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Reference values of fetal atrioventricular time intervals derive from antegrade late diastolic arterial blood flow (ALDAF) from 14 to 40 weeks of gestation

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Background: Congenital heart defects are the most common of birth defect, which leads to neonatal death after birth. Early diagnosis during prenatal period would be a benefit for precaution and treatment. Antegrade Late Diastolic Arterial blood Flow (ALDAF) was reported to measure fetal atrioventricular (AV) time intervals (FAVTI) at an early gestational ages (GA) of 6 weeks. There has been no previous studies reporting reference value of fetal atrioventricular time intervals (FAVTI) derive from ALDAF technique. *Methods*: Using fetal echocardiogram, this cross-sectional study was performed on 528 healthy fetuses between 14 and 40 weeks. Pulsed wave Doppler-derived FAVTI (milliseconds) were measured from ALDAF-AO and ALDAF-PA and left ventricle (LV) In/Out. Correlations between these three Doppler measurement techniques were examined with the Bland-Altman analysis and Pearson correlation coefficient. GA was used as specific reference value and its correlation with FAVTI was examined with linear regression. *Results*: We establish reference values of fetal atrioventricular (AV) time intervals (FAVTI) from antegrade late diastolic arterial blood flow (ALDAF) aorta (AO) and pulmonary artery (PA) from 14 to 40 weeks of gestation (GA). A positive correlation between FAVTI and GA was identified when using each of the three measurements (ALDAF-AO/ALDAF-PA and LV In/Out) ($R2 = 0.177–0.272$; $P < 0.001$). GA had the strongest impact on ALDAF-AO FAVTI, which was estimated to have a predicted FAVTI of $1.02 \times$ GA (weeks) + 87.82. Bland-Altman analysis showed FAVTI of ALDAF-AO and ALDAF-PA were also significantly correlated ($R2 = 0.573$, $P < 0.001$). Intra-observer and inter-observer reliability coefficients showed good reproducibility (ICC >0.90) for all methods. *Conclusions*: This is the first study to establish reference ranges for FAVTI obtained from ALDAF-AO/ALDAF-PA for each week of gestation from 14 to 40 weeks. Our findings inform clinical practice by establishing GA-specific ALDAF-AO/PA cut-off values for the diagnosis of congenital heart block. FAVTI from ALDAF-AO/ALDAF-PA is a more practical measurement to use in the clinical setting because it is easier to investigate than LV In/Out. Good reproducibility in FAVTI measurements and a lack of fetal heart rate influence underpin the strength of our findings.

Keywords

Fetal echocardiography; Atrioventricular time; Congenital heart block; Pulse wave Doppler; Prenatal diagnosis

1. Introduction

Congenital heart disease (CHD) is a major cause of death in the first year after birth [1] with a perinatal mortality prevalence of about 0.4% [2]. Most infants born with CHD come from families without risk factors for this disease. However, general screening of low-risk populations shows variation in CHD detection rates, ranging from 5 to 14% [3–5]. In view of these variations, screening of whole populations may be warranted in order to achieve high prenatal detection rates. Detailed fetal echocardiography screening, which is widely used for prenatal diagnosis of CHD, has been reported to generate a pooled detection rate of 45.1% [6]. Screening of pregnant women between the gestational ages (GA) of 18–22 weeks has been recommended by the American Institute of Ultrasound in Medicine using fetal echocardiography for heart rate and rhythm assessment [7]. The clinical procedure is especially required for those with high risk of congenital heart block. In pregnant women with systemic lupus erythematous, rheumatoid arthritis, or positive blood test for anti-SSA/Ro or anti-SSB/La, fetal congenital heart block occurs in as many as 2–5% of pregnancies at GA of 18–24 weeks [8].

The pathogenesis of congenital heart block includes transplacental passage of maternal autoantibodies which may trigger an inflammatory process resulting in AV node damage and progressive prolongation of the electrical AV conduction. The inflammatory destruction of the AV node may be preventable if recognized at an early stage before leading to third-degree AV block. A first or second-degree AV block in a fetus is rectified with intrauterine fluorinate steroids. Late diagnosis comes with severe pathological conditions resulting in a third-degree AV block, where the block is considered complete. At this phase, the fetus may die *in utero*, or it may be necessary to install a pacemaker to control the heart rhythm. Thus, this provides a strong rationale for detecting AV blocks at the first or second-degree levels [9].

Of the several methods for diagnosing congenital fetal heart block, pulsed wave Doppler measurements of fetal atri-

