Review

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Investigation of cardiac remodeling and cardiac function on fetuses conceived via artificial reproductive technologies: a review

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Abstract: The prevalence of artificial reproductive technologies (ART), such as intra-uterine insemination (IUI), *in vitro* fertilization (IVF), and intracytoplasmic sperm injection (ICSI), has surged in response to the global increase in infertility rates, now impacting 17.5 % of couples. With over nine million babies born through ART, the safety and efficacy of these methods are largely recognized; however, emerging concerns regarding their association with prenatal and long-term health risks, especially cardiovascular disease (CVD), necessitate a thorough examination. This review synthesizes recent findings on the cardiac remodeling observed in ART-conceived fetuses, highlighting the potential for sub-clinical dysfunction and subsequent cardiovascular anomalies that may extend into adolescence. It delves into the perinatal complications linked to ART and examines the contribution of the renin-angiotensin system, epigenetic modifications, and altered microRNA expressions to fetal cardiovascular development. The analysis further differentiates the cardiac effects of fresh vs. frozen ART cycles and investigates the enduring nature of these changes beyond birth. Addressing the elevated CVD risk among ART individuals, the review suggests proactive measures, including lifestyle adjustments initiated early in life, to mitigate potential adverse outcomes. It emphasizes the critical need for ongoing research and intervention strategies to safeguard the cardiovascular health of the increasing number of ART-conceived individuals.

Keywords: IVF; ART; cardiac remodeling; cardiac reprogramming; fetal cardiac function; fetal cardiac morphology

Introduction

It is a well-established fact that the use of artificial reproductive technologies (ART), including intra-uterine insemination (IUI), *in vitro* fertilization (IVF), and intracytoplasmic sperm injection (ICSI), has met an enormous worldwide expansion, as infertility rates are raising globally. According to WHO (World Health Organization), infertility affects one out of six couples [1]. Infertility rates globally have been calculated to be 17.5 % in 2023, while previous studies estimated that infertility affected 8–12 % of the whole earth's population [2]. WHO also stated that affordable and high-quality fertility care has to be provided in couples desire childbearing [1]. As a result, more than nine million babies have been born through ART, as the safety, popularity and accessibility of these technologies are following a skyward trend [3].

There is no doubt that ART have helped millions of infertile couples to create their family. Although the use of ART is considered safe for both the mother and her child, there are some concerns regarding prenatal, perinatal, postnatal and also long-term risks for the children or adults conceived via ART. Preterm birth, low birth weight and congenital malformations are some of the perinatal risks that have been associated with ART [4]. Recent data from the worldwide literature provided evidence that ART fetuses present also cardiac remodeling and sub-clinical dysfunction *in utero,* resulting in alterations in vasculature which persist postnatally and until adolescence. Therefore, ART individuals might be in an increased risk for cardiovascular disease (CVD) in adulthood [5].

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This review presents an overview of the expanding knowledge about the main patterns leading to cardiac remodeling in ART fetuses, the data from the current literature occupied with this hot topic are summarized and finally, some of the strategies that have the potential to prevent CVD in postnatal life are also suggested.

Pathophysiology of cardiac remodeling of ART fetuses

Heart has an essential role in embryonic and fetal life and our knowledge about its contribution in fetal life is now starting to expand. Heart is the first organ which develops and its role is crucial for blood, nutrients and oxygen supply to the developing fetus. Heart is also a kinetic organ which has the potential to alter its structure, size and form, according to a sub-optimal environment or disease, in order to maintain an efficient function, ensuring an optimal supply to the fetal organs [6].

Therefore, intrauterine environment during fetal life plays the most significant role for the optimal cardiac structure and function [6]. Factors which have to be ideal in order to have an excellent cardiac function are; myocyte contractility, fiber orientation, tissue elasticity, pressure and volume load, electrical activation and myocardial perfusion. Additionally, as fetal cardiac remodeling happens during early development, cardiac shape and micro-structure are still prone to changes [6].

Another interesting fact is that fetal cardiac remodeling is more evident in the right heart, reflecting its dominance during fetal life, as the right heart has the responsibility to supply the fetal organs and the placenta with blood, nutrients and oxygen. Also, a higher sensibility of the right fetal heart to loading conditions is another factor that causes the right heart to be more prone to cardiac remodeling. Most of the changes that the fetal heart undergoes in order to maintain an optimal function, are leading to sub-clinical cardiac dysfunction. Nevertheless, under persistent circumstances, cardiac remodeling might result to clinical cardiac failure, which *in utero* is presented as fetal hydrops [6].

