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Fetal cardiac function at midgestation and conception by *in-vitro* fertilization

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KEYWORDS: cardiac function; deformation; fetal echocardiography; speckle tracking; sphericity index

CONTRIBUTION

What are the novel findings of this work?
In pregnancies conceived via *in-vitro* fertilization (IVF), compared with those conceived spontaneously, the fetal heart was globular and left ventricular systolic function assessed by speckle tracking was mildly reduced. These differences were independent of the type of embryo transfer (fresh or frozen).

What are the clinical implications of this work?
In IVF pregnancies, compared with those conceived spontaneously, there is evidence of fetal cardiac remodeling at midgestation, which is not related to the use of fresh or frozen embryo transfer or changes in placental perfusion and function. These findings suggest an independent effect of IVF on the fetal heart.

ABSTRACT

Objective *To assess differences in cardiac morphology and function at midgestation in fetuses conceived by* in-vitro *fertilization (IVF), using fresh or frozen embryo transfer, compared with those conceived naturally.*

Methods *This was a prospective study of 5801 women with a singleton pregnancy attending for a routine ultrasound examination at 19 + 0 to 23 + 6 weeks' gestation, including 343 that conceived by IVF. Conventional and more advanced echocardiographic modalities, including speckle-tracking analysis, were used to assess fetal cardiac function in the right and left ventricles. The morphology of the fetal heart was assessed by calculating the right and left sphericity index. Placental perfusion and function were assessed by measurement of uterine artery pulsatility index (UtA-PI) and serum placental growth factor (PlGF), respectively.*

Results *Fetuses that were conceived by IVF, compared with those conceived spontaneously, had significantly lower right and left ventricular sphericity index, higher left ventricular global longitudinal strain and lower left ventricular ejection fraction. There were no significant differences in any of the cardiac indices within the IVF group between the fresh and frozen embryo transfers. In the IVF group, compared with spontaneously conceived pregnancies, UtA-PI was lower and PlGF was higher, suggesting better placental perfusion and function.*

Conclusions *Our study demonstrates that, in IVF pregnancies, compared with those conceived spontaneously, there is evidence of fetal cardiac remodeling at midgestation, which is not related to the use of fresh or frozen embryo transfer. In the IVF group, compared with naturally conceived pregnancies, fetal heart was globular and left ventricular systolic function was mildly reduced. Whether these cardiac changes are accentuated later in pregnancy and remain in the postnatal period remains to be established.* © *2023 International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

Epidemiological studies have reported that pregnancies conceived by *in-vitro* fertilization (IVF) are associated with a higher risk of adverse perinatal outcomes, including preterm birth and low birth weight[1,2]. Long-term cardiovascular and metabolic risks for the offspring have also been reported in both animal and human studies[3–5]. For instance, children conceived by IVF are prone to elevated systolic blood pressure and impaired cardiovascular function compared with their naturally conceived counterparts[6,7]. Research studies performed in the third trimester of pregnancy showed that cardiovascular remodeling and suboptimal cardiac

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function are already present in the fetal period and persist after delivery, supporting the theory of the fetal cardiovascular programming effect of IVF treatment[8–10].

Given these associations and the increasing uptake of IVF treatment, a more comprehensive investigation is necessary to provide insight into the timing of fetal cardiac remodeling and explore factors, such as maternal characteristics, placental function and perfusion, and type of embryo transfer (fresh *vs* frozen), which may affect this process.

We performed a large fetal cardiac screening study in women attending for a routine ultrasound examination at midgestation to assess whether fetal cardiac morphology and function differ in fetuses conceived via IVF compared to those conceived spontaneously and to explore potential determinants of these differences.

METHODS

Study design and participants

This was a prospective observational cohort study of women who attended for a routine hospital visit at 19 + 0 to 23 + 6 weeks' gestation at King's College Hospital, London, UK, between August 2019 and December 2021. Gestational age was determined by the measurement of fetal crown–rump length at 11–13 weeks' gestation[11].

The visit included recording of maternal demographic characteristics and medical history, ultrasound examination for fetal anatomy and growth, measurement of left and right uterine artery pulsatility index (UtA-PI) by transvaginal or transabdominal pulse Doppler ultrasound and calculation of the mean value of the two arteries[12], measurement of maternal serum placental growth factor (PlGF) in pg/mL by an automated biochemical analyzer (BRAHMS KRYPTOR compact PLUS; Thermo Fisher Scientific, Hennigsdorf, Germany) and fetal cardiac functional assessment.

