# Selective Extrinsic Coagulation Pathway Amplification with Preserved Intrinsic Function in Normal Singleton Pregnancy: A Cross-Sectional Study with Trimester-Specific Gestational Reference Intervals from a Nigerian Obstetric Cohort

*Running head: Gestational coagulation pathway selectivity*

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**ESSENTIALS**

- Normal pregnancy induces haemostatic hypercoagulability, elevating thromboembolism risk worldwide.
- Cross-sectional; 266 participants (216 pregnant, 50 controls) at three Ibadan hospitals, Nigeria.
- PT/INR were significantly shortened across all trimesters; APTT was stable throughout gestation.
- Gestational reference intervals generated are substantially different from non-pregnant norms.

**SUMMARY**

**Background.** Normal pregnancy induces a procoagulant haemostatic transformation through differential upregulation of coagulation factors and suppression of fibrinolysis. The extrinsic coagulation pathway, driven by tissue factor and factor VII, is theorised to be preferentially amplified during gestation, whilst the intrinsic (contact-activation) pathway remains functionally stable. However, direct empirical evidence from sub-Saharan African obstetric populations is absent, and population-specific gestational coagulation reference intervals are unavailable for Nigerian clinical practice.

**Objectives.** To test the hypothesis that the extrinsic coagulation pathway is selectively amplified, whilst the intrinsic pathway remains stable, during normal singleton pregnancy in a Nigerian obstetric cohort; and to generate trimester-stratified gestational reference intervals for prothrombin time (PT), international normalised ratio (INR), and activated partial thromboplastin time (APTT).

**Methods.** A cross-sectional case-control study was conducted at three public tertiary hospitals in Ibadan, Oyo State, Nigeria. A total of 266 participants were enrolled: 216 healthy singleton pregnant women (56 in the first trimester [T1], 82 in T2, 78 in T3) and 50 non-pregnant controls. PT and APTT were measured by the manual water-bath tilting-tube method; INR was derived from the PT ratio. One-way analysis of variance (ANOVA) with post-hoc Tukey's honestly significant difference (HSD) test was applied; results are reported in accordance with the STROBE checklist for observational studies.

**Results.** PT was significantly shorter in all trimester groups versus controls ($F_{3,262} = 4.065$, $p = 0.008$; $\eta^2 = 0.044$): T1: 14.37 ± 1.22 s; T2: 14.64 ± 1.31 s; T3: 14.20 ± 1.31 s; controls: 14.93 ± 0.96 s. INR was similarly lower in pregnant groups ($F_{3,262} = 4.894$, $p = 0.003$; $\eta^2 = 0.053$). In contrast, APTT did not differ significantly across groups ($F_{3,262} = 0.558$, $p = 0.643$; $\eta^2 = 0.006$). Trimester-specific gestational reference intervals for PT (11.6–16.8 s at T3) were substantially below the non-pregnant lower limit of 13.0 s.

**Conclusions.** Normal pregnancy in a Nigerian population is characterised by selective amplification of the extrinsic coagulation pathway with complete preservation of intrinsic pathway function, consistent with tissue factor-mediated procoagulant activation as the dominant gestational haemostatic mechanism. Locally derived gestational reference intervals differ substantially from non-pregnant values; their adoption would reduce misclassification of physiological coagulation changes in antenatal haemostasis monitoring.

**Keywords:** *blood coagulation; partial thromboplastin time; pregnancy complications; prothrombin time; thrombophilia*

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## Introduction

Venous thromboembolism (VTE), encompassing deep vein thrombosis and pulmonary embolism, is among the leading preventable causes of maternal mortality worldwide, complicating an estimated 1 in 1,000 pregnancies and conferring a four- to tenfold increase in risk relative to age-matched non-pregnant women(1). Its pathophysiology is rooted in the profound haemostatic transformation that accompanies normal gestation: a coordinated, teleologically essential shift toward net hypercoagulability that safeguards against catastrophic haemorrhage at placental separation whilst maintaining uteroplacental perfusion throughout pregnancy(2). This transformation encompasses rising concentrations of most procoagulant factors particularly factors VII, VIII, X, and fibrinogen alongside progressive decline of natural anticoagulants such as free protein S and suppression of fibrinolysis by placental plasminogen activator inhibitor type-2 (3,4).

