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Development of Gestational Age-Specific Nomograms for Fetal Splenic Artery Doppler Parameters Between 18 and 40 Weeks of Gestation

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ABSTRACT

OBJECTIVE: To characterize the gestational age-related behavior of fetal splenic artery (SA) Doppler parameters and to establish appropriate reference models for the systolic/diastolic (S/D) ratio, pulsatility index (PI), peak systolic velocity (PSV), and time-averaged maximum velocity (TAMV) in normal singleton pregnancies between 18 and 40 weeks of gestation.

STUDY DESIGN: This observational cross-sectional study included 103 normal singleton pregnancies undergoing routine obstetric ultrasonographic examination between 18 and 40 weeks of gestation. SA Doppler waveforms were obtained using a standardized technique, and the S/D ratio, PI, PSV, and TAMV were recorded. We modeled the relationship between gestational age and each parameter using linear and quadratic regression analysis and derived gestational age-specific Z-score equations and percentile reference values (5th, 10th, 50th, 90th, and 95th). Gestational age-specific modeling was applied to PSV and TAMV; PI, which showed no gestational age dependence, was reported as a single pooled reference interval, and S/D, which could not be adequately modeled parametrically, was reported descriptively. Correlations between SA Doppler parameters and gestational age, fetal biometric parameters, and umbilical artery (UA) Doppler indices were assessed using Spearman correlation analysis.

RESULTS: SA PSV and TAMV increased significantly with advancing gestational age (PSV=−5.651+1.587×GA, R^2=0.399, p<0.001; TAMV=−10.900+0.929×GA, R^2=0.401, p<0.001), whereas the SA S/D ratio and PI showed no significant association with gestational age. SA PSV and TAMV correlated positively with abdominal circumference and estimated fetal weight (r=0.632-0.674, all p<0.001) and negatively with UA Doppler indices. In contrast, SA S/D correlated positively with the UA S/D ratio (r=0.405, p=0.006).

CONCLUSION: This study provides gestational age-specific nomograms and Z-score equations for SA PSV and TAMV, a pooled reference interval for SA PI, and descriptive values for SA S/D in normal pregnancy. These reference values may serve as a normative foundation for future research examining SA hemodynamics in relation to fetal growth, anemia, and pancreatic development.

Keywords: Doppler ultrasonography; Fetal splenic artery; Nomogram; Pregnancy; Reference range

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Introduction

Doppler ultrasonography is widely used for fetal surveillance, offering a non-invasive assessment of fetal hemodynamics in both normal and complicated pregnancies (1). Its clinical use has primarily focused on a limited number of vessels, namely the umbilical artery, middle cerebral artery, and ductus venosus, which are used to detect placental insufficiency, brain-sparing responses, and cardiac decompensation

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(1). Even for these established vessels, measurement variability remains a concern. A recent reproducibility study reported considerable intra- and interobserver variability in fetal Doppler indices, even among experienced sonographers (2), underlining the need for standardized technique and properly validated reference ranges before any Doppler parameter can be applied clinically.

In recent years, Doppler assessment has expanded beyond these classic vessels to other visceral arteries. The fetal hepatic artery has been studied for a hepatic arterial buffer response similar to that described postnatally, in which decreased pulsatility index (PI) and resistance index (RI) together with increased peak systolic velocity (PSV) reflect compensatory vasodilation when portal or umbilical venous flow decreases. However, gestational age-specific reference ranges are not yet available for this vessel (3). Longitudinal reference values for the fetal inferior adrenal artery were also published only recently (4). These vessels illustrate that the field continues to expand, but that reference data for most newly studied vessels remain incomplete compared with the classic Doppler indices.

The fetal splenic artery (SA) arises from the celiac trunk and courses along the superior border of the pancreas before entering the splenic hilum, and this course is commonly used as a sonographic landmark for identifying the fetal pancreas on color or power Doppler (5,6). The fetal spleen also functions as a transient site of extramedullary hematopoiesis during the second and third trimesters, giving the splenic circulation a role beyond that of a simple visceral branch vessel (7).

The SA has drawn additional attention because of its relationship with the fetal pancreas, particularly in pregnancies complicated by gestational diabetes and other metabolic conditions related to fetal programming of adult disease (8,9). As fetal pancreatic size and echogenicity are increasingly studied as prenatal markers of maternal metabolic status, the vessel supplying this organ has become a relevant companion hemodynamic marker.

While gestational age-specific reference ranges have previously been reported for SA PI and PSV, no study to date has provided reference values for the SA systolic/diastolic (S/D) ratio or time-averaged maximum velocity (TAMV), or derived Z-score equations for any SA Doppler parameter. Available data are mostly limited to a single parameter, derived from narrow gestational age windows, or expressed as multiples of the median rather than as percentile curves or Z-score equations. We therefore aimed to construct gestational age-specific regression equations, Z-score formulas, and percentile reference values for fetal SA Doppler parameters including the S/D ratio, PI, PSV, and TAMV, in normal singleton pregnancies between 18 and 40 weeks of gestation and to evaluate their relationships with fetal biometric parameters and umbilical artery Doppler indices.

Material and Method

Study Design and Setting: This observational cross-sectional study was conducted at the Department of Perinatology, Izmir City Hospital, University of Health Sciences, a tertiary referral center, between February 2026 and June 2026. The local Non-Interventional Ethics Committee approved the study protocol (2026/108; Approval Date: February 2026), and the study was conducted in accordance with the ethical principles of the Declaration of Helsinki (as revised 2024). We obtained written informed consent from all participants before enrollment.

