Does carbon dioxide pneumoperitoneum altering pressure levels lead to ultrastructural damage of fallopian tube and ovary?

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Summary

Aim: To assess carbon dioxide pneumoperitoneum and its different pressure levels related to cellular injury on ovarian surface epithelium, endothelium, and fallopian tube ciliated epithelium in laparoscopic rat model. *Materials and Methods:* Twenty-four Wistar-Albino female rats were randomized into three groups. Laparotomy was applied for Group 1 (control). Groups 2 and 3 had laparoscopy with pneumoperitoneum pressures at 10 mmHg and 15 mmHg, respectively. After 150 minutes (last 30 minutes was after desufflation for Group 2 and 3) in all groups, bilateral ovariectomy and salpingectomy were performed. The ultrastructures of ovarian surface epithelium, ovarian endothelium, and fallopian tube ciliated epithelium were evaluated by transmission electron microscope. Ovarian surface epithelium changes were divided into three groups, apical surface changes, lateral surface chances, and organelle modification/damage. *Results:* No apical or lateral surface changes or organelle modifications in ovarian surface epithelium were observed in the control group. Apical ovarian surface epithelium changes were statistically significant in Groups 2 and 3 in comparison to the control group. No significant differences were observed with regards to lateral surface changes in all groups. The organelle modification was only significant in Group 3 compared to the control group. The authors revealed that the ultrastructures of the ovarian endothelium and fallopian tube epithelium were not affected by pneumoperitoneum. *Conclusions:* Pneumoperitoneum may cause ischemia-reperfusion damage in ovarian cortex correlated with the amount of pressure.

Key words: Pneumoperitoneum; Laparoscopy; Transmission electrone microscope; Rat; Fallopian tubes; Ovaries.

Introduction

For the induction of pneumoperitoneum, the pressures required to provide adequate intra-abdominal operational space (10-15 mmHg) during the laparoscopic surgery are usually higher than the normal physiological portal system circulation pressure (7-10 mmHg). This causes a decrease in micro- and macro-circulation of the abdominal organs and tissues, leading to hypoxia-anoxia especially in splanchnic organs, including the small intestine, liver, and kidneys [1]. In addition to this ischemic-hypoxic period, following deflation, which restores visceral perfusion of organs with oxygenated blood, the generation of reactive oxygen free radicals causes a second-hit to the cell, leading to cell death by both apoptosis and necrosis [1, 2]. As a consequence, laparoscopic surgery may cause ischemia-reperfusion (I/R) injury in the abdominal organs and tissues in a time- and pressure-dependent manner [3].

Hence, during the initial ischemic period, cells may die, which is known as necrosis; after that, following reperfusion of blood, apoptotic loss of cells will take place, requiring energy substituted from the blood stream [4]. Subsequently, cells undergo specific changes in enzyme activities, mitochondrial function, cytoskeletal structure, membrane transport, and antioxidant defenses in response to hypoxia, which then collectively predispose them to reoxygenation injury [5]. A number of mitochondrial enzymes decrease in activity, and expression of the multisubunit cytochrome oxidase, and cytoskeletal changes could likely alter endothelial and epithelial permeability that can be observed as damaged ultrastructure [5]. All of these structural and morphological changes, owing to oxidative stress and inflammation, can only be correctly ascertained by a transmission electron microscope and not by a light microscope in the early stage, as in the present study. The light microscopic histologic findings are regarded as late stage [6].

To date, no study has investigated the effect of carbon dioxide (CO_2) pneumoperitoneum and different intraperitoneal pressures on the ovarian surface epithelium, ciliated fallopian tube epithelium, and ovarian endothelium. Moreover, studies investigating the effect of capnoperitoneum on the ultrastructure of parietal and visceral peritoneum were evaluated by scanning electron microscope (SEM) only and not by transmission electron microscope [7-11]. Intracellular organelles and DNA cannot be evaluated with SEM. Therefore, the ovarian surface epithelium (being a part of the peritoneum), ovarian endothelium as a surrogate of ovarian microcirculation, and ciliated epithelium of the fallopian tube were evaluated according to the structural configuration.