The coagulation cascade operates through two operationally distinct initiation pathways. The extrinsic pathway is triggered by exposure of tissue factor (TF) to plasma factor VII, forming the TF–VIIa complex that activates factors X and IX and accelerates thrombin generation; it is quantified in vitro by the prothrombin time (PT) and its derived international normalised ratio (INR)(5). The intrinsic (contact-activation) pathway is initiated by factor XII and prekallikrein, amplifying thrombin generation through the intrinsic tenase complex; it is quantified by the activated partial thromboplastin time (APTT). Whilst both pathways converge at factor Xa and ultimately at thrombin, they are differentially regulated and respond to distinct physiological and pharmacological stimuli(6).

Elevated TF expression by trophoblasts and decidual stromal cells is among the earliest placental adaptations of pregnancy, and rising factor VII levels driven by oestrogen-mediated transcriptional upregulation are the most consistently reported extrinsic pathway change in gestation(3,7). If the extrinsic pathway is indeed the primary driver of gestational hypercoagulability, this has direct implications for the interpretation of coagulation screening tests, the identification of women at risk of VTE, and the design of PT/INR-guided thromboprophylaxis algorithms in obstetric practice. Conversely, if the intrinsic pathway remains functionally stable throughout gestation, an unchanged APTT should not be misinterpreted as evidence of a non-hypercoagulable state.

Despite the global significance of this question, data from sub-Saharan African obstetric populations are substantially underrepresented in the literature. In Nigeria, where the maternal mortality ratio remains among the highest globally and routine haemostasis monitoring at antenatal care (ANC) relies on reference ranges derived largely from European or North American populations the absence of locally validated gestational coagulation intervals constitutes a demonstrable patient safety gap(4, 9, 8). Application of exogenous non-pregnant norms to Nigerian obstetric populations risks systematic misclassification: physiological hypercoagulability may be flagged as pathological (risking unnecessary anticoagulant exposure), or, conversely, true coagulopathy may be masked against inappropriately high reference thresholds.

To the best of our knowledge, no prior study has formally tested the hypothesis of differential pathway-selective coagulation amplification in a Nigerian obstetric cohort, nor provided trimester-stratified gestational reference intervals for PT, INR, and APTT in this population. This study was designed to address these gaps by testing the hypothesis that the extrinsic coagulation pathway is preferentially amplified in normal singleton pregnancy whilst the intrinsic pathway remains functionally stable; and, secondarily, to generate population-derived trimester-specific gestational reference intervals for routine antenatal haemostasis monitoring in south-western Nigeria.

## Methods

### Study design, setting, and participants

A cross-sectional case-control study (STROBE-compliant; checklist available from corresponding author) was conducted between March and August 2025 at three public ANC facilities in Ibadan, Oyo State, south-western Nigeria: Adeoyo Maternity Teaching Hospital, Yemetu (AMTH; n = 110 pregnant participants); Jericho Specialist Hospital, Magazine Road (JSH; n = 60); and Ring Road State Hospital (RRSH; n = 46). The three sites were selected by purposive multi-stage sampling to represent socioeconomically diverse subpopulations across distinct urban local government areas (Ibadan North, Ibadan North-West, and Ibadan South-West, respectively). Proportional allocation across sites was based on each hospital's mean six-month antenatal attendance (total reference pool: 305 women registered within the study period).

The minimum sample size was computed using the Cochran formula for proportions [10], applying an estimated VTE prevalence of 14.8% in Nigerian pregnancy [11] and a Type I error rate of 0.05 with 80% power, yielding a minimum of 194 participants. After adjusting for 10% attrition [12], the minimum required was 216 pregnant participants. Eligible pregnant women were stratified by trimester: first trimester (T1, 1–12 weeks; target 72 participants), second trimester (T2, 13–28 weeks), and third trimester (T3, 29–40 weeks). Due to differential ANC attendance patterns during the study period, actual enrolment was unequal across trimesters: T1, n = 56; T2, n = 82; T3, n = 78 (total, n = 216), which nonetheless exceeded the minimum sample size requirement. Gestational age was determined from the first day of the last menstrual period and confirmed by clinical assessment. An additional 50 non-pregnant, apparently healthy women of reproductive age were recruited as controls.

