ART and 80 spontaneously conceived (controls) recruited from fetal life and followed up to 3 years of age (see supplementary material for details of the prenatal(10) and follow-up cohorts). Preimplantation genetic diagnosis, oocyte donation, multiple pregnancies, any maternal medical disease, fetal malformations, chromosomal anomalies or any pregnancy complications leading to delivery before 34 weeks of gestation were considered as exclusion criteria. The study protocol was approved by the Institutional Review Board at Hospital Clinic, and written parental consent was obtained for all study participants.

Child's follow-up evaluation was scheduled at 3 years of corrected age including anthropometric data, echocardiography and vascular assessment. Examiners were blinded to conception type. Anthropometric data included the child's height, weight and body mass index measured at the time of the examination.

Echocardiography

Echocardiography was performed following a standardized protocol(14) using a Vivid q (General Electric Healthcare. Horten. Norway) with 2-10 MHz phased-array transducer. Children were studied when resting quietly. A complete two-dimensional M-mode and Doppler echocardiographic examination was performed initially to assess structural heart integrity and *morphometry*. Left and right atrial planimetric areas were measured on a 2D image from an apical four-chamber view at end-systole (greatest dimension, just before mitral or tricuspid valve opening). Ventricular base-to-apex length and transverse diameter were measured on a 2D image from an apical four-chamber view at end-diastole. Left and right ventricular sphericity indexes were calculated as base-to-apex length/mid-transverse diameter, Ventricular end-

diastolic septal and lateral free wall thicknesses were measured by M-mode from a parasternal long-axis view.(14, 15)

Systolic function of both ventricles was evaluated using shortening fraction, cardiac output, tricuspid and mitral annular plane systolic excursion (TAPSE and MAPSE) and annular systolic peak velocities (S').(16) Left shortening fraction was calculated from internal ventricular diameters obtained from a parasternal long-axis view by M-mode, using the equation (end-diastolic dimension – end-systolic dimension)/ end-diastolic dimension. Left and right stroke volumes were calculated as $\pi/4$ x (aortic or pulmonary valve diameter)^2 x (aortic or pulmonary artery systolic flow velocity-time integral). Left and right cardiac outputs were calculated as stroke volume x heart rate. Diameters of the aortic and pulmonary valves were measured in frozen real-time images during early to mid-systole by the leading-edge-to-edge method; aortic diameter was obtained from the parasternal long-axis view, while the pulmonary artery diameter was obtained in a parasternal short-axis view.(16) Ascending aorta flow velocity integral was measured with pulsed Doppler from an apical five-chamber view, and the pulmonary artery flow velocity integral was recorded from a standard parasternal short-axis view with the sample volume placed immediately distal to the pulmonary valve. Velocity-time integrals were calculated by manual trace of the spectral Doppler area. TAPSE and MAPSE were measured real time in an apical four-chamber view, by placing the M-mode cursor at the atrioventricular junction, marked by the tricuspid valve rings at the right free wall. Maximum amplitude of motion was taken as the extent of displacement between end-systole and end-diastole, and measured in millimeters. Tissue Doppler was applied at tricuspid and mitral lateral annuli from an apical four-chamber view, to record S' in centimeters/second.(16)

Diastolic function of both ventricles was evaluated by atrioventricular peak velocities at early diastole and atrial contraction (E/A ratios), E deceleration time, diastolic annular peak velocity (E') and left isovolumic relaxation time (IRT). Atrioventricular flow were obtained from an apical four-chamber view, placing the pulsed Doppler sample volume just below the valve leaflets. E

deceleration time was measured as the time from the maximum mitral/tricuspid velocity to the baseline. Tissue Doppler was applied at tricuspid and mitral lateral annuli from an apical four-chamber view to obtain E'. Left IRT was obtained from the pulsed Doppler waveform of the aortic blood flow, from the end of the aortic wave to the beginning of the mitral early filling wave.

Vascular assessment

Vascular assessment included blood pressure and carotid wall thickness by ultrasound.

*Systolic and diastolic blood pressure*s were obtained at the beginning of the medical evaluation from the brachial artery using a validated ambulatory automated Omron 5 Series device, while the child was resting. Mean blood pressure was calculated as [(2 x diastolic)+systolic] / 3.

