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First-trimester bleeding: Can ultrasound and biochemical developmental markers deepen our understanding of threatened miscarriage?

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ABSTRACT

Introduction: Understanding the gestational behaviour of ultrasound and biochemical markers in early pregnancy is essential for improving clinical prediction. This study applies statistical modelling to explore how these markers evolve across gestation, with a particular focus on comparing their trajectories in women presenting with threatened miscarriage (TM) versus those with asymptomatic pregnancies.

Methods: A prospective case–control study was conducted with 177 participants, divided into a study group (TM group) and a control group. Dynamic markers were modelled against gestational age using linear, quadratic, and segmented linear regressions. Statistical comparisons of model coefficients were used to evaluate differences both within pregnancy outcome groups (live birth vs loss) and between study and control cohorts.

Results: Several markers, including fetal heart rate (FHR), trophoblast thickness, yolk sac (YS) wall thickness, beta-human chorionic gonadotropin (β-hCG), and mean amniotic sac diameter (MASD), were best represented by segmented linear models, reflecting distinct phase-dependent changes across gestational age. Linear models fit other markers such as progesterone, mean gestational sac diameter, and the soluble fms-like tyrosine kinase-1 to placental growth factor ratio. Statistically significant differences in regression coefficients were observed within the study group between outcome categories, as well as across groups, particularly for FHR, β-hCG, YS wall thickness and MASD. These differences support the notion that TM follows a physiologically distinct developmental trajectory compared to normal pregnancy.

Conclusion: First-trimester markers in women with TM follow gestational trajectories that diverge from those in asymptomatic pregnancies. This physiological distinction underscores the need for tailored modelling to better reflect the unique risk profile in TM.

Implications for practice: TM-specific models are crucial for accurate risk assessment and personalised care. Applying general thresholds may misclassify risk in TM, leading to unnecessary intervention or missed opportunities for reassurance.

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Introduction

Threatened miscarriage (TM) is a common first-trimester complication, affecting approximately 10–30 % of pregnancies globally.[1–11] It presents as vaginal bleeding (VB) in an otherwise viable pregnancy in early gestation and is associated with a significantly increased risk of pregnancy loss, particularly within the first 12 weeks. Although over half of pregnancies affected by TM continue to term, affected women are at an increased risk of adverse obstetric outcomes, including spontaneous pregnancy loss, preterm labour, fetal growth restriction (FGR), placental abruption, and preeclampsia.[12,13] The pathophysiological mechanisms underlying these complications are complex, involving factors such as impaired placentation, suboptimal maternal–fetal immune interactions, and inflammatory processes that contribute to poor pregnancy outcomes.[14,15]

Despite its prevalence and clinical significance, accurately predicting outcomes in TM remains challenging. The physiological trajectory of early pregnancy is marked by rapid changes in both ultrasound (US) and biochemical (BC) markers, many of which reflect fetal development and placental function.[16,17] These markers, such as fetal heart rate (FHR), mean gestational sac diameter (MGSD), serum progesterone, β-human chorionic gonadotropin (β-hCG) and others, evolve with gestational age (GA) in ways that are not always linear or uniform.

In clinical practice, these markers are often interpreted using thresholds (cut-offs) or ranges. However, modelling changes in these markers over time with respect to GA has been shown to better distinguish between typical and high-risk pregnancies. The rate and shape of marker progression can differ markedly between healthy pregnancies and those complicated by TM. For instance, FHR typically rises sharply in early weeks,[18,19] reaching a peak at eight weeks, before plateauing for the remainder of the trimester. β-hCG follows a similar non-linear pattern with an early rapid rise that flattens as placental function takes over.[20,21] In pregnancies complicated by TM, these patterns are often altered, either blunted, delayed, or irregular, indicating underlying physiological disruption.[22,23] These trends reflect key processes such as cardiac maturation, placental development, and hormonal regulation.[24,25]

This study aims to statistically model the gestational behaviour of both US and BC markers in a prospective cohort of women, comparing symptomatic (TM) and asymptomatic groups. By applying linear, quadratic, and segmented linear regression models, we seek to identify the most accurate representations of marker behaviour across pregnancy outcomes and between clinical cohorts. The ultimate goal is to establish whether distinct modelling approaches are necessary for TM versus normal pregnancies, and to explore the potential implications of such differences.