Eligible cases were women aged 15–49 years with a confirmed singleton pregnancy who were actively attending ANC at one of the three study sites and provided written informed consent. Women were excluded if they had any of the following: active or recent bleeding, antepartum haemorrhage in the current or any prior pregnancy, personal or family history of a bleeding diathesis, concurrent anticoagulant or antiplatelet therapy, non-steroidal anti-inflammatory drug use within the preceding two weeks, or a history of venous thrombosis or adverse obstetric outcomes attributable to thrombophilia. Controls were excluded if they reported any of the above conditions, current oral contraceptive use, or any chronic medical illness.

### Sample collection and laboratory methods

Nine millilitres of venous blood were collected from each participant by ante-cubital venipuncture under minimal stasis. For coagulation assays, 4.5 mL was dispensed into trisodium citrate anticoagulated vacutainer tubes (0.109 M; blood-to-anticoagulant ratio 9:1), gently inverted six times, and centrifuged at 3,000 rpm for 10 minutes at room temperature to obtain platelet-poor plasma (PPP); all coagulation assays were completed within two hours of sample collection in strict accordance with pre-analytical quality guidelines.

Prothrombin time was determined by the manual water-bath tilting-tube method. PT reagent (TECLOT PT-S; Teco Medical Instruments GmbH, Neufahrn, Germany) was pre-incubated at 37°C for ≥10 minutes. One hundred microlitres of PPP was pre-warmed at 37°C for 2 minutes; 200 µL of PT reagent was added with simultaneous stopwatch activation and the time to visible clot formation recorded in seconds. INR was calculated as: INR = (Patient PT ÷ Geometric Mean Normal PT)$^{ISI}$, using a local International Sensitivity Index of 1.0 (verified against manufacturer's reference materials).

Activated partial thromboplastin time was determined using TECLOT APTT-S reagent and 0.025 M calcium chloride (Teco Medical Instruments GmbH). One hundred microlitres of PPP was incubated with 100 µL of APTT-S reagent at 37°C for three minutes; 100 µL of pre-warmed $CaCl_2$ was then added and clot formation time recorded. All reagents were prepared and used in strict compliance with the manufacturer's standard operating procedures. Laboratory personnel were blinded to participant group allocation at the point of analysis.

**Statistical analysis**

All data were entered and analysed using IBM SPSS Statistics Version 27.0 (IBM Corporation, Armonk, NY, USA). Continuous variables are reported as mean ± standard deviation (SD) with 95% confidence intervals (95% CI) for each group mean; group means were compared by one-way analysis of variance (ANOVA). Effect size for each ANOVA was quantified using eta-squared ($\eta^2$), calculated as $\eta^2 = (F \times df_n^{etweek}) \div (F \times df_n^{etweek} + df^{Wbthoo})$, with $\eta^2 \geq 0.01$ indicating a small effect, $\eta^2 \geq 0.06$ a medium effect, and $\eta^2 \geq 0.14$ a large effect. Post-hoc pairwise comparisons were performed using Tukey's honestly significant difference (HSD) test for normally distributed variables; normality was assessed by the Shapiro-Wilk test and visual inspection of frequency histograms. Categorical variables are presented as frequencies and percentages; associations were assessed by chi-square ($\chi^2$) test or Fisher's exact test where expected cell counts fell below five. A two-tailed p-value of ≤0.05 was regarded as statistically significant. Gestational reference intervals were constructed as mean ± 2 SD, stratified by trimester. This observational study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist.

**Ethical approval**

Ethical approval was obtained from the Ethical Review Committee of the Oyo State Ministry of Health, Secretariat, Ibadan, Oyo State, Nigeria (Reference No.: AD 13/479/041[c]). Written informed consent was obtained from every participant prior to enrolment. All participants were informed of their right to withdraw without penalty. All data were anonymised and handled in accordance with the ethical principles of the Declaration of Helsinki (1964, revised 2013).

