STUDY PROTOCOL WITH INFORMED CONSENT FORM

Official Title:

Single-Layer Versus Double-Layer Uterine Closure After Primary Cesarean Section: Impact on Residual Myometrial Thickness and Cesarean Scar DefectFormation — A Prospective Randomized Controlled Trial

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Contents of This Document: Part 1 — Full Study Protocol

Part 2 — Informed Consent Form

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Ethics Committee Approval: Ethics Committee of the Faculty of Medicineof Sfax, Tunisia

Approval Number: 31/26

STUDY PROTOCOL

1. GENERAL INFORMATION

Full Title:
Single-Layer Versus Double-Layer Uterine Closure (Hysterorrhaphy) After Primary Cesarean Section: Impact on Residual Myometrial Thickness and Cesarean Scar Defect Formation — A Prospective Randomized Controlled Trial

Protocol Date: January 2025
ClinicalTrials.gov Identifier: [To be assigned upon registration]

Principal Investigator: [Larbi Nizar. MD]
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2. BACKGROUND AND RATIONALE

Cesarean section (CS) is one of the most frequently performed surgical procedures worldwide, with global rates steadily rising over the past three decades. While CS is a lifesaving intervention when appropriately indicated, it is associated with both short- and long-term complications, particularly those related to uterine scar integrity. Among these, the **cesarean scar defect (CSD)** — also referred to as uterine niche, isthmocele, or uterine dehiscence — has emerged as a clinically significant sequela that may compromise subsequent reproductive outcomes and maternal health.

A CSD is defined, in accordance with the **European Niche Task Force criteria (Jordan et al., 2019)**, as a triangular anechoic indentation **≥ 2 mm** of the anterior

uterine wall at the site of the cesarean scar, detected by transvaginal ultrasonography (TVUS). The **residual myometrial thickness (RMT)**, measured as the minimum thickness of myometrial tissue persisting at the scar site — defined as the distance between the base of the niche (or the scar line when no niche is present) and the outer edge of the uterine serosa, measured at the thinnest point — is considered a reliable surrogate marker for scar healing quality. An **RMT of less than 2.5 mm** is widely accepted as the threshold below which the risk of uterine rupture in subsequent pregnancies is substantially increased.

The technique of uterine closure at the time of cesarean section has long been debated as a modifiable surgical factor influencing scar quality. Two principal closure techniques are currently practiced:

- **Single-layer closure (SLC):** The uterine incision is closed with a single non-locking continuous suture incorporating the full thickness of the myometrium in one pass, including the decidua.
- **Double-layer closure (DLC):** The uterine incision is closed using two successive non-locking continuous extra-mucosal sutures, excluding the decidua: a first layer approximating the inner half of the myometrium, and a second layer burying the first, incorporating the outer half of the myometrium and the uterine serosa.

Despite extensive debate, the literature remains **conflicting and methodologically heterogeneous**. Several randomized controlled trials and meta-analyses have examined these two techniques, yet no definitive consensus has been reached regarding their comparative impact on RMT and CSD formation. Some studies suggest that DLC results in superior RMT and lower CSD rates, while others demonstrate no statistically significant difference, or even suggest potential disadvantages of DLC due to tissue ischemia from excessive suture tension.

Furthermore, the majority of existing studies suffer from significant methodological limitations, including heterogeneous patient populations with prior uterine surgeries, variable follow-up durations, inconsistent ultrasonographic measurement protocols, lack of standardized surgical technique within trial arms, and insufficient sample sizes to detect clinically meaningful differences.

Given the **primary cesarean section** context — where the uterine tissue is native and unaltered by previous scarring — this population provides the ideal setting in which to evaluate the de novo impact of closure technique on scar formation, free from the confounding influence of pre-existing scar pathology.

The present trial is therefore designed to address this evidence gap by conducting a well-powered, prospective, randomized, controlled trial in women undergoing **primary cesarean section** at CHU Hédi Chaker, Sfax, Tunisia, with standardized surgical technique, blinded ultrasonographic assessment, and rigorous follow-up at 6 weeks and 6 months postoperatively.

3. OBJECTIVES

3.1 Primary Objective

To compare the **residual myometrial thickness (RMT)** at the uterine scar site between single-layer and double-layer hysterorrhaphy techniques, and to compare the **prevalence of cesarean scar defect (CSD)**, at **6 weeks and 6 months** following primary cesarean section, as measured by transvaginal ultrasonography, in order to assess both early scar formation and the stability of the effect over time.

3.2 Secondary Objectives

1. To compare **intraoperative parameters** between groups, including hysterorrhaphy duration, total operative time, visually estimated blood loss (PSTEV), calculated total blood loss (PSTC), number of additional hemostatic sutures, and intraoperative complications.
2. To assess **immediate postoperative outcomes**, including hemoglobin variation (ΔHb), transfusion requirement, early postoperative complications (endometritis, wound infection, parietal hematoma, thromboembolic events, surgical revision), and hospital length of stay.
3. To evaluate **medium-term gynecological symptoms** potentially attributable to CSD at 6 weeks and 6 months, including postmenstrual spotting, prolonged menstruation (> 7 days), dysmenorrhea, chronic pelvic pain, menometrorrhagia, and dyspareunia.
4. To compare **CSD morphological characteristics** between groups, including defect depth, length, width, and estimated volume using the ellipsoid formula.
5. To assess the **adjacent myometrial thickness (AMT)** and the **myometrial ratio (RM = RMT/AMT × 100)** at both evaluation time points.
6. To identify **clinical and surgical predictors** of CSD formation and reduced RMT.