Methods

This study employed a prospective case–control design to explore the gestational behaviour of US and BC markers in early pregnancy. Participants were recruited from the national, state hospital in Malta over an 18-month period and were classified into two groups: women presenting with symptoms of TM (study group, S) and non-bleeding women presenting with pregnancy-related complaints such as abdominal pain and/or hyperemesis (control group, C). All participants had a confirmed singleton, live, normally sited first-trimester pregnancy (5^{+0} to 12^{+6} weeks' gestation). Outcomes were determined through hospital records and supplemented via telephone follow-up where necessary. These were categorised as live birth (1) or pregnancy loss (2) for both groups, with subgroups S1 and S2 (study group) and C1 and C2 (control group) denoting outcome-based classifications.

Data collection included transvaginal US measurements of developmental markers, as well as pathological structures such as intrauterine haematomas and uterine leiomyomas (fibroids), which may also exhibit dynamic growth alongside fetal development. Other markers which are static with respect to fetal growth were also taken. To mitigate inter-practitioner variability and enhance the reliability of the data, all USSs were performed by one experienced US practitioner. Furthermore, to maintain accuracy and standardisation, all participants were scanned using the same General Electric (GE) Voluson S10 US machine, which undergoes annual quality assurance testing. BC markers were assessed through blood sampling, collected via venipuncture and tested using standard clinical assays. The complete list of markers is summarised in Table 1.

Each dynamic marker was modelled independently against GA using three approaches: linear regression, quadratic regression, and segmented linear regression (piecewise linear fits). GA was calculated based on crown-rump length (CRL), due to its established accuracy in early pregnancy dating.[26,27] Linear models assumed a constant rate of change, while quadratic models accounted for curvature. Segmented models allowed different linear slopes before and after a fixed cutoff at 56 days (eight weeks' gestation), a time-point chosen to reflect a biologically meaningful milestone in early embryonic development. This approximately corresponds to the transition from the embryonic to fetal phase, during which multiple physiological processes shift in pace or trajectory, including a peak in FHR,[18,19] rapid expansion in CRL,[26] regression of the yolk sac (YS),[28] early changes in placental perfusion and villous development,[16,17,29] and inflection in β-hCG rise.[20,21] While the fixed breakpoint allows comparability across groups and markers, it is acknowledged that marker-specific inflection points may vary. Future work may incorporate data-driven or adaptive breakpoint estimation to refine model accuracy. Higher order polynomial fits were not considered due to their inherent risk of divergence, especially at the limits of the dataset range.

The goodness-of-fit of each model was assessed using the coefficient of determination (R^2) and residual distribution plots. Predictor significance was assessed via F-tests, with P-values <0.05 considered statistically meaningful. To formally evaluate whether more complex models offered statistically superior fits, Chi-square based likelihood ratio tests (LRT) were conducted. LRT compared nested models to determine whether improvements in model fit justified additional complexity. Final model selection was based on both statistical significance ($P < 0.05$) and parsimony, with

Table 1
The complete list of markers measured.

Variable	Variable Name	Units
Static		
Cervical length	CervicalL	mm
Max corpus luteum diameter	MCLD	mm
Body-mass index	BMI	kg/m^2
Dynamic		
Fetal heart rate	FHR	bpm
YS diameter	YSD	mm
YS wall thickness	YS wall	mm
Mean GS diameter	MGSD	mm
Crown-rump length (gestational age)	CRL	mm (days)
Trophoblast thickness	TT	mm
Mean AS diameter	MASD	mm
Max IUH diameter	MIUHD	mm
Mean fibroid diameter	MUFD	mm
Beta human chorionic gonadotropin	BHCG	IUL
Progesterone	Prog	ngml
fms-like tyrosine kinase 1/Placental growth factor	sFIT-1:PlGF	

preference given to simpler models unless higher-order models provided significantly better explanatory power.

Z-tests with *P*-values <0.05 were employed to compare model coefficients both within pregnancy outcome groups and across study cohorts, providing a detailed assessment of how early pregnancy markers varied in different clinical contexts. Within-group comparisons (i.e., live birth vs. pregnancy loss within the study or control group) were conducted to evaluate whether the relationship between each marker and GA differed according to pregnancy outcome. This allowed for identification of markers that may be predictive of pregnancy loss within each population. Across-group comparisons (i.e., study vs. control group) were performed to determine whether pregnancies affected by TM displayed distinct developmental trajectories compared to clinically normal pregnancies. This helped assess whether TM was associated with broader physiological disruptions, independent of pregnancy outcome.