The aim of the experimental study was to analyze ultrastructural alterations to the integrity of the ovarian surface and fallopian tube epithelium generated by increased intra- abdominal pressure due to capnoperitoneum.

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Materials and Methods

Animals: This study was performed at the Experimental Research Center of Baskent University. The Ethical Committee approval was obtained. Twenty-four mature (four months old) female, non-pregnant Wistar Albino rats weighing between 170 and 304 g were used as an experimental model. All rats were provided by Animal Laboratory of Baskent University. They were caged in a controlled environment of 22°C with 12 h light/dark cycles. Standard rat feed and reverse-osmosis-purified water were provided ad libitum. All rats were allowed to have one week of acclimation to this environment before the experiment. Female Wistar rats were fasted overnight with free access to water containing 20% glucose.

The rats were randomized into three groups each one consisting of total of eight rats: Group 1 (control) had laparotomy and were left for 150 minutes after the incision. Groups 2 and 3 had laparoscopy and were left for 120 minutes under at 10 mmHg and at 15 mmHg of pressure, respectively. Thirty minutes after desufflation, laparotomy was also performed in Groups 2 and 3. In all groups, bilateral ovariectomy and salpingectomy were performed. The ultrastructures of the ovarian surface epithelium, ovarian endothelium, and fallopian tube ciliated epithelium were evaluated by transmission electron microscope.

The Baskent University Committee on the Use and Care of Animals approved the experiments, and all investigations complied with the 1996 National Academy of Science's Guide for Care and Use of Laboratory Animals.

Surgical procedures: All the rats were anesthetized with an intraperitoneal administration of 50 mg kg^{-1} ketamine hydrochloric acid and five mg kg^{-1} xylazine hydrochloric acid. They were immobilized on a standard rat surgery board. Before surgery, the abdominal skin was shaved and antisepsis was achieved with 10% povidone iodine solution. All the animals were kept on a warming mat. Five cm ventral vertical incision was made and covered with a sterile sponge soaked with saline and left for 150 min ın Group l. Groups 2 and 3 were insufflated with CO_2 under a pressure of 10 mmHg and 15 mmHg, respectively using a CO_2-pneu-Automat 2245 laparoscopic insufflator via an 18-gauge arterial catheter inserted into the peritoneal cavity through the right lower abdominal wall. The pneumoperitoneum was maintained for 120 min. The rats were left for 30 min for the occurrence of the ischemia-reperfusion injury. Then five-cm ventral vertical incision was made to expose the reproductive organs. The ovaries and tuba uterine of each rat in all the groups were removed after the 150 minutes. Specimens were fixed in 10% formalin and 2.5% glutaraldehyde for transmission electron microscopy examination. Two surgeons blinded to the groups performed all the operations and measurements.

Histologic examination: The specimens were fixed in 2.5% glutaraldehyde in 0.1l of phosphate buffer, pH 7.3, for six hours. The fixative was washed out in buffer for two x 15 min, post-fixed in one percent osmium tetroxide (OsO4) in the same buffer for 120 min, washed twice in buffer for two x 15 min, and dehydrated in a graded series of ethanol concentrations (25%, 50%, 75%, and 95% absolute alcohol) embedded with araldite 2-dodecenyl succinic anhydride (CY 212, DDSA), benzyldimethyl amine (BDMA), and dibutyl phthalate. They were polymerized for 48 h at 56°C in an incubator. Uranyl acetate and lead-citrate dyed ultrathin sections were studied in a transmission electron microscope *(LEO 906E EM).*

Analysis of transmission electron microscopy: In accordance with literature, the normal findings of ultrastructural evaluation of the Ovarian Surface Epithelium (OSE) are described as follows: OSE is heterogeneous and shows deep invagination, and serous-villous like papillary projections. Usually OSE is composed of a single layer of cubic epithelium covered with short uniform villi and differentiated from each other by significant intercellular borders. Golgi apparatus, endoplasmic reticulum at apical cytoplasm, scattered polysomes in the perinuclear cytoplasm, and various numbers of mitochondria are located in the basal and apical zones of cells. Intercellular lateral connections are formed as interdigitation, and in some areas large, asymmetric, irregular gaps are observed. These gaps fill with a pale amorphous substance (intracellular liquid?).