ART fetuses present distinct patterns of cardiac remodeling

Although evidence regarding cardiac remodeling is novel, recent data suggest that fetal heart undergoes distinct patterns of remodeling in response to different insults [6]. Also, the pattern that will be followed is in regard with the duration, type and timing of the insult.

During ART procedures, several steps of the physiological process of human conception is interrupted, namely gametogenesis, fertilization and early embryonic maturation. Also, gametes are manipulated, embryos are exposed to culture fluids and altered pH, temperature, oxygen pressure, light or hormonal concentration. Expectantly, during these very early stages of peri-conceptional period, the embryo is extremely sensitive to signals emerging from its surroundings, resulting to an altered development as a response to a changing environment [7].

Conception via artificial reproductive techniques leads to a pressure overload type of cardiac remodeling *in utero*. Therefore, fetal heart alters its shape to a more globular shape in order to bear with the increased wall stress. If the insult persists (the pressure overload), myocytes are forced to be hypertrophic leading to myocardial hypertrophy with simultaneously decrease of the diameter of cardiac ventricles [8]. This is also called increased relative wall thickness with normal outer heart shape [6]. Moreover, ART fetuses experience more prominent right pressure overload resulting to a cardiac phenotype with concentric ventricular remodeling accompanied with atrial dilatation, decreased longitudinal motion and impaired relaxation. The atria in ART fetuses are dilatated in an effort to overcome a ventricular early filling hassle that has to be compensated by a larger atrial confluence. In addition, impaired relaxation, expressed as shortened E and E′ deceleration time, seems to be caused by a decrease in ventricular compliance, leading to higher end-diastolic pressures [8, 9].

The above findings are also in line with a very interesting trial of Valenzuela – Alcaraz and colleagues, who investigated the different and independent patterns of cardiac remodeling that ART and small for gestational age (SGA) fetuses present, resulting to different cardiac phenotypes [5]. The prime thought behind this inquiry is that ART fetuses are more likely to be also SGA and until now the patterns of cardiac remodeling in these fetuses were unclear. In this study the fetuses under investigation were sub-grouped in four groups; appropriate for gestational age (AGA), spontaneously conceived fetuses (AGA-SC), AGA-ART fetuses, SGA spontaneously conceived fetuses (SGA-SC) and SGA-ART fetuses. ART fetuses presented dilatated atria and more globular ventricles, without any effect on cardiac size. Concerning cardiac function, ART and SGA fetuses shared a decreased longitudinal motion with tricuspid ring displacement and impaired relaxation time. Deceleration time was also shortened to AGA-ART fetuses and this was more often noted secondary to increased ventricular end-diastolic pressures. Expectantly, SGA-ART fetuses presented a mixed cardiac phenotype. Nevertheless, as cardiac remodeling that SGA-ART fetuses were presented was more similar to that of AGA-ART fetuses, the authors concluded that pressure overload caused by ART was stronger than the SGA insults [5].

Nevertheless, infertility *per se*, variations of parental holistic health and nutrition, genetic background, surrounding environment, perinatal complications and postnatal

factors might all play their role to cardiovascular remodeling of ART fetuses and the determining factor for this phenomenon cannot be set comprehensibly.

Evidence based on studies in animal models

Renin-angiotensin system (RAS) is a well-studied pathway which has a crucial role in the pathogenesis of cardiovascular disease in adults. One of its essential roles is to regulate blood pressure and also balance fluids and electrolytes concentration within the human body. New evidence provides proof that RAS is also a major contributor in the development of congenital heart defects during fetal life, as the heart, great vessels and also the kidneys are targeted by RAS [10].

A very interesting study, run by Wang et al., investigated the expression of different aspects of the RAS in mice conceived by IVF. Their findings are in line with the hypothesis that RAS plays an essential role in cardiac remodeling in ART. They concluded that expression of the angiotensin II receptor type 1 (AGTR1), connective tissue growth factor (CTGF) and collagen 3 (COL3) in the myocardial tissue of IVF-conceived mice, were all statistically significant increased at 3 weeks, 10 weeks, and 1.5 years of age, when compared to non-IVF fellows. The increased expression of AGTR1 ensues the increased expression of collagen 1 (COL1) and COL3, which are both crucial macro-modules for cardiac rigidity and elasticity [11]. Concerning CTGF, its role is to promote fibrosis and, during the early embryonic period, its over-activation results in vascular fibrosis and myocardial dysfunction. These findings suggest that RAS might act as an autocrine or paracrine system [12].