Carotid ultrasound assessment was performed by skilled sonographer using a Vivid q (General Electric Healthcare. Horten. Norway). Longitudinal clips of the far wall of both carotid arteries were obtained approximately 1 cm proximal to the bifurcation using a 3.33-10.0MHz linear-array transducer. Carotid intima-media thickness (IMT) was measured offline according to a standardized protocol based on a trace method with the assistance of a computerized program EchoPAC Software Only. To obtain IMT, three end-diastolic still frames were selected across a length of 10 mm and analyzed for mean and maximum IMT, and the average reading from these three frames was calculated.(17)

RESULTS

Baseline and perinatal characteristics

Baseline and perinatal characteristics of the study are shown in Table 1. Parental baseline characteristics were similar among the study groups, with the exception of older parental age and higher rate of nulliparity in the ART group as compared to spontaneously conceived ones. As expected, the ART group showed a worse perinatal outcome with earlier gestational age at delivery, lower birthweight and birthweight centile as compared to controls. There was also a non-significant trend for higher prevalence of preeclampsia, gestational diabetes, prenatal

corticoid exposure, cesarean section and admission to neonatal intensive care unit in the ART group as compared to controls.

Study protocol in early childhood

Anthropometric and cardiovascular results at 3 years of age are shown in Table 2. Both groups showed similar age and anthropometric characteristics at evaluation. ART children showed larger right atrium together with more spherical ventricles and similar myocardial thickness as compared to spontaneously conceived ones. While cardiac output and tissue Doppler values were similar among groups, ART children showed decreased shortening fraction and mitral/tricuspid ring displacement together with prolonged IRT. In addition, blood pressure and carotid IMT were significantly higher in the ART group as compared to spontaneously conceived children (Figure 1). Most cardiovascular changes remained statistically significant even after adjustment for potential confounding factors such as parental age, gestational age at delivery and birthweight centile.

DISCUSSION

Main Findings

This study demonstrates the persistence of cardiovascular changes associated to ART from fetal life to early childhood, supporting the concept of primary cardiovascular programming in ART offspring.

Children conceived by ART showed signs of cardiac remodeling such as larger right atrium and more spherical ventricles, and also cardiac dysfunction demonstrated by decreased ring displacement and prolonged relaxation time. These changes are consistent with our previous report in the same cohort during fetal life also demonstrating larger atria, more globular ventricles and signs of systolic and diastolic biventricular dysfunction.(10)

Strength and limitations

Our major strength is the study design of a well-phenotyped cohort recruited from conception up to childhood including complete fetal, infant and children echocardiographic data.(10) As limitations, we acknowledge that the present study might be underpowered as sample size was not estimated at this evaluation in childhood and there was a 20% lost in follow-up. Detailed information regarding the population lost in follow-up is shown in Appendix S1. We also acknowledge the presence of potential underlying confounding factors that are almost impossible to rule out due to the intrinsic baseline (such as parental infertility per se, socioeconomic status, nutrition and lifestyle habits or maternal comorbidities) and perinatal factors (mainly coexistence of prematurity or placental disease) usually associated to ART pregnancies. For example, in our study we could observe differences in parental age, parity, gestational age and weight at birth; statistical analysis was adjusted for these potential confounders, even though, this correction might be suboptimal for comparing cardiac characteristics. The mechanisms driving these changes in ART children remain to be elucidated; confounding factors such as advanced maternal age are well-known contributors for adverse pregnancy outcomes(18) including low birthweight, condition that has a high prevalence in ART population and is also directly related with fetal cardiovascular programming.(19, 20) Future studies including subfertile couples conceiving spontaneously could help in better control for confounders and provide new insights on the mechanisms underlying cardiovascular changes observed in ART offspring.

Interpretation

Our results are also in line with other recent studies also suggesting cardiovascular differences in childhood or adolescence of subjects conceived by ART.(11-13) Zhou et al.(11) reported signs of left ventricular hypertrophy and dysfunction in ART children at 2-6 years of age. Liu et al.(12) reported left ventricular reduced motion and diastolic dysfunction in ART children at 5 years of age, together with non-significant trend for more spherical ventricles. Von Arx et al.(13) demonstrated right ventricular dysfunction under stressful conditions of high-altitude exposure in

ART preadolescents who were born at term with normal birth weight. Although most studies report significant cardiovascular changes associated to ART, the pattern of cardiac remodeling and dysfunction differs among populations. We report dilated atria and more spherical and less efficient ventricles (both left and right), while Zhou et al. described left ventricular hypertrophy, Liu et al. left ventricular dysfunction, and von Arx et al. right ventricular dysfunction under stressful conditions.