The ultrastructural evaluation of the OSE was categorized into three main groups: apical surface specializations, lateral surface specializations, and organelle modifications. All results were recorded as positive or absent.

Staging the damage in ovary epithelial cells by means of transmission electron microscopy:

Stage 0: Normal cells, no damage. Ovary epithelial cells (germinal epithelium) usually consist of a single line of cuboidal cells (simple cuboidal epithelium) separated from each other by clear intercellular borders and covered with short, uniform microvilli (M). The lateral face junctions between cells are observed to be in the form of interdigitation. There is a terminal bar in the apical section. A large number of mitochondria are located in apical and basal. The nucleus is covered with double-membrane nucleolemma and has made indentation in some cells. It has a clear nucleolus. The cells are located on the basement membrane.

Stage 1: Deterioration of lateral face junctions, disordered microvilli distribution, no microvilli observed in the apical surface (M), deletion of mitochondria cristae (cristolysis) in 25% of the cells, swelling in the mitochondria, and vacuolization (V) formation inside the cell.

Stage 2: Cristolysis of mitochondria cristae, presence of residual bodies (R) in the cell, V formation in the cell, and observation of changes as presence of lipid droplets in more than 50% of the cells.

Stage 3: Cristolysis of mitochondria cristae, swollen mitochondria, presence of R bodies in the cell, V formation in the cell, and observation of changes like presence of lipid droplets in more than 50% of the cells.

Stage 4: No remnants of amorphous bodies between the cells, separation of large cytoplasmic bodies from the cell, formation of projections and blebs (B), and complete separation of the cells from the basement membrane.

Statistical analysis

The categorical data was evaluated by Chi-Square test. Because the case number for each cell was not sufficient, *p* value could not be given. Therefore the groups were compared in doubles. Each time point was evaluated separately, and *p* values less than 0.05/3 = 0.017 was considered significant. SPSS (Statistical Package for the Social Sciences, version 11.0) was used for all analysis.

Results

No apical or lateral surface changes or organelle modifications in ovarian surface epithelium were observed in the control group (Figure 1). Apical ovarian surface epithelium changes were statistically significant ($p < 0.001$) in Groups 2 and 3 in comparison to the control Group (Figures 2-4), but no significant difference was found between Groups 2 and 3 according to the apical