Additional investigation of microRNA (MiRNAs) micro-array analysis of the myocardial tissue of aged IVF-conceived mice, supported the data above; miR-100, miR-297, and miR-758, which interact with COL3, AGTR1, and COL1 respectively, were upregulated when compared to naturally mated mice of the same age. MiRNAs are small fragments of RNA, containing 22–24 nucleotides which are non-coding. However, they participate in the expression of genes significant for blood pressure control, lipid and glucose metabolism and also endothelial function [10].

Interestingly, decreased methylation of CpG sites in COL1 was also encountered on IVF-mice [10]. Epigenetic alterations in IVF mice are also proven by Rexhaj et al. The authors concluded that in this mice population, the methylation at the promoter of the gene encoding nitric oxide synthase (NOS) in the aorta was altered, leading in decreased vascular NOS expression and nitric oxide synthesis. As a result, endothelium was dysfunctional and also premature vascular senescence was also noticed [13].

All these findings reinforced the hypothesis that amended expression of RAS in myocardial tissue might contribute to cardiovascular dysfunction in IVF-conceived offspring and these cardiovascular abnormalities might be the result of shifted DNA methylation and abnormal regulation of microRNAs [10].

What current literature has proven so far

Studying fetal heart is a challenging task. Conventional ECG (electrocardiogram) and CT (computerized tomography) have no role in intrauterine life. Regarding MRI, it is also difficult to provide conclusive results due to the small size of fetal heart and the increased fetal heart rate. Consequently, for the assessment of cardiac remodeling *in utero*, fetal echocardiography presents the most attractive characteristics; it has high reproducibility and it is highly feasible for measuring cardiac dimensions, structure and shape [6].

Nevertheless, fetal cardiac function is even a more demanding and complex procedure. Myocardial shortening, longitudinal, radial and circumferential contraction have to be evaluated for cardiac function. A recently introduced technique which really succors cardiac function evaluation is speckle-tracking echocardiography (STE). With this technique, myocardial mechanism is directly analyzed by tracking tissue pixels in the myocardium throughout the cardiac cycle [4]. Another parameter which is frequently evaluated for fetal cardiac remodeling and mainly ventricular diastolic and systolic function *in utero* is the Myocardial Performance Index (MPI). MPI is measured with Doppler assessment and it is the result of the lowing equation: MPI=(IVCT + IVRT)/ET (IVCT, isovolumetric contraction time: ET, ejection time) [14]. An overview of the studies that have been occupied with cardiac remodeling on ART fetuses is presented in Table 1.

One of the pioneers investigating cardiac remodeling in ART fetuses is Valenzuela- Alcaraz and her associates [8]. These authors, on their very first study occupied with this subject, published in 2013, investigated cardiac remodeling in ART fetuses. They evaluated cardiac shape and function in 100 ART and 100 spontaneously conceived fetuses. Their data proved that ART fetuses have dilated atria, increased myocardial wall thickness and lower ventricular sphericity indexes. Regarding cardiac function, ART fetuses presented statistically significantly decreased right longitudinal function and impaired isovolumic relaxation time (decrease in tricuspid E′ and E deceleration time). Additionally, left ejection fraction and ring displacement was also statistically

Table 1: List of the articles in current literature and their conclusions.