We hypothesize that cardiac changes observed in ART offspring are mainly secondary to increased vascular stiffness leading to increased cardiac pressure that needs to be compensated by adapting ventricular shape (more spherical and/or more hypertrophic) and dilating the atria. Then, differential cardiac patterns could be observed according to the age at assessment: more evident and predominant right changes in fetal life evolving to less prominent changes -as myocardium becomes more mature and compliant and pulmonary pressures decrease- or shifted to the predominant left ventricle in postnatal life. Differences among studies could also be explained by heterogeneity in the factors potentially involved in the pathophysiological origins of these findings: 1) parental parameters and the cause and severity of infertility; 2) ovarían stimulation and its effects on the oocyte and endometrium; 3) ART laboratory procedures (manipulation of gametes and embryos, culture conditions, transfer at the blastocyst stage, vitrification techniques); 4) maternal environment and perinatal comorbidities (such as prematurity and fetal growth restriction).(21) Improvements and changes made to ART techniques over the time, could also be the possible cause of the observed differences in initial studies that have not been reproducible or vary widely.

Regarding vasculature, we could demonstrate increased blood pressure and carotid wall thickness in ART children as compared to naturally conceived ones. These data is consistent with our vascular neonatal results in this same cohort.(10) It is also in agreement with the previously reported systemic and pulmonary vascular dysfunction in late childhood.(8, 22) However, results on systemic blood pressure in ART offspring are controversial. Dissimilarities

among studies have been recently analyzed in a metaanalysis including 872 IVF-ICSI offspring from 10 different studies. They concluded an overall increase of 1.88 mmHg in systolic blood pressure among ART children as compared to those spontaneously conceived. Interestingly, blood pressure was statistically significantly higher in those ART children born 1990-1999 than in those born during 2000-2009, suggesting an improvement over the years most likely due to changes and maturation of ART techniques.(23) The pathophysiology underlying the vascular impairment observed in ART remains unsolved although experimental research suggests premature vascular aging and arterial hypertension probably related to epigenetic alterations in vascular key factors such as the endothelial nitric oxide synthase gene.(24)

Conclusions

With children conceived through ART now forming a sizeable subgroup of the population, further large clinical follow-up studies are required to establish the clinical relevance of evaluating cardiovascular health in those born after ART. The long-term consequences of cardiovascular changes observed in fetal life and childhood are not fully understood. Observations in adolescence suggest that cardiovascular dysfunction appears only under stress conditions -such as high altitude- allowing us to speculate that ART programs individuals with subclinical changes more susceptible to develop disease under certain stressful circumstances later in life.(8, 22) The ability to determine which individuals may be more susceptible to develop subsequent cardiac and/or vascular disease would be of great importance to apply public health strategies. Hence, the clinical relevance of continuing long-term studies in this population. The challenge here is that ART techniques keep on changing rapidly over time evolving relevant improvements in ovarian stimulation protocols, oocyte and embryo vitrification techniques(25) or culture conditions.(26, 27) Improvements applied to these techniques through time in order to achieve pregnancy transferring the “best” embryo in the optimal conditions could explain discrepancies among studies. The real impact of these factors on the embryo’s phenotype

(morphology, developmental kinetics, physiology and metabolism) still needs to be understood; in both, animal and human models. Therefore, large collaborative studies over time are warranted to address the impact on offspring health complexity of different ART techniques.

As final conclusion, our and previous studies support the concept of primary cardiovascular programming associated to ART. Further research is warranted to determine the exact mechanisms underlying these cardiovascular changes and its potential long-term consequences on the health of these children and future adults.

From a clinical perspective and regardless of the need to clarify the specific mechanisms, the existence of fetal programming in these infants presents important opportunities to improve cardiovascular health in a relevant proportion of the population. Our study demonstrates significant changes in cardiovascular structure and function in ART children as compared to those naturally conceived. These changes are subclinical, with most cardiovascular indexes lying within normal ranges explaining that most children are asymptomatic without clinical signs of disease. However, subclinical changes in cardiovascular structure and function in the early stages of life might represent an underlying mechanism for increased cardiovascular risk later in life. In fact, some of cardiovascular differences, such as those reflected by significant increases in blood pressure and IMT, are recognized as potential risk factors for subsequent cardiovascular disease. Future studies are warranted to evaluate the long term persistence of these cardiovascular changes throughout life and better assess the potential risk consequences of these findings.