7. To evaluate the **mediating role** of CSD at 6 weeks on RMT at 6 months.

4. STUDY DESIGN

4.1 Design Overview

This is a **prospective, randomized, controlled, parallel-group, single-blind** trial comparing single-layer versus double-layer uterine closure following primary cesarean section. Blinding applies to participants and ultrasonographers performing outcome assessments. The operating surgeon and principal investigator are not blinded due to the nature of the surgical intervention and the necessity of data centralization, respectively.

4.2 Setting

The trial is conducted exclusively at the **Department of Obstetrics and Gynecology, CHU Hédi Chaker, Sfax, Tunisia** — a monocentric study at a tertiary-level university hospital with an established cesarean section program and a dedicated ultrasonography unit.

4.3 Study Timeline

- **Recruitment period:** 01 January 2025 to 31 December 2025 (12 months)
- **Follow-up period per participant:** 6 months (last follow-up visit: 6–7 months after delivery)
- **Primary completion date:** 01 January 2026
- **Study completion date:** 30 April 2026

5. ELIGIBILITY CRITERIA

5.1 Inclusion Criteria

Participants are eligible for enrollment if ALL of the following criteria are met:

Demographic criteria:

1. Female patients aged **18 to 45 years**

Obstetric criteria:

2. **Singleton ongoing pregnancy**
3. Gestational age **> 37 weeks of amenorrhea**, confirmed by **first-trimester**

ultrasonography
4. **Non-scarred uterus** (no prior uterine surgery of any kind)

Administrative criteria:
5. Written **informed consent** obtained
6. Patient affiliated with a **social security scheme**
7. Willingness and ability to comply with scheduled **follow-up visits** at 6–8 weeks and 6–7 months postoperatively

5.2 Non-Inclusion Criteria

Participants are not included if ANY of the following criteria are present:

General maternal criteria:

1. **BMI ≥ 35 kg/m²** (morbid obesity)
2. **Pre-gestational diabetes mellitus** (type 1 or type 2)
3. **Autoimmune or chronic systemic diseases**
4. **Hereditary connective tissue disorders**
5. **Constitutional or acquired hemostasis disorders**, or ongoing curative anticoagulation therapy

Obstetric criteria:
6. Suspected or documented **intrauterine infection** (chorioamnionitis)
7. **Abnormal placental insertion** (placenta previa or accreta spectrum)
8. **Retroplacental hematoma**
9. **Multiple gestation**

Uterine criteria:
10. Prior **uterine surgery** or known **uterine malformation**
11. **Documented adenomyosis**

Fetal criteria:
12. **Severe intrauterine growth restriction**
13. **Major known fetal malformation**
14. **Intrauterine fetal death**

5.3 Secondary Exclusion Criteria (Post-Randomization)

Participants are secondarily excluded if the following events occur:

During the intervention:

1. Intraoperative discovery of an **undiagnosed uterine anomaly**

2. Necessity of a **corporeal (vertical) hysterotomy**
3. **Major intraoperative hemorrhage** requiring non-standardized hemostatic procedures

During follow-up:
4. **Loss to follow-up**
5. **Withdrawal of consent**
6. **Occurrence of a new pregnancy before the 6-month evaluation**

6. RECRUITMENT MODALITIES

6.1 Scheduled (Elective) Cesarean Sections

Patients are identified at the preoperative consultation, generally conducted **48 to 72 hours** before the planned intervention. The investigator or a trained team member presents the study, answers questions, and provides the participant information document. Informed consent is obtained on the **morning of the intervention**, after verification of full understanding of the study modalities.

6.2 Cesarean Sections During Labor

Recruitment in the intrapartum context requires particular care. Only patients whose **clinical and emotional condition** permits serene and informed communication are approached. Patients in active distress, pain, or hemodynamically compromised are not solicited for enrollment.

7. RANDOMIZATION AND BLINDING

7.1 Randomization

Eligible participants are randomized in a **1:1 ratio** to receive either:

- **Group A:** Single-Layer Uterine Closure (SLC)
- **Group B:** Double-Layer Uterine Closure (DLC)

Randomization is performed using the **Clinical Trial Randomization Tool application**, generating a computer-based allocation sequence using **permuted blocks of variable sizes (4, 6, and 8)**, ensuring an unpredictable sequence and minimizing selection bias.

7.2 Allocation Timing and Procedure

The group allocation is revealed to the operating surgeon **on the day of the intervention only**, under the following sequential conditions:

1. **Verification of all eligibility criteria**
2. **Signed informed consent obtained**
3. **Fetal delivery and placental delivery completed**
4. **Immediately before performance of hysterorrhaphy**

This sequential allocation procedure ensures that randomization occurs as late as operationally feasible, maximizing allocation concealment and minimizing contamination.

7.3 Blinding

Actor	Blinding Status	Justification
Operating surgeon	**Non-blinded**	Must know the technique to be performed
Participants	**Blinded**	Not informed of the technique used
Ultrasonographers (evaluators)	**Blinded**	No access to allocation data
Principal Investigator	**Non-blinded**	Responsible for data centralization; holds both clinical and allocation data

8. INTERVENTIONS

8.1 Common Standardized Surgical Elements (Both Groups)

All cesarean sections are performed by **senior gynecologist-obstetricians or fourth- and fifth-year obstetric residents**, all experienced in both hysterorrhaphy techniques, according to a **strictly standardized operative protocol** from anesthesia to parietal closure. The only variable between groups is the hysterorrhaphy technique; all other operative steps are conducted uniformly.