Static variables, which did not require regression modelling, were analysed using a two-step approach to compare differences between pregnancy outcome groups. Normality was assessed via the Shapiro–Wilk test. If normal, Welch's t-test compared means; if not, the Mann–Whitney U test compared ranks to test for association between groups. Non-normal distributions were summarised using median and interquartile range.

Variables that could not be reliably fitted due to sparse data or non-significance were excluded from further modelling. All statistical analyses were performed in R (version 4.4.3).

Results

Data were collected from 181 participants, resulting in 177 valid records after exclusions due to loss of follow-up. Of these, 118 women presented with signs of TM and were assigned to the study group, while 59 asymptomatic women with viable first-trimester pregnancies formed the control group. In the study group, 91 participants (77 %) progressed to live birth, while 27 (23 %) experienced pregnancy loss. In the control group, 53 participants (90 %) had a live birth and six (10 %) experienced pregnancy loss. With this information, the odds of pregnancy loss in the study group were found to be 2.62 times those in the control group (OR = 2.62, 95 % CI: 1.02–6.76, $P = <0.05$). This statistically significant association suggests that women who present with signs of TM during the first trimester are significantly more likely to experience pregnancy loss compared to those who do not.

To explore potential contributors to this difference in outcomes, group comparisons were made for the static variables. These were all found to have a non-normal distribution leading to the use of the Mann–Whitney U-test to check for association across outcomes and groups. Among the variables assessed, only cervical length showed a statistically significant difference within the study group, with a shorter median length of 37.05 mm observed in cases leading to pregnancy loss, as against 40.8 mm (Table 2). No significant associations were found for BMI or MCLD in either group. Additionally, comparison between study and control groups revealed no significant differences, suggesting overall homogeneity across the study and control group for these static variables (Table 3).

Table 3
Summary of the Mann–Whitney U test for homogeneity between the study and control group.

Variable	Study Group (S1+S2)	Control Group (C1+C2)	*P*-value
	Median (IQR)	Median (IQR)	
cervicalL (mm)	40.20 (36.60–46.70)	42.00 (37.61–47.40)	0.164
MCLD (mm)	19.40 (15.98–23.70)	18.75 (15.75–22.03)	0.412
BMI (kg/m^2)	24.87 (22.28–28.78)	25.28 (22.36–29.28)	0.620

Distinct progression patterns were observed among the dynamic markers when analysed using regression modelling. Representative regression plots and residuals for FHR are shown in Fig. 1, with corresponding model statistics presented in Table 4. Model comparisons via LRT across groups (S1, S2, C1, C2) indicated that at least one cohort significantly benefited from the more complex model, supporting its uniform application for comparability. Table 5 highlights FHR as an example, confirming segmented regression as the optimal model.

Table 6 summarises the findings for all dynamic variables measured, with the final parameters of the optimally suited regression model being shown. YSD, MGSD, MIUHD, progesterone and sFlt-1:PlGF ratio were found to be best modelled using a linear relationship with GA, whilst FHR, the YS wall thickness, TT, MASD and β-hCG were found to be best modelled using a segmented linear model. In cases marked as Not Significant (NS) in Table 6, insufficient data points or weak correlations prevented model fitting with statistical significance, making cross-group comparisons unfeasible. MUFD, in particular, had too few valid measurements to allow any meaningful modelling.

The strength of each model's fit was evaluated using the R^2 statistic, which varied considerably across variables. MGSD exhibited a high R^2 (≈0.8–0.9), indicating a strong and consistent relationship with GA and, by extension, with pregnancy progression. Conversely, the sFlt-1:PlGF ratio had a much weaker fit ($R^2 \approx 0.14$–0.17), highlighting the limited proportion of its variance explained by GA in this context. These R^2 values not only reflect the model's ability to capture each marker's gestational pattern, but also offer insight into effect-size of the marker for predicting pregnancy outcome. Higher R^2 values suggest stronger developmental consistency and potentially greater prognostic value, while lower R^2 values may indicate less gestational dependency, or a more complex relationship not well captured by the tested models.

