

# Long-Term Effects of Elevated Intra-Abdominal Pressure on Testes

## An Experimental Model of Laparoscopy

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**Purpose:** To determine the long-term outcomes of pneumoperitoneum on the testes in an experimental laparoscopy model.

**Materials and Methods:** Twenty-four rats were divided into three groups: Group A, the control group; Group B, exposed to a 10 mmHg intra-abdominal pressure (IAP); and Group C, exposed to a 20 mmHg IAP with CO<sub>2</sub> pneumoperitoneum for 60 minutes. After 6 weeks, the testes were removed, and testicular injury score and Johnson score were examined histologically. Germ cell apoptosis was also detected using flow cytometry.

**Results:** A significant difference was determined between all groups in terms of testicular injury scores, Johnson scores, and germ cell apoptosis percentages. For the testicular injury score, there were significant differences between the groups for the right testis (group A versus B,  $P = .009$ ; group A versus C,  $P < .0001$ ; and group B versus C,  $P = .001$ ) and for the left testis (group A versus B,  $P = .001$ ; group A versus C,  $P < .0001$ ; and group B versus C,  $P = .002$ ). Significant differences were determined in the Johnson scores for the right testis between all groups (group A versus B,  $P = .001$ ; group A versus C,  $P < .0001$ ; and group B versus C,  $P = .008$ , respectively). Percentage of apoptotic testis cells were significantly differed between all groups ( $P = .001$  for each).

**Conclusion:** This study shows that increased IAP during pneumoperitoneum causes histopathology and apoptotically-evident damage to the testes in the long-term, depending on the magnitude of IAP increase, which may cause sub/infertility. Considering the experimental nature of this study, further clinical studies are needed for a more decisive conclusion.

**Keywords:** laparoscopy; pneumoperitoneum; fertility; testis; treatment outcome

## INTRODUCTION

Despite many potential advantages for the patients, laparoscopic surgery is not completely free of complications. Increased intra-abdominal pressure (IAP) is one of the main potential causes of laparoscopy-associated complications, which are subject to current investigations.<sup>(1-4)</sup> Studies have focused on changes in blood flow to intra- and extra-abdominal organs, including the intestine, liver, kidney, spleen, bladder, and even the testes and ovaries, during pneumoperitoneum caused by laparoscopic procedure and its effects in both the early postoperative period and the long-term.<sup>(1-10)</sup>

During the laparoscopic procedure due to increased venous resistance, blood flow to the organs is reduced, which restores following desufflation of the abdominal cavity. This ischemia/reperfusion condition promotes the generation of various reactive oxygen species (ROS), causing oxidative tissue damage. In tissues subject to the ischemic-reperfusion, the increase in ROS production is suggested to involve two phases. Immediately after reperfusion, the first phase occurs, which extends for a few hours. This is a typical oxidative stress situation, “reversible” in terms of cellular injury. The first phase is followed by the second phase, which extends from hours to days. “Irreversible” tissue damage occurs during the second phase.

This is the most important outcome, because the long-term consequences of these changes may affect organ functions. Since there is evidence of laparoscopic procedure-associated ischemia, which may underline pathogenesis of some early or long-term adverse clinical outcomes after laparoscopic procedures.<sup>(11-14)</sup> Therefore, various strategies, such as minimization of the IAP, ischemic preconditioning, and pretreatment with erythropoietin, mesna and/or antioxidants, are needed and indeed currently under consideration for the prevention of these laparoscopy-associated adverse outcomes.<sup>(15-18)</sup>

The blood supply of the testis is through the “testicular artery”, which has a high vascular resistance. Therefore capillary hydrostatic pressure in the testis low and is prone to affected from increase in venous pressure. Oxygen tension in the testis is low. The seminiferous tubules are apparently adapted to this condition of low oxygen, low vascular per-

fusion pressure, and high metabolic activity, and normally this vasculature is sufficient to supply the testis with adequate amounts of perfusion. This suggests that moderate disturbances in the blood supply to the organ may affect testicular function, resulting in sub/infertility.<sup>(19,20)</sup>