Standard protocol elements include:

- **Anesthesia:** Spinal or general (recorded)
- **Skin incision:** Pfannenstiel or Joel-Cohen technique (recorded)
- **Fascial incision:** Transverse
- **Peritoneal entry:** Sharp or blunt (recorded)

- **Uterotomy:** Low transverse hysterotomy
- **Uterine exteriorization:** At surgeon's discretion (recorded)
- **Placental delivery:** Standardized technique
- **Peritoneal closure:** Not performed in either group
- **Fascial closure:** Continuous absorbable suture
- **Skin closure:** Subcuticular absorbable suture
- **Prophylactic antibiotics:** Standard institutional protocol

8.2 Group A — Single-Layer Closure (SLC)

The uterine incision is closed with a **single non-locking continuous suture (surjet continu non verrouillé)**, incorporating the **full thickness of the myometrium in one pass**, with **inclusion of the decidua**. Suture bites are of approximately 8–10 mm in depth and spacing, applied with appropriate tension to ensure hemostasis without tissue strangulation.

8.3 Group B — Double-Layer Closure (DLC)

The uterine incision is closed using **two successive non-locking continuous extra-mucosal sutures (surjets continus non verrouillés extra-muqueux)**, **excluding the decidua** in both layers:

- **First layer (deep):** Continuous non-locking suture incorporating the **inner half of the myometrium**, ensuring primary hemostasis and deep wall apposition
- **Second layer (superficial):** Continuous non-locking suture burying the first layer, incorporating the **outer half of the myometrium and the uterine serosa**

9. OUTCOME MEASURES

9.1 Primary Outcomes

1. **Residual Myometrial Thickness (RMT)** at the uterine scar site, measured by **transvaginal ultrasonography (TVUS)** at **6–8 weeks** and **6–7 months** postoperatively (millimeters)
2. **Prevalence of Cesarean Scar Defect (CSD)** at **6–8 weeks** and **6–7 months** postoperatively, defined per **European Niche Task Force**

criteria (Jordan et al., 2019) as a triangular anechoic indentation **≥ 2 mm** of the anterior uterine wall at the scar site

9.2 Secondary Outcomes

Ultrasonographic outcomes:

- CSD morphological dimensions: depth, length, width (mm)
- CSD volume: estimated by ellipsoid formula — **V (mm³) = (π/6) × Length × Width × Depth**
- **Adjacent Myometrial Thickness (AMT):** measured 5–10 mm from the scar on both sides (mm)
- **Myometrial Ratio (RM):** calculated as **RM (%) = RMT / AMT × 100**
- Uterine position (anteverted / retroverted)

Intraoperative outcomes:

- **Hysterorrhaphy duration** (minutes and seconds)
- **Total operative time** (minutes and seconds)
- **Visually Estimated Blood Loss (PSTEV):** volume of bloody fluid in suction canister at end of surgery, minus estimated amniotic fluid volume (mL)
- **Calculated Total Blood Loss (PSTC):**

$$PSTC(mL) = \frac{VST \times (Hct_{preop} - Hct_{postop})}{35} \times 100 + Compensated\ losses$$

Where: VST (Total Blood Volume) = Pre-pregnancy weight (kg) × 65 × 1.4; Compensated losses = Number of packed red blood cell units transfused × 500 mL; 35 = reference hematocrit of one packed RBC unit

- Number of **additional hemostatic sutures**
- **Intraoperative complications:** hysterotomy extension, ureteral injury, bladder injury, intestinal injury, hemorrhage requiring transfusion

Immediate postoperative outcomes:

- **Hemoglobin variation:** ΔHb (g/dL) = Hb preoperative − Hb at Day 1 postpartum
- **Transfusion requirement** (packed RBC units)

- **Early postoperative complications:** postpartum endometritis, surgical site infection, parietal hematoma, surgical revision, thromboembolic events
- **Hospital length of stay** (days)

Medium-term symptom outcomes (at 6–8 weeks and 6–7 months):

- Postmenstrual spotting (characteristic of CSD)
- Prolonged menstruation (> 7 days)
- Dysmenorrhea requiring medication
- Chronic pelvic pain (outside menstruation)
- Menometrorrhagia
- Dyspareunia

10. DATA COLLECTION AND FOLLOW-UP SCHEDULE

Time Point	Assessment
Preoperative	Eligibility verification, informed consent, baseline NFS (CBC), demographics
Intraoperative	Randomization, surgical parameters, hysterorrhaphy duration, operative time, blood loss, complications
Postoperative Day 1	NFS (hemoglobin), vital signs, wound assessment
Discharge	Wound assessment, clinical evaluation, length of stay
6–8 Weeks postpartum	TVUS (RMT, CSD assessment), AMT, RM, clinical examination, standardized symptom questionnaire
6–7 Months postpartum	**TVUS (RMT, CSD — PRIMARY ENDPOINT)**, AMT, RM, CSD dimensions, volume, standardized symptom questionnaire

Ultrasonographic Examination Protocol

All TVUS examinations are performed:

- By a **dedicated, trained ultrasonographer per center, blinded to group allocation**

