The mouse as a model to study adhesion formation following endoscopic surgery: a preliminary report

N.Yesildaglar[1,3], J.L.Ordoñez[1], I.Laermans[1] and P.R.Koninckx[1,2]

[1]Centre for Surgical Technologies (CHT) and [2]Department of Obstetrics and Gynaecology, University Hospital Gasthuisberg, K. U. Leuven, Minderbroederstraat 17, B-3000, Leuven, Belgium

[3]To whom correspondence should be addressed

Our aim was to investigate the feasibility of a mouse model to study adhesion formation following endoscopic surgery. Following preliminary studies to establish anaesthesia and pneumoperitoneum pressure, a prospective randomized study was carried out to investigate the effect of CO_2 pneumoperitoneum on postoperative adhesions. In group I (control group), the duration of pneumoperitoneum was shorter than 5 min. In groups II, III and IV, pneumoperitoneum was maintained for 60 min without flow, with a continuous low flow (1 ml/min) and a continuous high flow (10 ml/min) through the abdominal cavities of the mice using non-humidified CO_2, respectively. Adhesions were scored after 7 days by laparotomy. The total adhesion scores were 0.9 ± 0.8 ($n = 15$) in control group, 2.4 ± 0.8 ($n = 15$) ($P < 0.001$ versus control group) in group II with no flow, 2.6 ± 1.3 ($n = 15$) ($P < 0.001$ versus control group) in group III with a continuous low flow and 4.3 ± 0.9 ($n = 15$) ($P < 0.001$ versus control group and $P < 0.001$ versus group II and III) in group IV with a continuous high flow. In conclusion, the mouse can be used as a model to study adhesion formation following endoscopic surgery. Duration of CO_2 pneumoperitoneum is a co-factor in adhesion formation.
Key words: adhesions/endoscopy/mouse

Introduction

Postoperative peritoneal adhesions are an important clinical problem occurring in 55–100% of patients following intra-abdominal surgery (Diamond *et al.*, 1987). Adhesions are estimated to be the cause in 6–64% of patients with small bowel obstructions (Ellis, 1982), in 13–26% of patients with chronic or recurrent pelvic pain (Goldstein *et al.*, 1980; Malinak, 1980; Rapkin, 1986; Renaer, 1986) and in 32% of women with infertility (Ryan, 1973). The mechanism of adhesion formation following a peritoneal injury is multifactor-ial, including an inflammatory reaction and capillary dilatation, fibrin deposition and fibrinolysis, tissue ischaemia and angiogenesis, fibroblast growth, migration of macrophages and tissue repair cells.

Laparoscopic surgery is claimed to be less adhesiogenic than laparotomy in humans, since it is less traumatic and more precise, with a lower postoperative morbidity. In experimental studies, the incidence of postoperative adhesions after laparotomy or laparoscopy is controversial. Filmar *et al.* (1987) concluded that adhesions following laparotomy and laparoscopy were not different. In 1989, Luciano *et al.* reported more postoperative adhesions after laparotomy than after laparoscopy in a rabbit model. According to the results of studies carried out by Marana *et al.* (1994) and Jorgensen *et al.* (1994), there was no significant difference between the adhesions after laparotomy and laparoscopy. Chen *et al.* (1998) reported that for para-aortic lymphadenectomy, the transperitoneal laparoscopic approach induced fewer adhesions than laparotomy (Chen *et al.*, 1998).

CO_2 is generally used for the pneumoperitoneum during laparoscopy, since its solubility in water is an important safety factor. CO_2 pneumoperitoneum, however, causes peritoneal acidosis, which is most severe at the site directly exposed to CO_2 (Volz *et al.*, 1997). Moreover, during CO_2 pneumoperitoneum especially with high flow rates, desiccation could occur which is a known co-factor in peritoneal injury and adhesiogenesis (Ryan *et al.*, 1973; Holmdahl *et al.*, 1997). Duration of CO_2 pneumoperitoneum in endoscopic surgery increased adhesion formation in rabbits (Ordonez *et al.*, 1997). In this study, we wanted to confirm and extend these observations in the mouse model.

