Effects of visceral peritoneal closure on scar formation at cesarean delivery

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1. Introduction

Cesarean delivery is the most common obstetric intraperitoneal operation, and the number of cesarean deliveries is increasing worldwide.

Although visceral peritoneal suturing is performed in many countries, it is no longer considered to be essential in abdominal wound closure [1]. Cheong et al. [2] concluded that short-term morbidity is not related to non-closure of the peritoneum. Studies on the effects of closure or non-closure of the peritoneum during cesarean delivery on adhesion formation have concluded that insufficient data were available and that adequately powered and designed trials were needed [3,4].

Limitations of studies on the effect of suturing the visceral peritoneum at cesarean delivery on scar formation concern the inclusion of both gynecologic and obstetric operations, not distinguishing between parietal and visceral peritoneum, combining results from scheduled and emergency cesarean deliveries, and not clearly differentiating between the short- and long-term effects of peritoneal suturing [1–4].

The aim of the present study was to assess the long-term effect of visceral peritoneal closure at cesarean delivery on adhesion formation either between the parietal peritoneum and visceral peritoneum or between the bladder and uterus. Adhesion formation was assessed at the time of a scheduled repeat cesarean delivery.

2. Materials and methods

The study was conducted at Santa Maria Hospital, Bari, and Vito Fazzi Hospital, Lecce, Italy, between September 2003 and September 2007. The study was approved by the institutions’ local ethics committees and consisted of 2 parts: the first part was enrollment to undergo an initial cesarean delivery performed by 1 of 2 methods; and the second part was to check the effects of visceral peritoneal closure on scar formation assessed during a repeat cesarean delivery.

The inclusion criteria for women undergoing an initial cesarean procedure, always after 37 weeks of pregnancy, were breech or other malpresentations, post-term pregnancy in women of advanced age, or cesarean delivery on request. The exclusion criteria for enrollment in this part of the study were women with any previous general or obstetric operation, and the number of cesarean deliveries is increasing worldwide.
gynecologic abdominal surgery and any of the following experienced during the pregnancy: macrosomia; infection; anticoagulation therapy; pre-eclampsia; HELLP syndrome; emergency cesarean delivery; ruptured membranes for more than 36 hours; placenta previa, and other placental pathologies.

A total of 129 women were eligible to participate and 112 women provided informed consent and were enrolled in the study. They all consented to undergo the first cesarean delivery and a repeat cesarean delivery in a subsequent pregnancy, avoiding a vaginal delivery after cesarean.

All women had negative vaginal swabs at 34–36 weeks (for Group B streptococcus, chlamydia, mycoplasma, and fungi).

Patients undergoing the initial cesarean procedure were consecutively allocated into 2 groups by the clinicians: group 1 underwent visceral peritoneal closure, while in group 2 the visceral peritoneum was not closed. Both groups of women were operated on by the same physician group either standardizing the surgical method for homogeneity or by alternating the patients consecutively.

Patients received combined spinal–epidural anesthesia, following an infusion of 500 mL of a plasma expander and 5 mg of intravenous ephedrine (to prevent the hypotension).

They also received a prophylactic antibiotic dose of 2 g of cefazoline intravenously. The initial cesarean procedure was performed using the Misgav Ladach method in all patients, with a Joel-Cohen laparotomy (JC-L), and a low uterine segment (LUS) incision using the Munro Kerr method, according to the following surgical description.

Anatomical forces were used to grasp the visceral peritoneum around the vesico–uterine peritoneal serosal fold; a scalpel was used to make a small midline incision in the visceral peritoneum, and both index fingers were used to stretch it, laterally, in both directions for about 4 cm and caudally for 3 cm to detach it transversely by the surface of the uterus. The bladder flap was pushed down using fingers or Doyen valves. A small transverse incision (about 2 cm) was made at the superior part of the LUS, and it was gently stretched, using index fingers, laterally in both directions, sufficiently enough to deliver the fetus and stopping before the lateral uterine blood vessels. No intrabdominal sponges, towels, or swabs were used to minimize future adhesions.

The fetus was delivered and the placenta was removed after it delivered spontaneously. The uterus was exteriorized for only a short period and the myometrium of the LUS was sutured in a single layer using extra-endometrial continuous absorbable stitches of polyglactin 910 (Vicryl 0; Ethicon, Somerville, NJ, USA).