Study Population: Pregnant women undergoing routine obstetric ultrasonographic examination between 18 and 40 weeks of gestation were prospectively enrolled. Gestational age was confirmed in all pregnancies using first-trimester crown–rump length (CRL) measurements. Only singleton pregnancies with a normal fetal anatomical survey were eligible for inclusion.

Inclusion criteria were as follows: gestational age between 18 and 40 weeks; a normal singleton pregnancy with an estimated fetal weight appropriate for gestational age (10th-90th percentile); technically adequate imaging allowing measurement of the fetal splenic artery (SA) Doppler waveform; and written informed consent for participation. We selected fetuses with an estimated fetal weight between the 10th and 90th percentiles to reduce the likelihood of undiagnosed growth abnormalities in the reference population. However, this restriction may have narrowed the observed biological variability relative to an unselected low-risk cohort.

Exclusion criteria were multiple pregnancy; major fetal structural or chromosomal anomaly; fetal growth restriction or small-for-gestational-age fetus (estimated fetal weight and/or abdominal circumference <10th percentile); large-for-gestational-age fetus (estimated fetal weight >90th percentile); pregestational or gestational diabetes mellitus; hypertensive disorders of pregnancy; oligohydramnios or polyhydramnios; abnormal umbilical artery Doppler findings; chronic maternal disease (renal, cardiac, autoimmune, or thyroid dysfunction) that could affect fetal circulation; insufficient image quality precluding SA Doppler assessment; and technical failure to obtain a reliable Doppler recording.

Each participant underwent a single ultrasonographic evaluation, and only one examination per pregnancy was included in the analysis, consistent with the cross-sectional study design.

Ultrasonographic and Doppler Assessment: All examinations were performed using a Voluson E8 ultrasound system (GE Healthcare, Zipf, Austria) equipped with a 2-9 MHz convex transducer, following standard obstetric protocols. Routine fetal biometric parameters, including biparietal diam-

eter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL), were measured at each examination.

Following biometric assessment, the fetal SA was identified. The SA arises from the celiac trunk, courses along the superior border of the pancreas, and enters the splenic hilum (5,6). Doppler measurements were obtained in a transverse or slightly oblique plane of the fetal upper abdomen at the level of the splenic hilum, using the adjacent splenic parenchyma as an anatomical landmark to distinguish the vessel from umbilical and uterine artery flow (Figure 1) (5). All Doppler measurements were obtained at a constant sampling site, at the level of the splenic hilum, to minimize variability related to sampling location along the vessel. After confirming vessel identity and flow direction with color Doppler, the pulsed-wave Doppler sample volume was set at 1-2 mm, and the insonation angle was maintained below 30˚ (10). Angle correction was applied at the time of image acquisition, with the correction cursor manually aligned parallel to the vessel wall before each Doppler recording. A single experienced perinatology specialist performed all examinations to minimize inter-observer variability; because gestational age was determined from concurrent fetal biometry at the same examination, the operator was not blinded to gestational age, consistent with routine clinical practice. Waveforms were recorded during periods of fetal quiescence in the absence of fetal breathing movements, and only recordings containing at least three consecutive, technically uniform cardiac cycles were accepted for analysis. The mean of three consecutive waveforms was used for the final measurement.

From the SA Doppler waveform, the S/D ratio, PI, PSV, and TAMV were calculated using the ultrasound system's built-in software. TAMV, also referred to as TAMXV or TAMX depending on the ultrasound manufacturer, is used throughout this manuscript to denote the time-averaged maximum velocity output directly recorded by the system's built-in Doppler software.

Variables and Data Collection: The primary aim of the study was to construct gestational age-specific nomograms, regression equations, Z-score formulas, and reference ranges for fetal SA Doppler parameters (S/D ratio, PI, PSV, and TAMV) in normal singleton pregnancies between 18 and 40 weeks of gestation. The secondary aim was to evaluate the associations between SA Doppler parameters and fetal biometric measurements and umbilical artery Doppler indices.

The dependent variables were the fetal SA Doppler parameters (S/D ratio, PI, PSV, and TAMV) obtained during ultrasonographic evaluation. Gestational age was used as the independent variable. Maternal age, parity, and fetal biometric parameters (BPD, HC, AC, and FL) were recorded as covariates.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). A priori power calculation assumed a conservative weak-to-moderate correlation coefficient (r=0.25) between SA Doppler parameters and gestational age, informed by the comparatively modest associations previously reported for celiac and splenic artery Doppler indices (11), with a significance level of α=0.05 and a power of 80%. This indicated a minimum requirement of 100 cases; we included 103 cases with technically adequate SA Doppler recordings, meeting this target. We assessed the distribution of continuous variables using the Shapiro-Wilk test and visual inspection of histograms and Q-Q plots. Continuous variables are presented as mean±standard deviation (SD) or median (interquartile range [IQR]), as appropriate, whereas categorical variables are presented as number (percentage).