Materials and methods

Animals

Ico: NMRI (Naval Medical Research Institute) mice (Iffa Credo, Brussels, Belgium) 12 weeks old and weighing between 31 and 43 g were used. Mice were kept under standard laboratory conditions (temperature 20–25°C, relative humidity 40–70%, 14 h light and 10 h dark) at the Animalium of St Rafael Hospital for Laboratory Animal Care of the Catholic University of Leuven and fed a standard laboratory diet (Hope Farms, Woerden, The Netherlands) with free access to water and food before and after the endoscopic procedure.

The study was approved by the Institutional Animal Care Committee of K. U. Leuven.

Experiments

Preliminary studies were necessary to establish a suitable anaesthesia for mice and the intra-abdominal pressures to permit endoscopic

procedures up to 2 h. Since anaesthesia of long duration permitting endoscopic surgery, was difficult with i.m. and s.c. anaesthetics, inhalational anaesthesia was required and halothane (Fluothane$^\Gamma$, Zeneca, Destelbergen, Belgium) with mask was used. In the preliminary study for insufflation pressure, the mortality rate was evaluated with insufflation pressures of 2.5, 5, 7.5, 10 and 15 cm of water for 10 min ($n = 100$) and subsequently with an insufflation pressure of 2.5 cm of water for 15, 30, 60, 90 and 120 min, respectively ($n = 100$). To evaluate adhesion formation, six monopolar coagulation lesions using 5 W (Autocon, Karl Storz$^\Gamma$, Belgium) for 2 s, were inflicted endoscopically to all animals using a specifically designed ball electrode. Three 1 mm diameter lesions were made on the uterus (one on each horn and one on the midline) and three identical lesions in the opposing mid-lower abdominal wall. These lesions were at 1 mm distance from each other. This procedure took 3–4 min. To evaluate the effect of CO_2 pneumoperitoneum upon adhesion formation, four groups were studied ($n = 60$). In group I (control group) pneumoperitoneum was not prolonged after the surgical procedure and thus consisted of a pneumoperitoneum of 3–4 min ($n = 15$). In all other groups the pneumoperitoneum was prolonged for 60 min with non-humidified CO_2. In group II ($n = 15$), there was no flow through the abdominal cavities of the animals whereas in group III ($n = 15$), a continuous low flow rate of ~1 ml/min per mouse and in group IV ($n = 15$), a continuous high flow rate of ~10 ml/min per mouse was maintained. Assuming a weight of 30–40 g and a pneumoperitoneum volume of 2–3 ml in mice and a weight of 50–70 kg and of a pneumoperitoneum volume of 3–4 l in humans, a flow rate of 1 ml/min in mice is estimated to be comparable to 1–1.5 l/min in humans.

After 1 week, mice were killed and immediately afterwards a laparotomy was performed. Adhesions were scored blindly by two researchers under an operative microscope (Zeiss$^\Gamma$, Brussels, Belgium) using the registration numbers of the animals. Type of adhesions was scored as 0 (no adhesions), 1 (filmy adhesions), 2 (firm adhesions) or 3 (vascular firm or dense adhesions). Tenacity of adhesions was scored as 1 (adhesions which essentially fall apart), 2 (adhesions which require traction) or 3 (adhesions which require sharp dissection). Maximum total adhesion score (type + tenacity) thus was 6.