- Using **Samsung HS40 ultrasound system with EVN4-9 endovaginal probe (4–9 MHz)**
- With **standardized imaging parameters**
- With the **bladder empty**
- **Preferably in the follicular phase (Day 7–14 of the menstrual cycle)** to optimize defect visualization
- In the **mid-sagittal plane**: depth, length, and RMT measured; width measured in transverse plane
- **RMT measured perpendicular to the serosa at the thinnest point** of the scar

11. SAMPLE SIZE CALCULATION

The sample size was determined based on the primary outcome — **RMT at 6 months** — using the standard formula for comparison of two independent means:

$$n = \frac{2 \times (Z_{\alpha/2} + Z_{\beta})^2 \times \sigma^2}{\Delta^2}$$

Parameters:

- Two-sided significance level: α = 0.05 (Z α/2 = 1.96)
- Statistical power: 80% (Z β = 0.842)
- Conservative standard deviation: **σ = 3.5 mm**
- Minimum clinically meaningful difference: **Δ = 1.3 mm**

Base sample size: 114 participants per group

Adjustment for loss to follow-up:
Based on local postpartum follow-up data and published literature, a **30% loss to follow-up rate** was anticipated:

$$n_{adjusted} = \frac{114}{1 - 0.30} \approx 163 \text{ per group}$$

Total enrolled: 384 participants (192 per group)

12. ETHICAL CONSIDERATIONS

The trial is conducted in accordance with:

- The **Declaration of Helsinki** (2013 revision)
- **ICH Good Clinical Practice (GCP) Guidelines E6(R2)**
- Applicable Tunisian national regulatory requirements

Ethical approval was obtained from the **Institutional Review Board (IRB) / Ethics Committee of CHU Hédi Chaker, Sfax** prior to enrollment of the first participant. All protocol amendments are submitted for ethics review before implementation.

13. DATA MANAGEMENT AND CONFIDENTIALITY

- All data are recorded in a **standardized data collection form** at each stage: inclusion, intervention, hospital follow-up, and outpatient consultations
- Each participant is assigned a **unique identification number**; no identifying information appears in the analysis database
- The **principal investigator** is the sole person with simultaneous access to both participant clinical/epidemiological data and group allocation
- Ultrasonographers and data analysts have access only to anonymized data
- Source documents are retained for a minimum of **15 years** following study completion
- Data access is restricted to **authorized study personnel only**

14. DISSEMINATION POLICY

Results will be disseminated regardless of direction of findings through:

- Peer-reviewed scientific publications
- International obstetric and gynecological conferences
- ClinicalTrials.gov results posting within **12 months** of study completion

Single-Layer Versus Double-Layer Uterine Closure (Hysterorrhaphy) After Primary Cesarean Section: Impact on Residual Myometrial Thickness and Cesarean Scar Defect Formation

STUDY PROTOCOL

1. GENERAL INFORMATION

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Protocol Date: January 2025
ClinicalTrials.gov Identifier: [To be assigned upon registration]

Principal Investigator: [Larbi Nizar. MD]
Institution: Department of Obstetrics and Gynecology, Hedi Chaker University Hospital (CHU Hédi Chaker), Sfax, Tunisia
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Cesarean section (CS) is one of the most frequently performed surgical procedures worldwide, with global rates steadily rising over the past three decades. While CS is a lifesaving intervention when appropriately indicated, it is associated with both short- and long-term complications, particularly those related to uterine scar integrity. Among these, the **cesarean scar defect (CSD)** — also referred to as uterine niche, isthmocele, or uterine dehiscence — has emerged as a clinically significant sequela that may compromise subsequent reproductive outcomes and maternal health.

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defined as the distance between the base of the niche (or the scar line when no niche is present) and the outer edge of the uterine serosa, measured at the thinnest point — is considered a reliable surrogate marker for scar healing quality. An **RMT of less than 2.5 mm** is widely accepted as the threshold below which the risk of uterine rupture in subsequent pregnancies is substantially increased.

The technique of uterine closure at the time of cesarean section has long been debated as a modifiable surgical factor influencing scar quality. Two principal closure techniques are currently practiced:

- **Single-layer closure (SLC):** The uterine incision is closed with a single non-locking continuous suture incorporating the full thickness of the myometrium in one pass, including the decidua.
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Despite extensive debate, the literature remains **conflicting and methodologically heterogeneous**. Several randomized controlled trials and meta-analyses have examined these two techniques, yet no definitive consensus has been reached regarding their comparative impact on RMT and CSD formation. Some studies suggest that DLC results in superior RMT and lower CSD rates, while others demonstrate no statistically significant difference, or even suggest potential disadvantages of DLC due to tissue ischemia from excessive suture tension.

Furthermore, the majority of existing studies suffer from significant methodological limitations, including heterogeneous patient populations with prior uterine surgeries, variable follow-up durations, inconsistent ultrasonographic measurement protocols, lack of standardized surgical technique within trial arms, and insufficient sample sizes to detect clinically meaningful differences.

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The present trial is therefore designed to address this evidence gap by conducting a well-powered, prospective, randomized, controlled trial in women undergoing **primary cesarean section** at CHU Hédi Chaker, Sfax, Tunisia, with

standardized surgical technique, blinded ultrasonographic assessment, and rigorous follow-up at 6 weeks and 6 months postoperatively.

3. OBJECTIVES

3.1 Primary Objective

To compare the **residual myometrial thickness (RMT)** at the uterine scar site between single-layer and double-layer hysterorrhaphy techniques, and to compare the **prevalence of cesarean scar defect (CSD)**, at **6 weeks and 6 months** following primary cesarean section, as measured by transvaginal ultrasonography, in order to assess both early scar formation and the stability of the effect over time.