Participants completed a questionnaire, which was then reviewed by a doctor together with the woman. Patient characteristics included maternal age; race (white, black, South Asian, East Asian and mixed); method of conception (natural or assisted by *in-vitro* fertilization followed by fresh or frozen embryo transfer); cigarette smoking during pregnancy; medical history of chronic hypertension, diabetes mellitus, systemic lupus erythematosus (SLE) and/or antiphospholipid syndrome (APS); family history of PE (woman's mother affected); and obstetric history, which included parity (parous or nulliparous if no previous pregnancy at ≥ 24 weeks' gestation) and, for parous women, history of pregnancy with PE and interpregnancy interval. The maternal weight and height were measured, and the body mass index was calculated in kg/m^2.

Data on pregnancy outcome, including information on live-birth delivery, birth weight and development of pre-eclampsia and gestational hypertension, were collected from hospital maternity records or general medical practitioners of the women. The incidence of PE, as defined by the American College of Obstetricians and Gynecologists, and small-for-gestational age, defined as birth weight below the 10th percentile of the Fetal Medicine Foundation fetal and neonatal population weight charts, was determined[13].

The women gave written informed consent to participate in the study, which was approved by the NHS research ethics committee. The inclusion criteria for this study were a singleton pregnancy delivering a liveborn fetus without major congenital abnormalities. Pregnancies with aneuploidy or major fetal abnormality were excluded.

Fetal cardiac functional analysis

A comprehensive fetal cardiac functional assessment was carried out using Canon Aplio i900 machines with a convex transducer (i8CX1) (Canon Medical Systems Europe BV, Zoetermeer, The Netherlands). Measurements were performed using conventional pulsed-wave Doppler and M-mode as well as more advanced imaging modalities, including tissue Doppler imaging and speckle-tracking echocardiography. Right systolic ventricular function was assessed in the apical four-chamber view by measuring tricuspid annular plane systolic excursion. This was obtained by placing the M-mode line from the right ventricular apex to the anterolateral tricuspid valve annulus (Figure S1a). Right ventricular global longitudinal strain was measured using speckle-tracking echocardiography[14]. Left ventricular systolic function was assessed by calculating the myocardial performance index[15] and left ventricular global longitudinal strain.

Image acquisition for speckle-tracking analysis was performed in the four-chamber view at an 'apex up or down' projection[16]. A clip of 3–5 s with a minimum of 100 frames/s was obtained for each case and analysis was carried out using Vitrea software (Canon Medical Systems, Crawley, UK), as described previously[16,17]. Left ventricular diastolic function was assessed by measuring the mitral valve early (E) and late (A) diastolic filling peak Doppler flow velocities and calculating the E/A ratio and by calculating E/e′ from pulsed tissue Doppler imaging, as described previously[17,18] (Figure S1b and S1c). The morphology of the left and right ventricles was assessed in the apical or basal four-chamber view and the length and width of the left and right ventricles were measured in end-diastole. The sphericity index was calculated by dividing base-to-apex length by transverse diameter. Fetal cardiac examinations were carried out by seven trained fetal medicine fellows who also performed the analysis of the Doppler indices. Analysis of speckle tracking was carried out by two operators. Inter- and intraobserver reproducibility for the Doppler indices was assessed in 20 fetuses, and the results have been reported previously[16].

Statistical analysis

Data were expressed as mean (interquartile range (IQR)) for continuous variables and *n* (%) for categorical

variables. Student's *t*-test and chi-square test or Fisher's exact test were used for comparing outcome groups for continuous and categorical data, respectively.

Multiple linear regression models were fitted to each of the 12 cardiac indices, with terms for gestational age at measurement, maternal age, weight, height, racial origin, heart rate, method of conception, diabetes mellitus, history of gestational diabetes mellitus (GDM), history of chronic hypertension or APS/SLE and PE. Histograms were used to identify suitable data transformation, where appropriate, and backwards elimination was used for model selection. First, the regression models were used to assess the effects of gestational age, maternal characteristics and medical history, GDM and PE on each of the cardiac indices. Second, partial residuals from the fitted models, after excluding the contribution of GDM and PE, comprised the $\log_{10}$ multiples of the median (MoM) values or deviations from the median (deltas), depending on the transformation of the cardiac outcome variable in the original model fitting. Similarly, MoM values were calculated for UtA-PI and PlGF. Geometric mean MoMs or mean deltas with 95% CIs, excluding the contribution of IVF, according to IVF status were calculated and compared, and correlations of UtA-PI MoM and PlGF MoM with cardiac indices were assessed. The statistical software package R was used for data analysis[19].