Hemostasis was performed at the hysterotomy site in both methods; the peritoneum was handled using anatomical forces and the closure was done with continuous polyglactin 910 (Vicryl 00; Ethicon).

Hemostasis on the uterine scar was performed using single Vicryl 00 stitches, except in 7 patients (4 in group 1 and 3 in group 2) where hemostasis was done by application of Bengolea forceps and single Vicryl 00 threads on the visceral peritoneal flap, avoiding the use of an electro-scalpel.

In all patients the parietal peritoneum was closed with Vicryl 000; the abdominal wall was closed by suturing the fascia, without suturing the abdominal muscles.

The 112 women who consented to participate in the study subsequently became pregnant again and, based on their decision to avoid a vaginal birth after cesarean delivery, all underwent repeat cesarean delivery.

In the repeat cesarean delivery procedure, the JC-L was used for 103 patients and the Pfannenstiel incision was used in 9 patients owing to previous abdominal–pelvic adhesions found at the first cesarean delivery (even if the distance was far from the LUS).

In the majority of cases the laparotomy was made at the previous scar; the cesarean delivery was performed using the Misgav Ladach method, as described above. After opening the abdominal cavity and lyses of intraperitoneal subtle and connecting adhesions, the bladder flap was visualized, cut by scissors, and pushed down using fingers.

The severity of the adhesions observed at repeat cesarean was assessed using the Adhesion Scoring Method of the American Fertility Society [5]; adhesions were graded as: none, mild (a filmy vascular adhesion), or severe (a dense organized cohesive vascular adhesion).

The visceral peritoneal detachment was carried out just on the LUS to expose the previous uterine scar. The LUS incision was made directly on the previous cesarean uterine scar for histologic and morphologic analysis. Samples were taken by sterile scissors of 4 complete thickness sections (from serosa to mucosa) cutting superior and inferior edges: 2 samples (of approximately 5 mm depth) were obtained from the superior edge and 2 from the inferior edge. The delivery and closure techniques were as above.

The tissue samples were placed in Bouin solution for 24 hours; the specimens were prepared in successive preparations of alcohol solutions starting at 70% and, once the samples were clear of water, they were fixed in paraffin wax.

Sections of 5-µm thickness obtained for each sample were stained with hematoxylin-eosin, 0.05% periodic acid solution (PAS), and Masson trichromic solution. Different preparation and fixation methods were used to prepare the tissues for scanning electron microscopy for a quantitative analysis of images (QAI), which allows assessment of the quantitative morphology of the uterine visceral peritoneal microvessels.

The samples were stored for 6–10 hours in a 2% glutaraldehyde solution in 0.1 M cacodylate buffer at pH 7.35 and subsequently washed in tap water for 2–3 hours, dehydrated and treated with the critical point drying technique, gold coated by a Denton apparatus and then observed and photographed under a scanning electron microscope (JSM-35; JEOL, Tokyo, Japan) [6].

Quantitative analysis of images was performed to evaluate the total area of each sample and changes in these areas using a Quantimet Analyser (Leica, Cambridge, UK). Examinations were performed separately for each slice, allowing for evaluation of the standard error of the mean (SEM). The values given represent the surface of microvessels for each sample and are expressed in conventional units (CU) ± SEM. Other information on CU (reference values) are reported elsewhere [7].

The visceral peritoneal specimens were analyzed to evaluate the stromal relationship on 20 fields at ×200 magnification to detect the presence of adhesions between the parietal peritoneum and the visceral peritoneum, mesothelial hyperplasia, fibrosis involving the mesothelial stroma, and neoangiogenesis of the mesothelial stroma.

The presence of pericytes around the microvessel walls, at ×2000 magnification, was assessed by scanning electron microscopy.

For the initial cesarean procedure, assessment was made of intraoperative blood loss (measured using a sterile vacuum aspirator connected to a graduated container), operative time, bladder injuries during the procedure, postoperative urinary dysfunction, and postoperative pelvic pain. Postoperative pelvic pain was defined as a patient request for analgesics, which was managed by intravenous injection of 30 mg of ketorolac or 100 mg of tramadol.