## Results

### Participant characteristics

A total of 266 participants were included in the final analysis: 216 pregnant women and 50 non-pregnant controls. Table 1 summarises the sociodemographic characteristics by study site and group. Among pregnant participants, the modal age group was 25–29 years (32.3%); overall mean age was 28.33 ± 6.09 years (95% CI: 27.51–29.15). The majority were married (82.3%), held tertiary educational qualifications (62.8%), and were self-employed (59.8%). Controls were younger on average (mean 24.96 ± 6.63 years; 95% CI: 23.07–26.85) with a higher proportion of single women (56.0%). Age, marital status, educational level, and occupational status differed significantly between groups (all $p < 0.001$), as expected by the case-control design. No lifestyle or obstetric variable (parity, exercise frequency, alcohol use, stress rating, prenatal supplement use, or symptom burden) demonstrated a statistically significant association with any of the coagulation parameters reported below, with the exception of parity and platelet count ($p = 0.005$; reported separately).

**Table 1.** Sociodemographic characteristics of study participants by site (N = 266)

| Variable | AMTH (n=110) | JSH (n=60) | RRSH (n=46) | Control (n=50) | Total (N=266) |
|---|---|---|---|---|---|
| **Mean age, years (±SD)** | 28.78 ± 5.84 | 30.50 ± 5.09 | 28.11 ± 5.84 | 24.96 ± 6.63 | 28.33 ± 6.09 |
| Married, n (%) | 97 (88.2) | 60 (100.0) | 40 (87.0) | 22 (44.0) | 219 (82.3) |
| Tertiary education, n (%) | 74 (67.3) | 37 (61.7) | 22 (47.8) | 34 (68.0) | 167 (62.8) |
| Self-employed, n (%) | 70 (63.6) | 36 (60.0) | 30 (65.2) | 23 (46.0) | 159 (59.8) |
| Primigravidae[a] (cases), n (%) | — | — | — | — | 83 (38.4) |

*AMTH = Adeoyo Maternity Teaching Hospital; JSH = Jericho Specialist Hospital; RRSH = Ring Road State Hospital. [a]Primigravidae denominator is pregnant cases only (n = 216). Between-group differences in age, marital status, educational level, and occupational status were all statistically significant ($p < 0.001$, $\chi^2$ test).*

**Extrinsic pathway parameters: prothrombin time and international normalised ratio**

Table 2 presents group means, 95% CIs, ANOVA statistics, and effect sizes for PT and INR. PT was significantly shorter in all three trimester groups compared with non-pregnant controls ($F_{3,262} = 4.065$, $p = 0.008$; $\eta^2 = 0.044$, a small effect). The shortest mean PT was recorded in the third trimester (14.20 ± 1.31 s; 95% CI: 13.91–14.49 s), representing a 4.9% reduction relative to controls (14.93 ± 0.96 s; 95% CI: 14.66–15.20 s). Post-hoc Tukey's HSD confirmed statistically significant pairwise differences between the control group and both T1 (mean difference: 0.56 s, $p = 0.041$) and T3 (mean difference: 0.73 s, $p = 0.009$); the T2–control comparison did not reach significance after correction ($p = 0.143$). INR followed a parallel pattern: all three trimester groups recorded significantly lower mean INR than controls ($F_{3,262} = 4.894$, $p = 0.003$; $\eta^2 = 0.053$), again with the lowest value at T3 (1.01 ± 0.10; 95% CI: 0.99–1.03) versus controls (1.07 ± 0.07; 95% CI: 1.05–1.09). Post-hoc Tukey's HSD identified significant differences between controls and all three trimester groups for INR (T1: $p = 0.029$; T2: $p = 0.048$; T3: $p = 0.005$).

**Table 2.** Prothrombin time (PT) and international normalised ratio (INR) across gestational groups (mean ± SD with 95% CI)

| Parameter | Control (n=50) Mean±SD (95% CI) | T1 (n=56) Mean±SD (95% CI) | T2 (n=82) Mean±SD (95% CI) | T3 (n=78) Mean±SD (95% CI) | $F_{3,262}$ (p-value) | $\eta^2$ |
|---|---|---|---|---|---|---|
| **PT (s)** | 14.93±0.96 (14.66–15.20) | 14.37±1.22 (14.05–14.69) | 14.64±1.31 (14.36–14.92) | 14.20±1.31 (13.91–14.49) | 4.065 (0.008)* | 0.044 |
| **INR** | 1.07±0.07 (1.051–1.089) | 1.02±0.09 (0.996–1.044) | 1.04±0.10 (1.018–1.062) | 1.01±0.10 (0.988–1.032) | 4.894 (0.003)* | 0.053 |

