Original Article

A preliminary study on fetal cardiac morphology and systolic function of normal and anemic pregnant women by fetal heart quantification technology

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Background: Maternal anemia is a common nutritional problem during pregnancy. Fetal heart quantification (fetal HQ) technology is used to quantitatively evaluate the size, shape, and contractile function of the fetal heart, which can reflect the development of the fetus in the uterus.

Methods: We used fetal HQ technology to evaluate the basal-apical length (BAL), the transverse width (TW) and the area (A) of the four chamber view at end-diastole in 77 normal fetuses and 40 fetuses of women with anemia. We analyzed the changes of fetal heart size and measured the global sphericity index (GSI), the fraction area change (FAC), and the global longitudinal strain (GLS). The sphericity index (SI) and the fractional shortening (FS) of 24 segments were analyzed to identify any changes of fetal heart morphology and systolic function. The normal range of Z value was set at –2 to 2.

Results: Fetal BAL, TW, A, and gestational age (GA) were positively linearly correlated, while GSI, GLS, and FAC had no significant correlation with GA. There was no significant difference in fetal BAL, TW, A, GLS, and FAC between the two groups (P>0.05). There was no significant difference in the FS of the 24 segments of the left and right ventricles between the two groups (P>0.05). There was no significant difference in the SI of the 1-24 segments of the right ventricle between the two groups (P>0.05). The difference in fetal GSI between the two groups was statistically significant (P<0.05). There was no significant difference in SI between the 1–22 segments of the left ventricle between the two groups (P>0.05), but there was a statistically significant difference between the 23–24 segments (P<0.05).

Conclusions: The fetal HQ analysis technology can quickly and simply quantitatively assess the size, shape, and contractility of the fetal heart. Anemia in pregnant women has no significant effect on fetal heart size and systolic function; it only affects the morphology of the heart, showing that the heart tends to be spherical as a whole and some segments of the apical segment of the left ventricle are abnormal.

Keywords: Fetal heart quantification technology; 24 segments; fetal heart function; anemia in pregnant women

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Introduction

Anemia is a common clinical symptom of decreased peripheral blood red blood cell volume below the lower limit of the normal range (1), and is the most common nutritional problem throughout pregnancy. Anemia has been reported in about 50% of women during pregnancy (2,3). Studies have confirmed that anemia during pregnancy may lead to fetal hypoxia, and may also cause degenerative changes in the villi in the placenta, resulting in insufficient blood supply to the placenta, in turn causing fetal intrauterine asphyxia. In severe cases, premature birth and stillbirth may result (4,5). The fetal endocardium is the most sensitive to hypoxia, so assessing changes in fetal cardiac function can reflect fetal development in utero.

Fetal heart quantification (fetal HQ) technology (6) is a new technology based on a two-dimensional speckle tracking imaging technology that divides the fetal ventricle into 24 segments (7). Fetal HQ is used to quantitatively analyze and evaluate fetal heart morphology and function. The purpose of this study was to investigate the application value of fetal HQ technique in the fetal cardiac size, morphology, and contractility of non-anemic and anemic pregnant women. We present the following article in accordance with the MDAR reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-22-315/rc).

Methods

Study population

A total of 77 normal pregnant women [gestational age (GA) 26.6±3.6 w] and 40 anemic pregnant women (GA 26.2±4.9 w) in their second and third trimesters were selected from the Ningxia Maternal and Child Health Hospital (Ningxia Children's Hospital) and General Hospital of Ningxia Medical University from November 2021 to March 2022. Average age of all pregnant women is 30.03±0.85 y. The eligibility criteria were as follows: pregnant women with anemia: maternal peripheral blood hemoglobin (Hb) <110 g/L (8); single pregnancy; no other pregnancy complications or other diseases; fetus had no cardiac malformations and malformations of other systems; and no chromosomal abnormalities in the fetus. A normal pregnant woman was defined as a pregnant woman without complications during pregnancy and other inclusion conditions were consistent with the case group. Women in both groups had an accurate date of their last menstrual period (LMP), and the GA assessed by ultrasound was consistent with the GA calculated from the LMP history. All women were informed of the accuracy and limitations of fetal echocardiography before examination, and provided informed consent for fetal echocardiography. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of General Hospital of Ningxia Medical University (batch number: [2020]0520B), and the Ethics Committee of the Ningxia Maternal and Child Health Hospital (Ningxia Children's Hospital) (batch number: KJ-LL-2022-26). Informed consent was taken from all the participants for this study.

