British Journal of Surgery 1998, 85, 826–830

Experimental study of adhesion formation in open and laparoscopic fundoplication

L. KRÄHENBÜHL, M. SCHÄFER, V. KUZINKOVAS, P. RENZULLI, H. U. BAER and M. W. BÜCHLER

Department of Visceral and Transplantation Surgery, University of Berne, Inselspital, CH-3010 Berne, Switzerland
Correspondence to: Professor M. W. Büchler

Background The extent of adhesion formation following both open and laparoscopic surgery remains unclear. This study aimed to evaluate the extent of postoperative adhesion formation after laparoscopic and open fundoplication in a rat model.

Methods Fifty-two male Sprague–Dawley rats were randomized into four groups: laparoscopic fundoplication ($n = 20$), open fundoplication ($n = 20$), laparoscopy ($n = 6$) and laparotomy ($n = 6$). Blood as well as intraperitoneal fluid was sampled for tumour necrosis factor (TNF)α measurement by enzyme-linked immunosorbent assay. All the rats were killed 3 weeks after operation, and adhesion formation was evaluated using a standardized scoring system.

Results There were no intergroup differences in body-weight gain after surgery. The overall mortality rate was 19 per cent and death was observed only in the fundoplication groups. Animals that had open fundoplication developed significantly more adhesions than those that underwent laparoscopic fundoplication (34 *versus* 21). Laparoscopic surgery induced predominantly parietal adhesions, whereas open surgery was more associated with visceral adhesions. The adhesions observed in the laparoscopic groups were significant thinner than those after open surgery and the tenacity of adhesions was decreased in laparoscopic compared with open surgery. The peak plasma level of TNF-α was reached during laparoscopic fundoplication, whereas the peak level was observed 3 h after open fundoplication. Intraperitoneal TNF-α levels showed no significant differences at 3 h.

Conclusion These findings indicate that laparoscopic fundoplication in rats leads to less severe adhesions of a different type (parietal) compared with those seen in the open controls.

The increasing use of minimally invasive surgery has played an important role in the development of abdominal surgery at the end of the 20th century. Along with the wider application of minimally invasive surgical procedures[1–3], there is a growing interest in the physiological changes that occur compared with those during open surgery[4–9]. Open surgical procedures may cause postoperative adhesion formation, leading to delayed postoperative complications such as abdominal pain[10,11], infertility[12,13] and bowel obstruction[14–16]. There are few fundamental scientific data available in the literature concerning the pathophysiological changes (local and systemic) that occur during laparoscopic surgery[17].

The development of laparoscopic surgery was based on experimental animal research. The use of large animals, such as pigs or dogs, in surgical research is also associated with increased costs[4,18]. The rat model has been shown to be an excellent training model for performing microsurgical procedures[4,19], providing the opportunity to investigate physiological changes, adhesion formation and immune function during different laparoscopic procedures. Several studies suggested that tumour necrosis factor (TNF) α, an acute-phase cytokine, may be one of the markers of peritoneal adhesion formation[17,20–22]. The purpose of this study was to develop a standardized laparoscopic procedure (i.e. Nissen fundoplication) in the Sprague–Dawley rat model and to compare it with an open approach to investigate and correlate adhesion formation and TNF-α production during and after surgery.

Presented to the Society of American Gastrointestinal Endoscopic Surgeons meeting, San Diego, California, USA, March 1997 and published in abstract form as *Surg Endosc* 1997; 11: 204

Paper accepted 6 November 1997

Materials and methods

Animals and study design

The experiments were approved by the Animal Ethics Committee of the State of Berne and performed according to international guidelines. Fifty-two male Sprague–Dawley rats, weighing 300–460 g (Süddeutsche Versuchstierfarm, Tuttlingen, Germany), were used for all the experiments. They were kept under normal laboratory conditions and were fed a standard rat chow (Kliba Futter, Basel, Switzerland) with tap water *ad libitum* before and after surgery. The rats were divided into four main groups. Groups 1 ($n = 20$) and 2 ($n = 20$) underwent laparoscopic or open fundoplication respectively and determination of TNF-α levels in plasma and peritoneal fluid following surgery. Group 3 ($n = 6$) underwent diagnostic laparotomy and group 4 ($n = 6$) diagnostic laparoscopy.