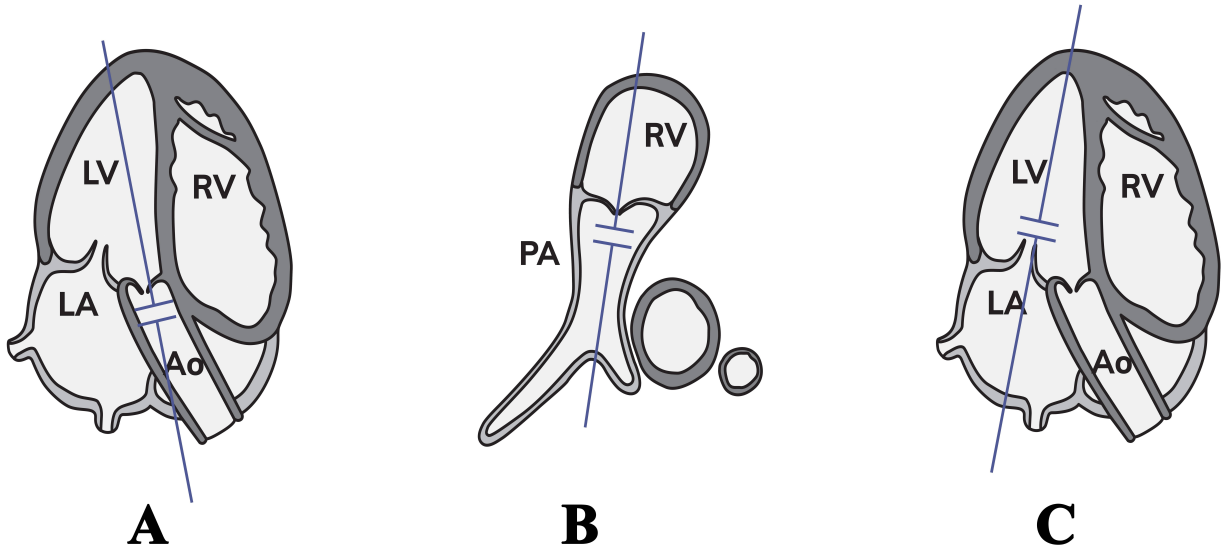

Fig. 1. Schematic drawing of appropriate positions of Doppler gate. (A) LVOT; (B) RVOT with appropriate positions of Doppler gate that can create waveform of outflow tract to identify ALDAF-AO/PA. (C) drawing of LV In/Out with appropriate positions of Doppler gate that create waveform of mitral valve inflow and aortic valve outflow to identify LV In/Out waveform.
LVOT, left ventricular outflow tract; RVOT, right ventricular outflow tract; ALDAF, antegrade late diastolic arterial blood flow; AO, aorta; PA, pulmonary artery; LV In/Out, left ventricular inflow/outflow.

oventricular time intervals (FAVTI) are the most commonly used [10–12]. Measurements are performed of the left ventricular inflow and outflow tracts (LV In/Out), the superior vena cava and ascending aorta (SCV/AO), or pulmonary artery and pulmonary vein (PA/PV). However, novel methods of detection that aim to raise efficiency are continually in development. In 2013, a method of using antegrade late diastolic arterial blood flow (ALDAF) was reported to measure FAVTI at an early GA of 6 weeks [13]. ALDAF detection occurs before the opening of the aortic and pulmonary valves at the end of diastole. Diastolic function in the immature fetus is reduced because the myocardium at this GA stage is less compliant with hindering efficient relaxation. For ALDAF detection, as a consequence of atrial contraction, ventricular end diastolic pressures must be sufficiently high. Thus, in atrial systole, the ventricles function as conduits, permitting forward blood flow through the semilunar valves in late diastole, resulting in augmented cardiac output. The ease of using ALDAF stems from a position that allowed measurement from both the aorta (AO) and pulmonary artery (PA). ALDAF FAVTI was found to strongly correlate with postnatal electrical PR interval [13], although this study did not specify the reference values of FAVTI based on GA for use in clinical diagnosis. We, therefore, conducted this study to establish reference values of FAVTI obtained by ALDAF/AO and ALDAF/PA methods at GA of 14–40 weeks.

2. Materials and methods

This cross-sectional descriptive study was undertaken between 1 November 2019 and 10 June 2020 at Phramongkutklao Hospital, Bangkok, Thailand. The study was approved by the institutional review board Royal Thai Army Medical Department. Eligible pregnant women were designated as low risk for a fetus with CHD, which we defined as a history of a normal complete anomaly ultrasound scan according to our protocol at the first trimester (GA 11–14 weeks) and second trimester (GA 16–20 weeks). Participants were recruited from the antenatal care clinic at Phramongkutklao Hospital, and written informed consent was obtained.

2.1 Inclusion and exclusion criteria

Inclusion criteria were as follows: (1) women aged 18 years and older, (2) GA of 14–40 weeks with normal anomaly scan, (3) no known medical or obstetric complications, (4) reliable GA based on regular menstrual cycle and certain last menstrual period consistent with sonographic fetal biometry in the first half of pregnancy and (5) normal fetal heart rate with no arrhythmia. Women at 14–15 weeks of pregnancy could be enrolled if first trimester scan was normal, but were then withdrawn from the study if the second trimester complete anomaly scan revealed any abnormalities.

Exclusion criteria were (1) multi-fetal pregnancies; (2) abnormal chromosomes in the fetus; (3) abnormal fetal growth (either restriction or macrosomia); (4) women with immune system disorders, including systemic lupus erythematosus, anti-phospholipid syndrome, rheumatoid arthritis, Sjogren syndrome, hyperthyroidism and undifferentiated autoimmune diseases; (5) women with a positive blood test for anti-SSA/Ro or anti-SSB/La auto-antibodies and (6) women taking medications (i.e., beta-adrenergic agonists) that affect fetal heart rate.