Instruments and steps

Instruments

A GE Voluson E8/E10 color Doppler ultrasound system (GE Healthcare, Chicago, IL, USA) was used, equipped with C1-6/C2-9/Em6C transducers and the fetal HQ software package (GE Healthcare).

Steps

General clinical data collection of pregnant women was conducted: maternal age, LMP, past medical history, gestational weeks, and so on. The woman was instructed to lie in a supine position. First, a routine two-dimensional ultrasound examination was performed to measure the fetal head circumference, biparietal diameter, abdominal circumference, humerus length, and femur length, and to determine the GA and weight of the fetus. Fetal echocardiography was then performed, followed by quantitative cardiac analysis using fetal HQ software.

Dynamic image acquisition of fetal apical four-chamber view

The operator was instructed to adjust the conditions of the ultrasound instrument, reduce the fan angle of image acquisition or increase the frequency of the probe, increase the frame rate of image acquisition, optimize the image quality, clearly display the boundary between the ventricular cavity and the endocardium, and keep a dynamic map of no less than 3 seconds. They also attempted to exclude the influence of the women's breathing and fetal movement.

Fetal HQ software analyzes fetal heart dynamic diagram

On the fetal apical four-chamber view (4CV), the frame before the closure of the atrioventricular valve was determined as end-diastole, and the basal - apical length (BAL) and transverse width (TW) of the four-chamber heart were measured on this view. BAL is the distance from the posterior base of the heart to the apical epicardium, TW is the distance between the epicardium of the largest TW of the heart; The software automatically calculated the area of the heart (A) and the global sphericity index (GSI=BAL/TW) (*Figure 1*). An end-systolic phase was then determined between the two end-diastolic phases of the ventricle in combination with the M-type curve (*Figure 2*).

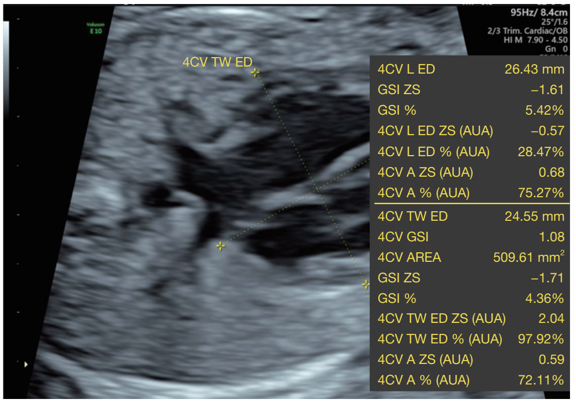

Figure 1 Fetal four-chamber end-diastole. 4CV L ED, four chamber view length end-diastole; 4CV TW ED, four chamber view transverse width end-diastole; GSI, global sphericity index; ZS, Z score; AUA, average ultrasound age; A, area of the heart; BAL, basal-apical length; TW, transverse width.

The systolic and diastolic ventricular endocardium curves of the left and right ventricles were traced, and the left and right ventricular end-systolic and end-diastolic phases were respectively divided into 24 segments. The software obtained the left and right ventricle area (LV/RV-A), the fraction area change (FAC), the global longitudinal strain (GLS), and the spherical index (SI) and fractional shortening (FS) of 24 segment. The Z score were used to analyze the whole fetal heart and cardiac function. In this study, Z-scores >2 or <−2 were considered abnormal.

Repeatability evaluation

A total of 20 fetuses were randomly selected and analyzed by fetal HQ after two weeks. Statistical analysis was performed on the two groups of data measured by the same examiner to evaluate the measurement consistency within the examiner.

Statistical analysis

The software SPSS 26.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis; the measurement data with normal distribution and homogeneous variance were expressed as $\bar{x}$±s; and two independent samples *t*-test was used for comparison between groups. The chi-square test was used to compare the abnormal rate of Z values. The repeatability test used the intraclass correlation coefficient (ICC) value to judge consistency. A P value <0.05 was considered statistically significant.