Significant differences in model parameters, including intercepts β_0, slopes β_1, and, for segmented linear models, slope changes β_2, were observed both within and across groups, reflecting distinct patterns of early pregnancy development. Within the study group, several variables demonstrated

Table 2
Summary of the Mann–Whitney U test for association between variables and pregnancy outcome for the study and control group.

Variable	Study Group			Control Group		
	S1 Live birth	S2 Pregnancy loss	*P*-value	C1 Live birth	C2 Pregnancy loss	*P*-value
	Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)	
cervicalL (mm)	40.80 (37.75–48.00)	37.05 (33.77–42.32)	**<0.05**	42.00 (37.60–47.50)	42.99 (38.66–45.24)	0.678
MCLD (mm)	19.45 (16.30–24.10)	18.75 (14.75–21.65)	0.712	18.20 (14.95–21.70)	21.40 (18.00–27.80)	0.444
BMI (kg/m^2)	24.85 (22.28–28.90)	24.97 (22.26–28.57)	0.873	25.28 (22.06–29.13)	25.79 (24.30–31.67)	0.394

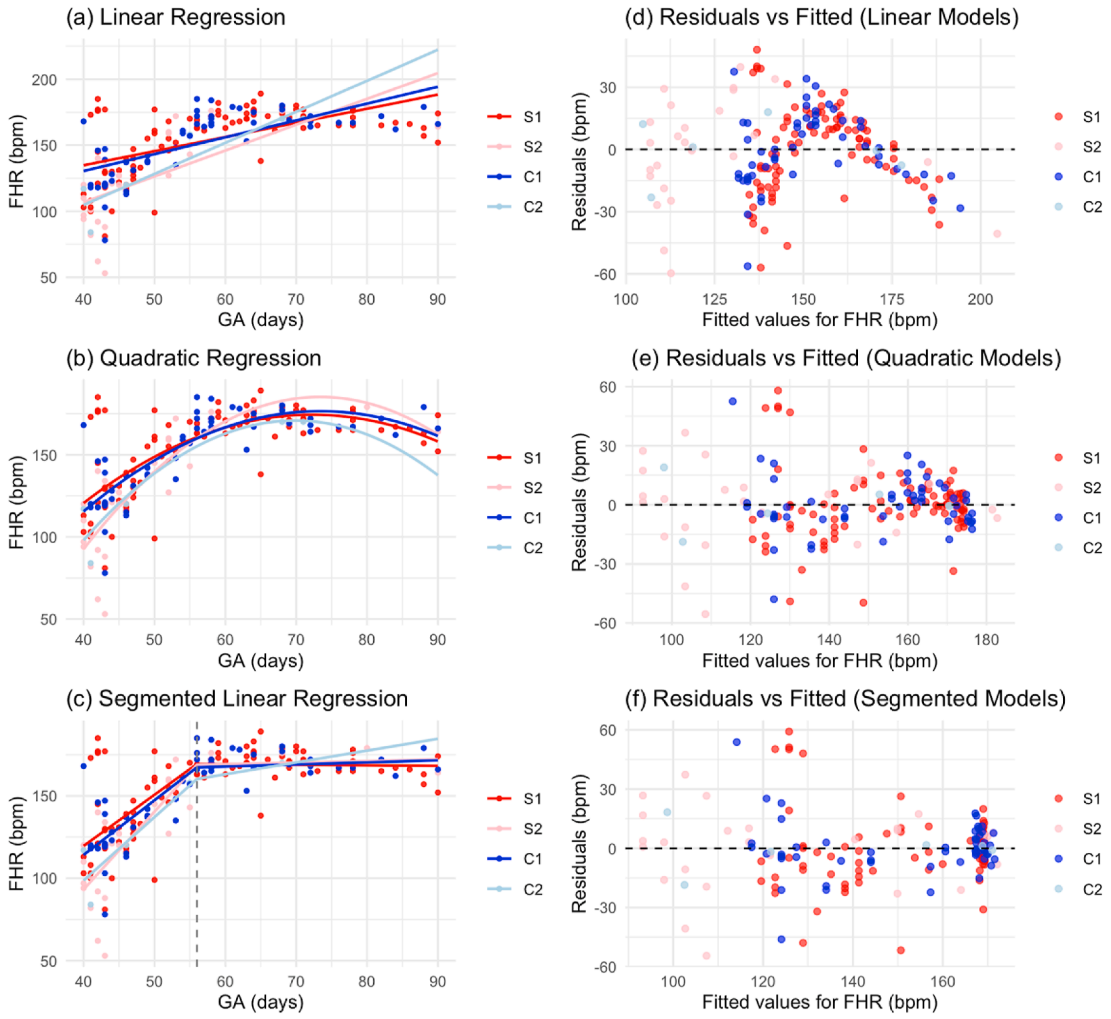

Figure 1. Regression model fits and residuals of FHR for the study and control group, for the live birth and pregnancy loss cases. S1 denotes the cohort within the study group that had a live birth, whilst S2 represents those within the study group that ended in pregnancy loss. C1 and C2 denote the control group with the same outcomes-based notation.

Table 4
Summary of the R^2 and *P*-values for linear, segmented linear and quadratic fits of FHR, for each of the four groups.

Group	R^2, *P*-value		
	Linear	Quadratic	Segmented Linear
S1	0.34, $P < 0.001$	0.50, $P < 0.001$	0.50, $P < 0.001$
S2	0.48, $P < 0.001$	0.67, $P < 0.001$	0.67, $P < 0.001$
C1	0.47, $P < 0.001$	0.64, $P < 0.001$	0.68, $P < 0.001$
C2	0.82, $P < 0.01$	0.88, $P < 0.05$	0.89, $P < 0.05$

Table 5
Summary of the LRT results comparing the various regression fits of FHR, for each of the four groups (chosen model fit in bold).