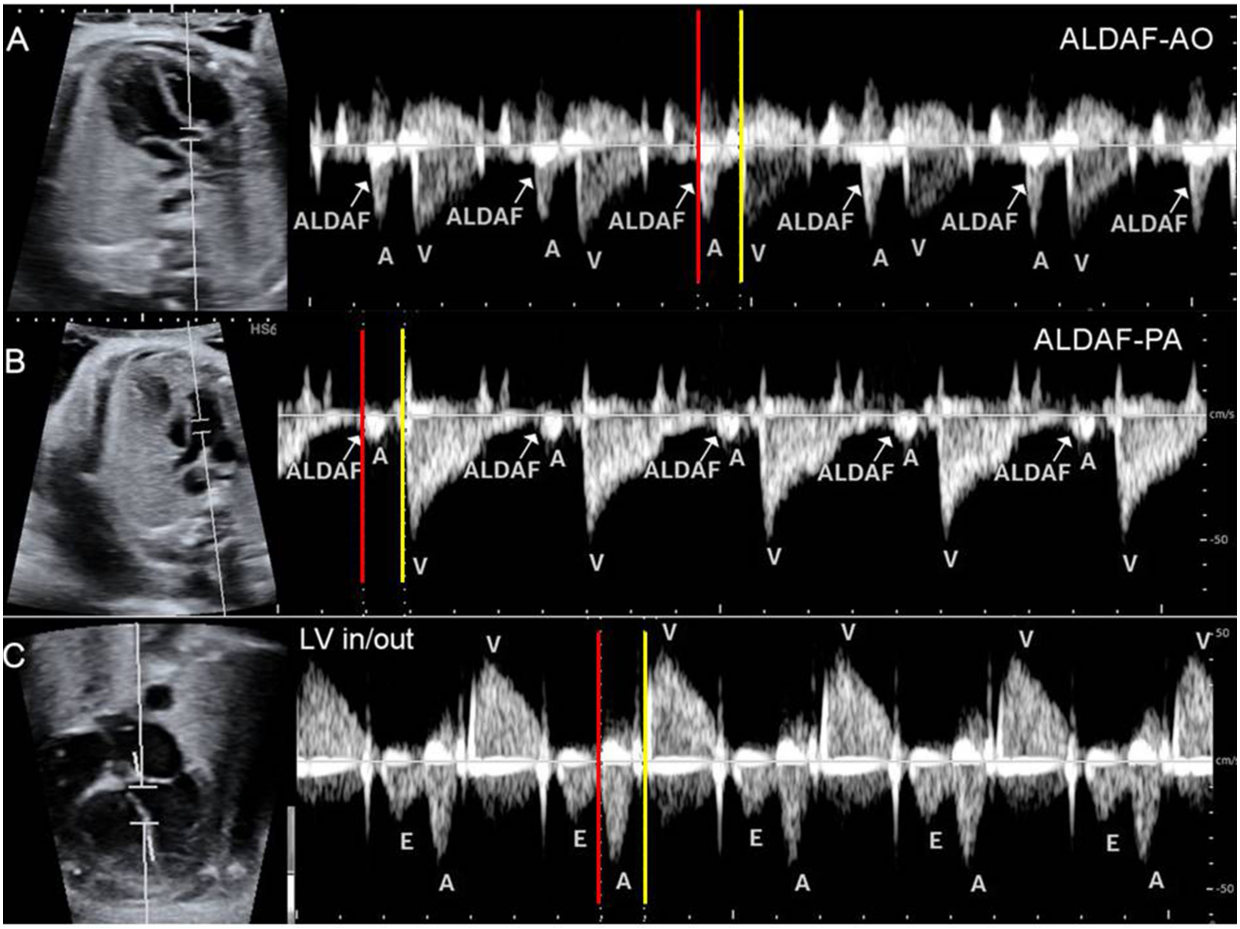

Fig. 2. Position of sample volume and pulse wave Doppler waveform patterns in each method, red line represent onset of A wave and yellow line represent onset of V wave. (A) ALDAF-AO; (B) ALDAF-PA; (C) LV In/Out.
ALDAF, antegrade late diastolic arterial blood flow; AO, aorta; PA, pulmonary artery; LV In/Out, left ventricular inflow/outflow; A, Atrial contraction peak velocity; V, ventricular systole; E, Early diastolic peak velocity.

2.2 Doppler measurements

All fetal echocardiogram were performed by specialists in maternal-fetal medicine (MFM) with qualified diploma of the Thai Subspecialty Board of Maternal and Fetal Medicine issued by the Medical Council of Thailand. At least 150 cases per month of fetal echocardiogram were performed in our MFM division. All pulse wave Doppler investigations were performed during fetal quiescence and apnea on Samsung HS60 abdominal 1–5 MHz curvilinear transducer (Samsung Medison, Korea). The setting of pulse wave Doppler included a wall motion filter of 120 Hz, a sweep speed of 117 mm/s to obtain 4–5 waveform images, pulse repetition frequencies of 5.5–6 kHz and an angle between the ultrasound beam and blood flow of less than 20°. In each woman, FAVTIs were measured using three different assessment (ALDAF-AO, ALDAF-PA and LV In/Out), repeated three times, all of which were averaged.

2.3 ALDAF-AO

In ALDAF-AO, we sought an apical view of the left ventricular outflow tract (LVOT). Transducer orientation was then adjusted to ensure an insonation angle <20 degrees along the direction of the aortic blood flow. A Doppler gate of 1–3 mm was selected and placed within the aorta and distal to the aortic valves (Fig. 1A) [13]. FAVTI measurement was set at onset of atrial systole (A-wave; red line) to onset of ventricular systole (V-wave; yellow line) during the same cardiac cycle (Fig. 2A).

2.4 ALDAF-PA

In ALDAF-PA, we sought a five-chambered view, with rotation or tilting of the transducer cephalad in order to view the PA arising from the RV. Transducer orientation was then adjusted to ensure an insonation angle <20 degrees along the direction of the pulmonary artery blood flow. A Doppler gate of 1–3 mm was selected and placed within the pulmonary artery and distal to the pulmonic valves (Fig. 1B) [13]. FAVTI measurement was set at onset of atrial systole (A-wave; red line) to onset of ventricular systole (V-wave; yellow line) during the same cardiac cycle (Fig. 2B).