Blood and peritoneal fluid sampling for tumour necrosis factor analysis

Venous blood was sampled from the external jugular vein of all the animals on induction of anaesthesia, during the operation and 1, 3 and 24 h after operation, and collected into 5·5-ml Monovette AH (Nümbrecht, Sarstedt, Germany) tubes containing ammonium-heparin (15 units heparin per ml blood). Plasma was obtained by centrifugation for 10 min at 2500*g* and 4°C, collected into 1·5-ml plastic snap-cap tubes and stored at −20°C until analysis. Peritoneal fluid was obtained 3 h after surgery by vacuum absorption from the peritoneal cavity through a polythene tube (catheter) 0·58 mm in diameter and 4–5 cm

long, which was inserted at the end of the open and laparoscopic procedure and stored at −20°C until analysis. The Cytoscreen Rat TNF-α kit (Cannon, Labor Service GMBH, Wiessbaden, Germany) was used for the TNF-α analysis.

Surgical procedures

All the animals used in the study were anaesthetized by intraperitoneal injection of a mixture (1 ml per kg body-weight) of 25 ml 5 per cent glucose, 5 ml azepromazin, 5 ml xylazin and 10 ml ketamin. The animals were shaved and secured in a supine position to the small-animal operating table[4]. The instruments used for all the laparoscopic procedures were a 2·7-mm arthroscope with a 3-mm trocar sleeve, two 3-mm trocars (working channels) and 2·7-mm instruments (scissors, straight and angled forceps, needle holder; Wolf, Tuttlingen, Germany). Ordinary small-sized hand surgery instruments were used for all open procedures. All procedures were performed without using swabs or wearing gloves. In the open procedure the abdominal wall was retracted with two sutures, so iatrogenic manipulation at this side was minimal.

Laparoscopy was performed through a 3-mm abdominal wall incision, midway as described by Berguer *et al.*[4]. Pneumoperitoneum was established with carbon dioxide insufflation up to 4 mmHg (abdominal pressure). Diagnostic laparoscopy (no procedure performed) lasted 30 min, after which all the trocar sleeves were removed and the wound closed with two single stitches. Laparoscopic fundoplication started with blunt dissection of the adhesions between the caudate liver lobe and the lesser curvature. The oesophagus, left gastric artery and anterior vagus nerve were identified and the oesophagus grasped and retracted anteriorly. Following this, an opening behind the oesophagus was created bluntly. The fundus of the stomach was grasped through the opening, pulled behind the oesophagus, turned ventrally and sutured extracorporeally with two 5/0 Vicryl sutures (Ethicon, Spreitenbach, Switzerland). A polythene tube 0·58 mm in diameter and 4–5 cm long was inserted through the left sleeve to the abdominal cavity for peritoneal fluid collection. The pneumoperitoneum was desufflated through the camera port and all the trocars were removed in order to prevent initial tissue attachments to the port sites.

All open procedures were performed using the same technique via a standardized 3-cm midline incision. Diagnostic laparotomy also lasted 30 min. Open fundoplication was performed with two 5/0 Vicryl single stitches. Before closure, a polythene catheter was inserted into the abdominal cavity for peritoneal fluid collection. The laparotomy was closed with a 3/0 Vicryl running suture in two layers.

Postoperative observation and determination of adhesion formation

The animals were observed in single cages for 24 h after operation until all the necessary blood and peritoneal fluid samples had been taken. After that, they were kept and observed under standard laboratory conditions. Three weeks after operation the animals were killed by decapitation and the abdominal cavity was opened by a wide inverted U-shaped laparotomy to investigate the extent and type of adhesion formation by two independent surgeons. The special criteria of Egea Moreno *et al.*[23], as shown in *Table 1*, were used to determine the extent of adhesions, as well as their localization, thickness, tenacity and vascularization.