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11. To compare **CSD morphological characteristics** between groups, including defect depth, length, width, and estimated volume using the ellipsoid formula.
12. To assess the **adjacent myometrial thickness (AMT)** and the **myometrial ratio (RM = RMT/AMT × 100)** at both evaluation time points.
13. To identify **clinical and surgical predictors** of CSD formation and reduced RMT.
14. To evaluate the **mediating role** of CSD at 6 weeks on RMT at 6 months.

4. STUDY DESIGN

4.1 Design Overview

This is a **prospective, randomized, controlled, parallel-group, single-blind** trial comparing single-layer versus double-layer uterine closure following primary cesarean section. Blinding applies to participants and ultrasonographers performing outcome assessments. The operating surgeon and principal investigator are not blinded due to the nature of the surgical intervention and the necessity of data centralization, respectively.

4.2 Setting

The trial is conducted exclusively at the **Department of Obstetrics and Gynecology, CHU Hédi Chaker, Sfax, Tunisia** — a monocentric study at a tertiary-level university hospital with an established cesarean section program and a dedicated ultrasonography unit.

4.3 Study Timeline

- **Recruitment period:** 01 January 2025 to 31 December 2025 (12 months)
- **Follow-up period per participant:** 6 months (last follow-up visit: 6–7 months after delivery)
- **Primary completion date:** 30 June 2026
- **Study completion date:** 30 April 2026

5. ELIGIBILITY CRITERIA

5.1 Inclusion Criteria

Participants are eligible for enrollment if ALL of the following criteria are met:

Demographic criteria:

2. Female patients aged **18 to 45 years**

Obstetric criteria:
2. **Singleton ongoing pregnancy**
3. Gestational age **> 37 weeks of amenorrhea**, confirmed by **first-trimester ultrasonography**
4. **Non-scarred uterus** (no prior uterine surgery of any kind)

Administrative criteria:
5. Written **informed consent** obtained

6. Patient affiliated with a **social security scheme**
7. Willingness and ability to comply with scheduled **follow-up visits** at 6–8 weeks and 6–7 months postoperatively

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Participants are not included if ANY of the following criteria are present:

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6. **BMI ≥ 35 kg/m²** (morbid obesity)
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Uterine criteria:
10. Prior **uterine surgery** or known **uterine malformation**
11. **Documented adenomyosis**

Fetal criteria:
12. **Severe intrauterine growth restriction**
13. **Major known fetal malformation**
14. **Intrauterine fetal death**

5.3 Secondary Exclusion Criteria (Post-Randomization)

Participants are secondarily excluded if the following events occur:

During the intervention:

4. Intraoperative discovery of an **undiagnosed uterine anomaly**
5. Necessity of a **corporeal (vertical) hysterotomy**
6. **Major intraoperative hemorrhage** requiring non-standardized hemostatic procedures

During follow-up:
4. **Loss to follow-up**
5. **Withdrawal of consent**
6. **Occurrence of a new pregnancy before the 6-month evaluation**

6. RECRUITMENT MODALITIES

6.1 Scheduled (Elective) Cesarean Sections

Patients are identified at the preoperative consultation, generally conducted **48 to 72 hours** before the planned intervention. The investigator or a trained team member presents the study, answers questions, and provides the participant information document. Informed consent is obtained on the **morning of the intervention**, after verification of full understanding of the study modalities.

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7.1 Randomization

Eligible participants are randomized in a **1:1 ratio** to receive either:

- **Group A:** Single-Layer Uterine Closure (SLC)
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Randomization is performed using the **Clinical Trial Randomization Tool application**, generating a computer-based allocation sequence using **permuted blocks of variable sizes (4, 6, and 8)**, ensuring an unpredictable sequence and minimizing selection bias.

7.2 Allocation Timing and Procedure

The group allocation is revealed to the operating surgeon **on the day of the intervention only**, under the following sequential conditions:

5. **Verification of all eligibility criteria**
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8. **Immediately before performance of hysterorrhaphy**

This sequential allocation procedure ensures that randomization occurs as late as operationally feasible, maximizing allocation concealment and minimizing contamination.

7.3 Blinding

Actor	Blinding Status	Justification
Operating surgeon	**Non-blinded**	Must know the technique to be performed
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Ultrasonographers (evaluators)	**Blinded**	No access to allocation data
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8.1 Common Standardized Surgical Elements (Both Groups)

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- **Uterotomy:** Low transverse hysterotomy
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- **Placental delivery:** Standardized technique

- **Peritoneal closure:** Not performed in either group
- **Fascial closure:** Continuous absorbable suture
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- **Prophylactic antibiotics:** Standard institutional protocol

8.2 Group A — Single-Layer Closure (SLC)

The uterine incision is closed with a **single non-locking continuous suture (surjet continu non verrouillé)**, incorporating the **full thickness of the myometrium in one pass**, with **inclusion of the decidua**. Suture bites are of approximately 8–10 mm in depth and spacing, applied with appropriate tension to ensure hemostasis without tissue strangulation.