2.5 LV In/Out

In LV In/Out, we sought an apical view of LVOT, then used pulsed Doppler with a sample volume wide enough to cover both diastolic inflow via the mitral valve and systolic outflow via the aortic valve. With a sample gate adjustment of 5–10 mm [14], the angle of insonation was fitted to get as close as possible to zero degrees (up to 20 degrees) (Fig. 1C). FAVTI measurement was set at onset of the mitral A-wave (red line) between the E-peak and A-peak to the beginning of the aortic V-wave (yellow line) in the aortic outflow tract (Fig. 2C).

2.6 Statistical analysis

We used SPSS version 26.0 (IBM Corp., Armonk, NY, USA) to analyze the data. FAVTI data in ALDAF-AO and ALDAF-PA of each GA were created at reference range percentile values of 2.5, 5, 10, 25, 50, 75, 90, 95 and 99. Descriptive values of the three Doppler measurements were expressed as means ± standard deviation. Data distribution from our large sample size (N = 528) tended towards normal [15, 16]. Categorical data were expressed in terms of number or percentage (%). Statistical significance was set at a two-tailed P value of <0.05.

Correlations between FAVTI with GA in ALDAF-AO, ALDAF-PA and LV In/Out methods were analyzed with linear regression. Bland-Altman analysis and Pearson correlation coefficient were used to compare the fetal AV time interval between measurement techniques. Assessing the reliability of FAVTI measurements from all three methods involved intraobserver and interobserver approaches with r values >0.80 indicating high accuracy.

3. Results

3.1 Quantitative features

A total of 528 pregnant women participated in the study. Table 1 quantifies the maternal demographic characteristics. This study found no neonatal structural heart anomalies or cardiac arrhythmias. The longest measured time was in LV In/Out: (118.83 milliseconds (ms) ± 13.27), shortest was in ALDAF-PA: (112.51 ± 15.03) and in between for ALDAF-AO (115.02 ± 14.98). The normal reference values for FAVTI measured by ALDAFAO and ALDAF-PA divided into GA from 14 to 40 complete weeks are shown in Table 2.

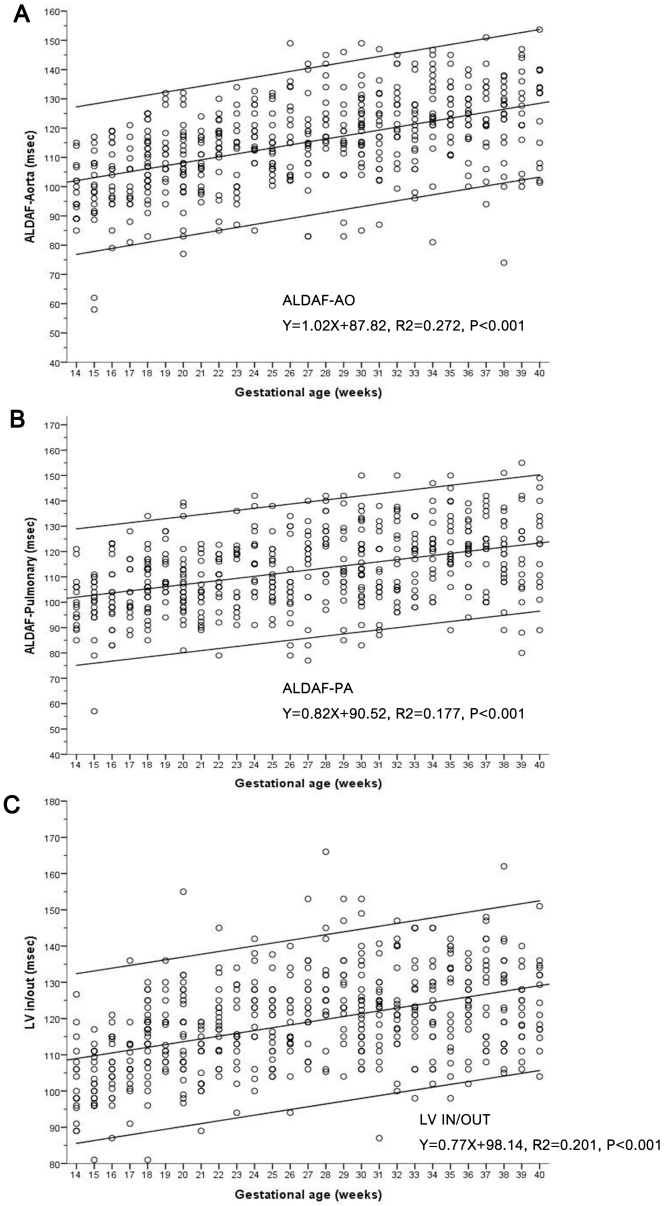

Fig. 3. Linear regression analyses between FAVTI and gestational age (weeks). (A) ALDAF-AO; (B) ALDAF-PA; (C) LV In/Out.
FAVTI, fetal atrioventricular time intervals; ALDAF, antegrade late diastolic arterial blood flow; AO, aorta; PA, pulmonary artery; LV In/Out, left ventricular inflow/outflow; R^2, square of the correlation. Lines denote regressions and 95% confidence limits for individual observations.

3.2 Correlation analyses

Linear regression analysis shows highly significant (P < 0.001) positive correlations between FAVTI outcome and GA in all three methods. The coefficients of determination (R^2) were 0.272, 0.177 and 0.201 with ALDAF-AO, ALDAF-PA and LV In/Out measurement, respectively (Fig. 3). The strongest correlation was identified in ALDAF-AO, in which the predicted estimation was 1.02 × GA (weeks) ± 87.82. Fig. 4A–C show outcomes of the Bland-Altman analysis with a FAVTI correlation strongest in ALDAF-AO versus ALDAF-PA (r = 0.57, P < 0.001) (Fig. 4C). This magnitude of correlation was confirmed in the mean FAVTIs difference values (95% CI) of 2.5 (–24.7, 29.7) between ALDAF-AO and ALDAF-PA, ALDAF-AO versus LV In/Out at –3.8 (–30.3, 22.7) and ALDAF-PA versus LV In/Out at –6.3 (–33.9, 21.3) (Table 3).