RESULTS

Study population

The study population consisted of 5801 women with a singleton pregnancy, of which 343 (5.9%) were conceived by IVF, including 112 after fresh embryo transfer and 231 after frozen embryo transfer. Compared with the non-IVF group, the IVF group had a higher median maternal age, median lower body mass index, higher incidence of white race, lower incidence of black race and higher incidence of nulliparity (Table 1).

In the IVF group, compared with the non-IVF group, UtA-PI was lower and PlGF was higher, suggesting better placental perfusion and function (Table 1). The incidence of small-for-gestational-age neonate was not significantly different between the two groups, but the incidence of PE was higher in the IVF group.

Fetal cardiac changes in IVF *vs* non-IVF pregnancies

Fetuses conceived by IVF, compared to those conceived spontaneously, had significantly lower right and left ventricular sphericity index, higher left ventricular global longitudinal strain and lower left ventricular ejection fraction (Tables 2 and S1, Figure 1). There were no significant differences in any of the cardiac indices within the IVF group between those conceived using fresh *vs* frozen embryo transfer.

The relationship of fetal cardiac indices with placental perfusion and function in the IVF and non-IVF pregnancies is summarized in Table 3. There were no significant associations between the cardiac indices and UtA-PI MoM or PlGF MoM in the IVF or non-IVF pregnancies, with the exception of one between left ventricular global longitudinal strain delta and PlGF MoM in the non-IVF group.

DISCUSSION

Main findings

In this large screening study at midgestation, we showed that fetuses conceived by IVF, compared with those conceived naturally, have more globular hearts and evidence of reduced left ventricular systolic function.

Table 1 Characteristics of study population according to method of conception

Characteristic	*No IVF (n = 5458)*	*IVF (n = 343)*	P
Maternal age (years)	33.0 (29.9–36.0)	37.6 (34.6–40.1)	< 0.0001
Maternal weight (kg)	71.0 (63.9–80.5)	71.0 (64.6–78.4)	0.182
Maternal height (cm)	166 (161–170)	167 (163–171)	0.006
Body mass index (kg/m^2)	25.7 (23.2–29.1)	25.3 (23.2–28.7)	0.005
Gestational age (weeks)	21.3 (20.9–21.6)	21.3 (20.7–21.6)	0.084
Race			< 0.0001
White	3912 (71.7)	287 (83.7)	
Black	858 (15.7)	17 (5.0)	
South Asian	339 (6.2)	23 (6.7)	
East Asian	149 (2.7)	10 (2.9)	
Mixed	200 (3.7)	6 (1.7)	
Medical history			
Chronic hypertension	97 (1.8)	5 (1.5)	0.822
DM type I	14 (0.3)	1 (0.3)	0.99
DM type II	17 (0.3)	1 (0.3)	0.99
SLE/APS	16 (0.3)	0 (0)	0.636
Smoker	69 (1.3)	0 (0)	0.066
Family history of PE	183 (3.4)	9 (2.6)	0.740
Parity			< 0.0001
Nulliparous	2901 (53.2)	252 (73.5)	
Parous, no previous PE	2406 (44.1)	89 (25.9)	
Parous, previous PE	151 (2.8)	2 (0.6)	
Interpregnancy interval (years)	2.5 (1.5–4.5)	2.4 (1.8–3.6)	0.567
Placental perfusion and function			
UtA-PI MoM	0.99 (0.82–1.21)	0.92 (0.75–1.12)	0.0001
Placental growth factor MoM	0.99 (0.73–1.33)	1.07 (0.78–1.44)	0.034
Pregnancy outcome			
BW < 10th percentile	697 (12.8)	40 (11.7)	0.607
PE	154 (2.8)	25 (7.3)	< 0.0001

Data are given as median (interquartile range) or *n* (%). APS, antiphospholipid syndrome; BW, birth weight; DM, diabetes mellitus; IVF, *in-vitro* fertilization; MoM, multiples of the median; PE, pre-eclampsia; SLE, systemic lupus erythematosus; UtA-PI, uterine artery pulsatility index.