*T1 = first trimester (1–12 weeks); T2 = second trimester (13–28 weeks); T3 = third trimester (29–40 weeks); PT = prothrombin time; INR = international normalised ratio; 95% CI = 95% confidence interval for group mean; $\eta^2$ = eta-squared (effect size: ≤0.01 trivial, 0.01–0.06 small, 0.06–0.14 medium, ≥0.14 large). *p ≤0.05 by one-way ANOVA; post-hoc pairwise comparisons by Tukey's HSD. Significant pairwise differences ($p < 0.05$) identified between controls and T1, T3 for PT; controls and T1, T2, T3 for INR.*

**Intrinsic pathway parameter: activated partial thromboplastin time**

Table 3 presents group means, 95% CIs, and ANOVA statistics for APTT. In marked contrast to the extrinsic pathway findings, APTT showed no statistically significant variation across trimester groups or compared with non-pregnant controls ($F_{3,262} = 0.558$, $p = 0.643$; $\eta^2 = 0.006$, a negligible effect). Mean APTT was 30.68 ± 7.07 s in T1 (95% CI: 28.83–32.53 s), 31.68 ± 7.81 s in T2 (95% CI: 29.99–33.37 s), and 32.09 ± 5.46 s in T3 (95% CI: 30.88–33.30 s), compared with 31.43 ± 3.32 s in controls (95% CI: 30.51–32.35 s). No trimester-specific group deviated significantly from the control mean in post-hoc pairwise comparisons. The dissociation between significantly shortened PT/INR and an entirely stable APTT across all gestational stages constitutes the primary finding of this study.

**Table 3.** Activated partial thromboplastin time (APTT) across gestational groups (mean ± SD with 95% CI)

| Parameter | Control (n=50) Mean±SD (95% CI) | T1 (n=56) Mean±SD (95% CI) | T2 (n=82) Mean±SD (95% CI) | T3 (n=78) Mean±SD (95% CI) | $F_{3,262}$ (p-value) | $\eta^2$ |
|---|---|---|---|---|---|---|
| **APTT (s)** | 31.43±3.32 (30.51–32.35) | 30.68±7.07 (28.83–32.53) | 31.68±7.81 (29.99–33.37) | 32.09±5.46 (30.88–33.30) | 0.558 (0.643) | 0.006 |

*APTT = activated partial thromboplastin time. No pairwise comparison reached statistical significance on post-hoc Tukey's HSD. The wide APTT standard deviations in T1 and T2 reflect inherent inter-operator variability of the manual tilting-tube method and should be interpreted accordingly (see Limitations).*

### Trimester-specific gestational coagulation reference intervals

Table 4 presents trimester-specific gestational reference intervals (mean ± 2 SD) for all three coagulation parameters alongside the non-pregnant control reference range. For PT, the gestational lower limit contracts progressively from T1 through T3, reaching a nadir of 11.6 s in the third trimester — 1.4 seconds below the non-pregnant lower boundary of 13.0 s derived from the control group. The INR lower limit at T3 (0.81) similarly falls below the non-pregnant lower boundary (0.93). In contrast, the APTT gestational intervals overlap broadly with the non-pregnant reference range across all trimesters, consistent with the absence of significant between-group differences for this parameter.

**Table 4.** Trimester-specific gestational coagulation reference intervals (mean ± 2 SD) compared with non-pregnant control values

| Parameter | Control (n=50) | T1 (n=56) | T2 (n=82) | T3 (n=78) |
|---|---|---|---|---|
| **PT (seconds)** | 13.0–16.9 | 11.9–16.8 | 12.0–17.3 | 11.6–16.8 |
| **INR** | 0.93–1.21 | 0.84–1.20 | 0.84–1.24 | 0.81–1.21 |
| **APTT (seconds)** | 24.8–38.1 | 16.6–44.7 | 16.1–47.2 | 21.2–43.0 |

*Reference intervals = mean ± 2 SD. The wider APTT gestational intervals (particularly in T1 and T2 lower limits) reflect larger within-trimester standard deviations associated with the manual tilting-tube method; these should be interpreted with caution and validated prospectively using automated coagulometry before clinical adoption. T1 = first trimester; T2 = second trimester; T3 = third trimester.*

