STATISTICAL ANALYSIS PLAN

Official Title:

Single-Layer Versus Double-Layer Uterine ClosureAfter Primary Cesarean Section: Impact on ResidualMyometrial Thickness and Cesarean Scar DefectFormation — A Prospective Randomized Controlled Trial

NCT Number: Pending

Protocol ID: 31/26

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Important Note: This Statistical Analysis Plan was finalized prior to database lock and unblinding.Any deviations from this pre-specified planwill be documented and justified in thefinal statistical report.

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Approval Number: 31/26

STATISTICAL ANALYSIS PLAN (SAP)

Date of Finalization: April 2026
Responsible Statistician: [Ben Dhaw Fedi, PhD]
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SAP-1. GENERAL PRINCIPLES AND PURPOSE

This Statistical Analysis Plan (SAP) has been **prospectively written and finalized prior to database lock and unblinding**. Its purpose is to pre-specify all planned statistical analyses to minimize the risk of outcome reporting bias and post-hoc analytical decisions. Any deviations from this pre-specified plan will be documented with justification and transparently reported in the final statistical report.

All analyses will be conducted using:

- **SPSS version 26.0** (IBM Corporation) — primary analyses
- **R version 4.3** (R Foundation for Statistical Computing) — complementary and advanced analyses

SAP-2. ANALYSIS POPULATIONS

SAP-2.1 Intent-to-Treat (ITT) Population — PRIMARY

All randomized participants, analyzed according to the **treatment group to which they were allocated**, regardless of the actual treatment received or protocol deviations. This constitutes the **primary analysis population**.

SAP-2.2 Per-Protocol (PP) Population — SENSITIVITY

All randomized participants who:

- Received the allocated intervention as specified in the protocol
- Completed the **6-month follow-up visit** with a valid TVUS measurement
- Had no major protocol deviations affecting outcome assessment

SAP-2.3 Safety Population

All randomized participants who underwent the surgical intervention (regardless of which technique was ultimately performed).

Protocol deviations will be prospectively classified as **major** (potentially affecting primary outcome validity) or **minor** (unlikely to affect outcomes) and documented in a deviation log.

SAP-3. BASELINE CHARACTERISTICS

Baseline characteristics will be summarized for the **ITT population** by treatment group:

Variable Type	Summary Statistic
Continuous, normally distributed	Mean ± Standard Deviation
Continuous, non-normal distribution	Median (Interquartile Range, IQR)
Categorical	n (%)

Normality of continuous variables will be assessed using both the **Kolmogorov-Smirnov test** and the **Shapiro-Wilk test**, complemented by visual inspection of Q-Q plots and histograms.

Variables to be summarized include: age, BMI at delivery, gestational age, parity, indication for cesarean section, type of anesthesia, skin incision type, uterine exteriorization status, pre-operative hemoglobin and hematocrit, and attending surgeon (anonymized).

No inferential statistics (p-values) will be calculated for baseline comparisons, as any imbalances in a randomized trial result from chance rather than systematic bias (CONSORT Statement 2010).

SAP-4. PRIMARY OUTCOME ANALYSIS

Outcomes:

1. **RMT** at 6–8 weeks and 6–7 months (continuous, mm)
2. **CSD prevalence** at 6–8 weeks and 6–7 months (binary)

SAP-4.1 RMT — Unadjusted Analysis

- **If normally distributed:** Independent samples **t-test** (or Welch's t-test if unequal variances)
- **If non-normally distributed: Mann-Whitney U test**

Reported statistics:

- Mean ± SD (or Median [IQR]) per group
- Mean difference (MD) with 95% Confidence Interval
- Two-sided p-value (significance threshold: $\alpha = 0.05$)
- Effect size: Cohen's d (t-test) or rank-biserial correlation (Mann-Whitney)

SAP-4.2 RMT — Adjusted Analysis (Primary)

A **multiple linear regression model** will be constructed:

Dependent variable: RMT at 6–7 months
Independent variables:

- Treatment group (primary predictor)
- Age (continuous)
- BMI (continuous)
- Gestational age (continuous)
- Uterine exteriorization (yes/no)
- Surgeon experience level (senior/resident Year 4–5)
- Duration of labor before cesarean section (if applicable)
- Indication for cesarean section

