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keting li
Tongji University
hongyu yang
Tongji University
chuanqing sun
Tongji University
fan huang
Tongji University
min ren
Tongji University
yu lu
luyu@51mch.com

Tongji University

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Associations of gestational diabetes mellitus with fetal renal artery Doppler parameters and MV-Flow Ratio in the second trimester

A baseline association analysis of a single-centre prospective study

Keting Li†, Hongyu Yang†, Chuanqing Sun, Fan Huang, Min Ren*, Yu Lu*

Department of Ultrasound, Shanghai First Maternity and Infant Hospital, School of Medicine, Tongji University, Shanghai, China

Shanghai Key Laboratory of Maternal Fetal Medicine, Shanghai Institute of Maternal-Fetal Medicine and Gynecologic Oncology, Shanghai First Maternity and Infant Hospital, School of Medicine, Tongji University, Shanghai 20009, China

† Keting Li and Hongyu Yang contributed equally to this work and share first authorship.

* Corresponding authors: Yu Lu (luyu@51mch.com) and Min Ren (renmin@51mch.com).

Abstract

Background: To investigate associations of maternal gestational diabetes mellitus (GDM) status with fetal renal artery Doppler parameters and MV-Flow Ratio (%) in the second trimester, and to explore whether the renal artery resistive index (RI) and MV-Flow Ratio provide complementary information on GDM group membership.

Methods: This single-centre prospective study was conducted at Shanghai First Maternity and Infant Hospital from January 2024 to August 2025. Ultrasound data obtained before initiation of GDM-related management were analysed for 84 singleton pregnancies at 20–28 weeks of gestation (29 GDM and 55 non-GDM). GDM was defined using the IADPSG 75-g oral glucose tolerance test criteria. Two blinded physicians independently examined both kidneys, obtaining three measurements per side. Under a prespecified rule, near-field measurements were averaged within each observer and then across observers. Peak systolic

velocity (PSV), S/D ratio, pulsatility index (PI), RI and MV-Flow Ratio were measured. Reliability was assessed using average-measures intraclass correlation coefficients. Associations were estimated using gestational-age-adjusted linear models with HC3 robust standard errors and Holm correction. A base model including gestational age and RI and an extended model adding MV-Flow Ratio were internally evaluated using 1,000 bootstrap resamples and leave-one-out cross-validation (LOOCV).

Results: The GDM group had lower S/D, PI and RI and higher MV-Flow Ratio than the non-GDM group. Adjusted mean differences were −0.065 for RI (95% CI −0.091 to −0.040) and 14.53 percentage points for MV-Flow Ratio (95% CI 10.57 to 18.48; both Holm-adjusted $P < 0.001$). The PSV difference was 0.15 cm/s (95% CI −5.45 to 5.75; $P = 0.958$). Apparent areas under the receiver operating characteristic curve (AUCs) were 0.826 and 0.924 for the base and extended models, respectively (difference 0.098, 95% CI −0.004 to 0.199; $P = 0.059$). Bootstrap-corrected AUCs were 0.815 and 0.910, and LOOCV AUCs were 0.800 and 0.902.

Conclusions: Maternal GDM status was associated with lower fetal renal artery S/D, PI and RI and higher MV-Flow Ratio in the second trimester. These findings support GDM-associated differences in renal haemodynamic and microvascular ultrasound phenotypes but do not establish increased renal perfusion, renal injury or causality.

Keywords: Gestational diabetes mellitus; Fetal kidney; Doppler ultrasonography; Microvascular flow imaging; MV-Flow Ratio; Baseline association analysis

Background

Gestational diabetes mellitus (GDM) is an important disorder of glucose metabolism during pregnancy and is closely associated with fetal growth and multiple adverse perinatal outcomes. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study demonstrated continuous associations between higher maternal glucose concentrations and

increased birth weight and cord-blood C-peptide concentrations, even below the threshold for overt diabetes, indicating a close relationship between the intrauterine glycaemic environment and fetal growth and metabolic responses [1]. Beyond fetal growth and perinatal outcomes, whether maternal glucose metabolism is accompanied by changes in organ-specific fetal circulatory phenotypes warrants investigation. The fetal kidneys can be visualised using prenatal ultrasonography. Renal Doppler waveforms and detectable intrarenal microvascular flow signals provide an imaging window for exploring these associations, characterising fetal renal blood flow and improving our understanding of GDM-associated fetal phenotypes.

Fetal renal artery Doppler ultrasonography provides a non-invasive method for assessing blood flow velocities and spectral characteristics in the arteries supplying the kidneys. The resistive index (RI), pulsatility index (PI) and peak systolic-to-end-diastolic velocity ratio (S/D) describe different aspects of the waveform; however, they do not directly measure renal blood flow volume or renal function [2]. Previous studies have used fetal renal artery Doppler to assess blood flow in GDM pregnancies. In a cohort examined at 37–40 weeks of gestation, Jamal et al. reported higher fetal renal artery RI, PI and S/D in the GDM group than in healthy controls, with some renal artery indices inversely associated with birth weight in the GDM group [3]. These findings suggest that renal artery spectral parameters may provide information on GDM-associated fetal blood flow. However, observations at term cannot establish whether similar changes occur in the second trimester. Evaluation at earlier gestational ages requires studies with clearly defined examination timing and clinical management contexts.

In addition to renal artery spectral analysis, microvascular flow imaging offers another approach to visualising intrarenal blood flow signals. MV-Flow can display microvascular flow and extract parameters within a region of interest (ROI). Using three-dimensional MV-Flow to quantify normal and abnormal fetal kidneys, Huang et al. found that the

vascularisation index and vascularisation-flow index increased with gestational age in normal kidneys, whereas the flow index was not significantly associated with gestational age. Different types of renal abnormalities also exhibited distinct microvascular parameter patterns [4]. These findings support the application of fetal renal microvascular imaging and indicate that gestational-age dependence varies among parameters. Earlier three-dimensional power Doppler studies also reported associations between volumetric vascular parameters of normal fetal kidneys and gestational age [5]. However, these three-dimensional volumetric parameters differ from the two-dimensional renal ROI-based MV-Flow Ratio used here in both acquisition and calculation; they are not interchangeable and cannot share reference values.

MV-Flow imaging displays detectable microvascular flow signals within a renal ROI. We used the MV-Flow Ratio (%) generated directly by the scanner's quantitative analysis interface to describe the relative proportion of blood flow signals within a manually delineated renal region. This parameter provides an additional dimension of imaging information beyond renal artery spectral parameters, but its relationships with actual tissue perfusion, renal function and long-term renal outcomes remain to be validated. Joint assessment of these parameter types may therefore help characterise GDM-associated fetal renal ultrasound phenotypes without assigning unvalidated physiological or diagnostic meanings to them. Placental MV-Flow studies suggest that the technique can characterise microvascular imaging features, but findings in other organs cannot directly validate the physiological meaning of fetal renal MV-Flow Ratio [6].