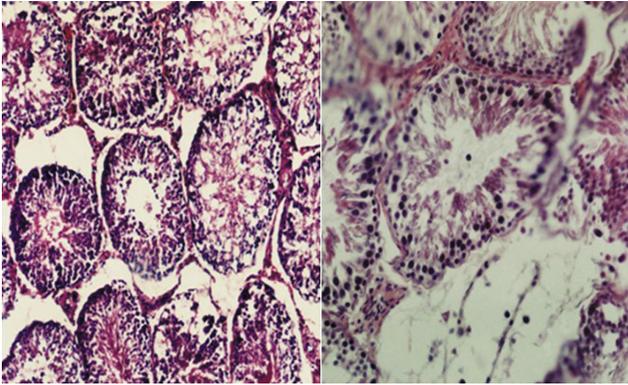
It has been well documented that elevated intraabdominal pressure during laparoscopic surgery may cause ischemia/reperfusion injury in primarily affected and also in distant organs and tissues; the effect is time- and pressure-dependent.<sup>(1,8,10,11,14,15,18)</sup> Thus, it is not surprising that testicular damage may develop and lead to, as a long-term consequence of laparoscopy, sub/infertility. Indeed, the experimental models have demonstrated that the increased IAP during laparoscopy reduces testis blood flow, which in turn causes a significant rise in oxidative stress in the testis tissue and, secondary to this, various degrees of damage occur in the parenchyma in the early stage.<sup>(19,20)</sup> However, the long-term effects of increased IAP on the testes have not been studied to date.

This study was planned with the aim of investigating whether damage arising in the early period had a negative impact on the testis function in the long term, which is the most important potential cause of sub/infertility. If it is determined that damage arises in the testis tissue in conventional pressures used in laparoscopy, such as 10 mmHg, then it may be necessary to review the range of pressures used during laparoscopy, especially in patients with conditions for subfertility.

## MATERIALS AND METHODS

### *Experimental Protocol and Operative Technique*

The study protocol was approved by the University Animal Care and Ethics Committee. Anesthesia was induced by intramuscular injection of ketamine hydrochloride 90 mg/kg (Ketalar, Parke-Davis, Berlin, Germany) plus xylazine 10 mg/kg (Rompun, Bayer, Germany). After tracheostomy, a 16 G cannula was inserted in the trachea. Thereafter, maintenance was performed by intramuscular injection of 10 mg/kg xylazine at hourly intervals. Continuous intravenous infusion was performed through cannula in the right femoral vein. The animals were continuously infused with physiological saline at 4 mL/kg/h. Muscle relaxation was attained and maintained by hourly intramuscular injections of 2.0 mg/kg pancuronium bromide (Pavulon; Organon Teknika, Boxtel,



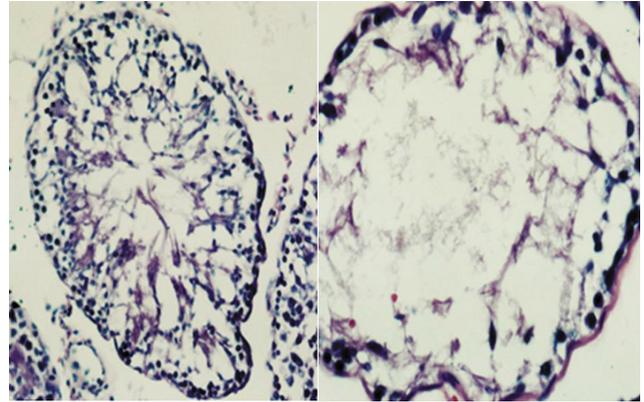
**Figure 1. A)** Histopathological image of grade 2 testis damage in group B (showing histological testis structure comprising a small number of closely packed, orderly, damaged seminiferous tubules and non-cohesive germinal cells) (Hematoxylin and Eosin stain, original magnification  $\times 4$ ); **B)** Histopathological image of grade 3 testis damage in group C (showing histological testis structure with mildly damaged seminiferous tubules and slightly damaged germinal cells and pyknotic cells in the tubules) (Hematoxylin and Eosin stain, original magnification  $\times 10$ )

the Netherlands). Then, the rats were mechanically ventilated with a pressure-controlled mode (peak inspiratory pressure of 12 cm H<sub>2</sub>O, a positive end-expiratory pressure of 8 cm H<sub>2</sub>O, a fraction of inspired oxygen of 1.0, a frequency of 50 beats per minute, a tidal volume of 10 mL/kg, and an inspiration/expiration ratio of 1:1).

By placing an 18-gauge angiocatheter caudally to the sternum a peritoneal cavity puncture was achieved for performing the pneumoperitoneum. Insufflation of carbon dioxide was also performed through this catheter. The targeted IAP was achieved and controlled for 60 min using an electronic laparoflator (KARL STORZ GmbH, Tuttlingen, Germany).

