

## **Pneumoperitoneum effect on testicular oxidative stress and histopathology –**

### **Systematic review**

**Efeito do pneumoperitônio no estresse oxidativo testicular e histopatologia – Revisão sistemática**

**Efecto del neumoperitoneo sobre el estrés oxidativo testicular y la histopatología - Revisión sistemática**

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### **Abstract**

Pneumoperitoneum is characterized by the presence of abdominal cavity gas. Thus, this is used as a tool to create a space in the abdominal cavity for video laparoscopic surgical procedures. However, insufflation of abdominal cavity is capable of causing damage induced by tissue ischemia and reperfusion, which is caused by hypoxia and an imbalance between free radical production and antioxidant defense system capacity. The objective of this study was to bibliographic review the negative effects of exposing healthy animals to different pneumoperitoneum settings by assessing oxidative stress and testicular histopathology, identifying intra-abdominal pressures that did not result in testicular alteration. A systematic search was carried out in three databases using the following terms: pneumoperitoneum AND testi\* or gonad. The survey conducted in the databases yielded 2209 scientific articles. After applying inclusion and exclusion criteria, six papers were selected. All the articles selected addressed the effects of pneumoperitoneum on testicular structure and used at least one scoring system to perform histopathological evaluation of the testis. Three studies verified the occurrence of changes in oxidative stress. According to this literature review, pneumoperitoneum used at intra-abdominal pressures equal to, or greater than, 9 mmHg caused testicular histological damage. According to the biomarkers used in studies, pressures greater than 10 mmHg were sufficient to cause testicular oxidative stress.

**Keywords:** Testis; Gonads; Injury.

### **Resumo**

O pneumoperitônio é caracterizado pela presença de gás na cavidade abdominal. Ainda, ele é usado como uma ferramenta para criar espaço na cavidade abdominal para procedimentos cirúrgicos de videolaparoscopia. No entanto, a insuflação da cavidade abdominal é capaz de causar danos induzidos pela isquemia e reperfusão tecidual, a qual é causada pela hipóxia e desequilíbrio entre a produção de radicais livres e a capacidade do sistema de defesa antioxidante. O objetivo deste estudo foi fazer uma revisão bibliográfica dos efeitos negativos da exposição de animais saudáveis a diferentes configurações de pneumoperitônio por meio da avaliação do estresse oxidativo e da histopatologia testicular, identificando pressões intra-abdominais que não resultem em alteração testicular. Uma busca sistemática foi realizada em três bases de dados usando os seguintes termos: pneumoperitônio E testi \* Ou gonad. O levantamento realizado nas bases de dados resultou em 2.209 artigos científicos. Após aplicação dos critérios de inclusão e exclusão, seis artigos foram selecionados. Todos os artigos selecionados abordaram os efeitos do

pneumoperitônio na estrutura testicular e utilizaram pelo menos um sistema de pontuação para a avaliação histopatológica do testículo. Três estudos verificaram a ocorrência de alterações no estresse oxidativo. De acordo com esta revisão da literatura, o pneumoperitônio utilizado em pressões intra-abdominais iguais ou superiores a 9 mmHg causou dano histológico testicular. De acordo com os biomarcadores utilizados nos estudos, pressões maiores que 10 mmHg foram suficientes para causar estresse oxidativo testicular.