Fig. 1

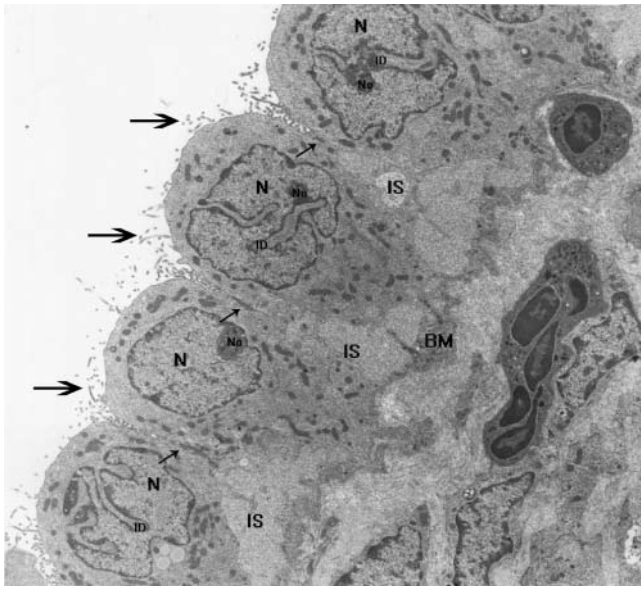

Fig. 2

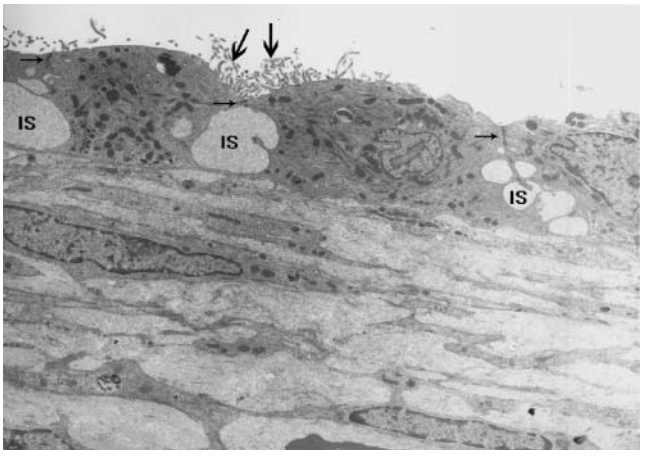

Fig. 3

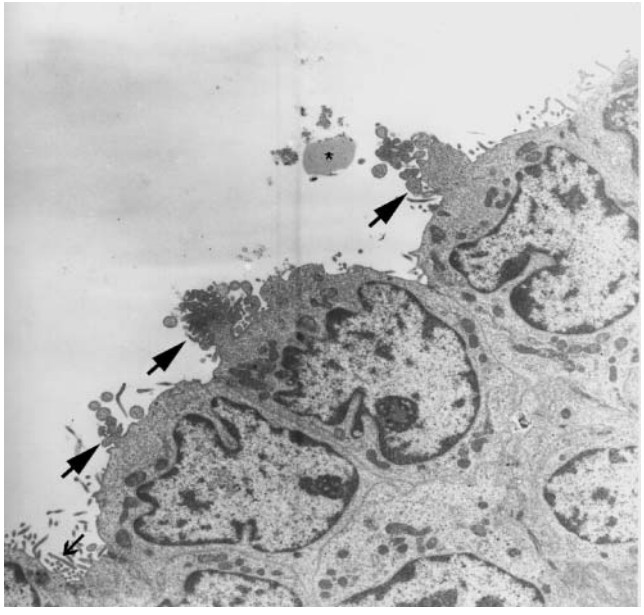

Fig. 4

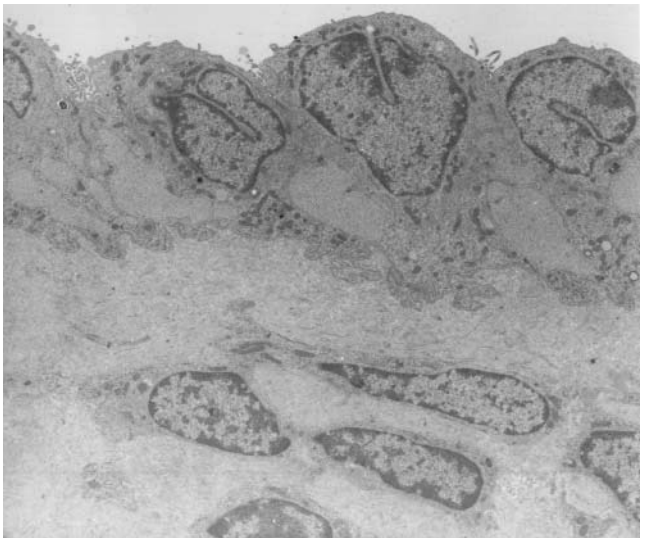

Figure 1. — Control group. Ovarian epithelial cells (germinal epithelium) usually consist of a single line of cuboidal cells (simple cuboidal epithelium) separated from each other by clear intercellular borders (small arrow) and covered with short, uniform microvilli (M, dark arrow). The lateral face junctions between cells are observed to be in the form of interdigitation (IS). There is a terminal bar in the apical section. A large number of mitochondria are located in apical and basal. The nucleus (N) is covered with double-membrane nucleolemma (No) and has made indentation(ID) in some cells. It has a clear nucleolus. The cells are located on the basement membrane (BM). [x2,784]

Figure 2. — Group 1 (10 mmHg). Disordered microvilli distribution (dark arrow), deterioration of apical border (small arrow) of lateral face junctions(IS). [x2,784]

Figure 3. — Group 1 (10 mmHg). Disordered and decreased microvilli distribution (arrows), gathered microvilli at the apical surface. [x3,597]

Figure 4. — Group 1 (10 mmHg). No microvilli observed in the apical surface. [x2,784]

surface changes. In terms of lateral surface changes in ovarian surface epithelium, no statistically significant differences were observed among the groups. The organelle modification was only significant ($p < 0.001$) in Group 3 compared to the control group (Figure 5). The ultrastructure of the endothelium under the surface epithelium of the ovaries and the isthmus epithelium of the fallopian tube were not affected by pneumoperitoneum (Figure 6).