Statistical analysis

Means were compared with an unpaired *t* test. $P < 0{\cdot}05$ was considered to be statistically significant.

Results

Operating time, body-weight, follow-up and death

The operating time ranged from 15 to 45 (mean 28) min for laparoscopic fundoplication and from 15 to 37 (mean 25) min for open fundoplication. All animals had a normal, proportional body-weight gain at 1, 2 and 3 weeks following surgery and there were no significant differences (data not shown). The overall mortality rate was 19 per cent and deaths occurred only in the two fundoplication groups (five deaths in each). Early death (from anaesthesia, bleeding, pneumoperitoneum) occurred in six animals and delayed death (due to septic complications) in four.

Table 1 Quantification of adhesion formation

Criterion	Comment
No. of adhesions	1 for each
Site of adhesions	
Pelvic fat body–abdominal wall	Parietal
Omentum–abdominal wall	Parietal
Intestine–abdominal wall	Parietal
Omentum–liver/stomach	Visceral
Intestine–intestine	Visceral
Liver–stomach	Visceral
Thickness of adhesions	
< 3 mm	
3–5 mm	
> 5 mm	
Tenacity	
Type I	Simple, without dissection
Type II	Dissection needed to separate adherent area
Type III	Dissection needed to cut the adhesion
Vascularization of adhesions	
Vascularized	
Avascularized	

Total adhesion score is the sum of each criterion (site of adhesions, thickness, tenacity and vascularization)

Adhesion formation

Number of adhesions. Five animals that underwent laparoscopic fundoplication, two that had diagnostic laparoscopy and two that had diagnostic laparotomy still had not developed adhesions 3 weeks after surgery, whereas adhesions were detected in all animals that had open fundoplication (*Table 2*). The 15 animals that had

Table 2 Adhesion formation after laparoscopic or open Nissen fundoplication

	Laparoscopic fundoplication ($n = 15$)	Open fundoplication ($n = 15$)
Animals with adhesions	10	15
No. of adhesions	21	34
Site of adhesions		
Parietal adhesions	13	5
Visceral adhesions	8	29
Intestine involved (bowel, stomach, liver)	11	30
Thickness of adhesions		
< 3 mm	12	3
> 3 mm	9	31
Tenacity		
Type I + II	12	6
Type III	9	28

There were no differences in vascularization between the groups

laparoscopic fundoplication developed 21 adhesions, whereas 34 adhesions were detected in the 15 animals that underwent open fundoplication. Rats that had diagnostic laparoscopy and laparotomy ($n = 6$ each) developed five and six adhesions respectively. The number of adhesions differed significantly ($P < 0{\cdot}05$) between laparoscopic and open fundoplication groups. No significant differences were observed in comparison with the other groups.

Site of adhesions. Animals that had laparoscopic surgery (laparoscopic fundoplication and diagnostic laparoscopy) developed predominantly parietal adhesions (13 of 21 after laparoscopic fundoplication and all five after diagnostic laparoscopy), mostly between the small intestine (three) or omentum (ten) and the scars of the optical or working trocars of the abdominal wall. There was no predominance of adhesion formation between the optic and working trocars. Animals that only underwent laparotomy also developed more parietal adhesions (five of six) between the small bowel or the omentum and the laparotomy scar. Conversely, animals that underwent open fundoplication developed predominantly visceral-type adhesions (29 of 34), especially between the intestine (two), liver/stomach and omentum (six) as well as between the stomach and liver (21). The distribution of adhesions after open fundoplication was significantly different from that after laparoscopic fundoplication ($P < 0{\cdot}01$) and diagnostic laparoscopy and laparotomy ($P < 0{\cdot}01$). No significant difference was seen between diagnostic laparoscopy and laparotomy. There was no significant difference in the distribution of visceral adhesions after laparoscopic fundoplication (six liver and stomach, three liver/stomach and omentum) and open fundoplication (23 and six respectively). However, a significant difference in adhesion formation involving intestine (liver, stomach, bowel) between laparoscopic fundoplication (11 of 21) and open fundoplication (30 of 34) was found ($P < 0{\cdot}05$).