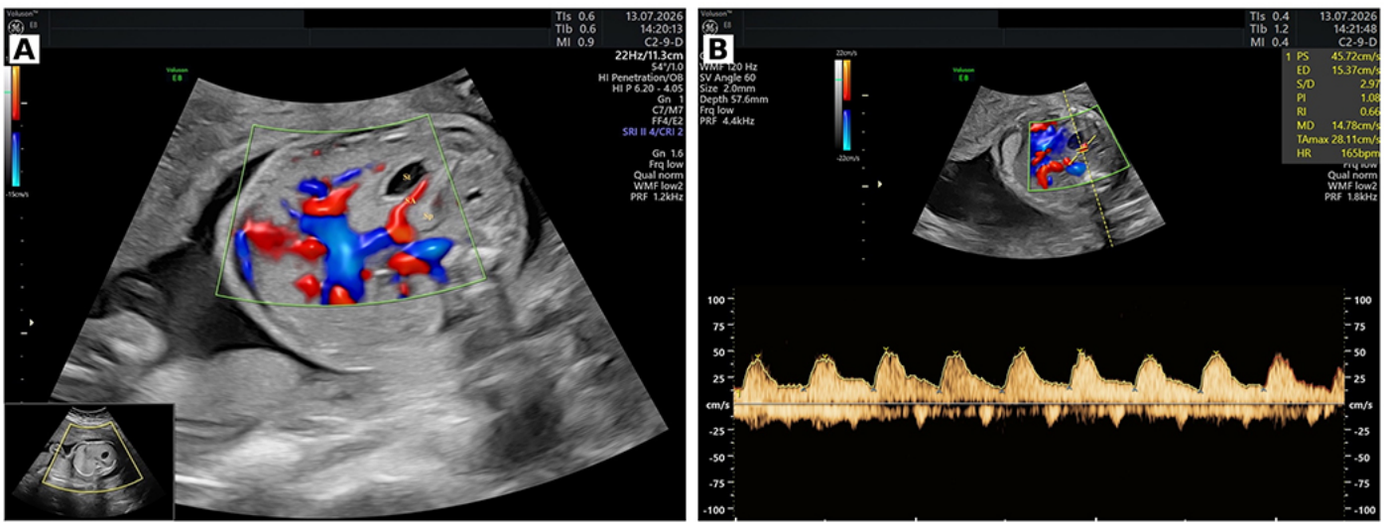

Figure 1: *Ultrasonographic visualization of the fetal splenic artery (SA). (A) Color Doppler image showing the SA arising from the celiac trunk and coursing along the superior border of the pancreas toward the splenic hilum (St, stomach; SA, splenic artery; Sp, spleen). (B) Pulsed-wave Doppler waveform of the SA obtained at an insonation angle <30˚, with corresponding velocimetric parameters.*

We evaluated the relationships between gestational age and fetal SA Doppler parameters using linear and quadratic regression analyses. We selected the best-fitting regression model for each parameter based on goodness-of-fit criteria, including the coefficient of determination (R^2) and adjusted coefficient of determination (adjusted R^2). We further supported model selection by visually inspecting residual plots to ensure an adequate fit across the gestational age range. We then used the selected regression equations to derive gestational age-specific predicted mean values.

Z-score equations were generated using the predicted mean and predicted standard deviation derived from the regression models. We internally validated the Z-score equations by calculating individual Z-scores for each observation using the corresponding gestational age-specific predicted mean and standard deviation. We evaluated the distribution of the calculated Z-scores by examining their mean and standard deviation, assessing normality using the Shapiro-Wilk test, and testing for residual association between Z-scores and gestational age using Spearman correlation analysis. We calculated gestational age-specific reference values for predefined gestational age groups (18-23+6, 24-27+6, 28-31+6, 32-35+6, and 36-40 weeks) and presented the 5th, 10th, 50th, 90th, and 95th percentiles. Because SA S/D and SA PI showed no significant association with gestational age, gestational age-specific modeling was not pursued for these two parameters. For SA PI, we derived a single gestational age-independent reference interval by pooling the mean and standard deviation across all five gestational age groups using standard formulas for combining subgroup summary statistics. For SA S/D, both the gestational age-specific Z-score model and the pooled parametric approach yielded implausible negative lower bounds, reflecting the pronounced right-skewness of this parameter; a valid parametric reference interval could therefore not be derived, and only descriptive statistics (median and interquartile range by gestational age group) are reported for SA S/D. The first gestational age band (18-23+6 weeks) was defined more broadly than the subsequent 4-week bands because reliable sonographic visualization and Doppler interrogation of the fetal splenic artery become technically feasible only from approximately 18 weeks of gestation onward, limiting the number of technically adequate measurements available at earlier gestational ages.

Correlations between fetal SA Doppler parameters and gestational age, fetal biometric measurements, and umbilical artery Doppler indices were initially evaluated using Spearman's rank correlation coefficient. Because both fetal biometric measurements and SA velocity parameters vary with gestational age, we also performed partial correlation analyses controlling for gestational age to assess associations between SA PSV and TAMV and abdominal circumference (AC) and estimated fetal weight (EFW). A two-sided p-value <0.05 was considered statistically significant.

Results

A total of 103 singleton pregnancies were included in the final analysis. The mean maternal height and weight were 162.95±5.45 cm and 69.22±11.46 kg, respectively, corresponding to a mean body mass index of 26.07±4.21 kg/m². The median gravidity was 2, and 59 women (57.3%) were multiparous. Two pregnancies (1.9%) were conceived via in vitro fertilization, and smoking during pregnancy was reported in 2 cases (1.9%). Examinations were performed at a mean gestational age of 27.37±6.46 weeks, with a mean abdominal circumference of 232.43±65.40 mm and a mean estimated fetal weight of 1345.87±1038.53 g. Table I presents the remaining demographic, maternal, and fetal characteristics of the study population.