### Groups

Twenty-four adult male Sprague-Dawley rats (weighing between 400 and 450 g) were randomly allocated to one of three groups: group A (n = 8), the gasless group (control group, an angiocatheter alone being inserted into the abdomen); group B (n = 8), 10 mmHg IAP with pneumoperitoneum for 60 min; and group C (n = 8), 20 mmHg IAP with pneumoperitoneum for 60 min. After six weeks, the testes were removed and longitudinally bisected. Half of the testes were fixed in Bouin's solution for histological examination. The other half



**Figure 2. A)** Histopathological image of testicular biopsy score 8 in group B (a large number of spermatid and spermatogenic cells appear undifferentiated in the seminiferous tubule) (Hematoxylin and Eosin stain, original magnification  $\times 25$ ); **B)** Histopathological testicular biopsy score 6 image in group C (with a small number of spermatogonia cells and spermatids) (Hematoxylin and Eosin stain, original magnification  $\times 40$ )

were fixed in 10% buffered formalin solution and then embedded in paraffin blocks for apoptosis evaluation.

### Histology

Light microscopic examination of the tissue sections was performed after Hematoxylin and Eosin (H & E) staining. Histological evaluations were performed by a histologist blind to the protocol of the study.

Two microscopic parameters were used for this evaluation; testicular injury score and maturation of the germinative epithelium.

Quantification of the histological injury was performed by a 4-level grading scale.<sup>(21)</sup> Testicular histology with orderly arrangement of germinal cells was rated as Grade 1. Injuries presenting less orderly, with noncohesive germinal cells and seminiferous tubules in the form of tightly-packed were rated as Grade 2. Tissues in the form of disordered sloughed germinal cells with shrunken pyknotic nuclei and unclear seminiferous tubule borders were considered as Grade 3. Injuries characterised by seminiferous tubules closely packed with coagulative necrosis of the germinal cells were rated as Grade 4. The mean injury score for each testis was then calculated.

Second, testicular biopsy score (TBS) modified by Johnson was used for grading the maturity of the germinative epithelium of the seminiferous tubules.<sup>(22)</sup> For this purpose evaluation of 25-50 tubules were performed for each section under 25×objective. For each testis the mean TBS was calculated by scoring system ranging from 1-10.

### **Apoptosis**

Tissue preparation was performed according to Hedley's method.<sup>(23)</sup> Material was treated with pepsin solution at room temperature, and thereafter, lysis and permeability reactive DNA-prep (PN 4238055-B) was added to lyse erythrocytes and increase the permeability of cell membranes. DNA prep stain (PN 4238055-BR) including propidium iodide was finally added to stain cell DNAs. Flow cytometric analysis was carried out with EPICS ELITE ESP (Coulter). For each histogram, 5000 to 10 000 cells were analyzed. Chicken erythrocyte nuclei (Coulter DNA prep PN 6604453) were used as DNA-diploid standard. Results of the histograms were analyzed using a multicycle DNA analysis program.<sup>(24)</sup>

### **Statistical Analysis**

Comparisons among the groups were performed using Kruskal-Wallis ANOVA (Mann-Whitney U test with Bonferroni correction as a post hoc test) for testicular injury score, TBS, and apoptosis values. Measured data are presented as median for testicular injury scores and arithmetic mean ± standard deviation for TBS and apoptosis, and data obtained by counting are shown as percentage.  $P < .05$  was considered statistically significant.

## **RESULTS**

### **Testicular Injury Score**

Group analysis revealed significant differences between all groups with regard to the degree of damage for both right and left testis (Table 1). For the right testis, this difference arose from the differences between group A versus group B and group A versus group C, and group B versus group C ( $P = .009$ ,  $P < .0001$ , and  $P = .001$ , respectively). For the left testis, this difference arose from the comparisons between group A versus group B and group A versus group C, and group B versus group C ( $P < .001$ , and  $P < .0001$ , and  $P =$

$.002$ , respectively).

Median values of damage was grade 1 in group A, grade 2 in group B (Figure 1A), and grade 3 in group C (Figure 1B). In the 10 mmHg IAP group specimens, seminiferous tubules were packed closely, and germinal cells were noncohesive and less orderly, particularly in the peripheral testicular parenchyma. In the 20 mm Hg IAP group, the specimens showed sloughed germinal cells with shrunken pyknotic nuclei and less distinct seminiferous tubule borders, these changes occurring diffusely in the testicular parenchyma.