Discussion

In literature, studies shows that CO_2 pneumoperitoneum and increasing the intra-abdominal pressure lead to ischemia and reperfusion damage and some dysfunctions of the organs. However ovarian surface epithelium, ovarian endothelium, and tubal sillier epithelium were not examined in such studies. In this study, the authors have shown that CO_2 pneumoperitoneum leads to alterations in

Fig. 5

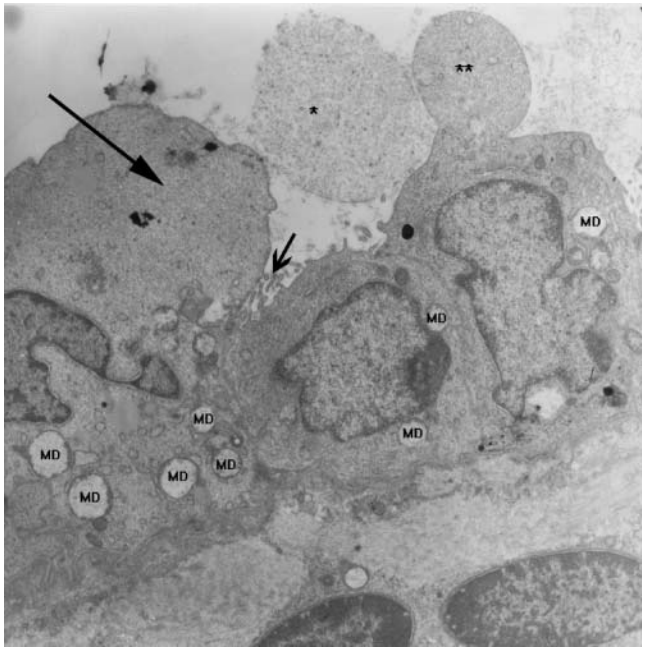

Fig. 6

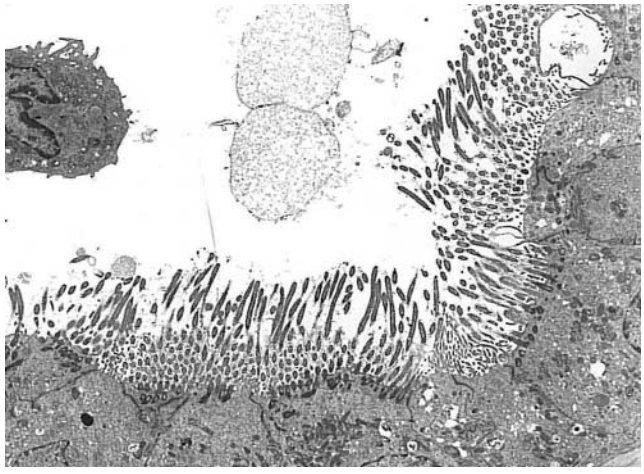

Figure 5. — Group 2 (15 mmHg). Separation of large cytoplasmic bodies from the cell (**), formation of projections, and blebs (arrow)), decreased microvilli (small arrow), and swollen mitochondria (MD). [x3,597]
Figure 6. — Group 2 (15 mmHg). The preserved isthmus epithelium of the fallopian tube. [x1,670].

ovarian surface epithelium's ultrastructure, the degree of which is well-dependent on intra-abdominal pressure. In pneumoperitoneum models where insufflations pressures were compared to each other although the intra-abdominal pressure was above 7 mmHg, fairly "lower" (i.e. 10 mmHg) and "higher" (i.e. 15 mmHg) intra-abdominal pressures were used. The general finding of these studies is that when high intra-abdominal pressure is used, there is increased tissue-organ hypo-perfusion and damage, increased metabolic effects, and increased formation of free oxygen radicals.