### Table 1

Baseline characteristics of the study participants.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Closure of VP (group 1, n=54)</th>
<th>Non-closure of VP (n=58)</th>
<th>P value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>31.6±1.8</td>
<td>32.3±2.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>BMI</td>
<td>25.1±2.3</td>
<td>24.7±2.4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Parity</td>
<td>1.2±1.9</td>
<td>1.4±1.2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Interval between 1st and 2nd cesarean delivery, y</td>
<td>2.3±1.8</td>
<td>2.6±1.1</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Abbreviations: VP, visceral peritoneum; BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters).

<sup>a</sup> Values are given as mean±SD.

<sup>b</sup> Statistical analysis used the Welch t test modified: α>0.05, DF = 110, P = 1.66.
Statistical analysis was performed using SAS software (SAS, Cary, NC, USA). \( P < 0.05 \) was considered significant. Qualitative variables were analyzed using the \( \chi^2 \) test, and comparison of quantitative variables was carried out using the \( t \) test and the Welch \( t \) test modified as the variances are significantly different between two groups or for matched samples, as appropriate.

### 3. Results

Of a total of 129 women eligible and invited to participate in the study, 112 provided consent and were enrolled. There were 54 women allocated to group 1 and 58 women to group 2 for the first cesarean delivery procedure. The clinical characteristics of the two groups of patients were similar (Table 1). All patients were white.

Closure of the visceral peritoneum was associated with a significant increase in adhesions between the parietal peritoneum and visceral peritoneum and between the bladder and uterus: 31 (57.4%) in group 1 had adhesions compared with 12 (20.6%) women in group 2 (\( P < 0.05 \)).

For the 31 women with adhesions in group 1, severity was mild in 22 women (70.9%) and severe in 9 patients (29.0%); whereas for the 12 women with adhesions in group 2, the severity was mild in 9 patients (75%) and severe in 3 women (25%).

The histologic characteristics of the samples are shown in Table 2. There was a significant difference in the presence of reactive mesothelial hyperplasia between patients in group 1 and group 2 (\( P < 0.05 \)). Fig. 1 shows reactive hyperplasia while Fig. 2 shows normal mesothelium. A significant difference was observed in the presence of fibrosis involving the mesothelial stroma between patients in group 1 and group 2 (\( P < 0.05 \)). Fig. 3 shows increased fibrosis seem in a group 1 patient and Fig. 4 shows a group 2 patient. There was also a significant difference in the presence of neoangiogenesis in mesothelial stroma between patients in group 1 and group 2 (\( P < 0.05 \)).

Scanning electron microscopy of microvessels in the visceral peritoneal samples showed pericytes around their walls in 26.3±1.4 patients in group 1 compared with 11.5±1.1 patients in group 2 (\( P < 0.05 \)). Fig. 5 shows scanning electron microscopy of microvessels in a group 1 patient, while Fig. 6 shows a group 2 patient.

Intraoperative assessment and postoperative follow-up after the first cesarean delivery showed significant differences between groups 1 and 2 (Table 3). More complications and adverse outcomes were observed in group 1 compared with group 2; these included intraoperative blood loss (286 mL vs 205 mL), operative time (43 min vs 34 min), bladder injuries during the procedure (3 vs 0 patients), postoperative urinary dysfunction (5 vs 2 patients), and postoperative pelvic pain (7 vs 1 patient), respectively. The intraoperative urinary injuries occurred during bladder flap dissection and they were bladder traumas with bladder opening (immediately sutured during cesarean delivery).

Most patients were discharged from hospital on the fourth postoperative day without major complications, except for 8 (7.1%)
women (6 in group 1 and 2 in group 2) who had fever (>38.5 °C) and were discharged on the seventh day.