	Model A vs Model B	LRT	df	*P*-value
S1	Quadratic vs linear	25.17	1	<0.001
	Segmented linear vs quadratic	−0.24	1	1
S2	Quadratic vs linear	12.17	1	<0.001
	Quadratic vs segmented linear	0.07	1	0.80
C1	Quadratic vs linear	21.47	1	<0.001
	Quadratic vs segmented linear	**5.67**	**1**	**<0.05**
C2	Quadratic vs linear	2.03	1	0.154
	Quadratic vs segmented linear	0.61	1	0.43

meaningful divergence in coefficient values between live birth and pregnancy loss cases. Notably, FHR, MGSD, TT, MASD, β-hCG, progesterone, and the sFlt-1:PlGF ratio showed statistically significant differences ($P < 0.05$) in at least one model parameter (Table 7), indicating that their relationships with GA varied depending on pregnancy outcome. For example, FHR exhibited an earlier rise in viable pregnancies, whereas this progression was delayed in cases of pregnancy loss.

Across-group comparisons for association further revealed differences in parameter estimates between the study and control cohorts. Significant variation was detected in model coefficients for FHR, YS wall thickness, MASD, MIUHD, and β-hCG (Table 8), suggesting altered developmental trajectories in pregnancies affected by TM. These parameter-level shifts, particularly in slopes and slope changes, point to disrupted physiological regulation during early gestation in symptomatic pregnancies. For instance, the rate of increase in β-hCG or the pattern of change in YS wall thickness appeared less stable in the study group, highlighting potential early markers of risk.

In contrast, variables such as YSD, MGSD, TT, and sFlt-1:PlGF showed no significant differences in model parameters between groups, making it unclear whether their gestational trajectories

Table 6
Summary of the parameters of the best regression fit for each of the four groups.