Using ultrasound data obtained before initiation of GDM-related management, this study compared fetal renal artery PSV, S/D, PI, RI and MV-Flow Ratio between GDM and non-GDM pregnancies, assessed interobserver reliability and gestational-age-adjusted associations, and examined the robustness of the main findings through sensitivity analyses. Logistic regression models with fixed predictor sets and internal validation were also used

to explore whether RI and MV-Flow Ratio provide complementary information on GDM group membership.

Methods

Study design and participants

This single-centre prospective study was conducted at Shanghai First Maternity and Infant Hospital from January 2024 to August 2025. Following a pilot study, the research team developed the final protocol and eligibility criteria. The study was implemented after approval by the hospital's ethics review committee (approval number KS22306). All participants provided written informed consent before the first study-related ultrasound examination and were followed until delivery. This report presents only a baseline association analysis of ultrasound data obtained before initiation of GDM-related management; intervention effects and longitudinal prognosis were not analysed. The restriction of the gestational-age range and the combined models with fixed predictor sets were exploratory analyses determined during manuscript revision and should not be interpreted as preregistered confirmatory analyses.

Participants were women with singleton pregnancies undergoing prenatal ultrasonography at the hospital during the study period. Potential participants were screened consecutively according to the protocol. Eligible GDM cases were enrolled consecutively, whereas non-GDM controls were randomly selected from contemporaneous eligible non-GDM candidates. Final group allocation was based on the results of a 75-g oral glucose tolerance test (OGTT).

GDM was diagnosed according to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria [7]. Women underwent a 75-g OGTT at 24–28 weeks of gestation. GDM was diagnosed if any glucose concentration met or exceeded the respective threshold: fasting, 5.1 mmol/L; 1 h, 10.0 mmol/L; or 2 h, 8.5 mmol/L. When the first study

ultrasound preceded the OGTT, final group allocation was determined by the subsequent OGTT result. When multiple study records were available, ultrasound data obtained before initiation of GDM-related management were used. Fetal ultrasound parameters were not used to diagnose GDM or determine group allocation.

Eligibility criteria for the GDM group were OGTT-confirmed GDM, singleton pregnancy and no fetal structural abnormalities detected on ultrasound screening. The non-GDM group comprised contemporaneous women whose OGTT results did not meet the GDM diagnostic criteria, who had no history of pregestational diabetes, and who had singleton pregnancies without detected fetal structural abnormalities. Neither group was subject to an additional 10th–90th percentile or other percentile-based eligibility restriction for biparietal diameter, head circumference, abdominal circumference, femur length or other fetal size measures. Fetal biometry was recorded as part of routine ultrasonography.

Exclusion criteria were multiple pregnancy, previously diagnosed type 1 or type 2 diabetes, fetal structural abnormalities, pregnancy complications that could substantially affect fetal haemodynamics, including pre-eclampsia, and inadequate image quality precluding reliable measurements because of maternal habitus, fetal position, amniotic fluid conditions or other technical factors. Exclusion criteria were assessed at the time points specified in the original protocol, and reasons for exclusion were recorded.

Analysis population and data handling

After exclusion of records obtained after 28 weeks of gestation, the analysis included 84 women and their singleton fetuses examined at 20–28 weeks: 29 in the GDM group and 55 in the non-GDM group. Each woman–fetus pair constituted one independent analysis unit, using the summary ultrasound values obtained before initiation of GDM-related management. Bilateral kidneys, repeated measurements within an examination and subsequent follow-up examinations were not treated as independent cases. GDM status,

gestational age at examination, PSV, S/D, PI, RI and MV-Flow Ratio were complete for all participants in the primary analysis, which therefore used a complete-case dataset.

Ultrasound equipment and examination protocol

Examinations were performed using a HERA W10 ultrasound system (Samsung Medison Co., Ltd., Seoul, Republic of Korea) with a CA2-9A convex transducer. The integrated MV-Flow function was used to assess fetal renal microvascular flow signals. Two attending physicians, each with more than 5 years of experience in prenatal ultrasound diagnosis, independently performed the examinations while blinded to final GDM status and the other physician's measurements. Routine fetal ultrasonography was performed first to determine fetal position and measure biparietal diameter, head circumference, abdominal circumference and femur length, followed by renal artery pulsed-wave Doppler and MV-Flow examinations according to a standardised protocol.

Fetal renal artery Doppler measurements

Doppler acquisition followed the technical principles of the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) practice guidelines [2]. Two-dimensional and colour Doppler imaging were used to visualise the fetal kidneys, abdominal aorta and course of the renal arteries. A 1.0–2.0-mm sample volume was placed in the proximal renal artery near its origin from the abdominal aorta, with the insonation angle maintained at <30°. Once at least five consecutive, stable waveforms of consistent morphology had been obtained, the image was frozen and PSV, S/D, PI and RI were recorded.

Each physician measured both renal arteries, obtaining three measurements per side and calculating the mean for each parameter. Under a rule fixed in advance in the study protocol, the near-field kidney was always used to obtain that physician's measurement. The arithmetic mean of the corresponding results from the two physicians was then used in the

analysis. Selection of the near-field side did not depend on parameter magnitude or post hoc comparisons.

Technical examples of renal artery pulsed-wave Doppler (PW) are shown in Fig. 1A and C, illustrating sampling, consecutive waveforms and the scanner's measurement interface. The interface also displays end-diastolic velocity (EDV); these individual readings were not included as an additional statistical outcome. Figure 1 illustrates imaging techniques and does not present cases as examples from the 84-participant analysis sample.

MV-Flow imaging and Ratio measurements

After renal artery Doppler assessment, MV-Flow mode was activated and images were acquired in a sagittal plane clearly displaying the entire renal contour. The observer manually traced the ROI along the complete renal boundary so that it encompassed the whole kidney in the selected plane. The scanner's quantitative analysis software automatically analysed the MV-Flow blood flow signals within the ROI and returned a Ratio value. The original parameter name displayed by the software was retained and is consistently reported here as MV-Flow Ratio (%).