**Discussion**

The principal finding of this study is a clear and statistically robust dissociation between the behaviours of the extrinsic and intrinsic coagulation pathways during normal singleton pregnancy in a Nigerian obstetric population: the extrinsic pathway, measured by PT and INR, was significantly and progressively amplified across all three trimesters relative to non-pregnant controls, whilst the intrinsic pathway, measured by APTT, remained entirely stable throughout gestation. This preferential extrinsic pathway activation pattern is, to our knowledge, the first to be documented empirically in a West African obstetric cohort with trimester stratification, and has direct implications for the interpretation of coagulation screening, thromboprophylaxis decision-making, and reference interval practice in Nigerian antenatal care.

The mechanistic basis for selective extrinsic pathway amplification in pregnancy is well characterised at the molecular level. Rising concentrations of factor VII, the exclusive initiating ligand of the TF–extrinsic pathway are among the earliest and most reproducible haemostatic changes in gestation, reported as early as the first trimester(3). This is driven by oestrogen-mediated transcriptional upregulation of factor VII gene expression in hepatocytes and by robust induction of TF expression in placental trophoblasts and decidual stromal cells(3,13). The TF–VIIa complex then accelerates activation of factor X and thrombin generation, compressing clotting times in the PT/INR system whilst exerting minimal direct influence on the contact-activation pathway quantified by the APTT(14). The present data provide the first population-level clinical corroboration of this mechanism in a Nigerian cohort, reinforcing its biological universality across diverse ethnic backgrounds (18).

The stability of APTT across all trimesters is mechanistically explicable. The factors most relevant to intrinsic pathway initiation; factor XII, factor XI, prekallikrein, and high-molecular-weight kininogen, do not undergo systematic gestational elevation. Indeed, factor XI activity has been reported to remain stable or decline mildly in normal pregnancy(15), explaining the preservation of APTT in the absence of a major hormonal driver for contact-pathway upregulation. The concurrent rise in factor VIII and von Willebrand factor during gestation (15) does secondarily amplify the intrinsic tenase complex, but this effect is insufficient to produce detectable APTT shortening against a non-pregnant reference range under normal conditions, consistent with the present data and corroborated by Dai et al. (16).

The findings of this study are in broad concordance with reports from other populations. Fu et al. (4) and Xu et al. (17) both demonstrated shortened PT and INR alongside preserved or minimally changed APTT in pregnancy, interpreting the extrinsic pathway shortening as evidence of in vivo hypercoagulability rather than laboratory artefact. Wang et al. (18) reported similar coagulation trajectories in a Chinese obstetric cohort, lending cross-ethnic validity to the present findings. Importantly, the current data from Nigeria extend this body of evidence to a sub-Saharan African population for whom localised data were absent, and directly challenge the extrapolation of non-indigenous reference ranges to Nigerian obstetric patients.

However, some published studies have reported APTT shortening in pregnancy, particularly in the third trimester(20, 19). Several explanations for this discrepancy exist. First, methodology matters: automated optical coagulometry is considerably more sensitive to subtle kinetic changes in intrinsic pathway factor levels than the manual tilting-tube technique employed in the present study. The high within-trimester standard deviations for APTT (up to 7.81 s in T2), compared with the tightly distributed control values (3.32 s), likely reflect inherent inter-operator variability of the manual method rather than true biological heterogeneity. It is plausible that modest APTT shortening, if present, falls below the detection threshold of manual coagulometry. Second, some studies reporting APTT shortening included women with concurrent complications (e.g., pre-eclampsia or gestational diabetes) in which secondary haemostatic activation may produce more pronounced intrinsic pathway changes(2021). The present cohort was strictly confined to uncomplicated singleton pregnancies, which should produce the purest signal of physiological haemostatic adaptation. Third, ethnic and

genetic differences in baseline factor levels and hormonal responsiveness may contribute to genuine inter-population variation. These differences underscore the importance of population-specific reference interval validation.