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Contribution to autorship: B.V.A. and F.C. were responsible for the conception, design and writing of the study; A.S., A.S-M. and L.G-O. collaborated in database collection, quality control and patient's recruitment. M.R-L and M.C-L. also assisted with statistical analyses. G.C., B.B., M.S., and J.B.contributed as Senior investigators in document's discussion and conclusions. E.G. also contributed as Senior Investigator and approval of final document.

Details of Ethics Approval: Study was approved by Hospital Clinic Barcelona Ethics Committee Registry: HCB/2011/6247 (September 2011).

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FIGURE LEGENDS

Figure 1. Ultrasound carotid images illustrating a thicker carotid intima media in children conceived by ART comparing to spontaneously conceived one.

TABLE LEGENDS

Table 1. Baseline and perinatal characteristics of the study populations.

Table 2. Anthropometric and cardiovascular results at 3 years of age.

SUPPLEMENTARY TABLE LEGENDS

Table S1. Baseline characteristics of controls and cases included in the fetal and follow-up studies.

Table S2. Fertility characteristic and perinatal outcomes of controls and cases included in the fetal and follow-up cohorts.

Table 1. Baseline and perinatal characteristics of the study populations.

	Spontaneously conceived (n=80)	ART (n=80)	*p*-value
Maternal characteristics			
Maternal age (years)	34 ± 4.4	36 ± 2.9	0.025
Body mass index (kg/m^2)	23.1 ± 4.5	25.0 ± 6.9	0.079
Smoking (%)	11	6	0.246
Caucasian (%)	88	93	0.173
Primiparity (%)	41	53	0.365
Paternal characteristics			
Paternal age (years)	35 ± 6.0	38 ± 4.5	0.047
Body mass index (kg/m^2)	25.4 ±3.1	26.0 ± 4.3	0.437
Smoking (%)	31	25	0.061
Caucasian (%)	90	95	0.222
Fertility and ART characteristics			
Infertility cause			
Male (%)	¶	36	¶
ICSI (%)	¶	82	¶
Frozen embryos (%)	¶	6	¶
Pregnancy complications			
Prenatal corticoid exposure (%)	2	1	0.321
Preeclampsia (%)	0	7	0.061
Gestational diabetes (%)	6	8	0.205
Perinatal data			
Gestational age at delivery (weeks)	40 ± 4.0	39 ± 2.2	0.010
Male gender (%)	45	52	0.263
Birthweight (g)	3403± 403	3020± 600	0.002
Birthweight centile	52 ± 28	39 ± 31	0.017
5 minutes Apgar score	10 ± 0.62	10 ± 0.12	0.091
Admission to neonatal intensive care unit (%)	1	2	0.377

Data are mean (SD) or percentage.

ART = pregnancies conceived by assisted reproductive technologies. IVF = in vitro fertilization. ICSI = intracytoplasmic sperm injection.

* P-value calculated by Student's t-test and Pearson Chi-Square test.

† BMI calculated as weight in kilograms divided by the square of the height in meters.

¶ Non-applicable.

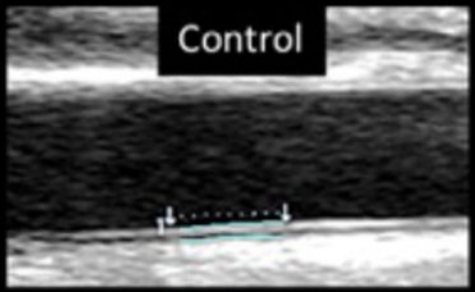
Table 2. Anthropometric and cardiovascular results at 3 years of age.