Surgical procedures

After preanaesthesia with 100 mg/kg of ketamine i.m. (Ketalin$^\circledR$ Apharmo, Arnhem, The Netherlands) and 16 mg/kg of xylazine s.c. (Rompun$^\circledR$ 2%, Bayer, Brussels, Belgium), anaesthesia was maintained with inhalational Halothane (Fluothane$^\Gamma$, Zeneca) at 0.5–1% using 1 l/min of O_2. The animals were secured to the table in supine position. A 5 mm abdominal skin incision on the midline 1 cm below the sternum, was made. The Veress needle together with a miniscope (Storz Miniscope, a 1.2 mm 0° fibroscope in a Veress needle, Karl Storz$^\circledR$) was introduced into the peritoneal cavity. The pneumoperitoneum was initiated and maintained with a conventional CO_2 insufflator (Thermoflator, Karl Storz$^\Gamma$) connected to a water valve (Koninckx and Vandermeersch, 1991), using non-humidified CO_2. This water valve was used, since it accurately permits insufflation pressures as low as 2.5 cm of water (1.9 mmHg). It also prevents extreme pressures due to the intermittent insufflation by the thermoflator. An elastic balloon was placed next to the water valve to eliminate virtually all pressure changes. Endoscopy was performed using a camera (Telecam, CCU, Karl Storz$^\circledR$) attached to the miniscope. Because of the small size of a mouse, the miniscope was held by the surgeon who was sitting and facing the monitor with his elbows stabilized. A secondary operating port was placed by the surgeon using his free hand in the left lower quadrant of the abdomen

using a 14-gauge angiocatheter (Insyte$^\Gamma$, Vialon$^\Gamma$, Becton Dickinson, Madrid, Spain) under direct laparoscopic vision. The needle was withdrawn and a monopolar home made ball electrode which was 10 cm long and 1.5 mm in diameter, was inserted through the angiocatheter. The animal was tilted to a 45° Trendelenburg position to explore the lower abdominal cavity and six lesions (5 W, 2 s) were applied. After this, the miniscope was withdrawn and replaced by a 2 mm introducer (MiniSite Introducers$^\Gamma$, Auto Suture, Mechelen, Belgium) through which pneumoperitoneum was maintained. The angiocatheter in the lower abdomen, was closed in group II with no flow and left open in group IV to maintain a high flow rate of ~10 ml/min per mouse. The lumen of the angiocatheter was reduced in group III to have a low flow rate of ~1 ml/min per mouse by inserting the needle of a 24-gauge angiocatheter through the angiocatheter in the lower abdomen.

Pneumoperitoneum can be maintained in 10 mice simultaneously using a multi-channelled-set-up (Figure 1). The pressure and flow rate settings of the insufflator, were 15 mmHg and 1 l/min respectively. Since the water valve limits the insufflation pressure to 2.5 cm of water, the insufflation pressure is identical in each mouse and virtually independent of the pressure setting of the insufflator in this multi-channelled-set-up because of the identical tubes with an internal diameter of 7 mm. In this set-up, the insufflation pressure depends only on the height of the water column in the water valve, since excess CO_2 escapes freely.

Desiccation experiments

A 200 ml Falcon dish with two holes of 7 mm on each side, containing 20 ml of water, i.e. covering the entire bottom surface, was used to evaluate water loss/desiccation during insufflation. The flow rates were adapted to the volume of the Falcon dish to induce measurable desiccation. Since pilot experiments had shown that the temperatures of the water and the gas were critical, great care was taken to keep the Falcon dish at exactly 36.5 ± 0.5°C using two heaters (Maquet Rastatt$^\circledR$, St-Pieters-Leeuw, Belgium and Ameda$^\circledR$, Zug, Switzerland). The insufflation was carried out with a thermoflator for insufflation which keeps the insufflated CO_2 at 35 ± 1°C. During the experiments, the temperature of the dish was monitored continuously using a temperature probe (Hewlett Packard$^\circledR$, Brussels, Belgium). This set-up was used to evaluate whether desiccation was linear with time and flow rate. In all experiments, observations were made in duplicate and averages were indicated. The standard deviation of the measurements, as estimated from duplicate experiments, was 0.018 g for the whole experiment.