Thickness of adhesions. The thickness of adhesions after laparoscopic and open fundoplication was significantly different ($P < 0{\cdot}05$) and inversely proportional. Animals that had laparoscopic surgery (laparoscopic fundoplication and diagnostic laparoscopy) mostly developed adhesions less than 3 mm thick (12 of 21 and three of five respectively), whereas those that had open surgery (open fundoplication and diagnostic laparotomy) mostly developed adhesions more than 5 mm thick (*Table 2*).

Tenacity. Animals that had a laparoscopic intervention mostly developed type I and II adhesions (12 of 21 after laparoscopic fundoplication and all five after diagnostic laparoscopy respectively), whereas open surgery was followed mainly by the development of type III adhesions (28 of 34 after fundoplication and four of six after diagnostic laparotomy) (*Table 2*). A small number of type I adhesions was noticed in all groups. Only type II adhesions developed after diagnostic laparoscopy. There were significant differences between open and laparoscopic surgery (laparoscopic *versus* open fundoplication, $P < 0{\cdot}01$; open fundoplication *versus* diagnostic laparoscopy, $P < 0{\cdot}05$).

Vascularization of adhesions. All animals primarily developed vascularized adhesions. However, after laparoscopic and open fundoplication one-fifth and one-quarter of the adhesions respectively were avascularized. All the adhesions that developed after diagnostic laparoscopy were vascularized, whereas half of those that developed after diagnostic laparotomy were avascularized. There were no significant differences between all the groups investigated. Overall adhesion score[23] is summarized in *Fig. 1*.

Tumour necrosis factor concentrations

There were different TNF-α ranges in the fundoplication groups (*Fig. 2*). Laparoscopic fundoplication induced an early intraoperative peak of TNF-α secretion in plasma and the preoperative level was reached 24 h after surgery. Open fundoplication induced only a slight rise in TNF-α secretion at operation and 1 h after operation; the peak

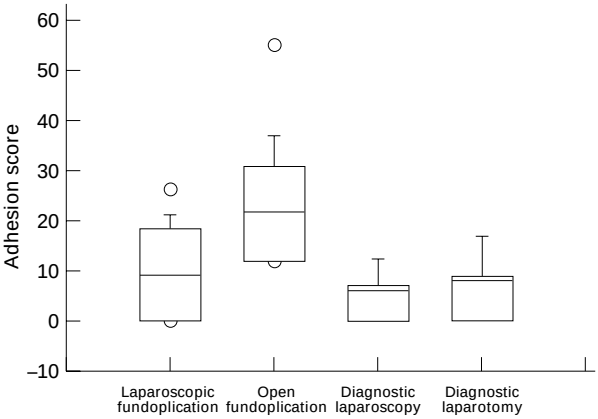

Fig. 1 Total adhesion scores in the four surgical groups. Boxes include values between the 5th and 95th percentile with the median values (horizontal line). T bars indicate standard deviations. Values outside the standard deviation are indicated as open circles. The adhesion score was significantly higher after open fundoplication than after the other procedures ($P < 0{\cdot}01$, unpaired *t* test)