Model assumptions verified:

- Linearity (partial regression plots)
- Homoscedasticity (Breusch-Pagan test)
- Independence of residuals (Durbin-Watson test)

- Absence of multicollinearity (Variance Inflation Factor < 5)
- Normality of residuals (Shapiro-Wilk on residuals)

Reported: Adjusted MD (β coefficient) with 95% CI, R^2, adjusted R^2, F-statistic, overall model p-value

SAP-4.3 CSD Prevalence — Analysis

Unadjusted:

- Chi-square test or Fisher's exact test (if expected cell count < 5)
- Relative Risk (RR) with 95% CI
- Absolute Risk Reduction (ARR) and Number Needed to Treat/Harm (NNT/NNH)

Adjusted:

- **Binary logistic regression** at 6–8 weeks and at 6–7 months
- Covariates: same set as RMT regression model
- Variables with $p < 0.20$ in univariate analysis AND a priori confounders (age, parity, BMI, indication) included
- Model fit: **Hosmer-Lemeshow test**
- Multicollinearity: **Variance Inflation Factors (VIF)**
- Discrimination: **Area Under the ROC Curve (AUC)**
- Results expressed as adjusted **Odds Ratios (OR)** with 95% CI

SAP-5. SECONDARY OUTCOME ANALYSES

SAP-5.1 Continuous Secondary Outcomes

- Unadjusted: t-test or Mann-Whitney U as appropriate
- Adjusted: Multiple linear regression (same covariate set)
- Results: Mean difference or median difference with 95% CI

SAP-5.2 Binary Secondary Outcomes (Complications, Transfusion)

- Unadjusted: Chi-square or Fisher's exact test
- Results: RR, ARR, NNT/NNH with 95% CI

SAP-5.3 CSD Morphological Dimensions

Analyzed within the **CSD-positive subgroup only**:

- Depth, length, width: Mann-Whitney U test
- **CSD Volume** (ellipsoid formula): Mann-Whitney U test

SAP-5.4 Repeated Measures — RMT at 6 Weeks and 6 Months

Method: Linear Mixed-Effects Model (LME)

Model structure:

- Fixed effects: treatment group, time point (6 weeks vs. 6 months), treatment × time interaction
- Random effect: participant-level intercept (accounts for within-subject correlation)
- Covariance structure: Unstructured (preferred); compound symmetry if convergence issues

Interpretation:

- Significant interaction ($p < 0.05$): treatment effect differs across time points
- Non-significant interaction: main effects interpreted independently

SAP-5.5 Symptom Outcomes (Ordinal / Binary)

- Binary symptoms (spotting, dyspareunia): Chi-square or Fisher's exact test; RR with 95% CI
- **Paired temporal evolution** (6 weeks → 6 months within each group): **McNemar's test**
- Adjusted: Binary logistic regression

SAP-5.6 Hemoglobin Variation and Blood Loss

- ΔHb: t-test or Mann-Whitney U; mean difference with 95% CI
- PSTEV and PSTC: Mann-Whitney U (likely non-normal); median difference with 95% CI

SAP-6. ADVANCED ANALYTICAL METHODS

SAP-6.1 Clinical Predictive Model for CSD

A **clinical predictive model** for CSD formation will be developed using **Firth penalized logistic regression**, justified by the ratio of events to variables approaching the threshold defined by Peduzzi et al.

Model development:

- Variable selection: univariate screening ($p < 0.20$) + a priori clinical variables
- Stability: **Bootstrap validation (10,000 replications)**
- Optimal threshold: **Youden Index**
- Internal validation: **10-fold cross-validation** and **70/30 data partition**
- Performance: AUC, sensitivity, specificity, positive and negative predictive values

SAP-6.2 Mediation Analysis

The **mediating role of CSD at 6 weeks** on the relationship between hysterorrhaphy technique and RMT at 6 months will be assessed using the **counterfactual mediation framework (Imai et al.)**.