Statistical analysis

Data were analysed using analysis of variance (ANOVA). Paired group comparisons were performed using the Tukey-HSD multiple range test (GraphPad, Prism^{TM}, San Diego, CA, USA). To estimate the accuracy of desiccation experiments, the SD of the whole experiment was calculated from all duplicate experiments using: SD = square root of the sum of the differences between the two measurements performed for each point, divided by the number of measurements. All data from the mice experiments are reported as means $\pm$ SD.

Results

In the preliminary studies, there was no mortality with insufflation pressures of 2.5 and 5 cm of water for 10 min. Mortality increased rapidly with higher pressures being 5, 15 and 40% for 7.5, 10 and 15 cm of water pressures respectively ($n = 20$
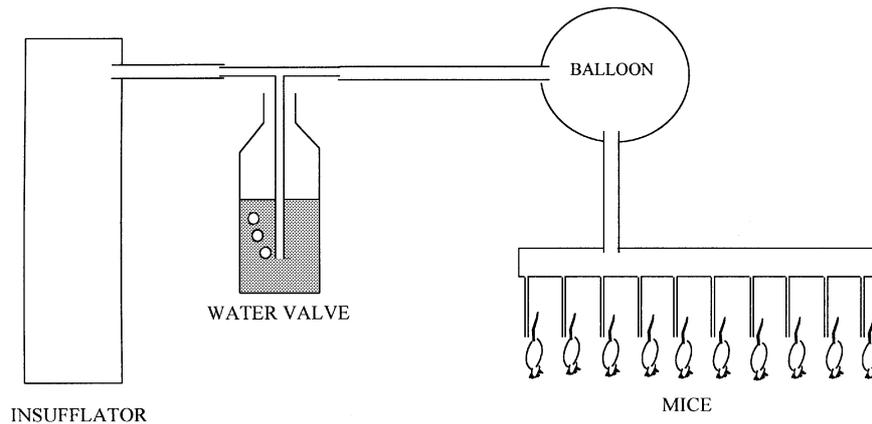
Figure 1. The set-up for pneumoperitoneum in mice. A water valve accurately limits the insufflation pressure to 2.5 cm of water and the system permits the maintenance of pneumoperitoneum simultaneously in 10 mice.

per group). With 2.5 cm of water pressure, not a single mouse out of 100 died after maintaining the pneumoperitoneum for 15, 30, 60, 90 and 120 min respectively ($n = 20$ per group).

In vitro, desiccation was linear with time and flow rate, being 1.28, 2.41, 3.50, 4.49, 5.48 and 6.44 g of water loss following 10, 20, 30, 40, 50 and 60 min of insufflation at 10 l/min flow rate respectively and being 1.61, 3.24, 4.83 and 6.53 g of water loss after insufflation at 5, 10, 15 and 20 l/min flow rates for 30 min (Figure 2a,b).

The total adhesion scores were 0.9 ± 0.8 ($n = 15$) in control group with some 5 min of pneumoperitoneum (Figure 3). The adhesion scores increased to 2.4 ± 0.8 ($n = 15$) ($P < 0.001$ versus control group) in group II with 60 min of pneumoperitoneum without flow and to 2.6 ± 1.3 ($n = 15$) ($P < 0.001$ versus control group) in group III with 60 min of pneumoperitoneum using continuous low flow ($P < 0.001$ versus control group). The total adhesion score increased further to 4.3 ± 0.9 ($n = 15$) ($P < 0.001$ versus control group, $P < 0.001$ versus group II and III) in group IV with 60 min of pneumoperitoneum using continuous high flow. There was no mortality in this study.