4. Discussion

Inflammation of the peritoneum was found in more patients who had visceral peritoneal closure (group 1) compared with those in whom the visceral peritoneum was not closed (group 2). Reactive and regenerative mesothelial hyperplasia and submesothelial fibrosis were observed, which are important factors in postoperative adhesion formation and are associated with an increase in complications and adverse outcomes. In group 2 patients, an increase in submesothelial formation and are associated with an increase in complications and regenerative mesothelia hyperplasia and submesothelial whom the visceral peritoneum was not closed (group 2). Reactive and had visceral peritoneal closure (group 1) compared with those in

Many studies on peritoneal suturing do not distinguish between the visceral and parietal peritoneum. In 1965, Ellis et al. [8] reported that the closure or non-closure of both layers of the peritoneum influences adhesion formation by effects on mesothelial repair. Recent studies in animals showed the short-term benefit of not suturing the visceral peritoneum [9,10] and other microscopic studies on peritoneal regeneration showed new peritoneal cells deriving from the mesodermal cells of the underlying granulation tissue, and mesenchymal multipotent cells are able to form fibroblast or mesothelial cells depending on environment [11,12].

In rats, suturing of peritoneal wounds significantly increased adhesions compared with non-closure and, in rabbits, adhesions occur after visceral peritoneal closure more frequently than after parietal peritoneal closure [9].

Torre et al. [10] showed that two different stimuli are necessary for adhesion formation: a direct lesion of the mesothelial layers and a solid substrate, such as a foreign body (stitches). The conclusion of these experimental studies is that the repair process is improved without peritoneal suturing owing to the lower inflammatory reaction, less tissue necrosis, and the absence of stitches [12].

The short-term positive clinical effects of non-closure of the visceral peritoneum, such as duration of operation, febrile morbidity, bowel functioning, requirement for drugs, less postoperative pain, and duration of hospital stay, have already been recorded [13–16]. Two studies found that suturing the visceral peritoneum does not seem to be an essential step in cesarean delivery [17,18], because closure frequently creates small fluid collections in the upper part of the LUS [19].

The incision and detachment of the bladder during cesarean delivery leads to a space (or pocket) between the anterior uterine wall and posterior bladder wall [20], which can contain fibrin, clots, exudates, edema or serous fluids.

Suturing of the visceral peritoneum can lead to a closed pocket known as a “bladder flap hematoma,” which is a common site for collection of non-draining fluid and fibrosis of the submesothelial stroma (both causes of inflammation), and a site for adhesion development between the bladder and uterus [16], a cause of possible bladder injuries [21] and a reason for neoangiogenesis [22].

Moreover, the neoangiogenesis of submesothelial stroma, evidenced at the repeat cesarean delivery, produces surgical bleeding and trauma during the detachment of the bladder from the LUS [20,23].

The delayed discharge of 7.1% of patients because of fever was more common in group 1 patients and was probably due to bladder flap hematoma, filled by blood and wound secretions from the uterine incision, which spontaneously drains in a few days [23].

<table>
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<th>Table 3</th>
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<tr>
<td>Surgical characteristics and clinical follow-up of 112 women to monitor postcesarean outcome.</td>
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<tr>
<td>Characteristics of cesarean delivery</td>
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<tr>
<td>Intraoperative blood loss, mL</td>
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<tr>
<td>Duration of procedure, min</td>
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<tr>
<td>Bladder injuries</td>
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<tr>
<td>Postoperative urinary dysfunction</td>
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<tr>
<td>Postoperative pelvic pain</td>
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</table>

* Values are given as mean ± SD or number (percentage).  
* For statistical analysis, differences among the percentages of positive patients were assessed using the χ² test, while differences among the means were assessed using the t test.
Therefore, visceral peritoneal closure at cesarean delivery causes tissue trauma resulting in regenerative mesothelial hyperplasia, fibrosis of the submesothelial stroma, and adhesions between the posterior bladder wall and the LUS [24]. In a repeat cesarean delivery, these adhesions can cause surgical difficulties and complications [25].

In conclusion, the preliminary results of the present study support the recommendation that the visceral peritoneum should be left open at cesarean delivery because closure produces inflammatory reactions, based on submesothelial fibrosis and enhanced by foreign body suture materials, with adhesions strengthened by regenerative mesothelial hyperplasia after the peritoneal suture. This investigation links histopathology to clinical outcomes, supports a mesenchymal reaction as the basis for adhesion formation, and is complementary to previous studies that have shown a short-term benefit of non-closure of the visceral peritoneum at cesarean delivery. Non-closure seems to be associated with less adhesion formation and neoangiogenesis at the hysterotomy site and fewer intraoperative complications, which is associated with improved postoperative outcome at repeat cesarean delivery.

References