Table 2 Fetal cardiac indices, expressed as delta values, in pregnancies conceived spontaneously *vs* those conceived by *in-vitro* fertilization (IVF), overall and according to type of embryo transfer

Cardiac index	*No IVF* (n = *5458*)	*IVF* (n = *343*)	*Fresh IVF* (n = *112*)	*Frozen IVF* (n = *231*)
Morphometric				
RV SI delta (*n* = 4922)	0 (−0.01 to 0.01)	−0.07 (−0.09 to −0.03)*	−0.06 (−0.12 to −0.003)	−0.07 (−0.11 to −0.03)
LV SI delta (*n* = 4919)	0 (−0.01 to 0.01)	−0.18 (−0.22 to −0.15)*	−0.18 (−0.24 to −0.13)	−0.19 (−0.24 to −0.14)
Diastolic				
MV E delta (*n* = 5760)	−0.01 (−0.16 to 0.13)	0.22 (−0.45 to 0.89)	0.78 (−0.27 to 1.84)	−0.06 (−0.90 to 0.78)
MV A delta (*n* = 5756)	0.001 (−0.20 to 0.20)	−0.016 (−0.84 to 0.81)	0.68 (−0.62 to 1.98)	−0.36 (−1.41 to 0.69)
MV E/A delta (*n* = 5697)	0.0003 (−0.0036 to 0.0043)	−0.005 (−0.021 to 0.010)	−0.003 (−0.03 to 0.03)	−0.007 (−0.03 to 0.01)
MV E/e′ delta (*n* = 5674)	−0.03 (−0.09 to 0.03)	−0.08 (−0.32 to 0.16)	0.26 (−0.11 to 0.62)	−0.24 (−0.54 to 0.05)
Systolic				
MPI delta (*n* = 5760)	-4×10^{-5} (−0.002 to 0.002)	6×10^{-4} (−0.007 to 0.009)	−0.005 (−0.02 to 0.01)	0.003 (−0.006 to 0.013)
TAPSE delta (*n* = 5640)	−0.003 (−0.023 to 0.017)	0.05 (−0.03 to 0.12)	0.03 (−0.08 to 0.15)	0.05 (−0.04 to 0.15)
Speckle tracking†				
RV GLS delta (*n* = 4379)	−0.03 (−0.17 to 0.11)	0.43 (−0.10 to 0.96)	0.59 (−0.38 to 1.57)	0.35 (−0.28 to 0.99)
LV GLS delta (*n* = 4388)	0 (−0.22 to 0.22)	2.32 (1.62–3.03)*	2.29 (1.16–3.42)	2.34 (1.44–3.24)
RV EF delta (*n* = 4379)	−0.0004 (−0.002 to 0.001)	0.005 (−0.002 to 0.012)	−0.003 (−0.02 to 0.01)	0.009 (0.0007–0.017)
LV EF delta (*n* = 4387)	0.001 (−0.003 to 0.006)	−0.048 (−0.067 to −0.030)*	−0.04 (−0.08 to −0.01)	−0.05 (−0.07 to −0.03)

Data are given as mean (95% CI). *Significant difference between IVF and non-IVF groups. †Median (interquartile range) frame rate for speckle-tracking analysis was 194 (163–226) frames/s. EF, ejection fraction; GLS, global longitudinal strain; LV, left ventricle; MPI, myocardial performance index; MV, mitral valve; RV, right ventricle; SI, sphericity index; TAPSE, tricuspid annular plane systolic excursion.