Author, year	Study design	Study population, number or participants	Statistically significant results (cases vs. controls)	Summary – conclusion
Huluta et al. (2023)	Prospective cohort (case/control study)	Cases: ART singleton fetuses with fresh ET, n=112 ART singleton fetuses with frozen ET, n=231 controls: SC fetuses n=5,458	**Epidemiological data:** maternal age, maternal height, maternal BMI, nulliparity **Placental factors:** PLGF MoM, UtA-PI MoM **Pregnancy complications:** preeclampsia **Cardiac morphometric data:** RV sphericity index, LV sphericity index **Speckle tracking:** LV global longitudinal strain, LV ejection fraction	**Positive correlation** During mid-gestation, ART fetuses present signs of cardiac remodeling **in utero**, regardless the method of embryo transfer (frozen or fresh). ART fetuses have more globular hearts with LV systolic dysfunction. More studies have to be held in order to investigate the persistence of this cardiac remodeling in the postnatal period.
Rizzo et al. (2020)	Prospective cross-sectional study	Cases: ART-ICSI singleton fetuses with fresh ET, n=101 ART-ICSI singleton fetuses with frozen ET, n=101 controls: SC fetuses n=120	**ART vs. SC:** **Cardiac morphometric data:** RV sphericity index, LV sphericity index, LA area, RA area **Frozen vs. fresh ET:** **Cardiac morphometric data:** RV sphericity index, LV sphericity index, LA area, RA area	**Positive correlation** ICSI fetuses present signs of cardiac remodeling **in utero** regardless fetal size and Doppler measurements. This pattern is more evident in the group of fresh embryo transfer (vs. frozen).
Boutet et al. (2021)	Prospective observational study	Cases: ART singleton fetuses with fresh ET, n=100 ART singleton fetuses with frozen ET, n=100 controls: SC fetuses n=100	**ART vs. SC:** **Epidemiological data:** nulliparity, PCOS, induction of labor, cesarean section **Pregnancy complications:** gestational diabetes **Neonatal outcomes:** minor neonatal morbidity **Cardiac morphometric data:** cardio-thoracic ratio, LA/heart area ratio, left free wall thickness, septal wall thickness, relative wall thickness **Diastolic function:** mitral E′, mitral A′ **Heart rate and timing:** left MPI **Fresh vs. frozen ET:** **Epidemiological data:** nulliparity, induction of labor, cesarean section **Pregnancy complications:** preeclampsia **Cardiac morphometric data:** LA/heart area ratio, left free wall thickness, relative wall thickness **Heart rate and timing:** left MPI	**Positive correlation** Although ART fetuses present signs of cardiac remodeling **in utero**, these changes are subclinical. More studies have to be held in order to investigate the long-term aspects of fetal cardiac remodeling in the postnatal period. Nevertheless, early lifestyle interventions in this population are important.
Boutet et al. (2023)	Prospective cohort study (case/control study)	Cases: ART singleton fetuses with fresh ET, n=96 SC singleton fetuses from subfertile couples[b], n=97 controls: SC fetuses from fertile couples n=96	**ART vs. SC (both fertile and subfertile):** **Epidemiological data:** nulliparity **Cardiac morphometric data:** cardio-thoracic ratio, LA/heart area ratio, RA/heart area ratio, LV sphericity index, RV sphericity index, left free wall thickness, septal wall thickness, relative wall thickness **Cardiac function:** mitral ring displacement, tricuspid ring displacement, heart rate, left MPI	**Positive correlation** Although ART fetuses present signs of cardiac remodeling **in utero**, these changes are subclinical. Subfertility per se is not associated with cardiac remodeling. More studies have to be held in order to investigate the long-term aspects of fetal cardiac remodeling in the postnatal period.
Bi et al. (2022)	Prospective observetional study	Cases: ART fetuses and infants, n=88 controls: SC fetuses and infants, n=85	**Epidemiological data:** nulliparity, maternal and paternal educational level (university education), cesarean section **Placental factors:** MCA PI, UtA-PI **Cardiac morphometric data**: left atria area, right atria area, relative LV wall thickness, LV sphericity index of mid-section,	**Positive correlation** ART fetuses present subclinical cardiac remodeling and dysfunction related to various ART procedures. Nevertheless, this pattern of remodeling and dysfunction is not evident in early infancy.

Table 1: (continued)

Author, year	Study design	Study population, number or participants	Statistically significant results (cases vs. controls)	Summary – conclusion
			cardio-thoracic ratio **Cardiac function:** mitral ring displacement, tricuspid ring displacement, mitral E′, LV GLS, LV GLS rate S, LV GCS, LV GLS rate A, LV GCS rate A	
Valenzuela-Alcaraz et al. (2015)	Prospective cohort study (case/control study)	Cases: ART fetuses and infants, n=100 controls: SC fetuses and infants, n=100 Both groups were followed- up durning the first and 6th month of life	**Perinatal characteristics:** vanishing twin, low birth weight, birth weight (g), birth weight (percentile), gestational age at delivery **Fetal assessment:** **Cardiac morphometric data:** left atria area, right atria area, LV sphericity index, RV sphericity index, septal wall thickness, LV free wall thickness, RV free wall thickness **Systolic function:** left ejection fraction, mitral ring displacement, tricuspid ring displacement, mitral S′ **Diastolic function:** mitral E′, tricuspid E′, mitral E deceleration time, tricuspid E deceleration time, left isovolumic relation time **1st month assessment:** **Blood pressure:** diastolic blood pressure **Vascular wall thickness:** aortic mean IMT, aortic mean IMT/weight, aortic maximum IMT, aortic maximum IMT/weight, carotid mean IMT, carotid mean IMT/weight, carotid maximum IMT, carotid maximum IMT/weight **6th month assessment:** **Cardiac morphometric data:** right atria area, RV sphericity index **Systolic function:** left shortening fraction, heart rate, mitral ring displacement, tricuspid ring displacement **Diastolic function:** mitral E deceleration time, tricuspid E deceleration time, left isovolumic relation time **Blood pressure:** systolic blood pressure **Vascular wall thickness:** aortic mean IMT, aortic mean IMT/weight, aortic maximum IMT, aortic maximum IMT/weight	**Positive correlation** ART fetuses present subclinical cardiac remodeling and dysfunction this pattern of remodeling and dysfunction is also present in infancy. These data suggest that early detection and lifestyle interventions in this population are important.
Valenzuela-Alcaraz et al. (2017)	Prospective cohort study (case/control study)	Cases: ART AGA fetuses, n=72 SC singleton fetuses ART SGA fetuses, n=31 SC SGA fetuses, n=28 controls: SC AGA fetuses, n=102	**ART AGA vs. controls:** **Epidemiological data:** maternal age, paternal age **Cardiac morphometric data:** Left atria/heart ratio, right atria/heart ratio, LV sphericity index, RV sphericity index, LV relative wall thickness, RV relative wall thickness **Systolic function:** mitral ring displacement, tricuspid ring displacement, mitral S′ **Diastolic function:** mitral E′, tricuspid E′, mitral E deceleration time, tricuspid E deceleration time, mitral A′, left isovolumic relation time	**Positive correlation** SGA and ART fetuses both present cardiac remodeling with different and distinct patterns, supporting that there are independent causes of cardiac programming in each group.