3.3 Reliability

Intraobserver reliability coefficients of FAVTI for ALDAF-AO, ALDAF-PA and LV In/Out were 0.967, 0.979 and 0.978, respectively (95% CI for the three values ranged from 0.96 to 0.98). Interobserver reliability coefficients of

Table 1. Maternal demographic characteristics and FAVTI measurements.

Characteristics	Descriptive statistical data
Age (years)	29.07 ± 5.77
Gravida	
1	250 (47.3)
2	176 (33.3)
3	70 (13.3)
4	28 (5.3)
5	4 (0.8)
Gestational age (weeks)	26.74 ± 7.69
Pre-pregnancy weight (kilograms)	56.47 ± 10.97
Height (centimeters)	158.47 ± 5.49
Pre-pregnancy body mass index (kg/m^2)	22.49 ± 4.24
Current weight (kilograms)	63.8 ± 11.79
FAVTI (millisecond)	
ALDAF-AO	115.02 ± 14.98
ALDAF-PA	112.51 ± 15.03
LV In/Out	118.83 ± 13.27

FAVTI, fetal atrioventricular time intervals; values are expressed as mean ± standard deviation except in gravida which were expressed as number of pregnant women (%). ALDAF, antegrade late diastolic arterial blood flow; AO, aorta; PA, pulmonary artery; LV, left ventricle.

FAVTI for ALDAF-AO, ALDAF-PA and LV In/Out were 0.991, 0.959 and 0.989, respectively (95% CI for the three values ranged from 0.923 to 0.996).

4. Discussion

4.1 Summary of findings

To our knowledge, the present study provides the first normal reference values for each week of gestation between 14 and 40 weeks of FAVTI derived from ALDAF-AO and ALDAF-PA. Here, we examined FAVTI in a large population (N = 528) between 14 and 40 weeks of gestation according to previous studies [9, 12]. We demonstrated GA specific normal reference values of FAVTI, which is different from previous investigations presenting with groups of GA [9, 11, 12, 17–19]. Detailed reference values of FAVTI for each week of gestation should be more accurate in diagnosing CHB than group GA. FAVTI is longest in LV In/Out and ALDAF-PA is shorter than ALDAF-AO, which was similar to findings observed by Howley and colleagues [13]. As a result of fetal cardiac cycle, ALDAF-AO/PA were obtained beyond aortic and pulmonic valve in proximal of vessels and the distance of RV apex to pulmonic valve is shorter than distance of LV apex to aortic valve [20]. Therefore, Doppler signal time in ALDAF-PA was shorter than ALDAF-AO.

We succeeded in obtaining FAVTI for ALDAF-AO/PA in 100% of examinations, as compared to 80% in a previous study [13]. Due to technical limitations, clear identification of the interrogated great artery is difficult in fetuses with gestational ages <11 weeks [13], which may have led to lower success rate of ALDAF AO/PA in this prior work. Therefore, in this study, pregnant women were selected with gestational age greater than or equal to 14 weeks to improve the success rate. Furthermore, ALDAF-AO/PA uses a single valve or vessel, in which pulse wave Doppler