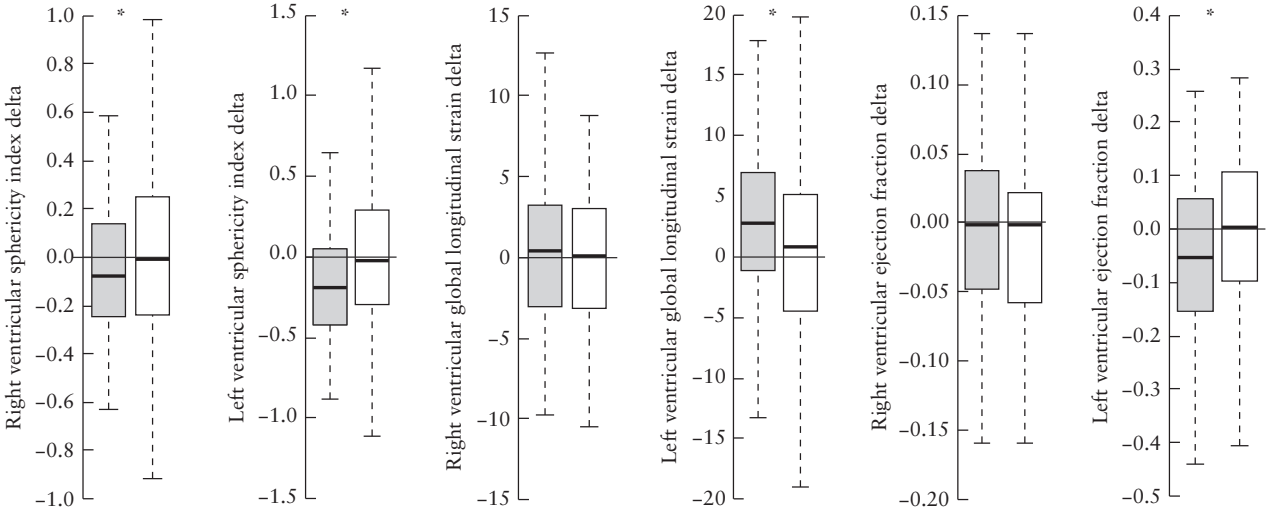

Figure 1 Box-and-whiskers plots showing quartiles, minimum and maximum for cardiovascular indices adjusted for maternal characteristics and medical history, expressed as difference from the median (delta), in fetuses that were conceived via *in-vitro* fertilization (▣), compared with those conceived spontaneously (□). *Significant difference between groups.

Table 3 Correlation coefficients of fetal cardiac indices with placental perfusion and function in pregnancies conceived spontaneously *vs* those conceived by *in-vitro* fertilization (IVF)

Correlation	*No IVF*	*IVF*
RV sphericity index delta *vs* UtA-PI MoM	−0.014 (−0.044 to 0.014)	−0.008 (−0.120 to 0.104)
LV sphericity index delta *vs* UtA-PI MoM	0.004 (−0.025 to 0.033)	0.077 (−0.035 to 0.187)
LV global longitudinal strain delta *vs* UtA-PI MoM	0.012 (−0.019 to 0.043)	−0.091 (−0.204 to 0.025)
LV ejection fraction delta *vs* UtA-PI MoM	−0.027 (−0.057 to 0.004)	0.047 (−0.069 to 0.161)
RV sphericity index delta *vs* PlGF MoM	0.009 (−0.020 to 0.038)	0.044 (−0.069 to 0.156)
LV sphericity index delta *vs* PlGF MoM	0.012 (−0.017 to 0.041)	0.051 (−0.062 to 0.162)
LV global longitudinal strain delta *vs* PlGF MoM	−0.037 (−0.067 to −0.006)	0.082 (−0.034 to 0.196)
LV ejection fraction delta *vs* PlGF MoM	0.030 (−0.001 to 0.060)	−0.008 (−0.120 to 0.104)

Values in brackets are 95% CI. LV, left ventricle; MoM, multiples of the median; PlGF, placental growth factor; RV, right ventricle; UtA-PI, uterine artery pulsatility index.

Cardiac changes were subclinical and did not differ between those conceived using fresh *vs* frozen embryo. Placental perfusion and function, reflected by the levels of UtA-PI and PlGF, were significantly better in the IVF group, and were not associated with cardiac indices. These findings suggest that fetal cardiac remodeling is present from midgestation in fetuses conceived via IVF, and this effect is not explained by changes in placental perfusion and function or differences in maternal characteristics.

Comparison with results of previous studies

A limited number of studies have investigated the influence of IVF treatment on fetal cardiac morphology and function. At 28 + 0 to 32 + 6 weeks' gestation, a cohort study of 111 fetuses conceived by assisted reproductive technology and 106 fetuses conceived naturally reported a reduction in left ventricular systolic function in the former group and no differences between different IVF procedures[20]. In another study, at 28–33 weeks', Boutet *et al.* compared fetal cardiac function between 200 fetuses conceived using IVF and 100 fetuses conceived spontaneously[10]. They demonstrated that IVF fetuses had subclinical derangements in both right and left ventricular systolic and diastolic functions. These findings complement a previous report from the same group, which showed that assisted reproductive technology was associated with biventricular functional and structural changes, which also persisted in the postnatal period[9]. However, in that study[9], a higher proportion of pregnancies with fetal growth restriction was noted, and this may have potentially impacted adversely the reported fetal cardiac changes.