8.3 Group B — Double-Layer Closure (DLC)

The uterine incision is closed using **two successive non-locking continuous extra-mucosal sutures (surjets continus non verrouillés extra-muqueux)**, **excluding the decidua** in both layers:

- **First layer (deep):** Continuous non-locking suture incorporating the **inner half of the myometrium**, ensuring primary hemostasis and deep wall apposition
- **Second layer (superficial):** Continuous non-locking suture burying the first layer, incorporating the **outer half of the myometrium and the uterine serosa**

9. OUTCOME MEASURES

9.1 Primary Outcomes

3. **Residual Myometrial Thickness (RMT)** at the uterine scar site, measured by **transvaginal ultrasonography (TVUS)** at **6–8 weeks** and **6–7 months** postoperatively (millimeters)
4. **Prevalence of Cesarean Scar Defect (CSD)** at **6–8 weeks** and **6–7 months** postoperatively, defined per **European Niche Task Force criteria (Jordan et al., 2019)** as a triangular anechoic indentation **≥ 2 mm** of the anterior uterine wall at the scar site

9.2 Secondary Outcomes

Ultrasonographic outcomes:

- CSD morphological dimensions: depth, length, width (mm)
- CSD volume: estimated by ellipsoid formula — **V (mm³) = (π/6) × Length × Width × Depth**
- **Adjacent Myometrial Thickness (AMT):** measured 5–10 mm from the scar on both sides (mm)
- **Myometrial Ratio (RM):** calculated as **RM (%) = RMT / AMT × 100**
- Uterine position (anteverted / retroverted)

Intraoperative outcomes:

- **Hysterorrhaphy duration** (minutes and seconds)
- **Total operative time** (minutes and seconds)
- **Visually Estimated Blood Loss (PSTEV):** volume of bloody fluid in suction canister at end of surgery, minus estimated amniotic fluid volume (mL)
- **Calculated Total Blood Loss (PSTC):**

$$PSTC(mL) = \frac{VST \times (Hct_{preop} - Hct_{postop})}{35} \times 100 + Compensated\ losses$$

Where: VST (Total Blood Volume) = Pre-pregnancy weight (kg) × 65 × 1.4; Compensated losses = Number of packed red blood cell units transfused × 500 mL; 35 = reference hematocrit of one packed RBC unit

- Number of **additional hemostatic sutures**
- **Intraoperative complications:** hysterotomy extension, ureteral injury, bladder injury, intestinal injury, hemorrhage requiring transfusion

Immediate postoperative outcomes:

- **Hemoglobin variation:** ΔHb (g/dL) = Hb preoperative − Hb at Day 1 postpartum
- **Transfusion requirement** (packed RBC units)
- **Early postoperative complications:** postpartum endometritis, surgical site infection, parietal hematoma, surgical revision, thromboembolic events
- **Hospital length of stay** (days)

Medium-term symptom outcomes (at 6–8 weeks and 6–7 months):

- Postmenstrual spotting (characteristic of CSD)
- Prolonged menstruation (> 7 days)
- Dysmenorrhea requiring medication
- Chronic pelvic pain (outside menstruation)
- Menometrorrhagia
- Dyspareunia

10. DATA COLLECTION AND FOLLOW-UP SCHEDULE

Time Point	Assessment
Preoperative	Eligibility verification, informed consent, baseline NFS (CBC), demographics
Intraoperative	Randomization, surgical parameters, hysterorrhaphy duration, operative time, blood loss, complications
Postoperative Day 1	NFS (hemoglobin), vital signs, wound assessment
Discharge	Wound assessment, clinical evaluation, length of stay
6–8 Weeks postpartum	TVUS (RMT, CSD assessment), AMT, RM, clinical examination, standardized symptom questionnaire
6–7 Months postpartum	**TVUS (RMT, CSD — PRIMARY ENDPOINT)**, AMT, RM, CSD dimensions, volume, standardized symptom questionnaire

Ultrasonographic Examination Protocol

All TVUS examinations are performed:

- By a **dedicated, trained ultrasonographer per center, blinded to group allocation**
- Using **Samsung HS40 ultrasound system with EVN4-9 endovaginal probe (4–9 MHz)**
- With **standardized imaging parameters**
- With the **bladder empty**
- **Preferably in the follicular phase (Day 7–14 of the menstrual cycle)** to optimize defect visualization

- In the **mid-sagittal plane**: depth, length, and RMT measured; width measured in transverse plane
- **RMT measured perpendicular to the serosa at the thinnest point** of the scar

11. SAMPLE SIZE CALCULATION

The sample size was determined based on the primary outcome — **RMT at 6 months** — using the standard formula for comparison of two independent means:

$$n = \frac{2 \times (Z_{\alpha/2} + Z_{\beta})^2 \times \sigma^2}{\Delta^2}$$

Parameters:

- Two-sided significance level: α = 0.05 (Z α/2 = 1.96)
- Statistical power: 80% (Z β = 0.842)
- Conservative standard deviation: **σ = 3.5 mm**
- Minimum clinically meaningful difference: **Δ = 1.3 mm**

Base sample size: 114 participants per group

Adjustment for loss to follow-up:
Based on local postpartum follow-up data and published literature, a **30% loss to follow-up rate** was anticipated:

$$n_{adjusted} = \frac{114}{1 - 0.30} \approx 163 \text{ per group}$$

Total enrolled: 384 participants (192 per group)

12. ETHICAL CONSIDERATIONS

The trial is conducted in accordance with:

- The **Declaration of Helsinki** (2013 revision)
- **ICH Good Clinical Practice (GCP) Guidelines E6(R2)**
- Applicable Tunisian national regulatory requirements

Ethical approval was obtained from the **Institutional Review Board (IRB) / Ethics Committee of CHU Hédi Chaker, Sfax** prior to enrollment of the first participant. All protocol amendments are submitted for ethics review before implementation.