We evaluated the relationship between gestational age and fetal splenic artery (SA) Doppler parameters using regression analysis, and Table II presents the best-fitting model for each parameter. SA peak systolic velocity (PSV) and time-averaged maximum velocity (TAMV) both showed a significant linear increase with advancing gestational age (PSV=-5.651+1.587×GA, R^2=0.399, adjusted R^2=0.393, $p<0.001$;

Table I: *Demographic, maternal, and fetal characteristics of the study population*

n=103	Mean±SD or n (%)	Median (IQR)
Maternal height (cm)	162.95 ± 5.45	163.00 (159.75 - 167.00)
Maternal weight (kg)	69.22 ± 11.46	68.00 (61.75 - 77.00)
Maternal BMI (kg/m²)	26.07 ± 4.21	25.62 (23.69 - 28.26)
Gravidity	2.21 ± 1.40	2.00 (1.00 - 3.00)
Parity	0.88 ± 1.04	1.00 (0.00 - 1.00)
GA at examination (weeks)	27.37 ± 6.46	25.50 (21.50 - 33.15)
AC (mm)	232.43 ± 65.40	235.0 (172.50 - 291.50)
EFW (g)	1345.87 ± 1038.53	931.0 (455.25 - 2069.25)
Nulliparity	44 (42.7%)	–
Multiparity	59 (57.3%)	–
IVF pregnancy	2 (1.9%)	–
Smoking	2 (1.9%)	–

SD: Standard deviation, IQR: Interquartile range, BMI: Body mass index, GA: Gestational age, AC: Abdominal circumference, EFW: Estimated fetal weight, IVF: In vitro fertilization

Table II: *Regression equations for fetal splenic artery Doppler parameters according to gestational age*

Parameter	Selected model	Regression equation	R^2	Adjusted R^2	p
SA S/D	Linear	S/D=11.486−0.139×GA	0.008	−0.002	0.380
SA PI	Quadratic	PI=0.762+0.069×GA−0.00130×GA²	0.025	0.005	0.293
SA PSV	Linear	PSV=−5.651+1.587×GA	0.399	0.393	<0.001*
SA TAMV	Linear	TAMV=−10.900+0.929×GA	0.401	0.395	<0.001*

*GA: Gestational age, SA: Splenic artery, S/D: Systolic/diastolic ratio, PI: Pulsatility index, PSV: Peak systolic velocity, TAMV: Time-averaged maximum velocity, R^2: Coefficient of determination, Adjusted R^2: Adjusted coefficient of determination. Regression equations were derived using the best-fitting regression model for each fetal splenic artery Doppler parameter. We evaluated linear and quadratic models and selected the optimal model based on goodness-of-fit statistics. Gestational age was expressed in weeks. *: p<0.05.*

TAMV=-10.900+0.929×GA, R^2=0.401, adjusted R^2=0.395, p<0.001). In contrast, the SA systolic/diastolic (S/D) ratio showed no significant linear association with gestational age (R^2=0.008, p=0.380), and SA pulsatility index (PI) showed no significant quadratic association with gestational age (R^2=0.025, p=0.293).

Table III presents Z-score equations derived from the predicted mean and gestational age-specific standard deviation values of the regression models, allowing standardized values to be calculated for each SA Doppler parameter at any gestational age. Assessment of the Z-score distributions showed adequate centering around zero and no significant residual association with gestational age for all parameters. However, the distributional performance differed among parameters. For SA PI, PSV, and TAMV, Z-score standard deviations were close to 1 (0.994, 1.058, and 1.022, respectively). In contrast, the SA S/D Z-score model showed substantial dispersion (SD=1.757) and persistent non-normality (Shapiro-Wilk p<0.001). Accordingly, the Gaussian Z-score framework was not considered valid for SA S/D, and this parameter is reported descriptively rather than as a validated Z-score reference model. Detailed results are presented in Table IV.

Gestational age-specific reference values for SA PSV and TAMV, expressed as the 5th, 10th, 50th, 90th, and 95th percentiles across five predefined gestational age groups (18-23+6, 24-27+6, 28-31+6, 32-35+6, and 36-40 weeks), are presented in Table V. Median PSV increased progressively from 26.43 cm/s at 18-23+6 weeks to 54.15 cm/s at 36-40 weeks, and median TAMV increased from 7.09 cm/s to 26.83 cm/s over the same period. Because SA PI and SA S/D showed no significant gestational age dependence, they are reported separately from the gestational age-specific PSV and TAMV values. The pooled, gestational age-independent reference interval for SA PI was 1.63±0.36 (parametric 5^{th}-95^{th} percentile range, 1.04-2.22). For SA S/D, median values by gestational age group ranged from 3.82 to 5.72 (Table V); given the pro-

Table III: *Z-score equations for fetal splenic artery Doppler parameters*

Parameters	Mean equation	SD equation	Z-score equation
SA S/D	Mean=11.486−0.139×GA	SD=5.465−0.031×GA	(Observed S/D − Mean)/SD
SA PI	Mean=0.762+0.069×GA−0.00130×GA²	SD=0.304−0.001×GA	(Observed PI − Mean)/SD
SA PSV	Mean=−5.651+1.587×GA	SD=0.271+0.315×GA	(Observed PSV − Mean)/SD
SA TAMV	Mean=−10.900+0.929×GA	SD=−5.720+0.402×GA	(Observed TAMV − Mean)/SD

GA: Gestational age, SA: Splenic artery, SD: Standard deviation, S/D: Systolic/diastolic ratio, PI: Pulsatility index, PSV: Peak systolic velocity, TAMV: Time-averaged maximum velocity. We derived predicted standard deviation equations from regression models of residual variability. Individual Z-scores were calculated as (observed value - predicted mean)/predicted SD. Internal validation of the proposed equations is presented in Table IV. The lower reference limit derived from the SA S/D mean and SD equations becomes physiologically implausible (negative) from approximately 28 weeks of gestation onward and is not recommended for clinical use; SA S/D is reported descriptively rather than as a validated Z-score model. The SA PI equations are presented here for completeness. Still, a single pooled gestational age-independent reference interval (reported in Results) is recommended over the gestational age-specific model, given the lack of a significant gestational age relationship for this parameter.