Discussion

A mouse model to study adhesion formation following endoscopic procedures was developed. The data clearly demonstrate the feasibility of inhalational anaesthesia for a period of 60–120 min following an induction using ketamine and xylazine. The pneumoperitoneum pressure is crucial, and should not exceed 5 cm of water in these small non-intubated animals during the endoscopic procedures with conventional equipment. Since insufflators designed for endoscopy in humans do not allow a precise insufflation pressure adjustment around 2.5 cm of water (1.9 mmHg), a water valve was necessary. For safety reasons, most of the insufflators stop insufflation of CO_2 intermittently in order to measure the intra-abdominal pressure during these periods. This intermittent delivery of small volumes of CO_2 gas does not cause pressure changes when delivered into a larger volume, e.g. human abdomen. Delivered into tiny abdominal cavities of mice, however, this causes important pressure changes which have to be damped.
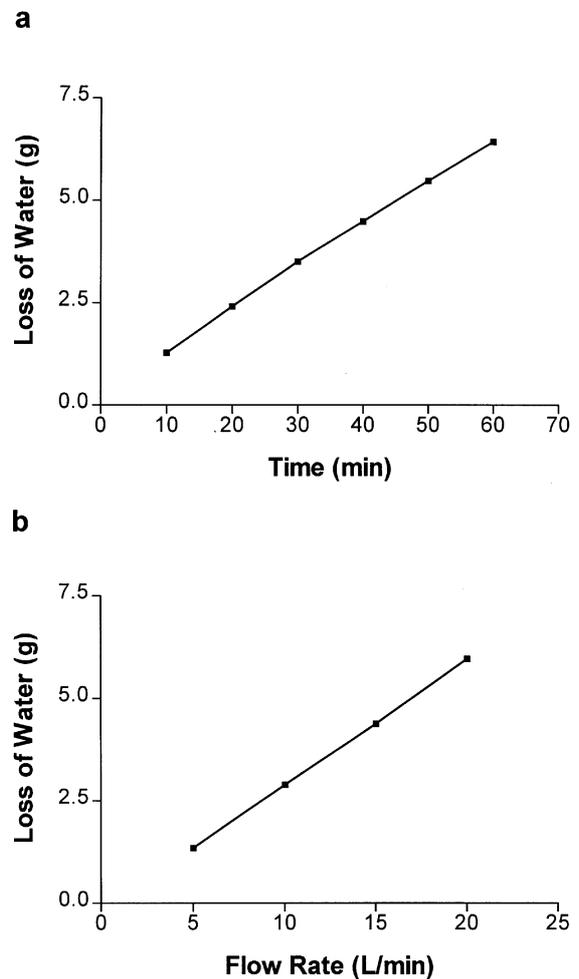

Figure 2. Water loss is linear with time (**a**: flow rate = 10 l/min) and flow rate (**b**: time = 30 min). Averages of duplicate experiments are indicated.

This is achieved mainly by the water valve and additionally by an elastic balloon placed next to the water valve to have a constant insufflation pressure. An electronic insufflator is not strictly necessary and the same results could be obtained with any simple CO_2 delivery system. We used the thermoflator
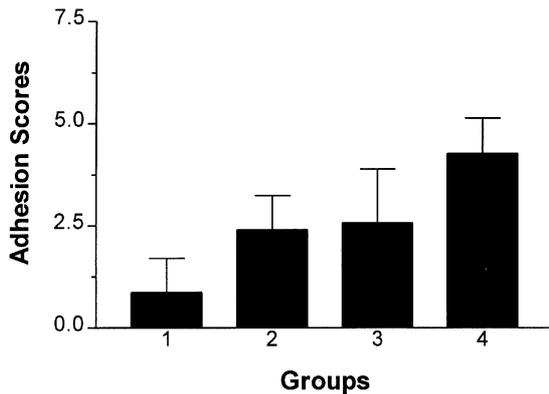
Figure 3. Total adhesion scores (versus control group $P < 0.001$ for groups II, III and IV; versus group IV $P < 0.001$ for groups II and III).

because heated and humidified CO_2 will be used in the further experiments.

