



## Effect of avoiding bladder flap formation in caesarean section on repeat caesarean delivery

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### ABSTRACT

**Objectives:** To evaluate the effect of bladder flap formation (BFF) during caesarean section (CS) on the uterine scar, assessed during repeat CS.

**Study design:** One hundred and fifteen women undergoing their first CS were divided into two groups: 58 women had a CS with BFF (Group 1) and 57 women had a CS without BFF (Group 2). During the repeat CS, four specimens from the uterine scar from the first CS were collected from each woman, and evaluated by light microscopy and transmission electron microscopy (TEM).

**Results:** Adhesions were found in 28 (48.3) women in Group 1 and 14 (24.1%) women in Group 2 ( $p < 0.01$ ). Of the women with adhesions in Group 1, 20 (71.4%) had mild adhesions and eight (28.6%) had severe adhesions. Of the women with adhesions in Group 2, eight (57.1%) had mild adhesions and six (42.9%) had severe adhesions. Light microscopy revealed significant differences in submesothelial fibrosis (39.6% vs 12.2%;  $p < 0.01$ ) and neo-angiogenesis of the mesothelial stroma (46.5% vs 21%;  $p < 0.01$ ) in Groups 1 and 2, respectively. TEM revealed more specimens with inflammatory cells in Group 1 compared with Group 2 {mean 29.7 [standard deviation (SD) 1.3] vs 18.2 (SD 1.9) patients;  $p < 0.01$ }.

**Conclusion:** BFF during CS leads to an inflammatory and fibrotic reaction, resulting in inflammation reactive and regenerative processes, mesothelial hyperplasia and submesothelial fibrosis. CS without BFF reduces the inflammatory processes and the subsequent intraperitoneal adhesions and adhesions between the bladder and uterus.

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## 1. Introduction

Bladder flap formation (BFF) is a common traditional surgical step during caesarean section (CS), although its necessity has never been established. Many authors have demonstrated that elimination of BFF during CS is preferable [1–3]. Current data support the International Medical Society's recommendations referring to the benefits of non-closure of the visceral peritoneum (VP) during CS [4], and a recent review on operative techniques in CS showed that non-closure of the VP is associated with significant benefits [5].

The aim of the present study was to compare the incidence of fibrosis in CS scars and clinically significant adhesions during repeat CS among women who had previously had a CS with or without BFF.

## 2. Materials and methods

The study was conducted at the Santa Maria Hospital, Bari and the Vito Fazzi Hospital, Lecce, Italy, between March 2005 and June 2009. The study consisted of two parts. In the first part, two groups undergoing their first CS were enrolled and subdivided into two groups. Group 1 had CS with BFF, and Group 2 had CS without BFF. The second part of the study compared the uterine scars of these women during repeat CS.

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The inclusion criteria were women undergoing a primary CS (pregnancies beyond 38 weeks of gestation) indicated due to malpresentations, post-term pregnancies, elderly primiparae or CS on demand. The exclusion criteria were previous gynaecological surgery and any of the following situations during pregnancy and/or delivery: infections, anticoagulation therapy, pre-eclampsia, HELLP syndrome, emergency CS, ruptured membranes for >36 h, placenta previae, other placental pathologies (as they change the normal lower uterine segment anatomy and could influence the surgical steps), and estimated fetal weight > 4.5 g (to avoid overdistension of the uterus).

In total, 134 women were eligible to participate, and 118 agreed to sign an informed consent form explaining the procedure and declaring that they would avoid a vaginal birth after CS during a possible second delivery.

The study was prospective and randomized. Patients were numbered; those with odd numbers were enrolled in Group 1 and those with even numbers were enrolled in Group 2. All results were assessed by an independent reviewer.

The same group of surgeons operated on both groups of women using a standardized surgical technique. Patients received combined spinal–epidural anesthesia plus a prophylactic dose of 2 g cefazoline intravenously.

The first CS was performed using the Misgav Ladach technique (Stark caesarean) in all patients, with a modified Joel-Cohen abdominal incision and a lower uterine segment (LUS) transverse incision according to Munro Kerr.

Intra-operative blood loss (measured using a sterile vacuum aspirator connected to a graduated container), operative time, bladder injuries, postoperative urinary dysfunction and postoperative pelvic pain were recorded. Postoperative pain was defined as the length of time a patient required intravenous injection of 30 mg Kerotolac or 100 mg Tramadol.

In Group 1, anatomical forceps were used to grasp the VP around the vesico-uterine peritoneal bladder flap. A scalpel was used to make a small transverse midline incision in the VP, and both index fingers were used to stretch it laterally in both directions for approximately 4 cm and caudally for 3 cm in order to separate the bladder from the LUS. A 2-cm transverse incision was made on the upper part of the LUS, and this was gently stretched laterally using the index fingers as wide as was necessary to deliver the baby.