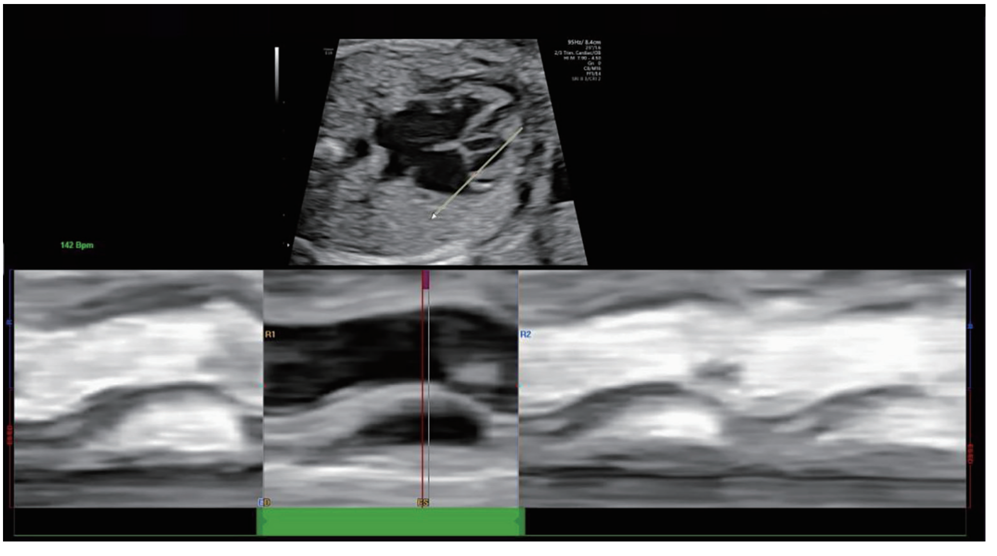

Figure 2 Determining an end-systolic phase between two end-diastolic phases of the ventricle combined with the M-curve. The arrow means right ventricular sidewall apex to bottom.