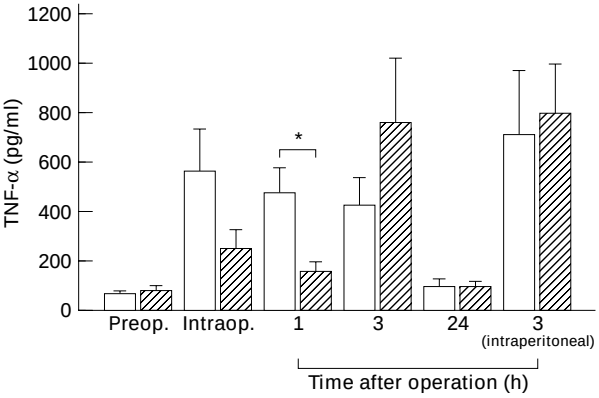

Fig. 2 Plasma and intraperitoneal tumour necrosis factor (TNF) α levels following laparoscopic (□) or open (▨) fundoplication. Values are mean(s.e.m.) *$P < 0{\cdot}05$, unpaired *t* test

TNF-α level occurred 3 h after operation. Some 24 h after surgery, the TNF-α level had again reached preoperative levels. TNF-α secretion was significantly different between laparoscopic and open fundoplication groups ($P < 0{\cdot}05$) 1 h after surgery, whereas all other plasma TNF-α values for the two fundoplication groups showed no significant differences. TNF-α concentrations, measured 3 h after operation in the peritoneal fluid, were increased after open fundoplication compared with laparoscopic fundoplication (*P* not significant). Comparison of peritoneal TNF-α concentrations with adhesion tenacity showed that no adhesions developed with TNF-α levels of 200 pg/ml or less.

Discussion

The present study demonstrates that laparoscopic and open Nissen fundoplication are technically feasible in the rat model and that a different extent (more after open surgery) and type of adhesion formation between laparoscopic fundoplication (parietal) and open fundoplication (visceral) are induced.

The development of peritoneal adhesions is an inevitable outcome of surgical operations in the abdominal cavity. It is one of the main complications, occurring some weeks or months after the initial operation[17], and it results in morbidity following abdominal, gynaecological, or cardiac surgical procedures. Such complications are visceral dysfunction, chronic abdominal pain, infertility and early or delayed bowel obstruction[10,14,15,23]. Morbidity due to adhesion formation is associated with increased socioeconomic costs, which reach up to $1.18 million in the USA per year[12]. Menzies and Ellis[14] published a prospective trial of 210 patients with a history of previous abdominal surgery who underwent a 'second-time' laparotomy; peritoneal adhesions were found in 93 per cent. Furthermore, in 115 patients who underwent first-time laparotomy, abdominal adhesions were demonstrated in 10 per cent.

Multiple factors may be responsible for the development of postoperative adhesions, for example the extent of surgical trauma and length of the procedure, bacterial contamination, allergic reactions to inserted foreign bodies and tissue ischaemia[17]. All of these factors cause peritoneal tissue injury, which leads to an inflammatory reaction and cytokine release, which are considered to be trigger mechanisms for further adhesion development. Thus, mesothelial, inflammatory and endothelial cells are activated to produce and release plasminogen activator inhibitors 1 and 2, which predispose to the loss of plasminogen activation in the injured area of the peritoneum. This process leads to impairment of fibrinolysis, fibrin coagulation and fibrin matrix formation[17,24,25].

Kaidi *et al.*[20,21] found a direct correlation between the grade of injury, TNF-α secretion and the extent of peritoneal adhesion formation. In the present series, during laparoscopic fundoplication there was a rapid rise in plasma TNF-α level, reaching its peak at operation and gradually decreasing to preoperative levels by 24 h after surgery. In contrast, there was only a slight TNF-α rise during and 1 h after open surgery, with a peak level 3 h after surgery. Again 24 h after surgery TNF-α levels were the same as those before operation. The overall TNF-α concentrations in the peritoneal fluid were higher than those in plasma 3 h after surgery and were slightly increased following open surgery (*P* not significant). These findings suggest that TNF-α may be produced primarily in the peritoneal cavity, secreted into the operative field and afterwards absorbed into the systemic circulation. This means that the TNF-α produced during the open procedure may well have been lost externally (open cavity, rinsing), and this may explain the fact that TNF-α levels were reduced during open fundoplication and 1 h afterwards. It also explains why no correlation between TNF-α levels and tenacity of adhesion formation was found and that any assessment of TNF as a measure of inflammatory response in the peritoneum may be inappropriate. A similar tendency was observed in other series which investigated the inflammatory cytokine response following abdominal surgical procedures[22].