Table IV: *Internal validation of gestational age-specific Z-score equations*

Parameter	Mean Z-score	SD	Shapiro-Wilk p	Correlation with gestational age (r)	p
SA S/D	−0.000	1.757	<0.001	−0.002	0.982
SA PI	−0.000	0.994	0.080	−0.000	0.999
SA PSV	0.001	1.058	<0.001	−0.006	0.953
SA TAMV	−0.005	1.022	<0.001	0.011	0.917

GA: Gestational age, SA: Splenic artery, SD: Standard deviation, S/D: Systolic/diastolic ratio, PI: Pulsatility index, PSV: Peak systolic velocity, TAMV: Time-averaged maximum velocity. Values are based on internally calculated Z-scores using the gestational age-specific regression equations. Normality was assessed using the Shapiro-Wilk test. Spearman correlation analysis was used to evaluate residual associations between Z-scores and gestational age. Despite adequate centering and no residual association with gestational age, the SA S/D Z-score model is not considered internally validated, given its standard deviation of 1.757 (substantially greater than 1) and persistent non-normality (Shapiro-Wilk p<0.001).

Table V: *Gestational age-specific reference values for fetal splenic artery Doppler parameters*

Gestational age (weeks)	n	Mean ± SD	Median (IQR)	P5	P10	P50	P90	P95
SA S/D	**SA S/D**	**SA S/D**	**SA S/D**	**SA S/D**	**SA S/D**	**SA S/D**	**SA S/D**	**SA S/D**
18-23+6	40	7.61±5.61	5.72 (4.48-9.04)	3.45	3.71	5.72	13.12	16.77
24-27+6	15	10.60±18.08	5.11 (4.00-6.88)	2.97	3.39	5.11	12.76	36.40
28-31+6	17	9.36±14.80	4.80 (4.33-7.82)	3.45	3.64	4.80	11.21	22.46
32-35+6	15	4.52±1.28	4.52 (3.75-5.13)	2.81	2.87	4.52	5.97	6.50
36-40	16	6.19±8.74	3.82 (3.38-4.47)	3.09	3.32	3.82	6.03	14.22
SA PI	**SA PI**	**SA PI**	**SA PI**	**SA PI**	**SA PI**	**SA PI**	**SA PI**	**SA PI**
18-23+6	40	1.62±0.33	1.60 (1.37-1.80)	1.22	1.26	1.60	2.02	2.24
24-27+6	15	1.68±0.35	1.66 (1.38-1.99)	1.17	1.29	1.66	2.05	2.07
28-31+6	17	1.74±0.35	1.62 (1.51-2.00)	1.37	1.41	1.62	2.25	2.30
32-35+6	15	1.60±0.33	1.62 (1.42-1.89)	1.07	1.12	1.62	1.94	1.98
36-40	16	1.52±0.41	1.40 (1.33-1.55)	1.11	1.21	1.40	2.12	2.23
SA PSV	**SA PSV**	**SA PSV**	**SA PSV**	**SA PSV**	**SA PSV**	**SA PSV**	**SA PSV**	**SA PSV**
18-23+6	40	28.10±8.87	26.43 (22.46-31.59)	14.82	18.06	26.43	41.68	44.66
24-27+6	15	33.75±12.67	36.49 (23.02-42.36)	16.72	18.73	36.49	49.24	51.81
28-31+6	17	41.32±10.60	39.80 (33.80-47.20)	29.18	31.04	39.80	57.61	58.96
32-35+6	15	42.12±9.67	41.01 (34.19-49.91)	30.18	32.84	41.01	53.40	55.75
36-40	16	58.66±21.79	54.15 (46.77-58.97)	37.45	41.13	54.15	89.83	108.53
SA TAMV	**SA TAMV**	**SA TAMV**	**SA TAMV**	**SA TAMV**	**SA TAMV**	**SA TAMV**	**SA TAMV**	**SA TAMV**
18-23+6	40	8.81±4.80	7.09 (5.44-11.25)	4.62	4.77	7.09	14.69	16.00
24-27+6	15	11.54±4.51	10.21 (8.63-12.62)	6.88	7.32	10.21	18.09	19.08
28-31+6	17	17.12±7.32	16.67 (11.58-19.22)	8.46	9.97	16.67	26.43	31.26
32-35+6	15	18.19±8.21	19.07 (11.08-24.07)	7.46	8.42	19.07	26.65	29.03
36-40	16	25.86±12.64	26.83 (15.29-30.91)	10.26	11.39	26.83	41.56	45.77

SA: Splenic artery, SD: Standard deviation, IQR: Interquartile range, S/D: Systolic/diastolic ratio, PI: Pulsatility index, PSV: Peak systolic velocity, TAMV: Time-averaged maximum velocity, P5: 5th Percentile, P10: 10th Percentile, P50: 50th Percentile (median), P90: 90th Percentile, P95: 95th Percentile. Values for SA S/D are presented for descriptive purposes only and do not constitute a validated reference range (see Statistical Analysis). The 24-27+6 and 32-35+6 gestational age groups contain relatively few observations (n=15 each), and the extreme percentiles (P5 and P95) within these and other small subgroups should be interpreted with caution given the wide confidence limits inherent to percentile estimation from limited sample sizes.

nounced right-skewness of this parameter, these are reported for descriptive purposes only and should not be interpreted as a validated reference range. Figure 2 illustrates these gestational age-specific trends.