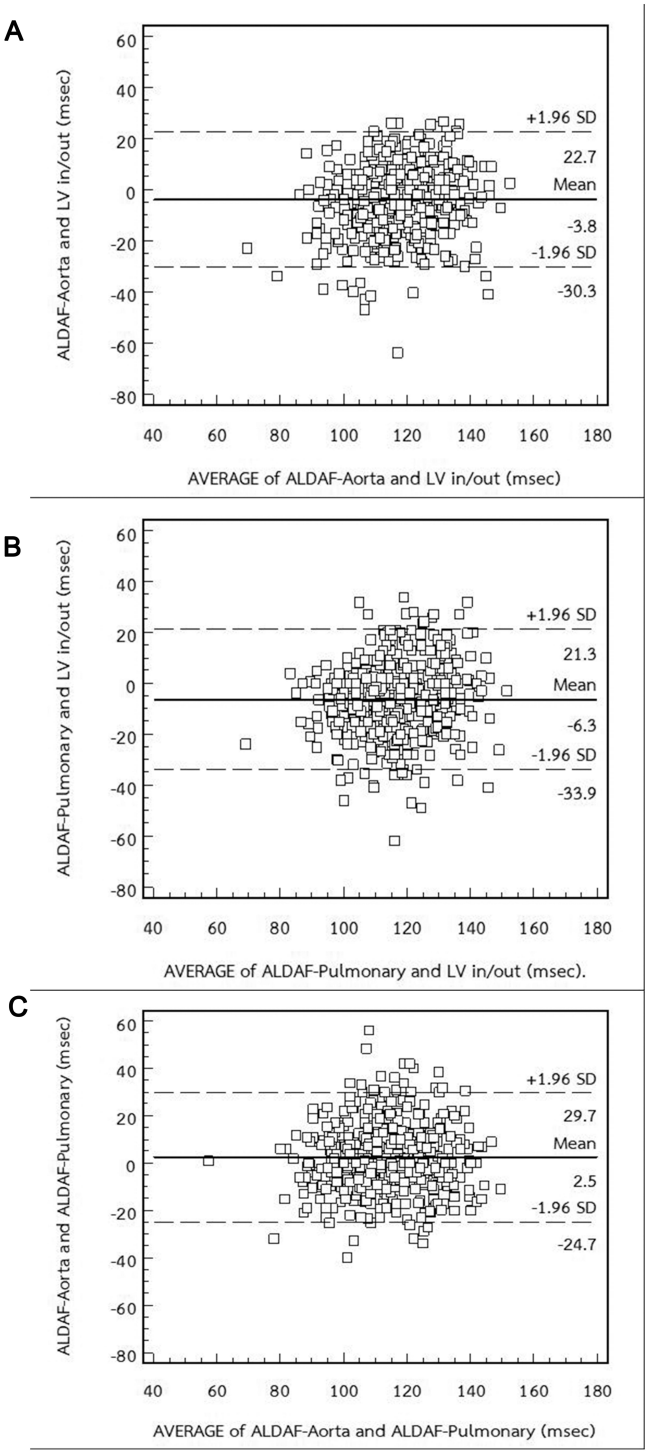

Fig. 4. Bland-Altman analysis of FAVTI. (A) ALDAF-AO versus LV In/Out; (B) ALDAF-PA versus. LV In/Out; (C) ALDAF-AO versus ALDAF-PA.

FAVTI, fetal atrioventricular time intervals; ALDAF, antegrade late diastolic arterial blood flow; AO, aorta; PA, pulmonary artery; LV in/out, left ventricular inflow/outflow.

Table 2. Normal reference values for FAVTI measured by ALDAF-AO and ALDAF-PA.

Gestational age (weeks)	N	ALDAF-Aorta (milliseconds)									ALDAF-Pulmonary artery (milliseconds)								
		P2.5	P5	P10	P25	P50	P75	P90	P95	P99	P2.5	P5	P10	P25	P50	P75	P90	P95	P99
14	17	85	85	89	89	94	102	114	115	115	85	85	89	94	99	106	119	121	121
15	20	58	60	75.3	91.3	99.2	108	113.7	116	117	57	68	82	95	100.3	107	110	110.5	111
16	20	79	82	89.5	96	105	115	119	119	119	83	83	86	95.7	99	111	122	123.2	123.3
17	16	81	81	88	95	99.2	107.8	117	121	121	87	87	89	97	99.3	113	117	128	128
18	30	83	91	94.7	102	109	115.7	124	125	125	85	89	91.8	98	107	113.7	119	123	134
19	17	94	94	98	106	111	115	130	132	132	94	94	100	106	108	117	128	128	128
20	28	77	83	85	100	106.8	116.5	128	130	132	81	91	93	99	104	112.8	134	138	139.3
21	18	94.7	94.7	97.3	100	107.5	116	119	124	124	89	89	90	94	103	111	121	123	123
22	20	85	86.5	91.7	107.5	114.2	119	124	127.5	130	79	85.5	94	104.7	109.5	117	119	120.8	122.7
23	17	87	87	94	100	113	119	128	134	134	91	91	94	98	117	119.7	122.3	136	136
24	17	85	85	108	112.7	117	125	130	132.7	132.7	91	91	102	108	115	123	138	142	142
25	21	100.3	102	104	106	112	117	130	131	132	91	93.3	100	102	108	117	121	128	138
26	17	102	102	102.3	104	117	127.3	136	149	149	79	79	83	99.3	104	113	130	134	134
27	19	83	83	83	108	114.7	121	140	142	142	77	77	83	102	119	123.3	132	140	140
28	17	104	104	104	115.7	121	128.7	142	145	145	97	97	100	111	125	130	140	142	142
29	17	83	83	87.7	111.3	115	121	134	146	146	85	85	89	106	112	125	139	142	142
30	29	85	104	106.7	114.3	121	126	134.7	138	149	83	91	98.3	105	113.3	123	136	138	150
31	20	87	94.5	102.2	107.7	119	126.5	134.2	141.7	147	87	88	90	104	110	121	131	135	138
32	20	99.3	102.7	107	117	123.2	131	142	143.5	145	96	96	97	104.8	116	133	137.2	143.8	150
33	19	96	96	98	108.7	119.3	128	140	142	142	98	98	98	104.7	120.3	125	134	134	134
34	20	81	90.5	106.5	121	124.5	137	143.5	145.8	146.7	100	100	101	112.2	121	125	138	143.5	147
35	19	110.7	110.7	110.7	117	125	134	142	145	145	89	89	106	115	119	134	145	150	150
36	20	100	101.8	105.8	116.7	125	130	136	139	140	94	98	104	117	122	129	134.3	137.5	139
37	18	94	94	101.7	115	122.2	134	142	151	151	100	100	100	104	122.7	134	140	142	142
38	21	74	100	103.3	117	128	130	136	137.7	138	89	96	108	111	119	130	136	137.3	151
39	16	100	100	102	118.5	126.3	137.7	145	147	147	80	80	88	105.7	116	128	142	155	155
40	15	101.3	101.3	102	108	132	139.7	140	153.7	153.7	89	89	101	108	123	134	145.3	149	149

FAVTI, fetal atrioventricular time intervals; N, number of subjects; P, percentile; ALDAF, antegrade late diastolic arterial blood flow; AO, aorta; PA, pulmonary artery.

measurements were easier to obtain than other techniques (LV In/Out, SVC/AO or PV/PA) requiring two valves or vessels for measurement. This led to high reliability coefficients (>0.9) by intraobserver and interobserver ratings in our study, indicating ALDAF shows high reliability and reproducibility method. The ability to obtain FAVTI is based on well-trained physician, high resolution ultrasound, and most importantly the ability to accurately position the sample volume and the angle of insonation is parallel to the signal of AO or PA outflow. Overall, an average time of 30 min was necessary to complete the echocardiographic assessment.