Characteristic	Spontaneously conceived (N=80)	ART (N=80)	*Crude P-value*	*Adjusted P-Value**
Age at evaluation (years)	3.0 ± 0.50	2.9 ± 0.30	0.149	0.075
Anthropometric data				
Height (cm)	98 ± 6.2	96 ± 5.0	0.088	0.101
Weight (Kg)	15.8 ± 2.7	15.1 ± 2.0	0.062	0.195
Body mass index (kg/m^2)	16.4 ± 1.6	15.9 ± 1.6	0.601	0.900
Body surface area (m^2)	0.34 ± 0.03	0.33 ± 0.06	0.935	0.700
Echocardiography				
Cardiac morphometry				
Left atrial area (cm^2)	5.29 ± 1.04	5.10 ± 1.01	0.307	0.468
Right atrial area (cm^2)	5.10 ± 0.87	5.54 ± 0.92	0.017	0.014
Left ventricular sphericity index	1.70 ± 0.20	1.62 ± 0.27	0.060	0.013
Right ventricular sphericity index	1.84 ± 0.29	1.70 ± 0.23	0.006	<0.001
Left ventricular wall thickness (mm)	6.11 ± 1.16	6.15 ± 1.31	0.830	0.654
Septal wall thickness (mm)	7.44 ± 1.39	7.61± 1.49	0.835	0.409
Systolic function				
Left shortening fraction (%)	38 ± 6.13	35 ± 4.61	<0.001	0.002
Heart rate (bpm)	106 ± 14	105 ± 15	0.968	0.837
Left cardiac output (mL/min)	43 ± 11.2	44 ± 12.0	0.460	0.382
Right cardiac output (mL/min)	33 ± 25.0	28 ± 10.8	0.163	0.132
Mitral ring displacement (mm)	11.19 ± 2.74	10.23 ± 2.01	0.026	0.048
Tricuspid ring displacement (mm)	18.28 ± 2.40	16.29 ± 2.74	<0.001	<0.001
Mitral lateral S' peak velocity (cm/s)	6.89 ± 1.90	6.64 ± 1.49	0.517	0.492
Tricuspid S' peak velocity (cm/s)	11.18 ± 2.22	11.37 ± 2.16	0.560	0.414
Diastolic function				
Mitral E/A ratio	1.68 ± 0.43	1.72 ± 0.50	0.729	0.399
Tricuspid E/A ratio	1.57 ± 0.44	1.68 ± 0.44	0.182	0.185
Mitral E deceleration time (ms)	138 ± 29.5	137 ± 37.3	0.971	0.547
Tricuspid E deceleration time (ms)	173 ± 52.3	174 ± 49.2	0.902	0.678
Mitral E' (cm/s)	15.9 ± 3.09	15.5 ± 2.68	0.442	0.421
Tricuspid E' (cm/s)	17.1 ± 3.10	16.3 ± 2.74	0.132	0.089
Mitral A' (cm/s)	6.95 ± 1.97	6.26 ± 1.67	0.030	0.125
Tricuspid A' (cm/s)	9.98 ± 9.08	9.05 ± 2.47	0.433	0.822
Mitral E/E' ratio	6.52 ± 1.51	6.42 ± 1.28	0.423	0.621
Tricuspid E/E' ratio	3.74 ± 1.76	3.64 ± 1.34	0.828	0.864
Left isovolumic relaxation time (ms)	67.06 ± 12.4	80.25 ± 13.4	<0.001	<0.001
Vascular assessment				
Systolic blood pressure (mmHg)	90 ± 7.2	95 ± 9.7	0.019	0.011
Diastolic blood pressure (mmHg)	62 ± 9.1	66 ±9.8	0.288	0.035
Mean blood pressure (mmHg)	76 ± 7.5	80 ± 9.2	0.012	0.007
Carotid mean IMT (mm)	0.45 ± 0.09	0.52 ± 0.03	<0.001	<0.001
Carotid maximum IMT (mm)	0.49 ± 0.10	0.60 ± 0.05	<0.001	<0.001

Data are mean (SD).

ART = pregnancies conceived by assisted reproductive technologies. S' = systolic annular peak velocity. E = ventricular inflow in early diastole. A = ventricular inflow during atrial contraction. E' = annular peak velocity in early diastole. IMT = intima-media thickness

* Adjusted P-value calculated by linear regression adjusted by parental age, gestational age at delivery, and birthweight centile.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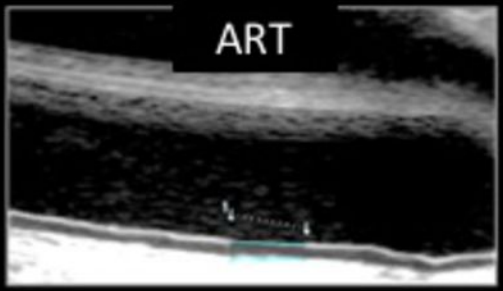
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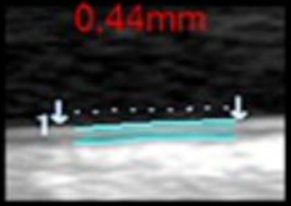
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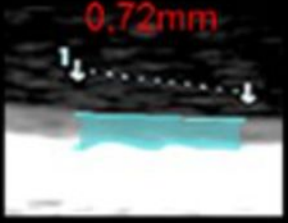
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