The in-vitro desiccation set-up showed that at $37°C$ desiccation is linear with time and flow rate, at least within the limits of the experiment. The amount of desiccation which was 0.674 g/(l/min flow rate)/h *in vitro*, can only give a rough estimation of the in-vivo situation. Indeed, surface area, temperature and transudation of the peritoneum are crucial factors, which will ultimately determine the drying of the peritoneum.

Many scoring systems have been used for postoperative adhesions. Generally extent, type, tenacity and inflammation were scored macroscopically and/or microscopically (Rijhwani *et al.*, 1995; Evrard *et al.*, 1996; Wallwiener *et al.*, 1998). We used the same scoring as in rabbits (Ordonez *et al.*, 1997), which is a scoring system essentially comparable to the scoring system of Fiedler *et al.* (1996) and Boyers *et al.* (1988). Because of the small dimensions of mice, adhesions have to be scored by laparotomy, preferentially using magnification such as an operating microscope. Inflammation was not scored since histological evaluation was not used in this study. Extent was not scored either, because in virtually all animals >75% of the lesion sites were covered with adhesions probably due to small lesion sites.

Our data clearly demonstrate that the duration of pneumoperitoneum using non-humidified CO_2, is an important co-factor in adhesion formation in the mouse model and also confirm the results of the study which showed that shorter operation times reduced adhesion formation in the rabbit model (Ordonez *et al.*, 1997). Pneumoperitoneum alone for 60 min without any flow through the abdomens of the mice, increased adhesion formation. This increase was probably mediated by acidosis (Volz *et al.*, 1997), hypoxaemia and/or desiccation. Desiccation was probably minimal, since there was no flow and since desiccation is flow related at least *in vitro*. Desiccation does increase adhesion formation, which was clearly apparent with higher flow rates through the abdomens of the mice, whereas a low flow rate had an effect similar to CO_2 alone without flow. Although the in-vitro observations can not be extrapolated to the situation *in vivo*, it is clear that high flow rates cause more desiccation being linear with time. For this reason, proper

humidification of CO_2, especially when used with a high flow insufflator, seems to be mandatory. The mouse model permitting surgery in 10 mice seems suited to investigate the exact correlation between flow rate, duration of surgery, degree of humidification, desiccation of the peritoneal lining and adhesion formation. Since most biological effects are not linear, this knowledge could be important to delineate the conditions when endoscopic surgery with high flow insufflation becomes dangerous for adhesion formation.

A mouse model for the studies of adhesion formation following endoscopic procedures, has some advantages. Firstly, the mouse is a known model in adhesion formation after a laparotomy. Secondly, this model permits the anaesthesia and maintenance of pneumoperitoneum even in 10 mice simultaneously, which is an obvious advantage to investigate the effect of variables such as the duration of CO_2 pneumoperitoneum. Thirdly, since implantation of human cells from endometriosis and malignant tumours is possible in some immunodeficient mice strains such as Nude and SCID (severe combined immunodeficiency) mice, this model opens new possibilities.

In conclusion, the mouse model can be used to study adhesion formation following endoscopic procedures. The duration of CO_2 pneumoperitoneum is a co-factor in adhesion formation.

References

Boyers, S.D., Diamond, M.P. and DeCherney, A.H. (1988) Reduction of postoperative pelvic adhesions in the rabbit with Gore-TexΓ surgical membrane. *Fertil. Steril.*, **49**, 1066–1070.

Chen, M.D., Teigen, G.A., Reynolds, H.T. *et al.* (1998) Laparoscopy versus laparotomy: an evaluation of adhesion formation after pelvic and paraortic lymphadenectomy in a porcine model. *Am. J. Obstet. Gynecol.*, **178**, 499–503.

Diamond, M.P., Daniell, J.F., and Feste, J. *et al.* (1987) Adhesion reformation and *de novo* adhesion formation after reproductive pelvic surgery. *Fertil. Steril.*, **47**, 864–866.

Drake, T.S. and Grunert, G.M. (1980) The unsuspected pelvic factor in the infertility investigation. *Fertil. Steril.*, **34**, 27–31.