13. DATA MANAGEMENT AND CONFIDENTIALITY

- All data are recorded in a **standardized data collection form** at each stage: inclusion, intervention, hospital follow-up, and outpatient consultations
- Each participant is assigned a **unique identification number**; no identifying information appears in the analysis database
- The **principal investigator** is the sole person with simultaneous access to both participant clinical/epidemiological data and group allocation
- Ultrasonographers and data analysts have access only to anonymized data
- Source documents are retained for a minimum of **15 years** following study completion
- Data access is restricted to **authorized study personnel only**

14. DISSEMINATION POLICY

Results will be disseminated regardless of direction of findings through:

- Peer-reviewed scientific publications
- International obstetric and gynecological conferences
- ClinicalTrials.gov results posting within **12 months** of study completion

INFORMED CONSENT FORM

Document Title: Participant Informed Consent Form — Standalone Version
Study Title: Single-Layer Versus Double-Layer Uterine Closure (Hysterorrhaphy)

After Primary Cesarean Section: Impact on Residual Myometrial Thickness and Cesarean Scar Defect Formation
Date: January 2025
ClinicalTrials.gov Identifier: [To be assigned]
Ethics Approval Number: [31/26] The Ethics Committee of the Faculty of Medicine of Sfax - Tunisia
Institution: Department of Obstetrics and Gynecology, CHU Hédi Chaker, Sfax, Tunisia

DEAR PARTICIPANT,

You are being invited to take part in a **medical research study** being conducted at the Department of Obstetrics and Gynecology of CHU Hédi Chaker in Sfax. This document has been prepared to give you a complete and clear understanding of what the study is about, what it involves, and what your rights are as a participant.

Please read this entire document carefully before making any decision.

You are free to take as much time as you need. You may take this document home, discuss it with your family or doctor, and ask any questions you wish. **There is no pressure to decide immediately.**

A. WHAT IS THIS STUDY ABOUT?

When a cesarean section (C-section) is performed, the surgeon makes a cut in the uterus (womb) to allow the baby to be born. Once the baby and placenta are delivered, the surgeon stitches this cut closed. There are currently **two accepted and safe methods** for doing this:

➤ Method 1 — Single-Layer Closure

The uterine incision is closed with **one continuous row of stitches** passing through the entire thickness of the uterine wall in a single pass.

➤ Method 2 — Double-Layer Closure

The uterine incision is closed with **two successive rows of stitches**:

- The **first row** closes the deep inner part of the uterine wall
- The **second row** reinforces the first, incorporating the outer part of the wall

Both methods are safe and widely used. However, the medical community has not yet reached a clear consensus on which method produces better healing of the uterine scar.

After a cesarean section, the scar on the uterus sometimes heals incompletely, creating what is called a **"scar defect"** (also known as a uterine niche or isthmocele). This is a thinned or hollow area at the scar site, which can sometimes cause:

- **Unusual spotting or bleeding** between periods
- **Painful periods** or pelvic pain
- In rare cases, **complications in a future pregnancy** if the scar is too thin

This study aims to find out which stitching method results in:

1. A **thicker, better-healed uterine scar**
2. A **lower risk of developing a scar defect**

Your uterine scar will be evaluated using an **internal vaginal ultrasound** at **6–8 weeks** and again at **6–7 months** after your cesarean section.

B. WHY ARE YOU BEING INVITED?

You are being invited because:

- This is your **first cesarean section** (your uterus has never been operated on before)
- You are pregnant with **a single baby**
- Your pregnancy is **at least 37 weeks** along
- You appear to meet **all the medical requirements** for safe participation

We plan to enroll **384 women** in this study.

C. MUST YOU PARTICIPATE?

No. Your participation is entirely your own choice.

Whether you agree or refuse to participate will have **absolutely no effect** on the care you receive — before, during, or after your cesarean section. Your surgeon

will continue to provide you with the same high standard of care regardless of your decision.

D. WHAT WILL HAPPEN IF YOU AGREE TO PARTICIPATE?

🔘 Step 1 — Enrollment and Randomization

If you agree to participate, you will be **randomly assigned** (by a computer program, like drawing lots) to receive either the single-layer or the double-layer closure technique.

Neither you nor your surgeon can choose which technique you receive. This random assignment ensures that the two groups are comparable and that the results of the study are fair and reliable.

This assignment will take place **after your baby and placenta have been delivered**, immediately before your surgeon closes your uterus.

You will not be told which technique was used during the study period. This ensures that the doctors performing your ultrasound assessments remain unaware of your group, making their measurements unbiased.

🔘 Step 2 — Your Cesarean Section

Your cesarean section will be performed **exactly as it would be** whether or not you participate in this study. The **only modification** is in how the uterine incision is stitched closed — using the randomly assigned technique. Everything else remains standard.

🔘 Step 3 — Hospital Stay

You will receive standard postoperative monitoring, including a blood test on the first day after surgery to check your hemoglobin level, wound monitoring, and routine medical follow-up until discharge.

🔘 Step 4 — Two Follow-Up Consultations

Visit	When	What Happens
Visit 1	**6–8 weeks** after surgery	Transvaginal ultrasound + clinical exam + symptom questionnaire
Visit 2	**6–7 months** after surgery	Transvaginal ultrasound + symptom questionnaire **(main assessment)**

🔘 What is a Transvaginal Ultrasound?