**Palavras-chave:** Testículos; Gônadas; Lesão.

### Resumen

El neumoperitoneo se caracteriza por la presencia de gas en la cavidad abdominal. Por lo tanto, se utiliza como una herramienta para crear un espacio en la cavidad abdominal para procedimientos quirúrgicos videolaparoscópicos. Sin embargo, la insuflación de la cavidad abdominal es capaz de causar daño inducido por isquemia y reperfusión tisular, que es causado por hipoxia y un desequilibrio entre la producción de radicales libres y la capacidad del sistema de defensa antioxidante. El objetivo de este estudio fue revisar la bibliografía sobre los efectos negativos de la exposición de animales sanos a diferentes escenarios de neumoperitoneo mediante la evaluación del estrés oxidativo y la histopatología testicular, identificando presiones intraabdominales que no resultaron en alteración testicular. Se realizó una búsqueda sistemática en tres bases de datos utilizando los siguientes términos: neumoperitoneo Y testi \* O gónada. La encuesta realizada en las bases de datos arrojó 2209 artículos científicos. Después de aplicar los criterios de inclusión y exclusión, se seleccionaron seis artículos. Todos los artículos seleccionados abordaron los efectos del neumoperitoneo sobre la estructura testicular y utilizaron al menos un sistema de puntuación para realizar la evaluación histopatológica del testículo. Tres estudios verificaron la ocurrencia de cambios en el estrés oxidativo. Según esta revisión de la literatura, el uso de neumoperitoneo a presiones intraabdominales iguales o superiores a 9 mmHg causó dano histológico testicular. Según los biomarcadores utilizados en los estudios, las presiones superiores a 10 mmHg fueron suficientes para provocar estrés oxidativo testicular.

**Palabras clave:** Testículo; Gónadas; Lesión.

## 1. Introduction

Pneumoperitoneum is the presence of gas in the abdominal cavity. This can occur as a pathological condition starting from visceral perforation and other thoracic, abdominal, and gynecological causes (Čečka et al., 2014). However, it is also used for creating space in the abdominal cavity for surgical procedures using video laparoscopy (Avtan, 2016). It is necessary to understand the physiologic alterations provoked by pneumoperitoneum and its possible risks with the intention of minimizing harmful effects.

Physiological changes caused by pneumoperitoneum include inflammatory and structural changes abnormalities in the peritoneum, which may reduce the capacity to protect against microorganisms, their products and components, and besides cardiovascular/hemodynamic, acid-base, and respiratory effects (Avtan, 2016). The hemodynamic effects of pneumoperitoneum are associated with intra-abdominal pressure (IAP) used in insufflation and results in the reduction of blood flow to the organs because of the relationship between increasing IAP, greater compression of the vena cava followed by venous blood damping, and a decrease in cardiac output (Deveney et al., 2006). The mechanisms of tissue injury following ischemia involve the reduction of blood and nutrient supply; reduced withdrawal of metabolic products (Kalogeris et al., 2012); increased capillary extravasation and leukocyte diapedesis; and there lease of inflammatory mediators by the endothelium with stimulation of cellular local response (Carden & Granger, 2000). Reperfusion-induced damage is caused by an imbalance between free radical production and antioxidant defense system capacity (Sammour et al., 2009).

Cellular lesion propagation is associated with the magnitude and duration of tissue ischemia (Kalogeris et al., 2012), which is associated with the level of vena cava compression; blood flow stasis caused by the IAP; and time of exposure (TE) to pneumoperitoneum. In minimally invasive therapeutic and exploratory procedures, pressure is chosen according to operative volumetric needs (Swanstrom, 2006), allowing for the visualization and manipulation of abdominal cavity structures.

Optimal pneumoperitoneum configuration can be chosen based on the knowledge of the harmful effects. The objective of this study was to bibliographic review the negative effects of exposing healthy animals to different pneumoperitoneum settings by assessing oxidative stress and testicular histopathology, identifying intra-abdominal pressures

that did not result in testicular alteration.

## **2. Methodology**

### *Search strategy*

We performed a bibliographic review using systematic search features to explore and describe information published in the databases (Pereira et al., 2018). The search was performed on March 20, 2017 in English without time restriction. The databases used were PubMed, ScienceDirect, and Web of Science. Full scientific articles found were assessed for their relevance to this study. The terms structure used for the search were: (pneumoperitoneum) AND (testi\* OR gonad).

### *Selection of Studies*

Two independent reviewers (BAA and VM) selected studies using the title, abstract, keywords, or full text by applying inclusion and exclusion criteria. Any differences were resolved through a discussion with a third reviewer (CDC).