The response of each tissue to ischemia and the entry into irreversible phase differs. Characteristically, it is noted that there are two phenomena which show that irreversible points are reached: mitochondria and plasma membrane damage. At this point, plasma membrane damage is central factor in pathogenesis. One of the important biochemical mechanisms having a role in membrane damage is a reactive oxygen particle, which causes ischemia and reperfusion damage. While reactive oxygen particles can be formed in the post-ischemic mitochondria by the insufficient reduction of oxygen or by the synthesis of superoxide ion by the ksantin oxidase on the vascular endothelium, it is in fact secreted by polymorphonuclear leukocytes. As a result of all these, there is calcium charge into the cell and the cells move towards the irreversible point [28].

If ischemia continues, there will be irreversible damage in the cell. The transition from irreversible status to cell death is not biochemically clear. While the degeneration of the membranes in the cell may result, intracellular calcium flow into the mitochondria may be observed as well. This will result in the vascularization of the mitochondria and the formation of mitochondrial density residual items. The calcium charge to the cell will increase especially if the ischemic area is reperfused. There will be constant outflow of enzymes, proteins, metabolites, etc. from the cell. At this point, lysosomal enzymes will be secreted in the cell and cell death occurs [28].

In the present study, while both in the 10 mmHg and 15 mmHg groups apical surface changes and membrane damage in the ovarian surface epithelium were observed, in the entire 15 mmHg group, in addition to the above, mitochondrial degeneration was also observed. It is logical that while ischemia occurred during pneumoperitoneum, it initially damages the plasma membrane and apical modifications in the cell, when the intra-abdominal pressure increases organelles, from which mitochondria is initially damaged. Because, after ischemia, oxidative phosphorylation in the mitochondria and the energy carrier of the cell, ATP decreases, which stops the activities associated with aerobic circulation. The sodium pump does not work; intracellular ion and water balance become disrupted. Furthermore, there will be calcium charge into the cell and potassium discharge of the cell. As a result, the cell swells, microvilli and cell skeleton disrupts, protrusions on the cell membrane are formed, mitochondria swells and expands, myelin figures are formed within and outside the cell [28].

Although statistically non-significant, especially in Group 2 (15 mmHg), higher trend for changes in lateral surface modifications and widening of the intercellular junctions were found. However it is not clear whether these changes are either attributable to an inherent property of CO_2 per se [12], leading to local acidosis or a direct pressure effect, leading to the temporary stretching and expansion of the peritoneal surface area by the pneumoperitoneum [13].

The other interesting finding was the mitochondrial degeneration that was found strikingly in high and in partly low pressures of pneumoperitoneum. In contrast to the above-mentioned findings, the degenerative changes in mitochondria were most likely related to post-ischemic reperfusion damage-second hit effect (surrogate of irreversible cell damage) leading to influx of calcium and H_2O, and affecting the cell skeleton [14-16].

The final deleterious effect that resulted from either local acidosis or the direct compression is disturbed microcirculation and hypoxemia [8]. Hypoxic tolerance of various cell types differs, depending on the metabolic rate and intrinsic adaptive mechanisms of the tissue. The deterioration of blood flow during pneumoperitoneum was more prominent in solid organs, such as the liver, pancreas, spleen, and kidneys, compared to that in hollow viscus organs such as the intestine, while it was non-significant in the stomach [17]. This discrepancy suggests a potentially varying degree of sensitivity to ischemic insult among different tissues. Although in literature various splanchnic organs have been tested for pneumoperitoneum-associated ischemia and reperfusion injury, only one study evaluated the ovarian tissue [18-21].