Table 1: (continued)

Author, year	Study design	Study population, number or participants	Statistically significant results (cases vs. controls)	Summary – conclusion
			ART SGA vs. controls: **Epidemiological data:** maternal age, paternal age, low socioeconomic status, birth weight (g), birth weight (percentile) **Pregnancy complications:** preeclampsia **Cardiac morphometric data:** left atria/heart ratio, right atria/heart ratio, LV sphericity index, RV sphericity index, LV relative wall thickness, RV relative wall thickness **Systolic function:** left ejection fraction, mitral ring displacement, tricuspid ring displacement, mitral S′ **Diastolic function:** mitral E′, tricuspid E′, mitral A′, tricuspid A′, left isovolumic relation time **ART AGA vs. ART SGA:** **Epidemiological data:** low socioeconomic status, birth weight (g), birth weight (percentile) **Pregnancy complications:** preeclampsia **Placental factors:** cerebroplacental ratio **Systolic function:** mitral S′ **SC SGA vs. controls:** **Epidemiological data:** birth weight (g), birth weight (percentile) **Pregnancy complications:** preeclampsia, prenatal cortisol exposure, admission to NICU, major neonatal morbidity **Placental factors:** cerebroplacental ratio, UtA-PI **Cardiac morphometric data:** Cardiothoracic ratio, LV sphericity index, RV sphericity index, LV relative wall thickness, RV relative wall thickness **Systolic function:** mitral ring displacement, tricuspid ring displacement, mitral S′ **Diastolic function:** mitral E deceleration time, tricuspid E deceleration time mitral E′, tricuspid E′, mitral A′, tricuspid A′, left isovolumic relation time **SC SGA vs. ART AGA:** **Epidemiological data:** maternal age, birth weight (g), birth weight (percentile) **Pregnancy complications:** preeclampsia, prenatal cortisol exposure, admission to NICU, major neonatal morbidity **Placental factors:** cerebroplacental ratio, UtA-PI **Cardiac morphometric data:** cardiothoracic ratio, left atria/heart ratio, right atria/heart ratio, LV sphericity index, RV sphericity index, LV relative wall thickness, RV relative wall thickness **Systolic function:** mitral ring displacement, mitral S′	

Table 1: (continued)

Author, year	Study design	Study population, number or participants	Statistically significant results (cases vs. controls)	Summary – conclusion
			Diastolic function: mitral E deceleration time, tricuspid E deceleration time mitral E′, tricuspid A′ **SC SGA vs. ART SGA:** **Epidemiological data:** birth weight (g) **Pregnancy Complications:** prenatal cortisol exposure, admission to NICU, major neonatal morbidity **Placental factors:** UtA-PI **Cardiac morphometric data:** cardiothoracic ratio, left atria/heart ratio, right atria/heart ratio, RV relative wall thickness **Systolic function:** mitral ring displacement **Diastolic function:** mitral E deceleration time, tricuspid E deceleration time mitral E′	
Valenzuela-Alcaraz et al. (2018)	Prospective cohort study (case/control study)	Cases: DC ART fetuses, n=50 controls: DC SC fetuses n=50	**Epidemiological data:** maternal age, paternal age, paternal BMI, cesarean section **Pregnancy complications:** preeclampsia **Neonatal outcomes:** admission to NICU, major neonatal morbidity[a] **Cardiac morphometric data:** left atria/heart ratio, right atria/heart ratio, RV sphericity index, septal wall thickness, RV free wall thickness **Systolic function:** TAPSE **Diastolic function:** mitral E′, tricuspid E′	**Positive correlation** ART twins present signs of cardiac remodeling in terms of shape and function. This remodeling pattern is similar to that of ART singleton pregnancies. This knowledge offers the opportunity of an early lifestyle intervention in this population.