### *Inclusion and Exclusion Criteria*

Inclusion criteria were (1) fully published in peer-reviewed journals; (2) using animal models; (3) comparing groups of animals exposed to pneumoperitoneum with an unexposed group; (4) results of the testicular damage were measured using the Cosentino et al. (1986) or Johnsen (1970) scoring system and performed by optical microscopy; (5) or testicular oxidative stress was assessed by free radical direct measurement, end products of damage by reactive oxygen species, or specific or total antioxidant levels, without technical restriction. The exclusion criteria were (1) the non-applicability of inclusion criteria on the study.

## **3. Results and Discussion**

A total of 2734 results were found in the databases. There were 113 from PubMed, 2574 from ScienceDirect, and 47 from Web of Science publications included. Subsequently, 2209 publications remained after publications that were complete scientific articles or duplicate items were excluded. Six full-text papers were selected after applying inclusion and exclusion criteria.

The Studies used pneumoperitoneum produced from abdominal cavity insufflation using carbon dioxide, however this information was not found in Aydin et al. (2014). Procedures were performed on pigs after pre-anesthetic medication with ketamine and xylazine, induction with propofol, and maintenance of inhalation anesthesia with the use of sevoflurane (Istanbulluoglu et al., 2011) in rats after general injectable anesthesia with ketamine hydrochloride and xylazine (Aydin et al., 2014; Imamoglu et al., 2013; İmamoğlu et al., 2006; Rifaioglu et al., 2014).

All six articles used at least one scoring system (Figure 1) for testicular histopathological evaluation and to address the effects of pneumoperitoneum on testicular structure. The articles are summarized in Table 1, showing their respective scores of histological damages, TE and IAP.

**Figure 1** – Cosentino and Johnsen histological degree criteria.

Cosentino et al. (1986) graduation		Johnsen (1970) graduation	
1	Normal testicular architecture with an orderly arrangement of germinal cells.	10	Complete spermatogenesis. Germinal epithelium organized in a regular thickness leaving an open lumen.
2	Less orderly and noncohesive germinal epithelium and packed seminiferous tubules.	9	Many spermatozoa present but germinal epithelium disorganized, with marked sloughing or obliteration of the lumen.
3	Disordered sloughed germinal cells with shrunken pyknotic nuclei and less distinct seminiferous tubule borders	8	Only a few spermatozoa
4	Seminiferous tubules that were packed with coagulative necrosis of the germinal epithelium.	7	No spermatozoa but many spermatids present.
		6	No spermatozoa and only a few spermatids.
		5	No spermatozoa and spermatids but spermatocytes present.
		4	No spermatozoa and spermatids but few spermatocytes
		3	Only spermatogonia present.
		2	Only Sertoli cells present.
		1	No cells in tubular cross-section,

Source: Authors.

**Table 1** – Summary of the selected studies with pneumoperitoneum TE, IAP and histological degree of injury findings.

Author	Pneumoperitoneum		Histological degree of injury			
	TE (h)	IAP (mmHg)	Cosentino (Median)		Johnsen (Mean)	
Aydin et al. (2014)	1	Control	1 (1-1)			
		6	1 (1-2)			
		9	2 (1-2)*			
		12	2 (1-2)*			
İmamoğlu et al. (2006)	1	Control	1 (1-1)	1 (1-1)		
		10	2 (2-2)*	2 (2-3)*		
		20	2 (2-3)*	2 (2-3)*		
Imamoglu et al. (2013)	1	Control	1 (1-1)	1 (1-1)	9.9±0.4	9.8±0.5
		10	2 (1-2)*	2 (1-2)*	7.9±0.8*	8±0.8*
		20	3 (2-3)*	3 (2-3)*	6.3±0.4*	6.8±1.0*
İstanbuluoğlu et al. (2011)	4	Control			10±0	
		20			8.66±0.8	
Ribeiro et al. (2013)	3	Control			8.2±0.4	
		8			8.4±0.4	
Rifaioglu et al. (2014)	1	Control			10	
		15			9*	

\* Groups that differed from their control, in the same column. IAP – Intra-abdominal pressure; TE – Time of exposure. Source: Authors.