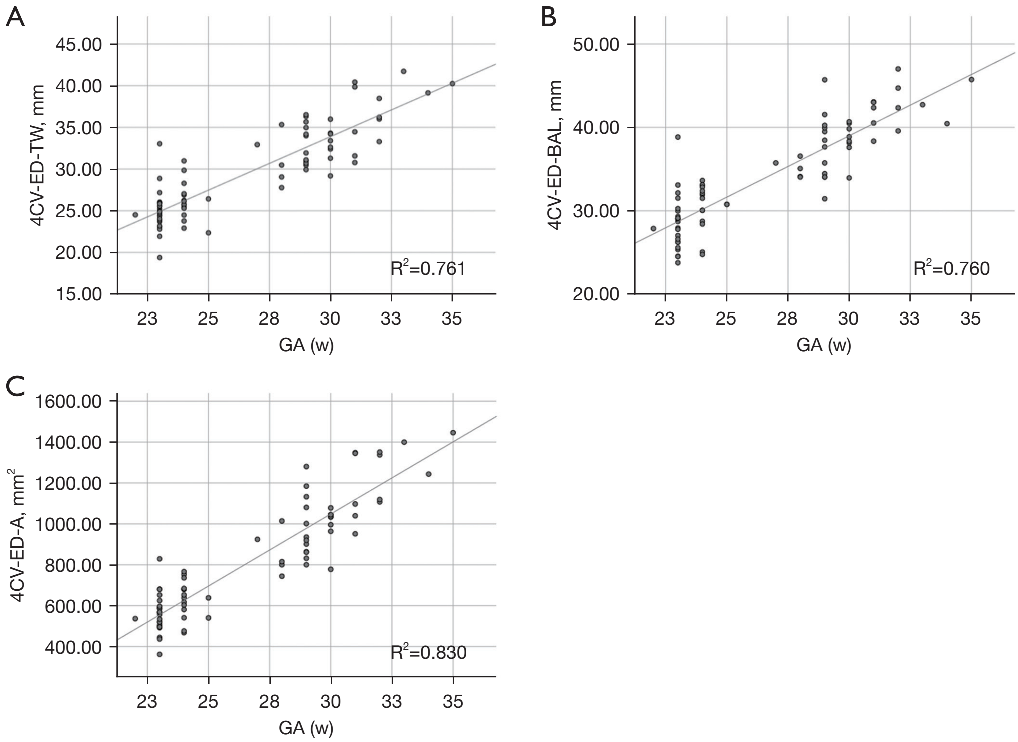

Figure 3 Correlation between fetal GA and heart size. (A) GA is positively linearly correlated with 4CV-ED-TW; (B) GA is positively linearly correlated with 4CV-ED-BAL; (C) GA is positively linearly correlated with 4CV-ED-A. GA, gestational age; 4CV, four chamber apical view; ED, end diastolic; TW, transverse width; BAL, basal-apical length; A, area of the heart.

Results

Comparison of fetal heart size between two groups

There was a linear correlation between BAL, TW, A, and GA at the end-diastolic stage of the four-chamber fetal heart (*Figure 3*). There was no significant difference in fetal GA between the two groups, and there was no significant difference in fetal BAL, TW, and A between the two groups (*Table 1*). Among the 77 cases in the normal group, 18 cases had abnormal BAL-Z values, and the abnormal rate was 23.4%; the number of abnormal TW-Z values was 8 cases, and the abnormal rate was 10.4%; and the number of abnormal A-Z values was 14 cases, and the abnormal rate was 18.2%. Among the 40 pregnant women with anemia, the number of abnormal fetal BAL-Z values was 6 cases, the abnormal rate was 15.0%; the number of abnormal TW-Z values was 1 case, the abnormal rate was 2.5%; and the number of abnormal A-Z values was 5 cases, the abnormal rate was 12.5%. There was no significant difference in fetal BAL-Z value, TW-Z value, and A-Z value between the two groups ($P>0.05$).

Comparison of fetal heart morphology between two groups

(I) The GSI was not significantly associated with GA (*Figure 4*). There was a statistically significant difference in GSI between the two groups ($P<0.05$) (*Table 1*). Among the 77 cases of normal fetal GSI, 14 cases had abnormal Z value, and the abnormal rate was 18.2%; a total of 3,696 segments of SI were measured: there were 120 cases with abnormal Z value, and the abnormal rate was 3.2%. Among the 1,848 SI segments of each ventricle, 52 cases in the left ventricle had an abnormal Z value, with an abnormal rate of 2.8%, and 68 cases in the right ventricle had abnormal Z value, with an abnormal rate of 3.7%. Among the 40 cases of pregnant women with anemia, 4 cases had abnormal Z value in fetal GSI, and the abnormal rate was 10.0%; a total of 1,920 segments of SI were measured, and 117 cases were abnormal, with an abnormal rate of 6.1%. Among the 960 segments of SI in each ventricle, 52 cases had abnormal Z values in the left ventricle, with an abnormal rate of 5.4%, and 65 cases in the right ventricle had abnormal Z values, with an abnormal rate of 6.8%. There was no significant difference in the abnormal rate of fetal GSI-Z between the

Table 1 Comparison of fetal gestational age and cardiac measurements between the two groups

Measurements	Case group ($\bar{x}$±s)	Normal group ($\bar{x}$±s)	*t*	P value
GA (w)	26.20±4.90	26.60±3.60	0.485	0.629
TW (mm)	27.35±7.50	29.54±5.25	−1.849	0.067
BAL (mm)	33.49±8.40	34.01±6.02	−0.385	0.700
A (mm²)	763.87±398.32	809.60±276.30	−0.727	0.469
GSI	1.24±0.13	1.16±0.12	3.451	0.001
LV-GLS	−19.36±6.08	−21.00±7.69	1.264	0.209
RV-GLS	−17.98±8.90	−15.62±7.79	−1.476	0.143
LV-FAC	37.86±9.76	40.15±11.22	−1.091	0.278
RV-FAC	32.48±12.79	28.49±12.10	1.659	0.100

GA, gestational age; TW, transverse width; BAL, basal-apical length; A, area of the heart; GSI, global sphericity index; LV-GLS, left ventricular global longitudinal strain; RV-GLS, right ventricular global longitudinal strain; LV-FAC, left ventricular fractional area change; RV-FAC, right ventricular fractional area change.