Correlations between SA Doppler parameters and gestational age, umbilical artery (UA) Doppler indices, abdominal circumference, and estimated fetal weight are summarized in Table VI. SA PSV and TAMV correlated positively with gestational age (r=0.632, p<0.001 and r=0.633, p<0.001, respectively), abdominal circumference (r=0.666 and r=0.668, both p<0.001), and estimated fetal weight (r=0.674 and r=0.672, both p<0.001), and correlated negatively with UA S/D ratio and UA PI. The SA S/D ratio correlated positively with the UA S/D ratio (r=0.405, p=0.006) but showed no significant correlation with UA PI, gestational age, abdominal circumference, or estimated fetal weight. SA PI showed no significant correlation with any assessed variable. Because gestational age was strongly associated with both fetal biometry and SA velocity parameters, we also performed partial correlation analyses controlling for gestational age. After adjustment for gestational age, the associations of SA PSV with AC (partial r=0.297, p=0.003) and EFW (partial r=0.301, p=0.003) remained significant. Similarly, SA TAMV remained significantly associated with AC (partial r=0.302, p=0.003) and EFW (partial r=0.286, p=0.004).

Discussion

In this observational cross-sectional study, we developed gestational age-specific regression equations and Z-score formulas for fetal SA PSV and TAMV, established a gestational age-independent reference interval for SA PI, and provided descriptive reference values for SA S/D in 103 normal singleton pregnancies between 18 and 40 weeks of gestation. Our findings demonstrated a significant linear increase in SA PSV and TAMV across gestation. In contrast, the SA S/D ratio and PI remained relatively stable and showed no significant association with gestational age. SA PSV and TAMV also correlated positively with fetal abdominal circumference and estimated fetal weight and correlated negatively with umbilical artery (UA) Doppler indices. In contrast, the SA S/D ratio showed a positive correlation with the UA S/D ratio. To our knowledge, this is the first study to provide descriptive reference values for SA S/D and gestational age-specific reference values for TAMV, while also deriving gestational age-specific Z-score equations for SA PSV and TAMV.