MV-Flow Ratio describes the relative proportion of detectable blood flow signals within the selected renal ROI. It is a device-derived relative quantitative imaging measure based on the image and ROI, rather than a direct measurement of absolute renal blood flow, volumetric flow per unit time or tissue perfusion. Each physician independently acquired images and measured both kidneys, with three repetitions per side averaged. Under the prespecified rule, near-field results were always selected, and the arithmetic mean of the two physicians' results was used for statistical analysis.

Technical examples of MV-Flow imaging are shown in Fig. 1B and D. The yellow outline delineates the renal ROI within which the scanner calculates and displays the Ratio. The individual sources and technical parameters of the panels in Fig. 1 do not substitute for the

analysis database or the acquisition records of the formal study. The GDM PW and MV-Flow panels are from different individuals and do not represent paired measurements.

Ratio/week was calculated by dividing the numerical percentage value of MV-Flow Ratio by gestational age at examination and was expressed as %/week. This exploratory derived measure was used only for supplementary description and exploratory analysis. It did not replace direct adjustment for gestational age in regression models and was not included in the main combined models.

Interobserver reliability

Both physicians independently acquired images and performed measurements in all 84 participants using the same protocol. Reliability was assessed using the average-measures intraclass correlation coefficient (ICC) from a two-way random-effects model with a consistency definition, together with its 95% confidence interval (CI) [8].

Statistical analysis

Continuous variables are presented as mean ± standard deviation, and unadjusted between-group comparisons used Welch's t test. Medians (25th and 75th percentiles) and Mann–Whitney U tests were used for supplementary description. Group sizes are reported as counts and percentages. Separate linear regression models were fitted for PSV, S/D, PI, RI and MV-Flow Ratio, each including GDM status and gestational age at examination. HC3 heteroscedasticity-robust standard errors [9] were used to estimate between-group mean differences, 95% CIs and P values. For the S/D model, the 95% CI and two-sided P value were calculated using a normal approximation. Differences were defined as GDM minus non-GDM, and absolute differences in MV-Flow Ratio were expressed in percentage points. P values for the five primary ultrasound parameters were adjusted using the Holm method [10]. The 95% CIs are parameter-specific pointwise intervals without simultaneous

confidence-interval adjustment. Because the models adjusted only for gestational age, the results were interpreted as gestational-age-adjusted associations.

Two logistic regression models with fixed predictor sets were developed using maternal OGTT-defined GDM status as the binary outcome. The base model included gestational age at examination (GA) and RI; the extended model additionally included MV-Flow Ratio. Odds ratios (ORs) were reported per 1-week increase in GA, 0.01 increase in RI and 1-percentage-point increase in MV-Flow Ratio. Model probabilities represent only the probability of GDM group membership within the sampled population.

Discrimination was assessed using the area under the receiver operating characteristic curve (AUC). Apparent AUCs and the 95% CI for their between-model difference were reported. Overall probability prediction error was assessed using the Brier score. Optimism and optimism-corrected performance were estimated using 1,000 bootstrap resamples. Resamples in which model fitting or performance calculation failed were excluded from correction, and the number of valid resamples was reported. Leave-one-out cross-validation (LOOCV) was also performed: each fixed model was refitted using the remaining 83 participants to generate a probability for the omitted participant, and these probabilities were pooled to calculate AUCs and Brier scores. A conventional same-sample DeLong significance test was not applied to the two LOOCV AUCs. Internal validation estimated optimism in the current modelling procedure and did not replace evaluation in an independent sample [11].

Internal calibration was assessed using recalibration intercepts and slopes; a slope <1 suggests that predicted probabilities may be too extreme [12]. LOOCV probabilities were divided into five groups of approximately equal size. Mean predicted probabilities were compared with observed GDM proportions within each group, with Wilson 95% CIs for the proportions. Grouped calibration describes performance only within the current sample.

Sensitivity analyses focused on RI and MV-Flow Ratio and were performed to assess the robustness of the primary findings. First, the primary multivariable regression models were repeated after restricting the analysis to pregnancies examined at 21–28 weeks of gestation (n = 82). Second, using the full study sample (n = 84), a quadratic term for gestational age (gestational age squared) was added to the original regression models to account for potential nonlinear associations with gestational age. The same covariates as those included in the primary adjusted models were retained in both sensitivity analyses. These analyses primarily assessed the stability of the direction and magnitude of the adjusted between-group differences and their 95% confidence intervals. All tests were two-sided, and P values for RI and MV-Flow Ratio were adjusted for multiple comparisons using the Holm method. A two-sided Holm-adjusted P value < 0.05 was considered statistically significant.

Results

Analysis population and distributions of ultrasound parameters

Of the 84 participants, 29 (34.5%) were in the GDM group and 55 (65.5%) in the non-GDM group. Gestational age at examination was 23.79 ± 1.90 and 23.42 ± 2.35 weeks, respectively (P = 0.432). Both participants examined at 20 weeks belonged to the non-GDM group. Unadjusted comparisons showed lower S/D, PI and RI and higher MV-Flow Ratio and Ratio/week in the GDM group, with no clear difference in PSV. Unadjusted mean differences were −4.04 for S/D, −0.065 for RI and 14.47 percentage points for MV-Flow Ratio (Table 1 and Fig. 2). Medians and Mann–Whitney U test results are presented in Supplementary Table S1.

Table 1 Gestational age at examination and ultrasound parameters by group

Parameter	Non-GDM (n=55)	GDM (n=29)	P value
Maternal age (years)	31.76 ± 4.79	32.84 ± 5.06	0.291
GA at examination (weeks)	23.79 ± 1.90	23.42 ± 2.35	0.432
Birth weight (g)	3231.5 ± 534.2	3153.8 ± 589.7	0.512
GA at delivery (weeks)	38.51 ± 1.42	38.12 ± 1.68	0.238
Renal artery Doppler parameters			
PSV (cm/s)	45.65±13.78	46.50±13.14	0.783
S/D	11.95±4.66	7.91±4.45	<0.001
PI	2.34±0.26	2.00±0.32	<0.001
RI	0.903±0.042	0.838±0.060	<0.001
MV-Flow Ratio (%)	40.13±8.64	54.60±8.48	<0.001
Ratio/week (%/week)	1.73±0.42	2.31±0.39	<0.001

Values are mean ± standard deviation. P values are from unadjusted Welch's t tests. Ratio/week is an exploratory derived measure. PSV, peak systolic velocity; S/D, peak systolic-to-end-diastolic velocity ratio; PI, pulsatility index; RI, resistive index; BMI, body mass index.