Earlier in pregnancy, at 20–24 weeks' gestation, Rizzo *et al.* showed that, in pregnancies conceived by intracytoplasmic sperm injection, right and left atria were increased and fetal hearts were more globular when compared to those in fetuses that were conceived spontaneously[21]. Our results complement those of previous reports and demonstrate that cardiac remodeling in fetuses conceived by IVF is apparent from midgestation. Although both the right and left ventricles were globular in shape, functional cardiac changes were noted only in the left ventricle. Similar changes in the shape of the fetal heart have been reported as a result of increase in the afterload due to placental dysfunction[22] but also in response to metabolic insults[18,23]. However, considering that markers of placental perfusion and function and incidence of small-for-gestational-age neonates were not increased in the IVF group in our study, our findings suggest an independent effect of IVF on fetal heart.

The impact of fresh *vs* frozen embryo transfer on fetal cardiac remodeling remains poorly defined. In the past, fresh embryo transfer was used for IVF, but recently, the use of frozen embryos has gained popularity[24]. Early systematic reviews and meta-analyses implied that the use of frozen embryo transfer was superior compared with fresh embryo transfer with regard to perinatal outcome, but this was not confirmed in more recent reports[25–28]. In addition, concerns were raised on whether the vitrification process or endometrial preparation required in anovulatory women who use frozen embryo transfer may have an adverse impact on the health of the offspring[24,29]. In our study, we found no differences in fetal cardiac remodeling according to type of embryo transfer in contrast to mild differences reported by the studies of Rizzo *et al.*[21] and Boutet *et al.*[10]. Unfortunately, in the current study, we had no information regarding specific protocols and procedures used in fresh or frozen embryo transfer; therefore, it is impossible to comment on methodological differences between studies and explain the noted discrepancy in results.

The mechanisms driving fetal cardiac changes in IVF pregnancies remain to be established. Several potential factors have been thought to contribute to this process, including, but not limited to, parental factors, placental function and type of embryo transfer technique. In our cohort, women who had an IVF pregnancy were older compared to those with spontaneous conception and were more often of white ethnic background, but these did not affect fetal cardiac measurements. In addition, although rates of PE were higher in the IVF group, these could not explain our findings.

Strengths and limitations

This is the largest screening study performed at midgestation in which detailed echocardiography was performed in all fetuses. We followed a strict protocol to characterize fetal cardiac morphology and function, aiming to minimize variability in measurements, which can arise due to technical issues. Our comprehensive functional assessment included not only conventional but also novel speckle-tracking analysis, and this allowed us to detect early fetal myocardial alterations. We performed detailed assessment of placental perfusion and function and obtained fetal cardiac outcomes. We showed fetal cardiac remodeling in IVF pregnancies irrespective of the method of embryo transfer and documented subclinical left ventricular functional alterations but no right ventricular changes. In addition, the incidence of small-for-gestational-age pregnancies did not differ between groups, and this provides further evidence that fetal cardiac remodeling in IVF pregnancies is not exclusively related to factors that contribute to development of fetal growth restriction. In IVF pregnancies, different medications and preparation protocols are used prior to conception to optimize the maternal environment and support the embryo transfer. In the current phenotyping study, we had no information about preconceptional treatment and, thus, it remains unknown whether differences in medical management may have impacted our findings. In addition, the contribution of infertility could not be differentiated from that of IVF procedure in this study. Finally, our study was cross-sectional and, therefore, we were unable to assess whether fetal cardiac remodeling is accentuated as pregnancy progresses and whether fetal cardiac alterations persist in the postnatal period.

Conclusion

Our study demonstrates that, in IVF pregnancies, compared to those with spontaneous conception, there is evidence of fetal cardiac remodeling at midgestation, which is not related to the use of fresh or frozen embryo transfer. In the IVF group, compared with naturally conceived pregnancies, the fetal heart was globular and left ventricular systolic function was mildly reduced, and these cardiac changes were not mediated by changes in placental function and perfusion. Although our findings support an independent early effect of IVF on fetal cardiac morphology and function, the clinical significance of these findings remains to be established.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Figure S1 (a) Measurement of tricuspid annular plane systolic excursion. (b) Measurement of pulse Doppler velocities in the mitral valve. (c) Tissue Doppler measurements.

Table S1 Comparison of unadjusted values of fetal cardiac indices in pregnancies conceived by *in-vitro* fertilization (IVF) *vs* those conceived spontaneously