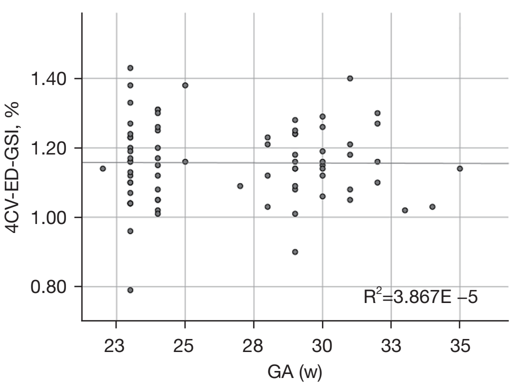

Figure 4 GA was not associated with 4CV-ED-GSI. GA, gestational age; 4CV-ED-GSI, four chamber apical view end diastolic global sphericity index.

two groups (P>0.05). The difference in the abnormal rate of SI-Z value of the 24 segments of the left and right ventricles was statistically significant (P<0.05).

(II) Comparing the SI of the 24 segments of the left and right ventricles of the two groups of fetuses, there was no significant difference in the SI of the 1–22 segments of the left ventricle between the two groups (P>0.05), and the comparison of the 23–24 segments of the left ventricle yielded a statistically significant difference (P<0.05). There was no significant difference in the SI of the 1–24 segments of the right ventricle between the two groups (P>0.05) (*Table 2*).

Comparison of fetal cardiac systolic function between two groups

(I) There was no significant correlation between fetal GLS, FAC, and GA (*Figures 5,6*), and there was no significant difference in GLS and FAC between the two groups of fetal left and right ventricles (P>0.05) (*Table 1*).

(II) Comparing the FS of the 24 segments of the left and right ventricles of the two groups of fetuses, there was no statistical difference between the 24 segments of the left and right ventricles (P>0.05) (*Table 3*).

Repeatability evaluation

The intra-measurer ICC of each fetal measurement value was 0.82–0.85, with good consistency.

Discussion

Pregnancy complications such as anemia, hypertension, and diabetes, fetal congenital heart disease, and fetal thoracic space-occupying lesions may affect the shape and systolic function of the fetal heart. Conventional two-dimensional echocardiography was used to measure cardiothoracic ratio, atrioventricular diameter, mitral valve, tricuspid valve peak early diastolic blood flow maximum peak velocity (E) and end-diastolic blood flow maximum peak velocity (A), aorta diameter, pulmonary artery diameter , flow rate, myocardial activity index, etc. to evaluate fetal heart function, but

Table 2 Comparison of the 24-segment spherical index between the two groups of fetal left and right ventricles

Location	Segment	LV-SI ($\bar{x}$±s)		RV-SI ($\bar{x}$±s)	
		Case group	Normal group	Case group	Normal group
Basal	1	1.29±0.33	1.79±0.33	1.59±0.36	1.52±0.34
Basal	2	1.79±0.34	1.79±0.34	1.58±0.36	1.50±0.33
Basal	3	1.79±0.34	1.79±0.34	1.57±0.37	1.49±0.32
Basal	4	1.79±0.35	1.79±0.35	1.56±0.37	1.48±0.32
Basal	5	1.80±0.36	1.80±0.36	1.56±0.38	1.49±0.32
Basal	6	1.81±0.36	1.81±0.36	1.56±0.37	1.50±0.32
Basal	7	1.83±0.35	1.83±0.35	1.57±0.36	1.52±0.33
Basal	8	1.86±0.35	1.85±0.35	1.58±0.36	1.55±0.34
Mid	9	1.89±0.35	1.88±0.35	1.61±0.36	1.58±0.34
Mid	10	1.92±0.36	1.91±0.36	1.64±0.36	1.62±0.35
Mid	11	1.95±0.36	1.95±0.36	1.68±0.37	1.67±0.36
Mid	12	2.00±0.37	1.99±0.37	1.74±0.38	1.72±0.37
Mid	13	2.05±0.39	2.04±0.39	1.80±0.40	1.78±0.38
Mid	14	2.10±0.40	2.10±0.40	1.88±0.42	1.86±0.40
Mid	15	2.17±0.41	2.17±0.41	1.98±0.44	1.95±0.43
Mid	16	2.24±0.42	2.24±0.42	2.09±0.47	2.06±0.46
Apical	17	2.31±0.42	2.31±0.42	2.21±0.49	2.20±0.49
Apical	18	2.39±0.43	2.39±0.43	2.37±0.51	2.36±0.52
Apical	19	2.50±0.45	2.50±0.45	2.56±0.52	2.58±0.57
Apical	20	2.70±0.51	2.70±0.51	2.85±0.56	2.91±0.66
Apical	21	3.08±0.02	3.07±0.62	3.32±0.63	3.43±0.83
Apical	22	3.83±0.82	3.82±0.82	4.19±0.80	4.39±1.13
Apical	23	5.49±1.24*	5.48±1.24	6.06±1.18	6.41±1.74
Apical	24	10.69±2.48*	10.68±2.48	11.85±2.34	12.61±3.54