Interobserver reliability

Measurements from both physicians and average-measures ICCs are presented in Supplementary Table S3. The ICC was 0.895 (95% CI 0.843–0.931) for MV-Flow Ratio and 0.903 (0.854–0.936) for RI. ICCs for PSV, S/D and PI were 0.831 (0.751–0.887), 0.874 (0.812–0.916) and 0.887 (0.831–0.925), respectively; the ICC for Ratio/week was 0.921 (0.881–0.948).

Gestational-age-adjusted between-group associations

After adjustment for gestational age at examination, RI was 0.065 lower in the GDM group than in the non-GDM group (95% CI −0.091 to −0.040), whereas MV-Flow Ratio was 14.53 percentage points higher (95% CI 10.57 to 18.48; both Holm-adjusted P < 0.001). Adjusted differences in S/D and PI were −4.15 (95% CI −6.23 to −2.07) and −0.331, respectively (both Holm-adjusted P < 0.001). The adjusted PSV difference was 0.15 cm/s (95% CI −5.45 to 5.75; P = 0.958) (Table 2).

Table 2 Between-group mean differences adjusted for gestational age at examination

Parameter and difference unit	Adjusted difference (95% CI)	P value	Holm-adjusted P value
PSV (cm/s)	0.15 (−5.45 to 5.75)	0.958	0.958
S/D	−4.15 (−6.23 to −2.07)	<0.001	<0.001
PI	−0.331 (−0.472 to −0.191)	<0.001	<0.001
RI	−0.065 (−0.091 to −0.040)	<0.001	<0.001
MV-Flow Ratio difference (percentage points)	14.53 (10.57 to 18.48)	<0.001	<0.001

Differences are GDM minus non-GDM. A separate linear model including GDM status and gestational age was fitted for each parameter, using HC3 robust standard errors. Holm correction was applied across the five primary ultrasound parameters. The 95% CIs were not adjusted for simultaneous coverage.

Exploratory combined models and internal validation

In the extended model, the OR was 0.850 per 0.01 increase in RI (95% CI 0.743–0.971; P = 0.017), 1.220 per 1-percentage-point increase in MV-Flow Ratio (95% CI 1.100–1.353; P < 0.001) and 1.160 per 1-week increase in GA (95% CI 0.847–1.589; P = 0.356) (Table 3).

Table 3 Extended logistic regression model with a fixed predictor set

Variable and OR increment	Coefficient on original scale	OR (95% CI)	P value
Intercept	0.760236	—	0.920
GA, per 1-week increase	0.148388	1.160 (0.847–1.589)	0.356
RI, per 0.01 increase	−16.274707	0.850 (0.743–0.971)	0.017
MV-Flow Ratio, per 1-percentage-point increase	0.199064	1.220 (1.100–1.353)	<0.001

The outcome is maternal GDM group membership. Coefficients are provided on the original scales for use in the model equation; the RI OR is additionally expressed per 0.01 increase. GA, gestational age at examination.

The extended model equation was logit(p) = 0.760236 + 0.148388 × GA − 16.274707 × RI + 0.199064 × Ratio. The base model equation was logit(p) = 19.165636 + 0.082691 × GA − 24.842539 × RI. For both models, probabilities were calculated as p = 1/[1 + exp(−logit(p))]. Ratio denotes the numerical percentage value of MV-Flow Ratio; for example, 54.60% is entered as 54.60. RI is entered on its original scale, and GA is expressed in weeks.

Apparent AUCs were 0.826 (95% CI 0.715–0.938) for the base model and 0.924 (95% CI 0.870–0.978) for the extended model. The increase in apparent AUC was 0.098 (95% CI −0.004 to 0.199; P = 0.059). Incremental discriminatory value was therefore not established, although these findings also do not establish equivalence between the models. Bootstrap optimism-corrected AUCs were 0.815 and 0.910, and LOOCV AUCs were 0.800 and 0.902, respectively (Table 4 and Fig. 3).

Table 4 Discrimination and probability prediction error of the fixed models

Performance measure	Base model GA + RI	Extended model GA + RI + Ratio
Apparent AUC (95% CI)	0.826 (0.715–0.938)	0.924 (0.870–0.978)
Bootstrap optimism-corrected AUC	0.815	0.910
LOOCV AUC	0.800	0.902
Apparent Brier score	0.147	0.112
Bootstrap optimism-corrected Brier score	0.158	0.131
LOOCV Brier score	0.158	0.132
Valid/planned bootstrap resamples	999/1000	998/1000

Ratio denotes MV-Flow Ratio (%). Correction refers to bootstrap optimism correction, not bootstrap confidence intervals. Lower Brier scores indicate lower overall probability prediction error. LOOCV, leave-one-out cross-validation.

Of the 1,000 bootstrap resamples, model fitting or calculation failed in one resample for the base model and two for the extended model. For the extended model, the bootstrap-corrected recalibration intercept was −0.070 and the slope was 0.831; the LOOCV recalibration intercept was −0.147 and the slope was 0.724. Slopes below 1 suggest a tendency towards overly extreme predicted probabilities. The model probability p represents GDM group membership within the current sampled population and cannot be directly extrapolated to absolute GDM risk in the general antenatal population. Grouped LOOCV calibration for the extended model is shown in Fig. 4.

Sensitivity analyses

Sensitivity analyses yielded results consistent with the primary analysis. After restricting the analysis to pregnancies at 21–28 weeks of gestation (n = 82), the adjusted between-group difference in renal artery RI was −0.065 (95% CI, −0.090 to −0.039; P <

0.001), while the adjusted difference in MV-Flow Ratio was 14.21 percentage points (95% CI, 10.22 to 18.20; P < 0.001). In a separate sensitivity analysis using the full sample (n = 84), a quadratic term for gestational age was additionally incorporated into the model. The corresponding adjusted between-group differences were −0.062 (95% CI, −0.093 to −0.030; P < 0.001) for RI and 14.61 percentage points (95% CI, 10.13 to 19.09; P < 0.001) for MV-Flow Ratio. These findings were consistent in direction and magnitude with the primary analysis, supporting the robustness of the results. (Supplementary Table S2).

Discussion

In this study of pregnancies examined at 20–28 weeks before initiation of GDM-related management, the GDM group had lower fetal renal artery S/D, PI and RI and higher MV-Flow Ratio, while no clear between-group difference was observed in PSV. These findings indicate that fetal renal ultrasound features associated with maternal GDM status involve both the spectral characteristics of the supplying artery and the relative proportion of detectable intrarenal blood flow signals. By extending observation to the fetal kidneys in the second trimester, this study provides additional evidence on GDM-associated fetal organ phenotypes.