### **Testicular Biopsy Score**

Mean TBS revealed almost full spermatogenesis in both right and left testes in group A, whereas disturbance of spermatid differentiation was determined in both right and left testes in groups B and C. For the right testis, this difference arose from Group A versus group B, group A versus group C, and group B versus group C ( $P = .001$ ,  $P < .0001$ , and  $P = .008$ , respectively). For the left testis, this difference arose from group A versus group B and group A versus group C ( $P = .001$  and  $P = .001$ , respectively; Table 1). Median TBS was 10 in group A and 8 in group B (Figure 2a), which was as low as 6 in group C (Figure 2b).

### **Apoptosis**

There was a statistically significant difference in both right and left testes apoptosis percentages between all groups (Table 1). For both the left and right testes, this difference arose from group A versus group B and group A versus group C, and group B versus group C ( $P = .001$  and  $P = .001$ , and  $P = .001$ , respectively).

In group A, apoptosis level was over 5% in both testes in two rats, and below 5% in both testes in others. Total apoptosis level was 4.4%. In group B, apoptosis level was over 20% in both testes in one rat, over 18% in both testes in two, over 14% in both testes in three, and below 14% in the right testis and above 14% in the left testis in one rat. Total apoptosis level was 22.4%. In group C, apoptosis level was above 30% in both testes in one rat, above 28% in both testes in three, above 24% in both testes in three, and below 24% in both testes in one rat. Total apoptosis level was 31.0%.

**Table.** Testicular injury score, Testicular biopsy score and Apoptosis results

Parametres and Groups		Right Testis		Left Testis	
Testicular injury scores		Median (min-max)		Median (min-max)	
A		1 (1-1)		1 (1-1)	
B		2 (1-2)		2 (1-2)	
C		3 (2-3)		3 (2-3)	
<i>P</i>		.0005		.0005	
Testicular biopsy scores		Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)
A		9.9 ± 0.4	10 (9-10)	9.8 ± 0.5	10 (9-10)
B		7.9 ± 0.8	8 (7-9)	8.0 ± 0.8	8 (7-9)
C		6.3 ± 0.4	6 (5-8)	6.8 ± 1.0	6 (5-8)
<i>P</i>		.0005		.0005	
Apoptosis		Mean ± SD	(min-max)	Mean ± SD	(min-max)
A		4.5 ± 0.7	(3.9-5.7)	4.6 ± 0.6	(3.5-5.5)
B		16.6 ± 3.3	(12.2-22.4)	16.7 ± 3.0	(13.4-21.5)
C		27.3 ± 2.8	(23.4-30.8)	27.2 ± 2.7	(22.8-31)
<i>P</i>		.0005		.0005	

## DISCUSSION

By aiming obtaining information on the long-term effects of laparoscopy-associated IAP increase on testicular function, this experimental study evaluated impacts of a low (10 mmHg reflecting conventional abdominal pressure during laparoscopy) and a high increase (20 mmHg) in IAP, obtained by pneumoperitoneum, on testicular morphology after 6 weeks, and documented pressure-dependent histopathological and apoptotic evidences implicating ischemia/reperfusion related damage in IAP increased groups compared to the control.

Although not investigated in detail, the obtained effects on testes are most likely due to reduction of its blood flow secondary to increased IAP occurring through two possible main mechanisms. The first is the direct effect of IAP on the testicular artery and vein, with their long course. Veins are subjected to greater pressure than arteries, and high venous resistance develops related to the increased hydrostatic pressure in the testes. The non-elasticity of the testis tunica albuginea also makes an additional contribution to the venous congestion, and then to impaired arterial circulation. Furthermore, this state of hypoperfusion may persist even after IAP has returned to normal. In a previous study, we determined, by color Doppler ultrasonography, that a level of 10 mmHg IAP at 30% to 35% and 20 mmHg IAP at 40% to 45% caused

a decrease in testicular blood flow.<sup>(19)</sup> In that study, we also showed that 10 minutes after desufflation, testicular reperfusion blood flow levels were still lower than basal testicular blood flow levels.