Variable	Group	Fit	Coefficient β_0	Coefficient β_1	Coefficient β_2	R^2, *P*-value
Linear models $Y = \beta_0 + \beta_1 GA$						
Segmented linear models $Y = \beta_0 + \beta_1 GA + \beta_2 (GA - 56) I(GA \geq 56)$						
FHR (bpm)	S1	Segmented linear	−4.6983	3.1070	−3.1398	0.50, ***P* < 0.001**
	S2		−95.5949	4.7209	−4.6228	0.67, ***P* < 0.001**
	C1		−18.6251	3.3188	−3.1925	0.68, ***P* < 0.001**
	C2		−55.0960	3.8440	−3.1250	0.89, ***P* < 0.05**
YSD (mm)	S1	Linear	0.374748	0.050049		0.5, ***P* < 0.001**
	S2		NS	NS		$P = 0.127$
	C1		0.688854	0.043258		0.35, ***P* < 0.001**
	C2		NS	NS		$P = 0.936$
YS_wall (mm)	S1	Segmented linear	0.3646	0.0322	−0.0194	0.11, ***P* < 0.05**
	S2		−2.1307	0.0902	−0.0943	0.25, ***P* < 0.05**
	C1		−1.1329	0.0624	−0.0758	0.28, ***P* < 0.001**
	C2		0.5327	0.0275	0.0068	0.9, ***P* < 0.05**
MGSD (mm)	S1	Linear	−22.3184	0.9640		0.82, ***P* < 0.001**
	S2		−23.5658	0.8678		0.88, ***P* < 0.001**
	C1		−27.8173	1.0489		0.86, ***P* < 0.001**
	C2		−15.4198	0.6991		0.92, ***P* < 0.05**
TT (mm)	S1	Segmented linear	1.1073	0.0822	0.1781	0.57, ***P* < 0.001**
	S2		−1.7665	0.1401	0.1288	0.69, ***P* < 0.001**
	C1		−4.1991	0.2027	0.0183	0.50, ***P* < 0.001**
	C2		−3.1576	0.1562	0.0517	0.64, ***P* < 0.05**
MASD (mm)	S1	Segmented linear	−34.0882	0.9500	0.7796	***P* < 0.001**
	S2		−34.8096	0.9476	−0.0032	***P* < 0.001**
	C1		−40.6504	1.0594	0.5848	***P* < 0.001**
	C2		NS	NS	NS	$P = 0.320$
MIUHD (mm)	S1	Linear	−8.6748	0.5716		0.19, ***P* < 0.05**
	S2		NS	NS		$P = 0.937$
	C1		NS	NS		$P = 0.860$
	C2		NS	NS		$P = 0.900$
MUFD (mm)	S1	Linear	NS	NS		$P = 0.073$
	S2		NS	NS		$P = 0.068$
	C1		NS	NS		$P = 0.055$
	C2		NS	NS		$P = 0.100$
BHCG (IUL)	S1	Segmented linear	−178894	4882	−6130	0.42, ***P* < 0.001**
	S2		−139019	3649	−4388	0.43, ***P* < 0.001**
	C1		−167848	4597	−5008	0.52, ***P* < 0.001**
	C2		NS	NS	NS	$P = 0.171$
Prog (ng/ml)	S1	Linear	22.3166	0.6110		0.12, ***P* < 0.001**
	S2		−2.0166	0.6872		0.18, ***P* < 0.05**
	C1		16.4331	0.5906		0.18, ***P* < 0.05**
	C2		NS	NS		$P = 0.106$
SFLT-1:PLGF	S1	Linear	4.5578	0.8129		0.17, ***P* < 0.05**
	S2		9.0055	4.9889		0.17, ***P* < 0.05**
	C1		13.7215	0.6041		0.16, ***P* < 0.05**
	C2		11.6333	0.2445		0.14, ***P* < 0.05**

differ meaningfully between pregnancies affected by TM and those without. Progesterone showed a borderline difference in slope ($P = 0.075$), indicating a trend that may not have reached significance, potentially due to limited statistical power.

Discussion

The results of this study provide novel insights into the temporal behaviour of both US and BC markers in early pregnancy, particularly among women with TM. By applying segmented, linear, and quadratic models, we were able to characterise distinct gestational trajectories across key markers, revealing parameter-level differences that differentiate between pregnancies that progressed to live birth and those that resulted in early loss.

FHR was one of the most consistently discriminative markers, echoing prior findings that slow heart rates in early gestation are strongly predictive of miscarriage. In our study, FHR in viable pregnancies exhibited a sharp increase in early gestation, which contrasted with a delayed and blunted trajectory in non-viable cases. This aligns with the predictive thresholds reported in previous literature,[18,19] which demonstrated that FHR <100 bpm before 6.3 weeks gestation is associated with poor prognosis. Reassuringly, our modelling approach captured these inflection points, validating FHR as a robust early predictor in the context of TM.

The YS wall thickness and MASD also exhibited significant parameter shifts between outcome groups. Prior literature has emphasised the diagnostic relevance of YS diameter and morphology,[28] and while wall thickness has been less extensively studied, our findings suggest that subtle changes may hold additional predictive value when assessed dynamically. Similarly, deviations in MASD have not been comprehensively researched in past research,[30,31] but our results show that when modelled with GA, it presents a possible indicator, especially in the context of TM.