Fallopian tube ciliated epithelial cells are extremely sensitive to hormones, in rat estrous cycle, such that their morphology can completely change in a 24-hour period. Constant change of morphology, especially ciliary movement after ovulation requires high energy and mitochondria activity. Hence, in the initial stages of study, they were assumed to be effected by ischemia and reperfusion damage and were included in the study. However, the response of the cells to damage depends on the type, duration, and intensity of the damage. Furthermore, the types of cells and their general condition are also important in this response. Each cell has a different response to ischemia and a different period of entry into the irreversible period. While this period is one to two hours for liver cells, it is three to five minutes for neurons. This may be the reason for the difference observed in the fallopian tube ciliated epithelial cells received from the isthmus, which is relatively inactive compared to ampulla. Another reason may be the observation of the internal epithelia, which is protected from the direct mechanical effect of the increased intra-abdominal pressure, contrary to the external fallopian tube epithelia. Furthermore, in contrast to ovarian surface epithelium, as these cells were not in direct contact with CO_2, intercellular hypercapni and acidosis may have occurred. If this experiment was conducted in the ampulla where ciliated cells are the most active, they may have less exposure to ischemic reperfusion damage (provided all subjects are in the estrus phase). The present authors revealed that in all groups ciliated tube epithelium was unaffected. Another explanations for these results may be avoiding exposure to direct CO_2 and stable intra-tubal pressure. Although SEM may be considered principally as an appropriate means for evaluating peritoneal surface changes, microvilli and organelles cannot easily be used for comparison because their number and appearance may vary greatly [23]. Hence transmission electron microscopy is more suitable for the evaluation of microvilli and intracellular organelles.

Although no standard CO_2 pneumoperitoneal pressures were identified in experimental studies, various studies used working pressure as low as four mmHg and as high as 20 mmHg [24-26]. In accordance with this finding, the present authors preferred to use high and low pressures in this study. In literature, nonetheless some studies proposed that pressures above eight to 10 mmHg in a rat model do not correlate well with working pressures in humans. Thus, the findings may not be applicable for humans. However, there were some methodological problems with the above mentioned recent study [26]. In this study, there was some variability in the end-tidal CO_2 baseline levels between the different pressure groups. This variability is the largest flaw of this study. The other criticism for this study is not measuring the central venous pressure, consequently lacking of close hemodynamic monitoring.

There are some limitations in the current study that must be acknowledged. There is a disadvantage in extrapolating data across species, as the immunologic properties of species are different. Additionally, rats were not mechanically ventilated due to technical constraints, as well as blood gas follow-up and close hemodynamic monitoring, especially in experiments in which a high intra-abdominal pressure model is used, in order to reduce evaluation errors that could result from differences in the insufflations system, and the intra-abdominal volume of the subject. Since the authors did not perform intubation and mechanical ventilation and did not follow up blood gases during the experiment, they cannot state whether hypercapnia or elevated intra-abdominal pressure influenced the results. Under full intubation, especially tissue perfusion being potentially different and effecting the results, comparison of low and high intra-abdominal pressure with regard to the present transmission electron findings are so significant that could not be disregarded even with such limitations.

A suggestion for a follow-up study and further analysis would be to examine the histological changes in ovaries under the same experimental conditions but one week later, to determine whether the changes are as significant and/or permanent.

The present authors found hazardous effects particularly ultrastructural damage on ovarian surface epithelium when the intra-abdominal pressure was set at 10 mmHg or 15 mmHg. They therefore planned a further study with lower intra-abdominal pressure (five mmHg) and different cytoprotective agents [27].

In literature, up until the period during which this study was conducted, no model on alternation of ovarian vascularization, the thin structure of the endothelium of the ovarian mucosa, due to increased pneumoperitoneum or intra-abdominal pressure, have been found; for this reason, it is not known how the ovarian microcirculation is affected from increased intra-abdominal pressure.

This experimental study demonstrated the depressed tissue blood flow and also prominent evidence of oxida-

tive stress injury in the ovaries during CO_2 pneumoperitoneum and proposed that the ovaries were also highly sensitive to ischemia. It was suggested that this hypoperfusion period may cause significant detrimental effects on the ovaries especially in critical conditions related to the ovary, such as unexplained infertility, in which subtle changes in follicle development, ovulation, and the luteal phase may be important etiologic factors [22]. The postoperative fertility studies should be undertaken to determine any long-term fertility effects. The clinical significance of the findings regarding humans has yet to be established. For this purpose, similar studies on the human ovary are imperative [22].

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