Second, it has been demonstrated that vena cava inferior pressure and vascular resistance significantly increased during prolonged periods of pneumoperitoneum, and remained so during the whole post pneumoperitoneum period.<sup>(25)</sup> Valveless testicular veins having to pump blood to a higher pressure venous system after a long course will further increase the hydrostatic pressure reflected in the testes. This high venous hydrostatic pressure contributes to the impairment of arterial circulation and deepening of the hypoxia. Sweeney and associates reported that in conditions of increased testicular venous pressure, microvascular fluid changes declines dramatically, and spermatogenesis and gametogenesis are harmed in consequence.<sup>(26)</sup>

In our previous study, oxidative stress response and histological outcomes of testes in 10 and 20 mmHg IAP groups were also compared with the control group. In that study, we demonstrated that 10 mmHg IAP, which is accepted as conventional abdominal pressure, in association with the testicular hypoperfusion, increased free radical production, and subsequent testicular damage occurred in the early period that is 30

minutes after pneumoperitoneum deflation.<sup>(19)</sup>

Recently, Istanbuluoğlu and coworkers also showed in a porcine model that laparoscopic nephrectomy caused ischemic changes in the testes in the acute stage.<sup>(20)</sup> Most importantly, they found that germ cell apoptosis was increased. No significant difference was noted in Johnson's scores between their two groups; however, congestion and necrosis, which were not documented in the control group, were observed in the increased IAP group. The key question is that whether these changes in the testicular tissue might be correlated with long-term testicular function. With respect to the consequences of sub-/infertility, long-term effects of different IAP levels in association with laparoscopy on spermatogenesis and testicular tissue histology was evaluated, and the results indicated the need for further studies. The evidence from these two studies leads us to design the present study to investigate the long-term effects of increased IAP on testes.

In the present study, two microscopic parameters were used for histological examination.

As can be seen in Table 1, compared with the control group (grade 1), the mean "testicular injury score" was significantly increased in both, the 10 (grade 2) and 20 mmHg IAP groups (grade 3). In the 10 mmHg IAP group, injury was observed, especially in the peripheral portion of the testicular parenchyma; however, in the 20 mmHg IAP group, the changes occurred diffusely in the testicular parenchyma. The testicular injury scores were also confirmed by TBS. Almost full spermatogenesis was observed in the control group, whereas disturbance of spermatid differentiation was determined in the 10 and 20 mmHg IAP groups. The mean TBS values of the study groups (the 20 mmHg group being even lower) were significantly lower than the control group (Table 1). Testicular injury and TBS scores are excellent parameters in the study of spermatogenesis, being widely used in the evaluation of testicular atrophy. Therefore, our experimental histopathological results indicate that increased IAP during laparoscopy may cause testicular damage, which could lead to sub-/infertility.

Khoury and colleagues recently suggested that the pneumoperitoneum induces apoptosis, increasing in parallel with rising IAP and pneumoperitoneum exposure duration, and this may be a mechanism involved in renal "delayed graft dys-

function" in recipients of laparoscopically harvested kidneys.<sup>(27)</sup> Bergh and associates observed that in conditions of testicular hypoperfusion, apoptosis may develop in Sertoli and germ cells in the seminal tubules, as a result of which fertility will be negatively affected.<sup>(28)</sup> Our study also indicates similar outcomes for apoptosis evaluation. In the 10 mmHg IAP group, total apoptosis level from the testis tissues was significantly higher than that of the control group (22.4% versus 4.4%). In the 20 mmHg IAP group, the level of apoptosis rose even higher (total apoptosis level of 31.0%; Table 1); indicating that the damage had a pressure-dependence. We therefore suggest that this importantly increased apoptosis levels exhibits an additional risk for impairment of fertility, considered in association with our histopathological results. One might wonder whether the scrotal pressure increased similar to intraperitoneal pressure and played a role in the obtained results. Although we did not perform any pressure measurements during the experiments, we did not notice any scrotal swelling and therefore we believe that the scrotal pressure remained normal during the abdominal insufflation. Furthermore, communication between the peritoneum and scrotum with patent processus vaginalis is necessary for increase in testicular pressure in parallel to intraperitoneal pressure. Therefore, the obtained results are more likely due to the increased pressure exerted on the vessels supplying the testes.

## CONCLUSION

This experimental study shows that increased IAP during pneumoperitoneum may cause potential adverse effects on fertility by causing gradual testicular hypoperfusion and related oxidative stress in the long-term, evidenced by histopathology and apoptosis index, depending on the magnitude of IAP increase. The results of this experimental study warrant further clinical studies to evaluate fertility of boys undergoing laparoscopy.

## CONFLICT OF INTEREST

None declared.

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