BC markers also played a pivotal role. The segmented modelling of β-hCG mirrored known biological patterns, that of a steep rise in early pregnancy followed by a plateau. In viable cases, the rise was timely and consistent, while in miscarriage cases, the trajectory was delayed or flattened, reflecting prior findings that a suboptimal rise in β-hCG is indicative of pregnancy failure.[32–34] Similarly,

Table 7
Summary of the Z-test results for association between models for the study and control group with respect to outcome.

Variable	Selected Model	Coefficient	Study Group (S1 vs S2)		Control Group (C1 vs C2)	
			z-score	*P*-value	z-score	*P*-value
FHR (bpm)	Segmented linear	β_o	2.14	< **0.05**	1.98	< **0.05**
		β_1	−2.01	< **0.05**	−2.03	< **0.05**
		β_2	1.98	< **0.05**	−1.97	< **0.05**
YSD (mm)	Linear	–	S2 NS		C2 NS	
YS_wall (mm)	Segmented linear	β_o	1.35	0.177	−1.97	< **0.05**
		β_1	−1.45	0.146	1.97	< **0.05**
		β_2	1.19	0.236	−2.30	< **0.05**
MGSD (mm)	Linear	β_o	0.29	0.769	−1.97	< **0.05**
		β_1	2.06	< **0.05**	2.90	< **0.05**
TT (mm)	Segmented linear	β_o	2.14	< **0.05**	0.60	0.550
		β_1	−1.99	< **0.05**	−0.40	0.691
		β_2	1.07	0.283	−0.03	0.978
MASD (mm)	Segmented linear	β_o	−0.10	0.920	C2 NS	
		β_1	0.46	0.642		
		β_2	0.03	0.979		
MIUHD (mm)	Linear	–	S2 NS		C1, C2 NS	
MUFD (mm)	None	–	–	–	–	–
BHCG (IUL)	Segmented linear	β_o	−1.15	0.251	C2, NS	
		β_1	2.09	< **0.05**		
		β_2	−2.11	< **0.05**		
Prog (ng/ml)	Linear	β_o	2.15	< **0.05**	C2 NS	
		β_1	−2.03	< **0.05**		
SFLT-1:PLGF	Linear	β_o	−0.15	0.881	0.16	0.87
		β_1	1.9	< **0.05**	0.76	0.45

Table 8
Summary of the Z-test results for homogeneity between models across the study and control group.

Variable	Selected Model (Study vs Control) (S1+S2 vs C1+C2)	Coefficient	z-score	*P*-value
FHR (bpm)	Segmented linear	β_o	−2.01	< **0.05**
		β_1	1.99	< **0.05**
		β_2	−1.98	< **0.05**
YSD (mm)	Linear	β_o	0.19	0.851
		β_1	0.05	0.958
YS_wall (mm)	Segmented linear	β_o	−2.22	< **0.05**
		β_1	2.05	< **0.05**
		β_2	1.97	< **0.05**
MGSD (mm)	Linear	β_o	0.44	0.659
		β_1	−0.45	0.650
TT (mm)	Segmented linear	β_o	−2.03	< **0.05**
		β_1	2.39	< **0.05**
		β_2	−0.48	0.635
MASD (mm)	Segmented linear	β_o	−1.97	< **0.05**
		β_1	0.83	0.407
		β_2	−0.47	0.058
MIUHD (mm)	Linear	β_o	2.01	< **0.05**
		β_1	1.93	< **0.05**
MUFD (mm)	None	–	–	–
BHCG (IUL)	Segmented linear	β_o	−1.37	0.172
		β_1	2.26	< **0.05**
		β_2	−2.11	< **0.05**
Prog (ng/ml)	Linear	β_o	−0.88	0.378
		β_1	1.78	0.075
SFLT-1:PLGF	Linear	β_o	−0.66	0.512
		β_1	0.71	0.769

progesterone levels were significantly lower in non-viable cases, consistent with previous evidence that low first-trimester progesterone is a strong predictor of early loss.[35,36]

The angiogenic ratio sFlt-1:PlGF, while better known for later-pregnancy applications, also demonstrated differential behaviour in our cohort. Though its effect size was modest, our findings support other work,[37–40] suggesting early placental imbalance may precede clinical symptoms. Its incorporation into early screening strategies may enhance the detection of high-risk pregnancies before clinical deterioration.[41,42] Importantly, these findings reinforce the value of combining US and BC markers,[43] to enhance diagnostic sensitivity.