ART, assisted reproductive technology; SC, spontaneously conceived; LV, left ventricle; RV, right ventricle; LA, left atria; RA, right atria; ET, embryo transfer; BMI, body mass index; MoM, multiple of median; UtA, uterine artery; PI, pulsality index; ICSI, intra-cytoplasmatic sperm injection; PCOS, polycystic ovarian syndrome; MPI, Myocardial Performance Index; MCA, middle cerebral artery; DC, dichorionic; TAPSE, tricuspid annular plane systolic excursion; E′, early diastolic peak velocity; A′, atrial contraction peak velocity; GLS, global longiditudinal strain; GCS, global circumferential strain; IMT, intima-media thickness; SGA, small for gestational age; AGA, appropriate for gestational age; NICU, Neonatal Intensive Care Unit. [a]Major neonatal morbidity: defined as at least one of the following: bronchopulmonary dysplasia, necrotizing enterocolitis, intraventricular hemorrhage, periventricular leukolamacia, retinopathy, persistent ductus arteriosus, sepsis. [b]Subfertile couples: time to pregnancy (TTP) over 12 months of trying to conceive.

significant decreased in ART fetuses compared to the controls. Expectantly, these changes were more prominent in the right side of the heart [8].

Boutet et al., gave an insight whether infertility *per se* or IVF procedure is the main cause of fetal cardiac remodeling [15]. They evaluated cardiac remodeling in 96 IVF fetuses, 97 spontaneously conceived fetuses whose parents were assumed as infertile (trying to conceived for more than 12 months) and 96 spontaneously conceived fetuses from fertile parents. IVF fetuses presented a similar cardiac phenotype presented by Valenzuela- Alcaraz et al. [8]; dilated atria, more globular right ventricles and thicker myocardial walls. Cardio-thoracic ration was also increased in IVF fetuses compared to controls. Cardiac function was also sub-optimal in IVF fetuses, as fetal heart rate was elevated, tricuspid ring displacement was reduced, accompanied by an increased left myocardial performance index (MPI). The authors concluded that infertility *per se* is not responsible for cardiac remodeling *in utero* [15].

A recent trial from Bi et al. [16], also endorsed the findings above. The authors investigated cardiac remodeling in 88 ART and 85 SC fetuses. ART fetuses had larger atria and more globular ventricles, following the same pattern as in the previous studies. In this trial, cardiac function of ART fetuses was extensively evaluated through the STE technology. Expectantly, ART fetuses presented sub-clinical cardiac dysfunction with reduced global longitudinal strain of the left ventricle indicating sub-optimal left systolic function. Tricuspid ring displacement was also lower in ART fetuses.

Twin pregnancies

With the expanding use of ART, multiple pregnancy incidence is also following an upward trend, as nowadays, in Europe the multiple pregnancy rate is 21.8 %. Nonetheless, multiple pregnancies carry significantly higher risk for perinatal complications such as gestational hypertensive disorders, gestational diabetes mellitus and preterm birth. Cardiac remodeling in twin pregnancies conceived by ART was evaluated in another trial of Valenzuela-Alcaraz and associates [17]. They investigated cardiac reprogramming in 50 SC dichorionic/diamniotic twins and in 50 dichorionic/diamniotic twins conceived via ART. Their findings demonstrated that ART twins presented the same alteration on cardiac phenotype as the singleton ART pregnancies; dilated atria, globular ventricles and increased myocardial thickness. In the ART group, cardiac function was also affected with reduced longitudinal motion, mainly of the tricuspid annular plane systolic excursion. Right cardiac side was again more stricken, with a right ventricular concentric remodeling [17].