These findings are of interest because the two types of ultrasound measures provide different dimensions of information. Renal artery Doppler describes blood flow velocity in the supplying artery and its variation throughout the cardiac cycle, whereas MV-Flow Ratio describes the relative proportion of detectable blood flow signals within the renal ROI. The absence of a clear PSV difference, alongside differences in resistance-related indices and Ratio, suggests that peak systolic velocity alone may not fully characterise fetal renal blood flow. Joint assessment may enable more detailed characterisation of GDM-associated fetal

renal ultrasound phenotypes, but the observed features cannot yet be defined as renal hyperperfusion or renal injury.

This study also adds evidence from an earlier gestational period. Brennan et al. established fetal renal artery Doppler reference ranges using longitudinal examinations of low-risk pregnancies, providing a gestational-age-specific context for interpretation [13]. Jamal et al. found higher fetal renal artery RI, PI and S/D in GDM pregnancies at 37–40 weeks than in controls, opposite to the directions observed here [3]. Differences in gestational age at examination, maternal metabolic status, clinical management and acquisition methods may affect comparability across studies. This inconsistency suggests that GDM-associated fetal renal features may depend on the context of observation, and findings from one stage should not be generalised to the entire pregnancy. Whether renal blood flow characteristics in the same fetus change with advancing gestation and maternal glycaemia is particularly worthy of further investigation. The present findings inform this longitudinal research question, but trajectories cannot be inferred from two independent cohorts.

MV-Flow Ratio extends assessment beyond conventional spectral Doppler. Huang et al. demonstrated different gestational-age patterns among microvascular parameters using three-dimensional MV-Flow of the fetal kidneys, supporting further investigation of intrarenal flow imaging [4]. However, three-dimensional parameters and the two-dimensional Ratio used here are measured differently and cannot share reference values. In addition, Hokke et al. found no clear difference in third-trimester fetal kidney volume between treated GDM pregnancies and controls [14]. This does not necessarily contradict our blood flow findings, because organ size, arterial spectra and intrarenal flow signals represent different levels of observation. Combining these measures may improve understanding of fetal renal phenotypes compared with assessment of a single parameter.

Song et al. reported differences in placental MV-Flow measurements between GDM and non-GDM pregnancies [15]. This supports the broader investigation of microvascular ultrasound phenotypes in GDM, but placental measurements cannot validate the physiological interpretation of fetal renal MV-Flow Ratio. Experimental evidence also provides a rationale for investigating renal development: in a mouse model, maternal glucose intolerance was associated with reduced offspring nephron endowment and greater glomerular volume in adulthood, despite preserved renal function [16]. These animal findings do not establish nephron loss or renal injury in the fetuses examined in the present study.

The potential value of this study lies in identifying candidate observational measures linking maternal glucose metabolism with fetal organ phenotypes. Future studies could examine whether RI and MV-Flow Ratio are associated with maternal glycaemic levels, fetal growth and postnatal renal measures, to determine which changes persist and which are related to clinical outcomes.

In the Generation R Study, fetal kidney volume was examined in relation to fetal growth and blood flow characteristics, supporting the value of assessing renal size alongside haemodynamic measures [17]. Subsequent longitudinal research found associations between fetal kidney volume and kidney size and function in school-aged children [18]. These observations support postnatal follow-up in future studies, but neither study establishes the prognostic significance of fetal renal MV-Flow Ratio.

This study is limited by its single-centre design, small sample and incomplete control of confounding. The principal associations remained directionally consistent after modification of the gestational-age range; nevertheless, the present findings are best regarded as a basis for further research. Standardised acquisition, larger samples and longitudinal follow-up are

needed to clarify the physiological meaning of these ultrasound features and determine whether they provide useful information for fetal surveillance.

Technical and modelling limitations also warrant consideration. In a flow-phantom study, quantitative three-dimensional power Doppler indices varied with machine settings, particularly gain, signal power and pulse repetition frequency [19]. Although that study did not evaluate MV-Flow, it underscores the need to investigate setting dependence and standardise acquisition in future MV-Flow studies. For the exploratory logistic models, future sample-size planning should account for the number of candidate predictor parameters, anticipated model performance and the need to limit overfitting [20]. Internal validation in this small sample does not establish clinical utility or replace independent validation.

Conclusions

In 84 singleton pregnancies examined at 20–28 weeks of gestation before initiation of GDM-related management, maternal GDM status was associated with lower fetal renal artery S/D, PI and RI and higher MV-Flow Ratio, with no clear difference in PSV. These findings suggest that GDM pregnancies may exhibit distinct second-trimester renal haemodynamic and intrarenal microvascular ultrasound phenotypes. They provide candidate measures and testable hypotheses for incorporating fetal renal ultrasonography into research on GDM-associated fetal phenotypes.

Abbreviations

AUC: Area under the receiver operating characteristic curve; BMI: Body mass index; CI: Confidence interval; EDV: End-diastolic velocity; GA: Gestational age at examination; GDM: Gestational diabetes mellitus; HAPO: Hyperglycemia and Adverse Pregnancy Outcome; IADPSG: International Association of Diabetes and Pregnancy Study Groups; ICC: Intraclass correlation coefficient; ISUOG: International Society of Ultrasound in

Obstetrics and Gynecology; LOOCV: Leave-one-out cross-validation; OGTT: Oral glucose tolerance test; OR: Odds ratio; PI: Pulsatility index; PSV: Peak systolic velocity; PW: Pulsed-wave Doppler; RI: Resistive index; ROC: Receiver operating characteristic; ROI: Region of interest; S/D: Peak systolic-to-end-diastolic velocity ratio.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Review Committee of Shanghai First Maternity and Infant Hospital (approval number KS22306). All participants provided written informed consent before the first study-related ultrasound examination.

Consent for publication

Written informed consent for publication was obtained from the patients.

Availability of data and materials

The datasets generated and/or analysed during the current study are available from the corresponding authors on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

KL contributed to study conception and design, data collection, data and statistical analyses, interpretation of the data, and drafting of the manuscript. HY and CS and FH contributed to data collection and ultrasound image acquisition. YL contributed to interpretation of the findings and revision of the manuscript for important intellectual content. MR supervised the study and critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript. KL and HY contributed equally to this work and share first authorship. YL and MR are co-corresponding authors.

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References

[1] HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med. 2008;358(19):1991–2002. doi:10.1056/NEJMoa0707943.

[2] Bhide A, Acharya G, Baschat A, et al. ISUOG Practice Guidelines (updated): use of Doppler velocimetry in obstetrics. Ultrasound Obstet Gynecol. 2021;58(2):331–339. doi:10.1002/uog.23698.