Our finding of a progressive increase in SA PSV across

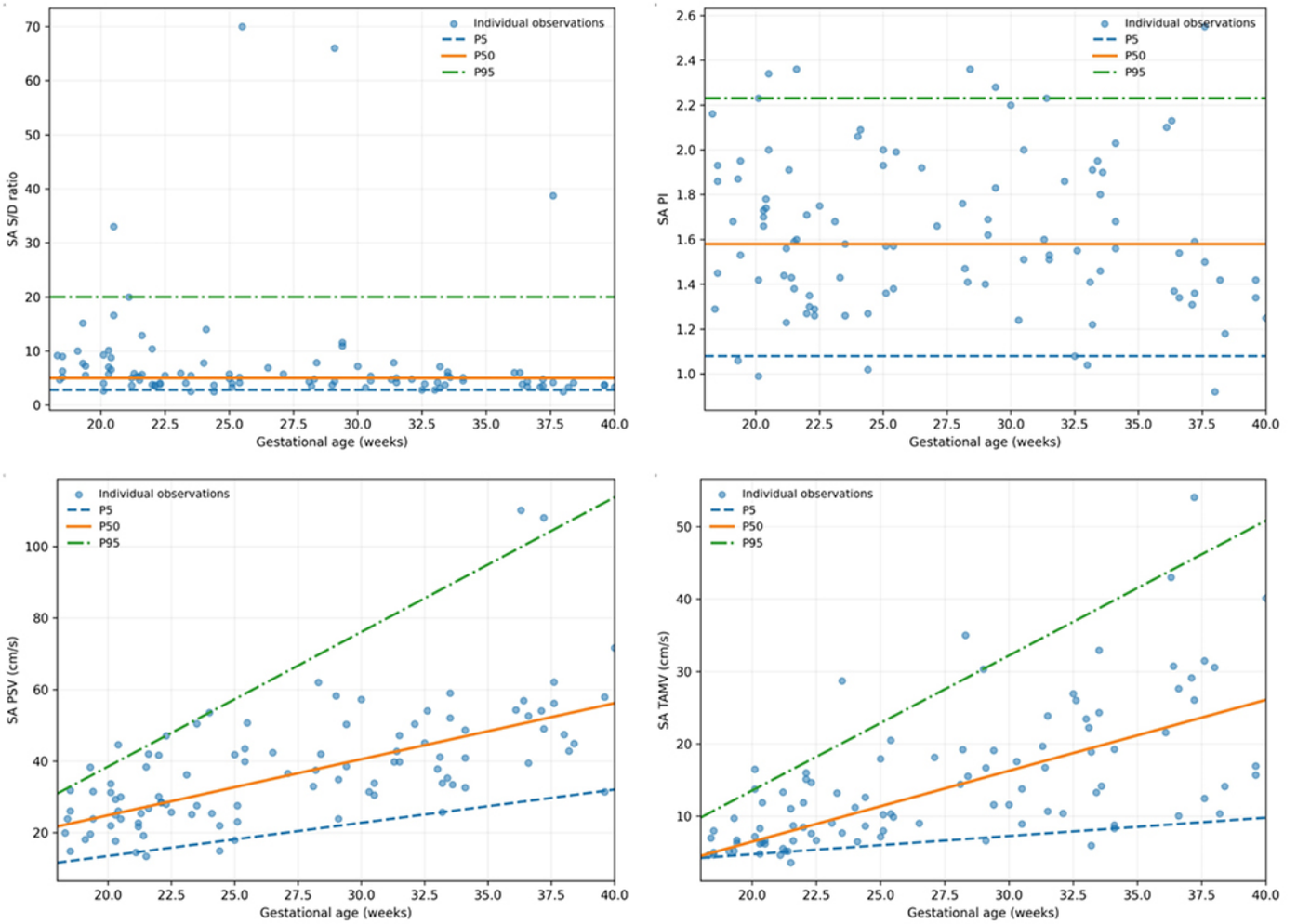

Figure 2: *Distribution of fetal splenic artery Doppler parameters and model-based reference curves according to gestational age. Individual observations are shown as scatterplots for (A) systolic/diastolic (S/D) ratio, (B) pulsatility index (PI), (C) peak systolic velocity (PSV), and (D) time-averaged maximum velocity (TAMV). Continuous curves represent model-based 5th (P5), 50th (P50), and 95th (P95) percentiles. Because S/D ratio and PI showed no meaningful gestational age dependency, their percentile estimates were modeled as gestational age-independent reference levels. In contrast, PSV and TAMV percentiles were modeled continuously across gestation.*

Table VI: *Correlations between fetal splenic artery Doppler parameters and clinical/ultrasonographic variables*

Variable	SA S/D	SA PI	SA PSV	SA TAMV
Gestational age	-0.089 (0.380)	-0.093 (0.360)	0.632 (<0.001)	0.633 (<0.001)
UA S/D	0.405 (0.006)	0.211 (0.164)	-0.376 (0.011)	-0.431 (0.003)
UA PI	0.208 (0.170)	0.113 (0.460)	-0.445 (0.002)	-0.430 (0.003)
Abdominal circumference	-0.129 (0.208)	-0.064 (0.532)	0.666 (<0.001)	0.668 (<0.001)
Estimated fetal weight	-0.116 (0.257)	-0.101 (0.320)	0.674 (<0.001)	0.672 (<0.001)
AC, adjusted for gestational age	-	-	0.297 (0.003)	0.302 (0.003)
EFW, adjusted for gestational age	-	-	0.301 (0.003)	0.286 (0.004)

SA: Splenic artery, S/D: Systolic/diastolic ratio, PI: Pulsatility index, PSV: Peak systolic velocity, TAMV: Time-averaged maximum velocity, UA: Umbilical artery, AC: Abdominal circumference, EFW: Estimated fetal weight. Data are presented as correlation coefficients (p values). Spearman's rank correlation was used for the unadjusted analyses. We additionally evaluated associations between SA PSV and TAMV and AC and EFW using partial correlation analysis controlling for gestational age.

gestation is consistent with the nomogram reported by Tongsong et al., who demonstrated a linear relationship between SA PSV and gestational age (14-40 weeks) and developed percentile-based reference ranges primarily for the surveillance of fetal anemia (12). Similarly, Ebbing et al. established longitudinal reference ranges for the fetal celiac and splenic arteries and reported that SA PI values remained comparatively low and stable across gestation, being more closely linked to the portocaval pressure gradient than to gestational age itself (11). Our observation that SA PI and the S/D ratio did not change significantly with gestational age aligns with these findings. It supports the concept that resistance-based SA indices may be less closely linked to gestational age than velocity-based parameters. However, this observation should

be interpreted cautiously given the non-normal distribution of S/D values observed in our cohort.

Several studies underscore the clinical relevance of establishing normal SA Doppler reference values by showing that SA waveforms change under pathological conditions. Abuhamad et al. and Oz et al. reported decreased SA resistance and PSV, respectively, in small-for-gestational-age fetuses, attributing these changes to redistribution of fetal blood flow (13,14). At the same time, Bahado-Singh et al. showed that SA PSV and deceleration-angle indices increase markedly in fetuses with severe anemia secondary to Rh alloimmunization, reflecting increased splenic flow related to compensatory erythropoiesis (15,16). More recently, Özalp et al. found that a reduced SA PI was associated with late-onset fetal growth restriction and adverse perinatal outcomes (17). At the same time, Albayrak et al. demonstrated that a low SA PI predicted the need for neonatal intensive care in gestational diabetes class A1 pregnancies (18). The gestational age-specific reference values generated in the present study may provide the normative background against which such pathological deviations can be more precisely quantified, since many earlier studies expressed SA Doppler values as multiples of the median derived from comparatively small or narrow-gestational-age control samples. The positive correlation we observed between SA PSV/TAMV and fetal biometric parameters is also consistent with the report by Ebbing et al., who found increased arterial PSV values, including in the SA, in macrosomic fetuses, supporting a link between SA flow velocity and fetal growth (19).