No adhesions were detected in the abdominal cavity following intraperitoneal anaesthesia and skin incision, which suggests that surgical trauma alone without opening the peritoneal cavity is not responsible for adhesion development ($n = 7$; data not shown). A minimal surgical trauma, such as diagnostic laparoscopy or diagnostic laparotomy without further surgical intervention or bowel revision, leads to less adhesion formation than upper gastrointestinal surgery. Laparoscopic Nissen fundoplication leads to less postoperative adhesion formation than the open procedure. Furthermore, adhesions in the laparoscopic group were of the parietal type, from omentum or small bowel to the parietal peritoneum, rather than the visceral type, developed between the abdominal organs themselves.

Similar observations were published by Tittel *et al.*[26] in a canine model, and by Semm[27] in humans. Semm clearly demonstrated in women who underwent diagnostic laparoscopy after previous abdominal operations (open or laparoscopic) that 84 per cent had adhesion formation after open surgery whereas only 40 per cent did after laparoscopic intervention. However, in his series it remained unclear which type of adhesions was responsible for the development of postoperative complications.

In conclusion, the present study demonstrates that laparoscopic surgery is technically feasible in the rat model with low morbidity and mortality rates at long-term follow-up. Postoperative adhesion formation occurred more frequently and severely after open surgery compared with the same operation performed laparoscopically. The TNF-α levels measured intraperitoneally did not differ between the two groups, and a positive correlation between the tenacity of adhesion formation and the intraperitoneal TNF-α level was not found. This animal model can be used for laparoscopic surgery and the study of adhesion formation. However, further studies are needed to evaluate the pathophysiological changes leading to postoperative adhesion formation and its prevention.

Acknowledgements

This work was supported by a Research Fellowship awarded to L.K. and V.K. from the Swiss Association of Laparoscopic and Thoracoscopic Surgery (SALTC) and a Grant in Aid from the Department of Clinical Research, University of Berne, to L.K.

References

1 Johnson A. Laparoscopic surgery. *Lancet* 1997; 349: 631–5.
2 Soper NJ, Brunt LM, Kerbl K. Laparoscopic general surgery. *N Engl J Med* 1994; 330: 409–19.