Ribeiro et al. (2013) observed a decrease in diameter of the seminiferous tubules in the 8 mmHg group when using light microscopy. The studies of İmamoğlu et al. (2013) and Ribeiro et al. (2013) extended the interval between disinflation and sampling time for histological evaluation and oxidative stress. Samples were collected six weeks after pneumoperitoneum exposure, unlike in other studies where material was collected after disinflation (İmamoğlu et al., 2006; Aydin et al., 2014;

Rifaioğlu et al., 2014; Istanbuluoğlu et al., 2011).

Three studies found changes in testicular oxidative stress in animals exposed to pneumoperitoneum. İmamoğlu et al. (2006) investigated levels of malondialdehyde (MDA) to estimate testicular tissue lipid peroxidation. The testes of the 10mmHg and 20mmHg groups presented higher levels of MDA compared to the control group. Using immunohistochemistry, Istanbuluoğlu et al. (2011) observed an increase in nitric oxide-producing enzymes (induced and endothelial) in animal testes that underwent 20 mmHg of IAP. The study conducted by Rifaioğlu et al. (2014) revealed the oxidative and total antioxidant (TOS and TAS) conditions of animals exposed to pneumoperitoneum, where the control group showed the highest TAS and lowest TOS while the pneumoperitoneum group with 15 mmHg showed the lowest TAS and the highest TOS.

The terms structure and inclusion criteria used in this study were broad, although there were defined histopathology scoring systems for inclusion in this systematic review. The Johnsen and Cosentino scores were chosen after an initial search because its use was observed in all journal articles included in the literature review. Nevertheless, it was impossible to perform a meta-analysis due to the small number of heterogeneous sample papers.

Using the information obtained from samples collected after testicular deflation and histological analysis (Table 1), it was observed that pressures equal to, or higher than, 9mmHg resulted in a greater degree of control according to Cosentino lesion scores. Using the Johnsen score, Istanbuluoğlu et al. (2011) found no difference between the groups even when using an IAP and an TE higher than those of Rifaioğlu et al. (2014), in which the group exposed to pneumoperitoneum differed from the control group. The study results of Istanbuluoğlu et al. (2011) may be explained by possible germ cell permanence in the injured testis after immediate disinflation because İmamoğlu et al. (2013) observed a marked fall in the Johnsen score in samples collected six weeks after pneumoperitoneum induction using the same IAP at a lower TE. Species-specific factors and different anesthetic protocols may explain the varied results found by Istanbuluoğlu et al. (2011) and Rifaioğlu et al. (2014). The first study was conducted on pigs while the second was conducted on rats. It is known that some drugs, such as ketamine, may induce anti-inflammatory activity, possibly conferring a protective effect on rat intestines (Guzmán-De La Garza et al., 2010) and guinea pig hearts (Al-Maghrebi et al., 2012) against ischemic-reperfusion injuries.

Testicular tissue changes we're not observed in short (Aydin et al., 2014) and long-term (Ribeiro et al., 2013) at pressures equal to, or lower than 8 mmHg. A pressure of 6 mmHg may be insufficient to perform minimally invasive procedures in small animals, where an IAP between 8 and 12 mmHg is usually used. This is even lower than the 15 mmHg pressure used in humans due to the thinness of the abdominal wall (Brun, 2015), resulting in greater compliance. The absence of changes in Johnsen scores in samples collected after 6 weeks of exposure to pneumoperitoneum (Ribeiro et al., 2013) may be indicative of germ cell restocking in the seminiferous tubules after injury since the mouse spermatogenic cycle is 48 to 52 days (De Kretser, 1982), rather than of injuries. Ribeiro et al. (2013) analyzed long-term studies and differed from Istanbuluoğlu et al. (2011) because their study not show testicular lesions. However, ischemic magnitude may explain the differences in results considering that the IAPs and TEs used by Ribeiro et al. (2013) were smaller. Seminiferous tubule lumen reductions observed by Ribeiro et al. (2013) may correspond to histological findings of graduation 2 of the Cosentino score. In addition, the compactness of the seminiferous tubules was analyzed based on their diameters. Based on these observations, it is suggested that an in-depth study on pneumoperitoneum using smaller IAP and with a similar TE be performed. It is also suggested that the Cosentino and Johnsen scoring systems be used to analyze short and long-term effects.