[3] Jamal AS, Naemi M, Eslamian L, Marsoosi V, Moshfeghi M, Nurzadeh M, et al. The association between fetal renal artery indices in late pregnancy and birth weight in gestational diabetes mellitus: A cohort study. Int J Reprod Biomed. 2022;20(1):21–28. doi:10.18502/ijrm.v20i1.10405.

[4] Huang C, Zhang L, Jiang Y, Zheng Q, Lei T, Du L, et al. Evaluation of normal and abnormal fetal renal microvascular flow characteristics of three-dimensional MV-flow imaging. Early Hum Dev. 2024;199:106149. doi:10.1016/j.earlhumdev.2024.106149.

[5] Chang CH, Yu CH, Ko HC, Chen WC, Chang FM. Quantitative three-dimensional power Doppler sonography for assessment of the fetal renal blood flow in normal gestation. Ultrasound Med Biol. 2003;29(7):929–933. doi:10.1016/S0301-5629(03)00886-X.

[6] Chen X, Wei X, Zhao S, Huang H, Wang W, Qiu J, et al. Characterization of placental microvascular architecture by MV-Flow imaging in normal and fetal growth-restricted pregnancies. J Ultrasound Med. 2021;40(8):1533–1542. doi:10.1002/jum.15531.

[7] International Association of Diabetes and Pregnancy Study Groups Consensus Panel. International Association of Diabetes and Pregnancy Study Groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care. 2010;33(3):676–682. doi:10.2337/dc09-1848.

[8] Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J Chiropr Med. 2016;15(2):155–163. doi:10.1016/j.jcm.2016.02.012.

[9] MacKinnon JG, White H. Some heteroskedasticity-consistent covariance matrix estimators with improved finite sample properties. J Econom. 1985;29(3):305–325. doi:10.1016/0304-4076(85)90158-7.

[10] Holm S. A simple sequentially rejective multiple test procedure. Scand J Stat. 1979;6(2):65–70.

[11] Steyerberg EW, Harrell FE Jr, Borsboom GJ, Eijkemans MJ, Vergouwe Y, Habbema JD. Internal validation of predictive models: efficiency of some procedures for logistic regression analysis. J Clin Epidemiol. 2001;54(8):774–781. doi:10.1016/S0895-4356(01)00341-9.

[12] Van Calster B, McLernon DJ, van Smeden M, Wynants L, Steyerberg EW. Calibration: the Achilles heel of predictive analytics. BMC Med. 2019;17:230. doi:10.1186/s12916-019-1466-7.

[13] Brennan S, Watson D, Schneider M, Rudd D, Kandasamy Y. Fetal renal artery blood flow – Normal ranges. Ultrasound. 2022;30(1):62–71. doi:10.1177/1742271X211022402.

[14] Hokke S, de Zoysa N, Carr BL, Abruzzo V, Coombs PR, Allan CA, et al. Normal foetal kidney volume in offspring of women treated for gestational diabetes. Endocrinol Diabetes Metab. 2019;2(4):e00091. doi:10.1002/edm2.91.

[15] Song J, Li X, Zhu C, Sun H, Ren Y. MV-Flow imaging for evaluating the placental function of gestational diabetes mellitus. Front Endocrinol (Lausanne). 2025;16:1674480. doi:10.3389/fendo.2025.1674480.

[16] Hokke S, Arias N, Armitage JA, Puelles VG, Fong K, Geraci S, et al. Maternal glucose intolerance reduces offspring nephron endowment and increases glomerular volume in adult offspring. Diabetes Metab Res Rev. 2016;32(8):816–826. doi:10.1002/dmrr.2805.

[17] Verburg BO, Geelhoed JJM, Steegers EAP, Hofman A, Moll HA, Witteman JCM, et al. Fetal kidney volume and its association with growth and blood flow in fetal life: The Generation R Study. Kidney Int. 2007;72(6):754–761. doi:10.1038/sj.ki.5002420.

[18] Kooijman MN, Bakker H, van der Heijden AJ, Hofman A, Franco OH, Steegers EAP, et al. Childhood kidney outcomes in relation to fetal blood flow and kidney size. J Am Soc Nephrol. 2014;25(11):2616–2624. doi:10.1681/ASN.2013070746.

[19] Raine-Fenning NJ, Nordin NM, Ramnarine KV, Campbell BK, Clewes JS, Perkins A, et al. Evaluation of the effect of machine settings on quantitative three-dimensional power Doppler angiography: an in-vitro flow phantom experiment. Ultrasound Obstet Gynecol. 2008;32(4):551–559. doi:10.1002/uog.6138.

[20] Riley RD, Snell KIE, Ensor J, Burke DL, Harrell FE Jr, Moons KGM, et al. Minimum sample size for developing a multivariable prediction model: PART II – binary and time-to-event outcomes. Stat Med. 2019;38(7):1276–1296. doi:10.1002/sim.7992.

Supplementary tables

Supplementary Table S1 Comparison of median gestational age and ultrasound parameters between groups

Parameter	Non-GDM (n=55)	GDM (n=29)	P value
GA at examination (weeks)	22.00 (22.00, 25.00)	23.00 (23.00, 25.00)	0.134
PSV (cm/s)	43.22 (34.95, 56.43)	47.00 (37.00, 54.99)	0.767
S/D	11.10 (9.59, 14.14)	6.33 (5.00, 8.50)	<0.001
PI	2.31 (2.19, 2.49)	2.00 (1.83, 2.16)	<0.001
RI	0.910 (0.895, 0.930)	0.830 (0.800, 0.880)	<0.001
MV-Flow Ratio (%)	40.00 (34.50, 45.55)	53.80 (48.80, 58.00)	<0.001
Ratio/week (%/week)	1.76 (1.45, 2.02)	2.31 (2.09, 2.52)	<0.001

Values are medians (25th, 75th percentiles). P values are from unadjusted Mann–Whitney U tests.

Supplementary Table S2 Sensitivity analyses of between-group differences in RI and MV-Flow Ratio

Sensitivity analysis	n	Adjusted difference in RI (95% CI)	Holm-adjusted P value	Adjusted difference in MV-Flow Ratio, percentage points (95% CI)	Holm-adjusted P value
Restricted to 21–28 weeks of gestation	82	−0.065 (−0.090 to −0.039)	<0.001	14.21 (10.22 to 18.20)	<0.001
Model including a quadratic term for gestational age	84	−0.062 (−0.093 to −0.030)	<0.001	14.61 (10.13 to 19.09)	<0.001

Note: RI, resistance index; CI, confidence interval. MV-Flow Ratio is expressed as a percentage; therefore, between-group differences are reported in percentage points. P values were adjusted for multiple comparisons using the Holm method.