3 Büchler MW, Frei E, Klaiber Ch, Krähenbühl L, eds. Gastroesophageal reflux disease (GERD): back to surgery? In: *Progress in Surgery*. Vol. 23. Basel: Karger, 1997.
4 Berguer R, Gutt C, Stiegmann GV. Laparoscopic surgery in the rat. Description of a new technique. *Surg Endosc* 1993; 7: 345–7.
5 Kragsbjerg P, Holmberg H, Vikerfors T. Serum concentrations of interleukin-6, tumour necrosis factor-α, and C-reactive protein in patients undergoing major operations. *Eur J Surg* 1995; 161: 17–22.
6 Lennard TWJ, Shenton BK, Borzotta A, Donnelly PK, White M, Gerrie LM *et al.* The influence of surgical operations on components of the human immune system. *Br J Surg* 1985; 72: 771–6.
7 Mealy K, Gallager H, Barry M, Lennon F, Traynor O, Hyland J. Physiological and metabolic responses to open and laparoscopic cholecystectomy. *Br J Surg* 1992; 79: 1061–4.
8 Redmond HP, Watson RWG, Houghton T, Condron C, Watson RGK, Bouchier-Hayes D. Immune function in patients undergoing open *versus* laparoscopic cholecystectomy. *Surg Laparosc Endosc* 1996; 6: 136–9.
9 Trokel MJ, Bessler M, Treat MR, Whelan RL, Nowygrod R. Preservation of immune response after laparoscopy. *Surg Endosc* 1994; 8: 1385–8.
10 Freys SM, Fuchs KH, Heimbucher J, Thiede A. Laparoscopic adhesiolysis. *Surg Endosc* 1994; 8: 1202–7.
11 Mueller MD, Tschudi J, Herrmann U, Klaiber C. An evaluation of laparoscopic adhesiolysis in patients with chronic abdominal pain. *Surg Endosc* 1995; 9: 802–4.
12 diZerega GS. Contemporary adhesion prevention. *Fertil Steril* 1994; 61: 219–35.
13 Stovall TG, Elder RF, Ling FW. Predictors of pelvic adhesions. *J Reprod Med* 1989; 34: 345–8.
14 Menzies D, Ellis H. Intestinal obstruction from adhesions – how big is the problem? *Ann R Coll Surg Engl* 1990; 72: 60–3.
15 Mucha P Jr. Small intestinal obstruction. *Surg Clin North Am* 1987; 67: 597–620.
16 Stewart RM, Page CP, Brender J, Schwesinger W, Eisenhut D. The incidence and risk of early postoperative small bowel obstruction. A cohort study. *Am J Surg* 1987; 154: 643–7.
17 Thompson JN, Whawell SA. Pathogenesis and prevention of adhesion formation. *Br J Surg* 1995; 82: 3–5.
18 Reissman P, Tioh TA, Skinner K, Burns JW, Wexner SD. Adhesion formation after laparoscopic anterior resection in a porcine model: a pilot study. *Surg Laparosc Endosc* 1996; 6: 136–9.
19 Bouvy ND, Marquet RL, Hamming JF, Jeekel J, Bonjer HJ. Laparoscopic surgery in the rat. Beneficial effect on body weight and tumor take. *Surg Endosc* 1996; 10: 490–4.
20 Kaidi AA, Gurchumelidze T, Nazzal M, Figert P, Vanterpool C, Silva Y. Tumor necrosis factor-α: a marker for peritoneal adhesion formation. *J Surg Res* 1995; 58: 516–18.
21 Kaidi AA, Nazzal M, Gurchumelizde T, Ali MA, Dawe EJ, Silva Y. Preoperative administration of antibodies against tumor necrosis factor-α (TNF-alpha) and interleukin-1 (IL-1) and their impact on peritoneal adhesion formation. *Am Surg* 1995; 61: 569–72.
22 Badia JM, Whawell SA, Scott-Coombes DM, Abel PD, Williamson RCN, Thompson JN. Peritoneal and systemic cytokine response to laparotomy. *Br J Surg* 1996; 83: 347–8.
23 Egea Moreno A, Aguayo Albasini JL, Zambudio Carmona G, Parrilla Paricio P. Adhesion response to different forms of treating a peritoneal lesion: an experimental study in rats. *Digestive Surgery* 1995; 12: 334–7.
24 Elkins TE, Stovall TG, Warren J, Ling FW, Meyer NL. A histologic evaluation of peritoneal injury and repair: implications for adhesion formation. *Obstet Gynecol* 1987; 70: 225–8.
25 Ellis H. The cause and prevention of postoperative intraperitoneal adhesions. *Surg Gynecol Obstet* 1971; 133: 497–511.
26 Schade DS, Williamson JR, The pathogenesis of peritoneal adhesions: an ultrastructural study. *Ann Surg* 1968; 167: 500–10.
27 Tittel A, Schippers E, Treutner KH, Anuroff M, Polivoda M, Ottinger A *et al.* Laparoscopy *versus* laparotomy. An animal experiment study comparing adhesion formation in the dog. *Langenbecks Arch Chir* 1994; 379: 95–8.
28 Semm K. Pelviscopic surgery: a key for conserving fertility. *Ann N Y Acad Sci* 1991; 626: 372–98.