The present study showed FAVTI obtained from ALDAF-AO, ALDAF-PA and LV In/Out FAVTI was significant correlated with advancing GA. This finding corroborates majority of previously published data [9, 12, 13, 17–19, 21, 22], although a few studies have not identified a correlation between FAVTI and GA [11, 23]. This correlation is likely attributable to enhancement of fetal cardiac size and chamber with progressive GA, which results in prolonged time of myocardium depolarization and repolarization leading to increasing FAVTI [19].

Regarding thresholds for FAVTI measurements, we established cut-off values for ALDAF-AO/PA for CHB diagnosis at >99th percentile for each specific GA between GA 14 to 40 weeks (Table 2) which was not similar to those with previous studies [9, 19, 21, 24, 25]. In this study, cut-off FAVTI value obtained from ALDAF-AO and ALDAF-PA are 115–153.7 milliseconds and 111–155 milliseconds respectively as more detail show in Table 2.

4.2 Advantages of FAVTI measurements

ALDAF-AO/PA has more advantages than LV In/Out in the aspect of fetal heart rate. At high heart rate, with no fusion of mitral E and A wave in ALDAF, FAVTI can be determined but by the LVI In/Out method [10, 26, 27]. It was reported that in 39% of moderate to severely prolonged FAVTI cases [28], the LV In/Out method could not identify A wave. However, ALDAF AO/PA can obtain FAVTI in all conditions.

Future studies are warranted to validate the ability of the ALDAF technique to diagnose congenital heart block in pregnancy with positive anti Ro/La autoantibody, and other preg-

Table 3. Bland-Altman analysis and Pearson correlation coefficient of FAVTI between ALDAF-AO, ALDAF-PA and LV In/Out.

Doppler measurement method	Mean difference (95% CI)	Pearson correlation coefficient (r) (*P*-value)
ALDAF-AO versus LV In/Out	−3.8 (−30.3, 22.7)	0.549 (<0.001)
ADDAF-PA versus LV In/Out	−6.3 (−33.9, 21.3)	0.511 (<0.001)
ALDAF-AO versus ALDAF-PA	2.5 (−24.7, 29.7)	0.573 (<0.001)

FAVTI, fetal atrioventricular time intervals; ALDAF, antegrade late diastolic arterial blood flow; AO, aorta; PA, pulmonary artery; LV In/Out, left ventricle inflow/outflow; CI, confidence interval.

nancies with risk factors for congenital heart block. Our study provides convincing evidence that ALDAF-AO and/or ALDAF-PA is a good technique, with minimal bias, high reliability, and high reproducibility for evaluation of prenatal congenital heart block. Both ALDAF-AO/PA can be used instead LV In/Out. Of note, we did not identify any differences in effectiveness of ALDAF-AO and ALDAF-PA. Additional studies should be done to compare FAVTI measured by ALDAF-AO/PA with other techniques such as SVC/AO or PV/PA. Larger sample sizes for each week of gestation may provide more accuracy in values of FAVTI with ALDAF-AO/PA.

We identified limitations in our study: (1) we did not compare our findings with electrical PR neonatal time intervals. However, the accuracy of FAVTI from ALDAF-AO/PA (mechanical PR interval) showed good correlation with neonatal (EKG) in a previous study [13]. (2) We were likewise unable to compare FAVTI obtained from Doppler measurement to other techniques such as RV tissue Doppler image, fetal EKG and fetal magnetocardiography because of supplier limitation.

5. Conclusions

In conclusion, this is the first study to establish the normal reference values of FAVTI measured by ALDAF-AO/PA for each week of GA between 14–40 weeks. Our findings will aid clinicians in early detection of fetal congenital heart block. Good accuracy, reliability, reproducibility and lack of fetal heart rate influence underpin the strength of our findings.

Abbreviations

ALDAF, antegrade late diastolic arterial blood flow; AO, aorta; CHD, congenital heart disease; CHB, congenital heart block; CI, confidence interval; FAVTI, fetal atrioventricular time intervals; GA, gestational ages; LV In/Out, Left ventricular inflow/outflow; LVOT, left ventricular outflow tract; PA, pulmonary artery.

Author contributions

TH and NIS contributed the study design and performed the experiments. TH, NP and TT performed data analysis and wrote the draft manuscript. All authors reviewed and approved final version of manuscript.

Ethics approval and consent to participate

The protocols and procedures were approved by the institutional review board Royal Thai Army Medical Department number IRBRTA 1182/2562. All subjects gave their informed consent for inclusion before they participated in the study.

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Conflict of interest

The authors declare no conflict of interest.

References

[1] Control CfD. Contribution of birth defects to infants' mortality—United States 1986. Morbidity and Mortality Weekly Report. 1989; 38: 633–635.

[2] Tongsong T, Luewan S. Fetal Cardiac Examination. Bangkok: Luksamerung. 2012.

[3] Hafner E, Scholler J, Schuchter K, Sterniste W, Philipp K. Detection of fetal congenital heart disease in a low-risk population. Prenatal Diagnosis. 1998; 18: 808–815.

[4] Stoll C, Alembik Y, Dott B, Meyer MJ, Pennerath A, Peter MO, *et al.* Evaluation of prenatal diagnosis of congenital heart disease. Prenatal Diagnosis. 1998; 18: 801–807.

[5] Boyd PA, Chamberlain P, Hicks NR. 6-year experience of prenatal diagnosis in an unselected population in Oxford, UK. Lancet. 1998; 352: 1577–1581.

[6] van Velzen CL, Ket JCF, van de Ven PM, Blom NA, Haak MC. Systematic review and meta-analysis of the performance of second-trimester screening for prenatal detection of congenital heart defects. International Journal of Gynecology & Obstetrics. 2018; 140: 137–145.