Cardiac remodeling in fresh and frozen cycles

Another question that had to be answered is whether the type of embryo transfer, i.e. fresh embryo transfer (ET) or frozen embryo transfer (FET), can influence the presence or the phenotype of cardiac remodeling *in utero*. Although nowadays frozen embryo cycles are gaining popularity with the "freeze-all" method, the safety and the overall influence on child and mother's health of this technique has to be determined. Data from recent studies provided evidence that FET cycles carry less perinatal complications, namely preterm birth, low birth weight and SGA. However, FET fetuses are in higher risk for being large for gestational age (LGA) and their mothers are in elevated risk for developing gestational diabetes mellitus, gestational hypertensive disorders and preeclampsia. These facts could be partially explained by the different laboratory conditions that FET embryos are undergone, as vitrification procedure includes an early supplementary manipulation. Also, FET embryos are exposed to higher amounts of cryoprotectants and finally they are thawed before transfer. On the other hand, however, FET embryos are transferred to an endometrium which has not been exposed to the hormonological changes of ovarian stimulation and, similarly, hormonal replacement in FET cycles reflects a more physiological condition of the endometrium. Finally, the higher incidence of gestational hypertensive disorders in FET cycles might be due to the lack of the hormone relaxin, which is secreted from the corpus luteum in normal circumstances. Interestingly, placental volume in ET pregnancies was significantly lower compared to FET pregnancies [18]. The fact that in another study uterine artery pulsatility index was lower and PlGF (Placental Growth Factor) was higher in FET pregnancies, is indicating that placental perfusion is better in FET fetuses [19].

Boutet et al., in 2020 investigated the influence of ET vs. FET cycles in terms of fetal cardiac remodeling [18]. They evaluated fetal cardiac structure and function in 100 spontaneously conceived (SC) fetuses, 100 fetuses conceived via fresh IVF cycles (IVF-ET) and 100 fetuses conceived via frozen IVF cycles (IVF-FET). The authors came to the conclusion that both IVF-ET and IVF-FET fetuses shared a similar cardiac phenotype as it has multiply described; IVF fetuses presented dilated atria, more spherical ventricles and increased myocardial wall thickness. Cardiac function and myocardial motility were also compromised in both groups of IVF fetuses, while tricuspid annular systolic peak velocity was also reduced in these groups. Fetal heart rate and both left and right MPI were increased in IVF fetuses compared to their counterparts, pointing out both systolic and diastolic dysfunction in IVF fetuses. Interestingly, these features were more pronounced in IVF-ET fetuses compared to IVF-FET fetuses [18].

Similarly, Rizzo et al. [20] agreed with the results of Boutet et al. [18]. The former, studied cardiac function and shape in 111 ICSI-FET, 101 ICSI-ET and 120 SC fetuses. The authors confirmed that both ICSI groups presented larger atria and more globular ventricles than SC fetuses. Atrial enlargement was more prominent to the ICSI-ET group, while sphericity index was higher in the ICSI-FET group [20].

Another interesting trial by Huluta et al. [19], also researched the influence of the different embryo transfer techniques to cardiac remodeling *in utero*. They assessed fetal cardiac structure and function with the STE technology in 5484 SC, 112 IVF-ET and 231 IVF-FET fetuses in mid-gestation (19–24 gestational weeks), including both FET and ET embryos. They noticed that both the right and the left ventricles were more spherical in IVF fetuses compared to the controls. Additionally, they noted only left cardiac sub-optimal cardiac dysfunction, as left ventricular global longitudinal strain was higher and left ventricular ejection fraction was lower in the IVF group. Conversely with the two previous studies, during mid-gestation, cardiac remodeling features are not different among the two subgroups of IVF fetuses. Importantly, the authors also highlighted the fact that cardiac remodeling in ART fetuses cannot be explained by alterations in placental function [19].

Persistence of cardiac remodeling in postnatal life

Evidence based on follow-up studies on IVF individuals during adult life, agree that cardiovascular remodeling and sub-clinical dysfunction is evident in this population. As cardiac remodeling takes place in a critical stage of development, some of the alteration on cardiac shape, structure and function have the potential to persist postnatally, making ART adults more prone to CVD in adulthood [6]. Increased arterial blood pressure, increased intima-media thickness, abnormal myocardial and vascular endothelial function due to altered lipid metabolism are also found in IVF adults [8, 13, 21–23]. Studies among late children and adolescents conceived by ART showed also dysfunctional changes in systemic and pulmonary circulation and premature sub-clinical atherosclerosis [24].

Ceelen et al., in 2008, were the first who provided evidence proving that ART children have elevated blood pressure [25]. Meister et al. [26], also investigated blood pressure in 54 apparently healthy and without any risk factor for CVD adolescences and young adults, born via ART. 46 matched controls were also included to the study. The authors concluded that ART group showed premature vascular aging and higher blood pressure compared to the controls [26]. This finding was also proved in an important meta-analysis run by Guo et al. in 2017 investigating the blood pressure of 2,112 children conceived by ART. The authors came to the conclusion that there was a small, though statistically significant increase on blood pressure measurements in ART children compared to their counterparts [27].