Supplementary Table S3 Measurements from the two physicians and average-measures ICCs

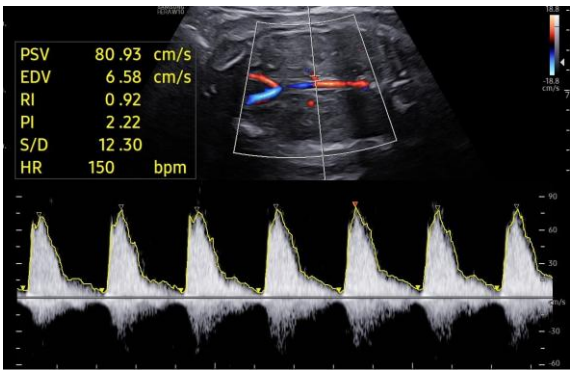
Parameter	Physician A	Physician B	ICC (95% CI)
PSV (cm/s)	45.93 ± 13.48	45.94 ± 13.49	0.831 (0.751–0.887)
S/D	10.54 ± 4.94	10.55 ± 4.95	0.874 (0.812–0.916)
PI	2.22 ± 0.33	2.22 ± 0.33	0.887 (0.831–0.925)
RI	0.882 ± 0.058	0.881 ± 0.057	0.903 (0.854–0.936)
MV-Flow Ratio (%)	45.13 ± 11.00	45.13 ± 10.99	0.895 (0.843–0.931)
Ratio/week (%/week)	1.93 ± 0.50	1.93 ± 0.49	0.921 (0.881–0.948)

Physician measurements are mean ± standard deviation. ICCs were calculated using a two-way random-effects model, a consistency definition and the average measurements of the two physicians, encompassing independent image acquisition and measurement.

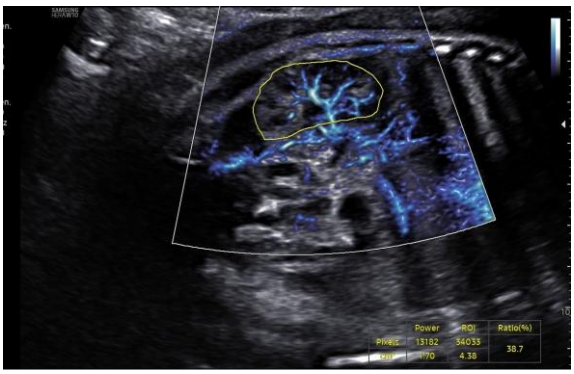
Figures and legends

Fig. 1 Technical examples of fetal renal PW Doppler and MV-Flow imaging

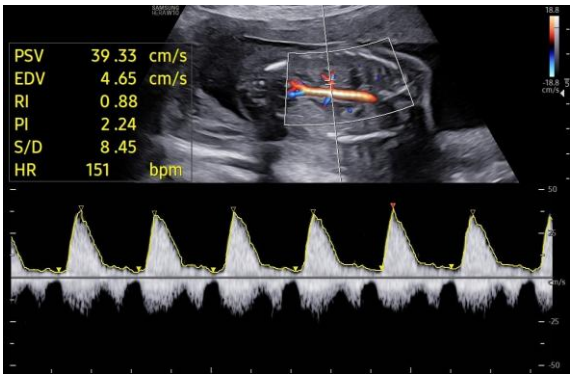
A Non-GDM renal artery PW Doppler



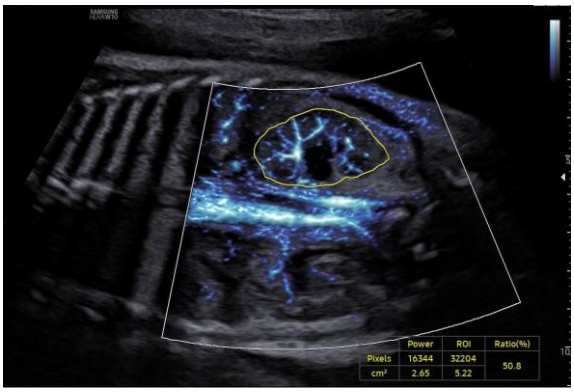
B Non-GDM MV-Flow



C GDM renal artery PW Doppler



D GDM MV-Flow



A and B illustrate non-GDM examinations; C and D illustrate GDM examinations. A: PSV 80.93 cm/s, EDV 6.58 cm/s, RI 0.92, PI 2.22 and S/D 12.30. B: Ratio 38.7%. C: PSV 39.33 cm/s, EDV 4.65 cm/s, RI 0.88, PI 2.24 and S/D 8.45. D: Ratio 50.8%. PW, pulsed-wave Doppler; EDV, end-diastolic velocity. The yellow contours in the MV-Flow images delineate the ROIs. Ratio is the relative proportion of blood flow signals reported by the scanner within the ROI.