In Group 2, a transverse incision was made 1 cm above the vesico-uterine peritoneal fold (identified using digital pressure or gentle traction of the VP with anatomical forceps). The direct transverse incision of the VP and, subsequently, the myometrium was performed without dissection of the bladder flap. No intra-abdominal sponges, towels or swabs were used in order to minimize future adhesions.

After delivery, the uterus was sutured without exteriorization. The myometrium was sutured in a single layer using continuous absorbable stitches of polyglactin 910 (Vicryl 0; Ethicon, Somerville, NJ, USA), avoiding the endometrium. Additional single haemostatic sutures were placed as necessary using single Vicryl 00 stitches. The parietal peritoneum was sutured in order to avoid any bias and to specifically examine the healing of the non-sutured VP with and without BBF. In all women, the parietal peritoneum was sutured with Vicryl 000. The abdominal wall was closed by suturing the fascia without suturing the abdominal muscles to each other, leaving the subcutaneous tissues unsutured and suturing the skin with intradermic sutures.

All the participants who became pregnant again underwent a repeat CS. The surgeons performing the repeat CS were unaware of whether the women had previously had a CS with BFF or a CS without BFF. The abdominal incision was made along the scar from the first CS, using the same method as before. After opening the

abdominal cavity and releasing adhesions when necessary, the bladder flap was exposed, dissected using scissors, and pushed down bidigitally to expose the previous uterine scar.

The severity of the adhesions observed during the repeat CS was assessed using the Adhesion Scoring Method of the American Fertility Society [6]. Adhesions were graded as: none, mild (a filmy, vascular adhesion) or severe (a dense, organized cohesive vascular adhesion).

The hysterotomy was made along the previous scar. After delivering the baby and removing the placenta, four complete thickness sections, approximately 5 mm in depth, were taken for histological and morphological analysis: two from the superior edge and two from the inferior edge. Delivery and closure of the uterus and abdomen were performed in the same way as in the first procedure.

The tissue samples were placed in Bouin solution for 24 h and then prepared in successive immersions of alcohol solution, starting with 70%. Once dehydrated, they were fixed in paraffin. Sections of 5- $\mu$ m thickness obtained from each sample were stained with haematoxylin and eosin, 0.05% periodic acid solution and Masson trichromic solution. Different preparation and fixation methods were used to prepare the tissues for electron microscopy scanning for quantitative analysis of images, which allows assessment of the quantitative morphology of the uterine wall and VP.

Fixation was performed by immediate immersion in 2.5% glutaraldehyde in 0.05 M cacodylate buffer at pH 7.2 for 2 h, and 2% osmium tetroxide in cacodylate buffer. The specimens were dehydrated using acetone. The specimens were embedded in EPON resin and cut using an ultratome (LKB V-2088, Bromma, Sweden). The semi-thin sections were stained with toluidine blue and examined under a light microscope. The contrast of the ultra-thin sections was performed using uranylacetate and lead citrate [27]. The ultra-thin sections were examined and photographed using a Tesla BS 500 electron microscope (Tesla, Brno, Czech Republic) [7]. Quantitative analysis of the images was performed in order to evaluate the total area and changes in each sample using a Quantimet Analyzer (Leica, Cambridge, UK). The evaluations were performed separately for each specimen, allowing evaluation of the standard error from the mean (SEM). The values given represent the surface of microvessels for each sample and are expressed in conventional units (CU)  $\pm$  SEM. More information on CU (reference values) is reported elsewhere [8].

The VP specimens were analysed to evaluate the stromal findings on 20 fields at 200 $\times$  magnification to detect adhesions between the parietal peritoneum and the VP, fibrosis involving the mesothelial stroma, and neo-angiogenesis in the mesothelial stroma. The presence of inflammatory cells in submesothelial tissue was assessed at 2000 $\times$  magnification using transmission electron microscopy (TEM).

Statistical analysis was performed using SAS software (SAS, Cary, NC, USA), and a *p*-value < 0.01 was considered to be significant. Qualitative variables were analysed using Chi-squared test. Quantitative variables were compared using the *t*-test and the modified Welch *t*-test, as the variances were significantly different between the two groups or between matched samples.

One aim of this study was to confirm the null hypothesis that the proportion positive is identical in the two populations. The criterion for significance ( $\alpha$ ) was set at 0.050. The test is two-tailed, which means that an effect in either direction will be interpreted.

With sample sizes of 58 and 57, the study will have power of 91.2% to achieve a statistically significant result. This calculation assumes that the difference in proportions is 0.29 (specifically, 0.50 vs 0.21).

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