A transvaginal ultrasound is a standard gynecological examination used to assess the uterus in detail. A small, smooth probe — about the width of a finger — is gently inserted into the vagina. The probe uses **sound waves, not radiation**, to produce internal images.

This examination:

- Is **safe and routinely performed** in gynecology
- Causes only **mild discomfort or pressure** in most cases, but is **not painful**
- Takes approximately **10–15 minutes**
- Is ideally performed around **Day 7–14 of your menstrual cycle** for optimal scar visualization
- Requires an **empty bladder**

🔘 Step 5 — Symptom Questionnaire

At each consultation, you will complete a **standardized questionnaire** asking about any symptoms you may have noticed, such as unusual bleeding, spotting between periods, pelvic pain, painful periods, or discomfort during intercourse.

E. WHAT ARE THE RISKS?

Risks of the Cesarean Section

Both closure techniques are equally safe and well-established. **Your participation does not add any surgical risk** compared to a standard cesarean section.

Risks that apply to all cesarean sections (whether or not you participate) include:

- Bleeding (occasionally requiring a blood transfusion)
- Wound or uterine infection
- Injury to nearby organs (bladder, bowel) — rare
- Blood clots — rare
- Anesthesia-related risks

Risks of the Transvaginal Ultrasound

- Mild, brief discomfort during insertion of the probe
- Very rarely, light vaginal spotting immediately afterward (resolves on its own)
- **No radiation exposure**

There are no additional risks associated specifically with your participation in this study.

F. WHAT ARE THE POTENTIAL BENEFITS?

For You

- You will receive **specialized monitoring of your uterine scar** at 6–8 weeks and 6–7 months — a level of follow-up not routinely offered after standard cesarean section
- If any scar abnormality is detected, you will be informed and referred for appropriate follow-up

For Future Patients

- Your participation will contribute to **important medical knowledge** that will help surgeons worldwide choose the best uterine closure technique, potentially improving outcomes for many future mothers

G. WHAT IF YOU DO NOT PARTICIPATE?

Your only alternative is to **not take part**. Your cesarean section will be carried out using your surgeon's standard preferred technique, and you will receive routine postoperative care with no additional scar monitoring.

H. HOW WILL YOUR PERSONAL INFORMATION BE PROTECTED?

All your personal and medical information is handled with the **strictest confidentiality**:

✅ You will be assigned a **unique anonymous study code** — your name will never appear in any research file or database
✅ Your data will be stored in a **secure, password-protected**

system accessible only to authorized study personnel
✅ Research publications will **never identify you personally**
✅ Your information will **not be shared** with any insurance company, employer, government agency, or third party
✅ Your data will be **kept for 15 years** following the end of the study (as required by law), after which it will be securely destroyed

Your anonymized data may be accessed by:

- The **Ethics Committee / Institutional Review Board** of CHU Hédi Chaker (for audit)
- **Health regulatory authorities** (if legally required)

I. CAN YOU WITHDRAW FROM THE STUDY?

Yes, at any time, for any reason, without consequence.

You may:

- **Refuse to participate** before signing this form
- **Withdraw your consent** after signing, at any point during the study
- **Request deletion of your data** from the study database before analysis

Withdrawal will have **absolutely no impact** on the medical care you receive at CHU Hédi Chaker.

J. WILL YOU BE PAID FOR PARTICIPATING?

There is **no financial compensation** for participating in this study. Study-related ultrasound examinations are provided **at no cost to you**. Transportation and other personal expenses are **not reimbursed**.

K. WHO CAN YOU CONTACT?

For any question, concern, or complaint regarding this study:

Principal Investigator:
◈ Dr. [Larbi Nizar], MD
◈ Department of Obstetrics and Gynecology, CHU Hédi Chaker, Sfax, Tunisia

◈ 📞 [+21693726]
◈ ✉ [dr.larbinizar.obgyn@gmail.com]
◈ Hours: Monday–Friday, 08:00–16:00

L. SIGNATURE AND CONSENT

Before signing, please confirm that:

- I have read and understood this entire document (ICF Version 2.0, January 2025)
- I have had sufficient time to consider my participation
- All my questions have been answered to my full satisfaction
- I understand that my participation is **voluntary** and may be withdrawn at any time
- I understand that I will **not be informed** of which surgical technique was used during the study
- I agree to attend **both follow-up visits** (6–8 weeks and 6–7 months postpartum)
- I consent to undergo **transvaginal ultrasound** at both follow-up visits
- I understand how my personal data will be **used, stored, and protected**

I give my free, informed, and voluntary consent to participate in this study

PARTICIPANT

Full Name (Print clearly):	_____
Signature:	_____
Date:	_____
Contact telephone:	_____

WITNESS ***(Required if participant is unable to read or write)***

Full Name (Print clearly):	_____
Relationship to participant:	_____
Signature:	_____
Date:	_____

INVESTIGATOR / PERSON OBTAINING CONSENT

I hereby confirm that I have clearly and fully explained to the participant the nature, purpose, duration, procedures, potential risks, and potential benefits of this study in language accessible to her. She has been given adequate time and opportunity to ask questions, and all questions have been answered truthfully and completely.

Full Name (Print clearly):	_____
Title / Role:	_____
Signature:	_____
Date:	_____

📋 A signed copy of this Informed Consent Form will be given to the participant to keep for her own records.
The original signed document will be retained in the official study file at CHU Hédi Chaker.