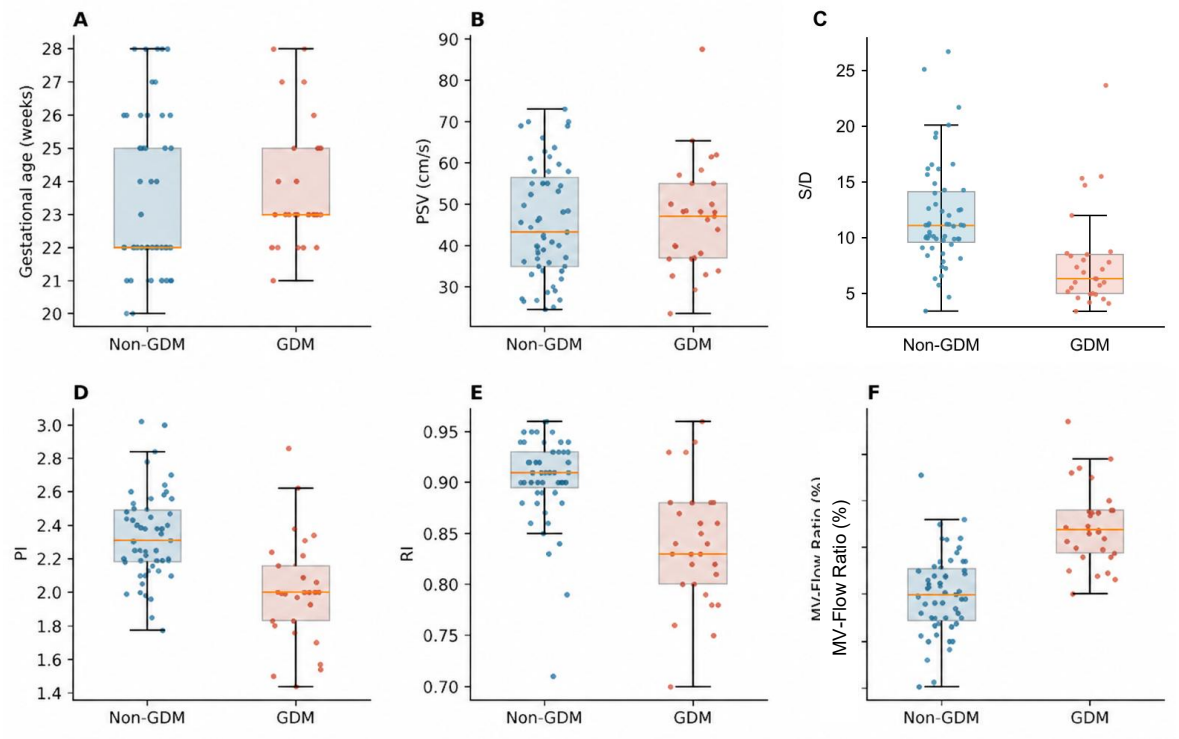


Fig. 2 Between-group distributions of gestational age and ultrasound parameters

Panels A–F show gestational age at examination, PSV, S/D, PI, RI and MV-Flow Ratio (%), respectively. Each point represents the final summary value for one woman and her singleton fetus. Boxes indicate the interquartile range, central lines indicate medians, and whiskers extend to observations within 1.5 times the interquartile range. GDM, n = 29; non-GDM, n = 55.

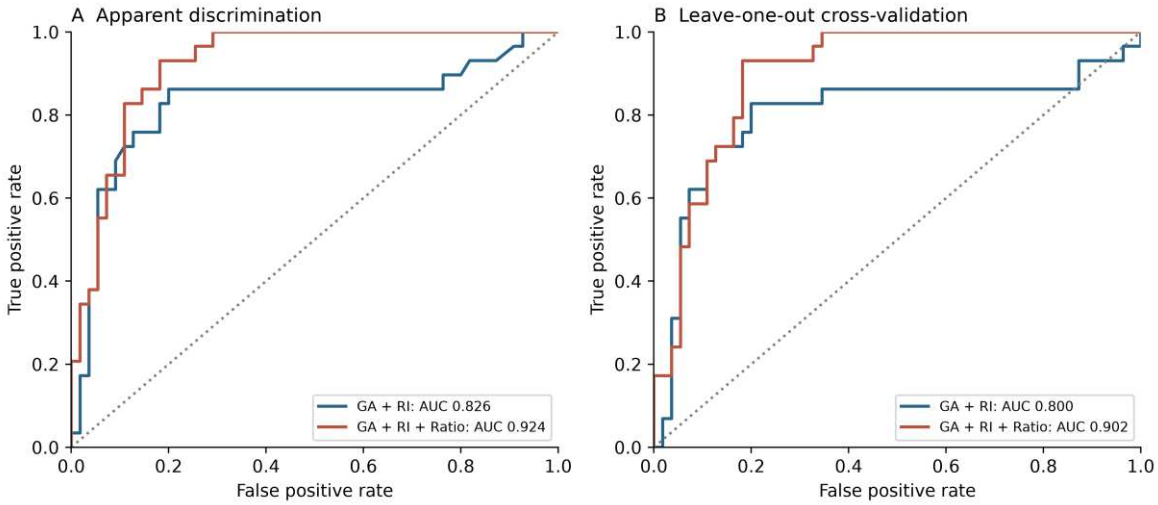


Fig. 3 ROC curves for the fixed models

A shows apparent discrimination; B shows leave-one-out cross-validated discrimination. The base model includes GA + RI, and the extended model includes GA + RI + MV-Flow Ratio. GA denotes gestational age at examination. Both models were refitted with fixed predictor sets, and all held-out predictions were obtained from training samples excluding the corresponding participant. The ROC outcome is maternal OGTT-defined GDM status. These curves do not represent clinical GDM screening performance or diagnostic performance for fetal renal hyperperfusion or renal injury.

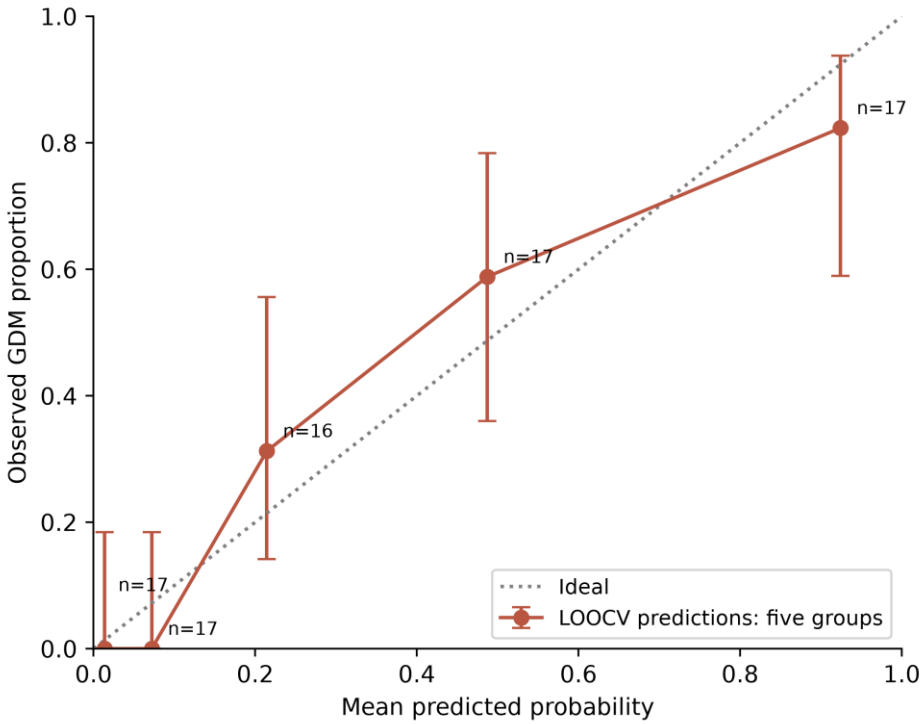


Fig. 4 Grouped leave-one-out calibration of the extended model

Leave-one-out predicted probabilities were divided into five approximately equal-sized groups. Points show mean predicted probabilities and observed GDM proportions; error bars indicate Wilson 95% CIs, and numbers indicate group sizes. The plot describes calibration within the current sampled population and cannot directly represent the general antenatal population.