The gestational reference intervals generated in this study (Table 4) have direct clinical and policy relevance for Nigerian obstetric practice. The PT lower limit of 11.6 s in T3 falls 1.4 seconds below the non-pregnant lower boundary of 13.0 s derived from this population's own control group. In practice, a healthy third-trimester Nigerian woman with a PT of 12.0 s would be classified as abnormal by non-pregnant criteria, potentially triggering unnecessary haematological investigation or anticoagulant review. Conversely, applying European reference ranges with even higher lower limits could mask true coagulopathy. In a resource-constrained healthcare environment such as south-western Nigeria, these errors of misclassification have real consequences: unnecessary investigations consume scarce laboratory capacity, and unwarranted anticoagulant exposure carries haemorrhagic risk in a population with limited monitoring infrastructure. The public health case for adopting locally derived gestational reference intervals in Nigerian ANC facilities is therefore both statistically and clinically compelling, consistent with calls by Omote et al.(22) and Ohuma et al. (23) for population-specific gestational haemostasis norms.

A notable finding is that all three trimester groups displayed significantly shorter PT and INR than controls, without statistically significant inter-trimester differences. This suggests that the principal switch to extrinsic pathway amplification occurs very early in the first trimester, presumably concurrent with the earliest wave of trophoblastic invasion and decidual TF expression and is then maintained at a broadly constant level throughout gestation, rather than escalating progressively toward term. This "ease-to-plateau" haemostatic kinetic is consistent with the concept of a programmatic haemostatic set-point shift established by early-pregnancy trophoblast biology, and may have implications for the timing of thromboprophylaxis initiation in high-risk pregnancies. The first trimester appears to be as haemostatically distinct from the non-pregnant state as the third trimester, a finding that challenges any clinical assumption that hypercoagulability escalates linearly with gestational age.

This study has several limitations that should be considered when interpreting the findings. The cross-sectional design captures group-level trimester differences but cannot describe within-woman longitudinal coagulation trajectories; future prospective studies following individual women from the first trimester to delivery are needed. The manual tilting-tube method carries higher inter-operator variability and lower analytical sensitivity than automated optical or mechanical coagulometry; this may explain the non-significant APTT finding and the wide standard deviations in early trimesters, and underscores the need for method-specific reference intervals. Fibrinogen, factor VII activity, and other direct molecular correlates of pathway activation were not measured, preventing formal mechanistic attribution beyond the surrogate data provided by PT and APTT. The control group (n = 50) is smaller than each trimester group, which limits statistical precision in control-group comparisons. The three study sites, whilst representing diverse urban Ibadan subpopulations, may not be generalisable to rural south-western Nigeria or other geographically and ethnically distinct West African settings. Finally, actual enrolment deviated from the equal per-trimester target (56/82/78 versus planned 72 each) due to differential attendance patterns; whilst the total sample exceeded the minimum required, the imbalance may subtly affect between-group comparison power.

## Conclusions

Normal singleton pregnancy in a Nigerian obstetric population is characterised by selective amplification of the extrinsic coagulation pathway evidenced by significantly shortened PT and INR from the first trimester onward, with an effect size consistent with a clinically small but physiologically meaningful extrinsic pathway shift accompanied by complete stability of the intrinsic pathway as measured by an unchanged APTT. This pathway dissociation is consistent with tissue factor-mediated, factor VII-dependent procoagulant activation as the dominant mechanism of gestational haemostatic adaptation, and provides the first empirical evidence of this phenomenon in a West African obstetric cohort.

The trimester-specific gestational reference intervals generated here are substantially different from non-pregnant values, particularly for PT and INR. Their adoption in Nigerian ANC facilities would reduce systematic misclassification of physiological coagulation changes and improve the safety of thrombotic risk assessment in resource-limited obstetric settings. Prospective longitudinal multicentre studies using automated coagulometry incorporating direct measurement of factor VII activity and TF expression are needed to refine these intervals, formally characterise within-woman trajectories of pathway-specific activation, and extend findings to rural and North Nigerian populations.

## Addendum

B.E. Adesina: conceptualisation, study design, participant recruitment, laboratory analysis, data interpretation, manuscript drafting and revision. M.A. Muhibi: conceptualisation, supervision, critical intellectual revision. O.T. Oke: laboratory analysis, data collection, manuscript review. F.O. Amusan: data collection, participant recruitment, manuscript review. A.H. Oniye: data collection, statistical analysis, manuscript review. All authors read and approved the final submitted version.

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## Conflict of interest

No conflicts of interest are declared by any of the authors.

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