*, P<0.05. LV, left ventricular; SI, sphericity index; RV, right ventricular.

fetal heart rate is fast, small, and easily affected by various factors, and the repeatability and accuracy of the above measurements are poor.

Fetal HQ analysis technology is a new technology based on two-dimensional speckle tracking technology for quantitative analysis of fetal heart function. This technology evaluates the size, shape, and function of the heart by tracking the endocardium and dividing the left and right ventricles into 24 segments, and can quantitatively analyze changes in cardiac morphology and cardiac function (9). Scholars at home and abroad use this technology to evaluate the morphology and systolic function of the fetal heart such as gestational diabetes mellitus, fetal growth restriction, coarctation of the aortic arch, patent ductus arteriosus, etc. It is critical for identifying subtle changes in the heart in the clinic and prompting timely intervention and management. In this study, the feasibility and accuracy of this technique were confirmed by analyzing the cardiac morphology and function of 77 normal fetuses and 40 fetuses of anemic pregnant women. The changes of fetal cardiac function measurements were compared between the two groups to analyze the influence of maternal anemia on

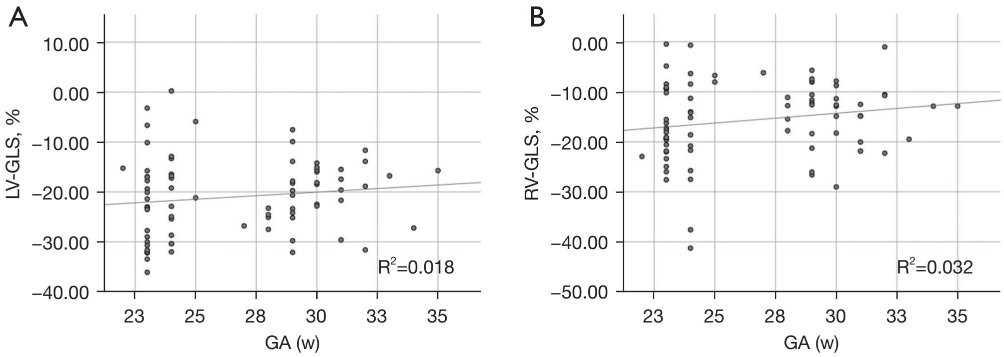

Figure 5 Correlation between fetal GA and ventricular GLS. (A) GA was not associated with LV-GLS; (B) GA was not associated with RV-GLS. GA, gestational age; LV-GLS, left ventricular global longitudinal strain; RV-GLS, right ventricular global longitudinal strain.