Ellis, H. (1982) *Intestinal Obstruction*. Appleton-Century-Crofts, New York, 197 pp.

Evrard, V.A.C., De Bellis, A., Boeckx, W. *et al.* (1996) Peritoneal healing after fibrin glue application: a comparative study in a rat model. *Hum. Reprod.*, **12**, 1877–1880.

Fiedler, E.P., Guzick, D.S., Guido, R. *et al.* (1996) Adhesion formation from release of dermoid contents in the peritoneal cavity and effect of copious

lavage: a prospective, randomized, blinded, controlled study in a rabbit model. *Fertil. Steril.*, **65,** 852–859.

Filmar, S., Gomel, V. and McComb, P.F. (1987) Operative laparoscopy versus open abdominal surgery: a comparative study on postoperative adhesion formation in the rat model. *Fertil. Steril.*, **48,** 486–489.

Goldstein, D.P., deCholoky, C., Emans, S.J. *et al.* (1980) Laparoscopy in the diagnosis and management of pelvic pain in adolescents. *J. Reprod. Med.*, **24,** 251–256.

GraphPad, PRISM™, version 2.0 (1995). 10855 Sorrento Valley Road, Suite, 203, San Diego, CA 92121, USA.

Holmdahl, L., Risberg, B., Beck, D.E. *et al.* (1997) Adhesions: pathogenesis and prevention-panel discussion and summary. *Eur. J. Surg. Suppl.*, **577,** 56–62.

Jorgensen, J.O., Lalak, N.J. and Hunt, D.R. (1994) Is laparoscopy associated with a lower rate of postoperative adhesions than laparotomy? A comparative study in the rabbit. *Aust. N. Z. J. Surg.*, **65,** 342–344.

Koninckx, P.R. and Vandermeersch, E. (1991) The persufflator: an insufflation device for laparoscopy and especially for CO_2-laserendoscopic surgery. *Hum. Reprod.*, **6,** 1288–1290.

Luciano, A.A., Maier, D.B., Koch, E.I. *et al.* (1989) A comparative study of postoperative adhesions following laser surgery by laparoscopy versus laparotomy in the rabbit model. *Obstet. Gynecol.*, **74,** 220–224.

Malinak, L.R. (1980) Operative management of pelvic pain. *Clin. Obstet. Gynecol.*, **23,** 191–200.

Marana, R., Luciano, A., Muzii, L. *et al.* (1994) Laparoscopy versus laparotomy for ovarian conservative surgery: a randomized trial in the rabbit model. *Am. J. Obstet. Gynecol.*, **171,** 861–864.

Ordonez, J.L., Dominguez, J., Evrard, V. *et al.* (1997) The effect of training and duration of surgery on adhesion formation in the rabbit model. *Hum. Reprod.*, **12,** 2654–2657.

Rapkin, A.J. (1986) Adhesions and pelvic pain: a retrospective study. *Obstet. Gynecol.*, **68,** 13–15.

Renaer, M. (1986) *Chronic Pelvic Pain in Women*. Springer-Verlag, New York, 78 pp.

Rijhwani, A., Sen, S., Gunesakaran, J. *et al.* (1995) Allopurinol reduces the severity of peritoneal adhesions in mice. *J. Pediatr. Surg.*, **4,** 533–537.

Ryan, G.B., Grobety, J., and Majno, G. (1973) Mesothelial injury and recovery. *Am. J. Pathol.*, **71,** 93–112.

Volz, J., Köster, S. and Leweling, H. (1997) Surgical trauma and metabolic changes induced by surgical laparoscopy versus laparotomy. *Gynaecol. Endosc.*, **6,** 1–6.

Wallwiener, D., Meyer A. and Bastert G. (1998) Adhesion formation of the parietal and visceral peritoneum: an explanation for the controversy on the use of autologous and alloplastic barriers? *Fertil. Steril.*, **68,** 132–137.