Our cross-group comparisons between the TM cohort and controls highlighted that several markers, including FHR, YS wall thickness, MASD and β-hCG, exhibited significantly different parameter values, suggesting that the physiological basis of TM may reflect broader disruptions in embryonic development and placental function. This supports the assertion that TM is not merely a transient symptom but may signify an altered gestational trajectory.[11,44]

By contrast, markers like MGSD, TT, and sFlt-1:PlGF showed no significant differences across groups, suggesting that their trajectories may not be well captured by the regression models applied. These findings highlight the need for marker-specific modelling and caution against assuming uniformity in early pregnancy development.[45,46]

Collectively, our results offer strong support for stratified risk models that account for dynamic marker behaviour over GA. Static cut-offs may mask subtle but clinically significant changes in trajectory. Instead, incorporating parameter-based insights, particularly from segmented modelling, can aid in the earlier identification of non-viable pregnancies and support more targeted management.

Limitations of this study include the moderate sample size, which may limit generalisability. Although this study employed a single US practitioner to ensure consistency in data collection, further research is needed to validate the generalisability of the results across multiple practitioners. Additionally, not all variables could be fitted across all groups due to sparse data, and residual confounding from unmeasured maternal or environmental factors cannot be excluded. Nonetheless, the consistency of key findings with established physiological knowledge strengthens confidence in the observed trends. Another limitation is the use of a fixed 56-day breakpoint in segmented regression models. While biologically informed, this uniform cut-off may not capture marker-specific inflection points. Future analyses could incorporate data-

driven or adaptive breakpoint estimation to optimise model accuracy.

Future research should aim to externally validate these modelling patterns across more diverse populations and explore their integration into clinical prediction frameworks. Incorporating gestational marker trajectories, rather than relying solely on static thresholds, within logistic regression or, preferably, machine learning algorithms, may enhance risk stratification and enable earlier, more informed decision-making for women presenting with TM.

Conclusion

This study demonstrates that pregnancies complicated by TM are associated with differing trends in physiological markers compared to those without symptoms. By modelling US and BC markers across GA using linear, quadratic, and segmented regression techniques, the analysis revealed meaningful differences in marker behaviour between viable and non-viable outcomes. Several markers, including FHR, β-hCG, YS wall thickness, TT, and MASD, displayed phase-dependent deviations best captured using segmented models, indicating non-linear growth patterns that correspond with known embryonic milestones.

These findings reinforce the limitations of relying on static thresholds in clinical practice and highlight the importance of evaluating gestational marker trajectories for early risk assessment. Although the sample size and data sparsity limited full subgroup modelling in some cases, the observed trends were physiologically consistent and clinically meaningful. Incorporating such trajectory-based approaches into clinical prediction frameworks may enhance early risk stratification, guide follow-up strategies, and improve decision-making for women presenting with TM during the first trimester. Furthermore, dynamic markers may be successfully utilised in well-calibrated multiple logistic regression models to strengthen predictive accuracy and support personalised care pathways.

Ethics approval and consent to participate

Ensuring data confidentiality and ethical integrity was a priority for this study. Ethical approval was obtained from Mater Dei Hospital's Data Protection Officer and Chief Executive Officer and the University Research Ethics Committee (UREC) (MED-2021-00032). Additionally, permission to conduct the study was obtained from the Chairperson and all the consultants within the Obstetrics and Gynaecology Department at Mater Dei Hospital. Written informed consent was obtained from all participants, with detailed information sheets provided in both English and Maltese to ensure clarity and comprehension. Participation was entirely voluntary, allowing individuals the right to withdraw from the study at any stage without consequences. Written informed consent was obtained for anonymised patient information to be published in this article.

Availability of data

Data required for this study may be made available by the author(s) upon reasonable request.

Author contributions

LS: Conceptualisation, Methodology, Data curation, Software, Formal analysis, Validation, Visualisation, Writing- Original Draft preparation.
PB: Supervision, Writing - Reviewing and Editing.
VG: Supervision, Writing - Reviewing and Editing.
BB: Investigation, Writing - Reviewing and Editing.
JCA: Supervision, Writing - Reviewing and Editing.

Generative AI use

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Conflicts of interest statement

The authors declare that they have no competing interests.

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