It is possible to estimate the damage caused by testicular blood flow occlusion and reperfusion through techniques such as 720° torsions or spermatic cord clamping. The Johnsen scores of the clamp group, applied for 1 hour, were lower than the short-term control group (4 hours after reperfusion, mean  $4.6 \pm 2.1$ ) (Al-Maghrebi et al., 2012), and long-term group (6 months after the procedure, mean  $6.87 \pm 0.37$ ) (Al-Maghrebi et al., 2010). The results found by Al-Maghrebi et al. (2010) were

similar to observations by İmamoglu et al. (2013), who evaluated a group after six weeks following the application of an IAP of 20 mmHg. These findings suggest that there is a point where the magnitude of injury established through the pneumoperitoneum, caused by a high IAP, appears to be similar to total occlusion of the testicular blood flow. However, the damage caused by short-term spermatic cord clamping is greater than any pneumoperitoneum configuration found in the studies, and could be greater than damages found in animals exposed to 20mmHg, which could explain similar morphological changes at different time points.

Only a few studies evaluated testicular oxidative stress, and these do not include IAPs less than 10 mmHg. In general, alterations were found in animals exposed to pneumoperitoneum with the pressures used (10, 15 and 20 mmHg) compared to controls. The study by İmamoglu et al. (2006) demonstrated an increase in MDA levels, similar to those found in total testicular torsion occlusion in other studies (Al-Maghrebi et al., 2010). According to İmamoglu et al. (2013), testicular tissue is highly sensitive to ischemia and can become hypoxic even after short-term microcirculatory disorders, leading to reduced tissue oxygenation, cell metabolism, acidosis, and the production of reactive oxygen species. Furthermore, Istanbuloglu et al. (2011) believe that reactive oxygen and nitrogen species are important mediators of ischemia-induced damage to tissues, and nitric oxide is a potent biological mediator that functions as a signal in physiological conditions. However, it can cause DNA damage and cell death in high concentrations. Nitric oxide-forming enzymes also showed an increase in concentration in animals submitted to testicular torsion of 720 ° for 1 hour (Fatih et al., 2015), as observed by Istanbuloglu et al. (2011). Although it was not the purpose of the study to examine the attenuate techniques for testicular damage during pneumoperitoneum, preconditioning studies of pneumoperitoneum were not found. Preconditioning uses brief exposure to IAP prior to the main procedure, where animals subjected to pneumoperitoneum carried out with pressure of 15 mmHg and TE of 1 hour showed lower degrees of ovarian lesions when compared to animals without pre-conditioning (Biler et al., 2014). Preconditioning the pneumoperitoneum can be an alternative for situations that require the highest IAPs, which result in testicular damage.

#### 4. Conclusion

According to this literature review, pneumoperitoneum with pressures equal to or greater than 9 mmHg caused the testicles to exhibit histological damage. In addition, pressures greater than 10 mmHg are sufficient to cause testicular oxidative stress, according to the biomarkers used in some included studies. However, oxidative stress evaluations were not observed in pneumoperitoneum at pressures lower than 8 mmHg.

In this study, we observed several methodological differences associated with, also different, biological responses. We suggest that pneumoperitoneal pressure and time be established for each species of interest. This species-specific information can also use methodological patterns observed in this study to ensure comparison.

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