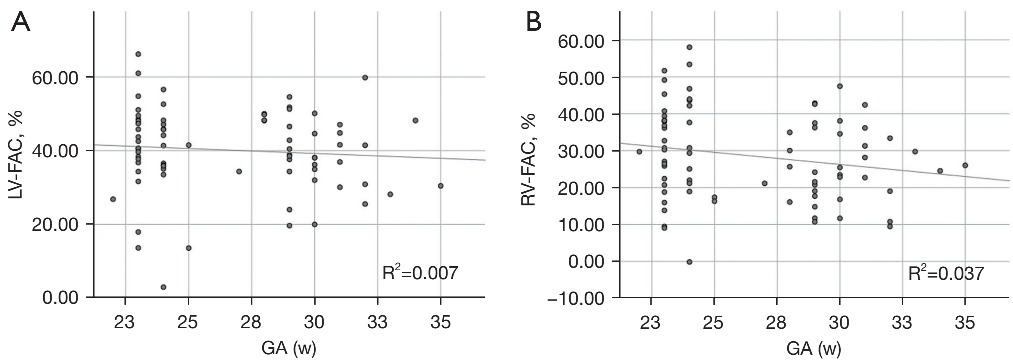

Figure 6 Correlation between fetal GA and ventricular FAC. (A) GA was not associated with LV-FAC; (B) GA was not associated with RV-FAC. GA, gestational age; LV-FAC, left ventricular fractional area change; RV-FAC, right ventricular fractional area change.

the morphology and function of the fetal heart.

The results of this study show that the quantitative analysis of fetal heart function has high accuracy, which is consistent with the results of previous studies (10-12). The intra-observer consistency was high, so the fetal heart morphology and cardiac function measurements measured by the quantitative fetal heart analysis technique have high repeatability. The most important aspect of using this technique is the acquisition of high-quality dynamic images of the four-chamber heart. The acquisition of the four-chamber cardiac dynamic image used in this study was acquired by a senior sonographer, and the acquired image was not less than 3 seconds. By reducing the fan angle or increasing the frequency, the frame rate of the acquired image was increased, and a high-quality four-chamber dynamic images was obtained, which enabled the endocardium, ventricle, and epicardium have a higher contrast, thereby improving the accuracy of this technique for endocardial tracking. The Z-score scoring system is a standardized tool, which has been reported in many studies in various aspects of fetal heart research (13,14). The critical value used in these studies was generally ±2, so Z-scores >2 or <−2 were considered abnormal in this study.

Heart area is a variable that reflects the size of the heart; according to the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) (15): "*A normal heart usually does not exceed one third of the area of the thoracic cavity*". However, these guidelines do not provide a standard for evaluating the fetal heart when it is larger than one-third of the thorax. The American Heart Association (AHA) statement recommends only measuring the apical diameter of the ventricle at end-diastole to assess ventricular size (16). The fetal HQ technology provides a simple double-diameter method to obtain the area of the heart, so that the changes in the size of the fetal heart can be more intuitively and quantitatively analyzed. The results of this

Table 3 Comparison of the fractional shortening of the 24 segments of the left and right ventricles of the two groups of fetuses

Location	Segment	LV-24-FS ($\bar{x}$±s)		RV-24-FS ($\bar{x}$±s)	
		Case group	Normal group	Case group	Normal group
Basal	1	12.51±8.72	12.48±8.06	15.59±9.32	15.3±8.23
Basal	2	13.52±7.89	13.90±7.73	15.82±8.88	15.48±7.80
Basal	3	14.55±7.35	15.32±7.63	16.03±8.82	15.65±7.54
Basal	4	15.71±7.15	16.80±7.74	16.21±9.02	15.79±7.44
Basal	5	17.13±7.25	18.40±8.02	16.36±9.26	15.88±7.43
Basal	6	18.88±7.64	20.18±8.43	16.49±9.38	15.91±7.50
Basal	7	20.83±8.26	22.06±8.98	16.63±9.46	15.87±7.69
Basal	8	22.79±9.01	23.93±9.59	16.83±9.59	15.79±8.05
Mid	9	24.58±9.71	25.67±10.12	17.12±9.82	15.65±8.53
Mid	10	26.05±10.22	27.14±10.48	17.52±10.18	15.45±9.09
Mid	11	27.23±10.59	28.37±10.70	17.97±10.79	15.24±9.74
Mid	12	28.21±10.89	29.46±10.88	18.42±11.61	15.04±10.49
Mid	13	29.07±11.17	30.48±11.08	18.82±12.50	14.89±11.30
Mid	14	29.88±11.42	31.52±11.34	19.14±13.29	14.84±12.11
Mid	15	30.68±11.69	32.58±11.66	19.34±13.96	14.79±12.91
Mid	16	31.46±12.01	33.65±12.03	19.31±14.53	14.64±13.73
Apical	17	32.20±12.33	34.66±12.40	18.89±14.95	14.25±14.52
Apical	18	32.86±12.64	35.56±12.76	17.97±15.24	13.48±15.28
Apical	19	33.37±13.01	36.28±13.23	16.69±15.70	12.33±16.26
Apical	20	33.67±13.51	36.81±13.85	15.29±16.62	10.89±17.79
Apical	21	33.81±14.09	37.17±14.54	14.00±17.95	9.35±19.85
Apical	22	33.86±14.63	37.39±15.10	13.05±19.22	8.08±21.89
Apical	23	33.86±15.03	37.51±15.49	12.43±20.15	7.18±23.49
Apical	24	33.85±15.27	37.58±15.72	12.07±20.72	6.64±24.50