Valenzuela-Alcaraz and her group, also followed up the ART fetuses within their first month of life and also at 6 months. Their results concerning infants and neonates were also very interesting. The authors noted that at the first follow-up visit (1 month old), ART neonates presented significantly higher diastolic blood pressure compared to spontaneously conceived fetuses. Systolic blood pressure did not show any statistically significant difference. Moreover, aortic and carotid intima-media thickness (IMT) were increased in ART neonates. During the second follow-up visit, within the sixth month of life, blood pressure and aortic IMT remained statistically significantly elevated in ART infants compared to controls. Additionally, ART infants had increased right atrial size, lower right sphericity index and thicker right ventricular wall. Cardiac output was preserved in ART infants. However, ART infants showed a significantly decreased shortening fraction and increased heart rate. Regarding cardiac function, ART fetuses also showed signs of both systolic and diastolic dysfunction as measured by significant decreases in ring displacement, E deceleration time and tissue Doppler velocities. Finally, isovolumic relaxation time was significantly elevated when compared to controls. Interestingly, even postnatally, cardiac remodeling was more pronounced in the right heart [8].

On the other hand, Mizrak et al., provided data from their study, held in 2022, which disagree with the studies above. The authors investigated arterial stiffness and blood pressure in 100 ART children and compared them with 50 NC (naturally conceived) children, using magnetic resonance imaging (MRI). All children in both groups were 8–9 years old. Their results are quite interesting; there was not any significant difference between ART and NC children in terms of blood pressure, left ventricular ejection fraction, cardiac output and aorta distensibility [28]. One other study which is in line with Mizrak's et al. trial, is this of Bi et al. [16]. In this study, 88 ART infants and 86 matched controls were followed up within the first two months of their life and they found only a slightly thinner relative wall thickness of the right ventricle and a higher tricuspid E/A ratio on ART infants. Importantly, these differences were not evident during the second follow-up, at 6 months. The authors assumed these findings as clinically insignificant [16].

Preventing measures to improve cardiovascular health in ART children and adults

Although there is definitive data from the literature proving clearly that ART provoke cardiovascular reprogramming, causing an advanced risk for CVD later in life, the role of other risk factors appearing later in life and their collaboration with the altered subclinical cardiovascular dysfunction resulting to CVD in adulthood is yet to be understood. The "second hit hypothesis" is well-established in biology of human oncology, indicating that a genetic or subclinical predisposition demands a second attack in order to be expressed into clinical disease. Concerning ART and CVD, the subclinical cardiovascular dysfunction requires environmental factors to result to clinical CVD. This "second hit" could be anything able to add an extra work to the subclinical affected myocardium, such as hypertension, dyslipidemia or arrhythmias [14].

Therefore, it is equitable to consider that in some degree, the subclinical dysfunction of the cardiovascular system of individuals conceived via ART could be reversed, ameliorate or undergone further contravention depending on life-style habits adopted in later life. Nevertheless, it is important to mention that alteration in everyday habits have the prospective to prevent CVD if they are adopted very early in life, giving

the "window of opportunity", where crucial interventions must be done in order to improve cardiovascular health [14].

Early identification of the children, adolescences and young adults in risk for CVD, and following preventing measures have major impact on their holistic health. Importantly, as the number of children born via ART is emerging, early interventions in this population carries also great benefits for public health [5]. Some measures that have the potential to decrease the cardiovascular risk in ART adults is breastfeeding, and maintaining a healthy diet with caution to conserve a healthy weight is crucial. More specifically, consumption of a diet rich in polyunsaturated fats, the use of omega-3 fatty acids, eicosapentaenoic and α-linolenic acids provide significant benefits in vascular and hemodynamic health of children and adolescences. Additionally, avoidance of smoking and frequent physical exercise are equally significant for CVD prevention [14].

Conclusions

There is no doubt that ART is a great medical achievement and has helped millions of couples to create their family. Nevertheless, ART is redefining biology and society and from a clinical point of view it is important to understand the potential impact and long-term health of the infants conceived via ART. In this review, the most recent data regarding cardiac remodeling and sub-clinical cardiac dysfunction in ART fetuses and infants have been presented. As ART adults are in greater risk for developing CVD, the identification of these fetuses which are at high risk for impaired cardiovascular health is vital, in order to offer them preventing measurements aiming to optimize their overall health.

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