[7] American Institute of Ultrasound in Medicine. AIUM practice parameter for the performance of fetal echocardiography. Journal of Ultrasound in Medicine. 2020; 39: E5–E16.

[8] Buyon JP, Hiebert R, Copel J, Craft J, Friedman D, Katholi M, *et al.* Autoimmune-associated congenital heart block: demographics, mortality, morbidity and recurrence rates obtained from a national neonatal lupus registry. Journal of the American College of Cardiology. 1998; 31: 1658–1666.

[9] Anuwutnavin S, Kolakarnprasert K, Chanprapaph P, Sklansky M, Mongkolchat N. Measurement of fetal atrioventricular time intervals: a comparison of 3 spectral Doppler techniques. Prenatal Diagnosis. 2018; 38: 459–466.

[10] Bergman G, Jacobsson LA, Wahren-Herlenius M, Sonesson SE. Doppler echocardiographic and electrocardiographic atrioventricular time intervals in newborn infants: evaluation of techniques for surveillance of fetuses at risk for congenital heart block. Ultrasound in Obstetrics and Gynecology. 2006; 28: 57–62.

[11] Glickstein JS, Buyon J, Friedman D. Pulsed Doppler echocardiographic assessment of the fetal PR interval. The American Journal of Cardiology. 2000; 86: 236–239.
[12] Nii M, Hamilton RM, Fenwick L, Kingdom JCP, Roman KS, Jaeggi ET. Assessment of fetal atrioventricular time intervals by tissue Doppler and pulse Doppler echocardiography: normal values and correlation with fetal electrocardiography. Heart. 2006; 92: 1831–1837.
[13] Howley LW, Yamamoto Y, Sonesson S, Sekar P, Jain V, Motan T, *et al.* Antegrade late diastolic arterial blood flow in the fetus: insight into fetal atrial function. American Journal of Obstetrics and Gynecology. 2013; 208: 490.e1–490.e8.
[14] Tongsong T, Luewan S. Five-Chamber view. In:Tongsong T, Luewan S, editors. Fetal Cardiac Examination. Bangkok: Luksamerung. 2012.
[15] Fagerland MW. *T*-tests, non-parametric tests, and large studies—a paradox of statistical practice? BMC Medical Research Methodology. 2012; 12: 78.
[16] Blanca MJ, Alarcón R, Arnau J, Bono R, Bendayan R. Non-normal data: is ANOVA still a valid option? Psicothema. 2017; 29: 552–557.
[17] Andelfinger G, Fouron JC, Sonesson SE, Proulx F. Reference values for time intervals between atrial and ventricular contractions of the fetal heart measured by two Doppler techniques. The American Journal of Cardiology. 2002; 88: 1433–6, A8.
[18] Gyenes DL, McBrien AH, Bohun CM, Serrano-Lomelin J, Alvarez SGV, Howley LW, *et al.* Evolution of the fetal atrioventricular interval from 6 to 40 Weeks of Gestation. The American Journal of Cardiology. 2019; 123: 1709–1714.
[19] Wojakowski A, Izbizky G, Carcano ME, Aiello H, Marantz P, Otaño L. Fetal Doppler mechanical PR interval: correlation with fetal heart rate, gestational age and fetal sex. Ultrasound in Obstetrics and Gynecology. 2009; 34: 538–542.
[20] Hill AJ, PA L. Comparative cardiac anatomy. In PA L. (ed.) Handbook of cardiac anatomy, physiology, and devices (pp. 89–111). 3rd edn. Switzerland: Springer international publishing. 2015.
[21] Mosimann B, Arampatzis G, Amylidi-Mohr S, Bessire A, Spinelli M, Koumoutsakos P, *et al.* Reference ranges for fetal atrioventricular and ventriculoatrial time intervals and their ratios during normal pregnancy. Fetal Diagnosis and Therapy. 2018; 44: 228–235.
[22] Tomek V, Janoušek J, Reich O, Gilík J, Gebauer RA, Skovránek J. Atrioventricular conduction time in fetuses assessed by Doppler echocardiography. Physiological Research. 2011; 60: 611–616.
[23] Bolnick A, Borgida A, Egan J, Zelop C. Influence of gestational age and fetal heart rate on the fetal mechanical PR interval. The Journal of Maternal-Fetal & Neonatal Medicine. 2004; 15: 303–305.
[24] Friedman DM, Kim MY, Copel JA, Davis C, Phoon CKL, Glickstein JS, *et al.* Utility of cardiac monitoring in fetuses at risk for congenital heart block: the PR Interval and Dexamethasone Evaluation (PRIDE) prospective study. Circulation. 2008; 117: 485–493.
[25] Van Bergen AH, Cuneo BF, Davis N. Prospective echocardiographic evaluation of atrioventricular conduction in fetuses with maternal Sjögren's antibodies. American Journal of Obstetrics and Gynecology. 2004; 191: 1014–1018.
[26] Phoon CKL, Kim MY, Buyon JP, Friedman DM. Finding the "PR-fect" Solution: what is the best tool to measure fetal cardiac PR intervals for the detection and possible treatment of early conduction disease? Congenital Heart Disease. 2012; 7: 349–360.
[27] Hornberger LK. Echocardiographic assessment of fetal arrhythmias. Heart. 2007; 93: 1331–1333.
[28] Mivelaz Y, Raboisson MJ, Abadir S, Sarquella-Brugada G, Fournier A, Fouron J. Ultrasonographic diagnosis of delayed atrioventricular conduction during fetal life: a reliability study. American Journal of Obstetrics and Gynecology. 2010; 203: 174.e1–174.e7.