Estimated quantities:

- **ACME** (Average Causal Mediation Effect): indirect effect through CSD at 6 weeks
- **ADE** (Average Direct Effect): direct effect of technique on RMT
- **Total Effect** and **proportion mediated**

Method: Bootstrap with **10,000 replications**

Sensitivity analysis: Critical ρ (rho) analysis to quantify robustness to unmeasured confounders

SAP-6.3 Inter-Operator Homogeneity

Breslow-Day test for homogeneity of treatment effects across operators (surgeons), to assess whether results are consistent regardless of the individual performing the procedure.

SAP-6.4 Measurement Reproducibility

Inter-observer concordance will be evaluated by **double-blind reading (n = 30 randomly selected examinations)**:

- Continuous variables (RMT, AMT): **Intraclass Correlation Coefficient (ICC)**
- Categorical variables (CSD presence): **Cohen's Kappa**

Temporal concordance of RMT measurements (6 weeks vs. 6 months):

- **Lin's Concordance Correlation Coefficient**
- **Bland-Altman analysis** (mean difference and limits of agreement)

SAP-7. SUBGROUP ANALYSES

Pre-specified subgroup analyses for the primary outcome (RMT at 6–7 months):

Subgroup Factor	Categories	Statistical Test
BMI category	< 30 vs. 30–34.9 kg/m²	Interaction term in regression
Gestational age	37–38+6 vs. ≥ 39 weeks	Interaction term
Indication for CS	Labor dystocia vs. Fetal indication vs. Maternal indication	Interaction term
Uterine exteriorization	Yes vs. No	Interaction term
Type of cesarean section	Scheduled vs. Intrapartum	Interaction term
Surgeon category	Senior vs. Resident Year 4–5	Interaction term

Test for interaction: Likelihood ratio test comparing models with and without the interaction term.

All subgroup analyses are exploratory and hypothesis-generating. No correction for multiple comparisons is applied to subgroup analyses; results will not drive primary conclusions.

Results will be presented in **forest plots**.

SAP-8. SENSITIVITY ANALYSES

Analysis	Method
SA-1: Per-Protocol population	Repeat primary analysis in PP population
SA-2: Intraoperative complication exclusion	Exclude participants with major intraoperative events (corporeal hysterotomy, hysterotomy extension > 2 cm)
SA-3: Multiple imputation for missing RMT	MICE — 5 scenarios: observed data, LOCF, best case, worst case, 6-week status carry-forward
SA-4: Inverse Probability Weighting (IPW)	Adjust for informative loss to follow-up
SA-5: Binary threshold analysis	Proportion with RMT < 2.5 mm and RMT < 3.0 mm; Chi-square test
SA-6: Complete case analysis	For comparison with imputed results

SAP-9. MULTIPLICITY AND TYPE I ERROR CONTROL

- **Primary outcomes:** α = 0.05, two-sided, for each co-primary endpoint (RMT and CSD prevalence at 6 weeks and 6 months)
- **Secondary outcomes:** Exploratory; **Holm-Bonferroni correction** applied to control family-wise error rate
- **Unmeasured confounders: E-value** calculated to quantify the minimum strength of an unmeasured confounder needed to explain away observed associations

SAP-10. MISSING DATA STRATEGY

Data Type	Strategy
Primary outcome (RMT at 6 months)	Five imputation scenarios (MICE)
Secondary continuous outcomes	Complete case + sensitivity with imputation if > 10% missing
Binary outcomes	Complete case analysis

Symptom questionnaires	Linear mixed model (full information maximum likelihood)

Missing data mechanism assessed by **Little's MCAR test**.

Participants lost to follow-up compared to completers on key baseline variables; reasons for dropout documented and reported.

SAP-11. PRESENTATION OF RESULTS

Results reported in accordance with **CONSORT 2010** guidelines:

- **Table 1:** Baseline characteristics (ITT population) — no p-values
- **Figure 1:** CONSORT flow diagram
- **Table 2:** Primary and secondary outcomes with between-group comparisons
- **Table 3:** Subgroup analysis (forest plots)
- **Table 4:** Multivariate analysis results
- **Table 5:** Safety and adverse event summary
- **Figure 2:** Bland-Altman plot for RMT reproducibility
- **Figure 3:** ROC curve for predictive model