The SA is also of particular interest because it constitutes the principal vascular supply of the fetal pancreas, and a growing body of literature, including several studies from our own research group, has examined the relationship between SA Doppler waveforms and fetal pancreatic size and echogenicity, particularly in the context of gestational diabetes mellitus (GDM). Golbasi et al. demonstrated that fetal pancreatic circumference and echogenicity are increased in GDM pregnancies and associated with adverse perinatal outcomes, that this increase in pancreatic size parallels significant elevations in SA S/D and PI reflecting increased vascular resistance, and that fetal pancreatic circumference in uncomplicated pregnancies correlates positively with gestational age, maternal body mass index, and birth weight (10,20,21). In parallel, Gilboa et al. demonstrated that fetal pancreatic circumference is increased in pregnancies complicated by GDM and pregestational diabetes and has moderate predictive value for the diagnosis of GDM (22,23). In our previous studies, we found that fetal pancreatic circumference and its vascular supply were already altered by the mid-second trimester in GDM pregnancies (24). Maternal body mass index was independently associated with increased fetal pancreatic circumference even in normoglycemic pregnancies, suggesting organ-specific, glycemia-independent effects of maternal obesity on the fetal pancreas (25). Taken together with a recent narrative review synthesizing this expanding body of evidence, these findings highlight the SA as a physiologically relevant vessel whose waveform characteristics may reflect not only general fetal hemodynamic adaptation but also organ-specific metabolic influences related to the pancreas (26). The gestational age-specific SA Doppler nomograms established in the present study may therefore serve as a normative foundation for future research examining SA hemodynamics in relation to fetal pancreatic development across a range of maternal metabolic conditions. These findings should be regarded as hypothesis-generating rather than immediately applicable to clinical decision-making, and external validation in larger, multicenter cohorts is required before use in this context.

Strengths and Limitations: This study has several strengths. It was conducted prospectively using a standardized Doppler technique performed by experienced perinatology specialists, and it provides, to our knowledge, the first gestational age-specific reference values for the SA S/D ratio and TAMV and the first Z-score equations for SA Doppler parameters, complementing previously published reference data for SA PI, velocity, and PSV. The application of strict inclusion and exclusion criteria, encompassing fetal growth abnormalities, maternal metabolic and hypertensive disorders, and abnormal UA Doppler findings, allowed the reference values to reflect a genuinely low-risk population.

Nevertheless, several limitations should be acknowledged. The cross-sectional design, in which each participant was evaluated only once, does not allow assessment of longitudinal, within-fetus changes in SA Doppler parameters across gestation, and, in this respect, our percentile curves may not be directly comparable to reference ranges derived from longitudinal cohorts such as that of Ebbing et al. (11). The sample size, although adequate based on the a priori power calculation, resulted in relatively small numbers within some of the five-week gestational age subgroups, particularly beyond 24 weeks, which may have reduced the precision of the percentile estimates in these intervals. In addition, although a standardized technique was used to minimize variability, this study did not assess formal intra-observer reproducibility (e.g., intraclass correlation coefficients or Bland-Altman analysis), which future validation studies should address before applying these reference values clinically. The EFW-based inclusion criterion, while intended to reduce the likelihood of undiagnosed growth abnormalities, may have narrowed the observed biological variability relative to an unselected low-risk cohort. Birth outcome data, including birth weight, gestational age at delivery, and APGAR scores, were not systematically collected in this cross-sectional cohort, limiting confirmation that the study population was uniformly free of undiagnosed growth or perinatal abnormalities. Because SA S/D and PI showed no significant association with gestational age, we reconsidered gestational age-specific modeling for these two pa-

rameters. For SA PI, we report a single, gestational age-independent reference interval instead. For SA S/D, neither the gestational age-specific nor the pooled parametric approach produced physiologically plausible reference bounds, given the pronounced right-skewness of this parameter; therefore, we report only descriptive statistics for SA S/D, and we could not establish a validated reference range from the present dataset. Establishing a valid reference range for SA S/D would likely require a non-parametric or transformation-based modeling approach (e.g., logarithmic transformation or a GAMLSS framework) applied to a larger, individual patient-level dataset, which was beyond the scope of the present analysis. Future studies with access to a fully accessible patient-level dataset should address both limitations. Finally, because this study was conducted in a single geographic population, future multicenter studies should confirm the applicability of these reference values to other populations.

Conclusion

In conclusion, this study provides gestational age-specific regression equations and Z-score formulas for SA PSV and TAMV, a pooled gestational age-independent reference interval for SA PI, and descriptive values for SA S/D, in normal singleton pregnancies between 18 and 40 weeks of gestation. SA PSV and TAMV increased significantly with advancing gestational age and correlated with fetal biometric parameters, whereas the S/D ratio and PI remained relatively stable across gestation. These findings extend the existing literature on fetal SA Doppler assessment, including studies evaluating its role in fetal growth restriction, fetal anemia, and gestational diabetes, and, given the SA's role as the principal vascular supply of the fetal pancreas, may provide a useful normative reference for future studies examining the relationship between SA hemodynamics and fetal pancreatic development. External validation in larger, multicenter, and longitudinal cohorts is warranted before these reference values are adopted into routine clinical use.

Declarations

Data Availability Statement: Data are not publicly available due to ethical restrictions. Further inquiries can be directed to the corresponding author.

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Author Contribution: IGA: Conceptualization, project development, data collection, data interpretation, manuscript drafting. HG: Study design, supervision, critical manuscript review and editing. BB: Statistical analysis, data management, methodological support. CSP: Data collection, manuscript review and editing. HAA: Manuscript review and editing. ZEC: manuscript review and editing.

Conflict of Interest Disclosure: The authors declare no conflicts of interest.

Ethics Approval Statement: This study complied with all relevant national regulations, institutional policies, and the principles of the Declaration of Helsinki (as revised in 2024). The local Non-Interventional Ethics Committee reviewed and approved the study protocol (2026/108, Approval Date: 18 February 2026).

Artificial Intelligence Statement: No artificial intelligence-based language model or tool was used in the preparation, drafting, analysis, or editing of this manuscript. The authors developed all content, interpretation, and conclusions and take full responsibility for the manuscript.

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