LV, left ventricular; FS, fractional shortening; RV, right ventricular.

study showed that fetal heart BAL, TW, A, and GA were positively linearly correlated; there was no significant difference in fetal GA between the two groups; and there was no significant difference in fetal BAL, TW, A, and Z values between the two groups (P>0.05). The obtained results indicated that maternal anemia had no significant effect on fetal heart size.

In this study, the changes of cardiac morphology were evaluated by obtaining the GSI of the heart and the 24-segment SI. It has been reported previously that GSI can effectively screen abnormal fetal heart morphology (7,17). The structure of the heart is very complex. This technique divides the left and right ventricles into 24 equally spaced segments by tracing the boundaries of the endocardium, and measures the SI and FS of the 24 segments (18-20). It can analyze the morphological and functional changes of each segment from the base of the ventricle to the apex in a more detailed and accurate manner; FAC and GLS of the left and right ventricles can also be obtained. Previous studies have demonstrated (21-23) that these indicators can accurately assess the systolic function of the left and right ventricles. The results of this study showed that fetal GSI, FAC, and GLS were not correlated with fetal GA. There was a statistically significant difference in fetal GSI between the two groups (P<0.05). The GSI of the fetuses in the anemia group was slightly larger than that of the fetuses in the normal group, indicating that the anemia caused the fetal four-chambered heart to be slightly spherical. However, there was no significant difference in the abnormal rate of fetal GSI-Z score between the two groups (P>0.05). There was a statistically significant difference in the abnormal rate of SI-Z values in the 24 segments of the left and right ventricles (P<0.05). There was a statistically significant difference in the SI of the 23–24 segments of the fetal left ventricle between the two groups (P<0.05). The SI of segments 1–22 of the left ventricle was not significantly different from that of the fetuses in the normal group (P>0.05). The SI of the 24 segments of the right ventricle and the FS of the 24 segments of the left and right ventricles were not significantly different from those of the fetuses in the normal group (P>0.05). There was no significant difference in GLS and FAC between the two groups of fetal left and right ventricle. The results showed that maternal anemia had no major impact on fetal heart morphology and function, but only slightly affected the overall four-chamber heart and left ventricular apical segment morphology at end-diastole.

Conclusions

This study confirms that quantitative fetal heart analysis technology can quickly and simply quantitatively assess the size, shape, and contractility of the fetal heart. It displayed a high inter-examiner consistency, confirming the good reproducibility of this technique. This study shows that maternal anemia has no significant effect on fetal heart size and systolic function, but only has a mild effect on morphology, manifested as abnormal SI in the whole and some segments of the left ventricle. This may have been because most of the pregnant women collected in this study had mild anemia and could still meet the normal development of the fetus in utero, so their fetal heart function did not change significantly. In the later stage, it is necessary to increase the sample size and collect samples of moderate and severe maternal anemia to analyze the impact of different degrees of anemia on fetal cardiac function.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tp.amegroups.com/article/view/10.21037/tp-22-315/rc

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of General Hospital of Ningxia Medical University (batch number: [2020]0520B), and the Ethics Committee of the Ningxia Maternal and Child Health Hospital (Ningxia Children's Hospital) (batch number: KJ-LL-2022-26). Informed consent was taken from all the participants for this study.

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