

Experimental model of the formation of pelvic adhesions by videolaparoscopic in female rabbits¹

Modelo experimental de formação de aderências pélvicas por videolaparoscopia em coelhas

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ABSTRACT

Purpose: To verify the frequency of postsurgical pelvic adhesion formation in an experimental animal model using videolaparoscopy. **Methods:** Experimental study in a sample of 11 non-pregnant female rabbits, aged 5 to 7 months. After general anesthesia, access to the abdominal cavity was performed by an open puncture technique, with 10mm optics, placing two other 5 mm trochars under direct visualization, in the iliac fossae. Then a fragment of peritoneum was resected, followed by electrocauterization. In 21 days, the videolaparoscopy was repeated, and adhesion formation and score was looked at, with biopsies at the surgical site. **Results:** 54 % of adhesion formation was observed, and the median score of adhesions was 6 (minimum of 3 and maximum of 10), all of them found in the bladder and the anterior abdominal wall. **Conclusion:** The method used presents a high frequency of intra-abdominal adhesion formation.

Key words: Adhesions. Laparoscopy. Surgery. Rabbits.

RESUMO

Objetivo: Verificar a frequência da formação de aderências pélvicas pós-cirúrgicas, em um modelo experimental animal, por videolaparoscopia. **Métodos:** Estudo experimental, em uma amostra de 11 coelhas, não prenhas, com idade entre cinco e sete meses. Após anestesia geral, o acesso da cavidade abdominal foi efetuado por técnica de punção aberta, com óptica de 10 mm, colocando-se outros dois trocateres de 5 mm, sob visão direta, nas fossas ilíacas. Realizou-se, então, ressecção de fragmento de peritônio, seguida de cauterização com eletrocautério. Em 21 dias, foi repetida a videolaparoscopia, verificando-se a formação e escore de aderências e realizando-se biópsias do local da cirurgia. **Resultados:** Observou-se 54,5% de formação de aderências, sendo o escore total mediano de aderências seis (mínimo de três e máximo de 10), todas encontradas na bexiga e na parede abdominal anterior. **Conclusão:** O procedimento utilizado apresentou alta frequência de formação de aderências intra-abdominais.

Descritores: Aderências. Laparoscopia. Cirurgia. Coelhos.

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Introduction

Surgical adhesion has been found in 56% to 100% of the patients at a second laparotomy, after gynecological surgery¹⁻³. The pathophysiology of adhesion formation is complex and unpredictable as to site and intensity^{1,3-7}.

Videolaparoscopic surgery has been considered more convenient for the patient than laparotomy, since it causes less trauma, less manipulation of the tissues, less exposure to foreign bodies, and is also associated with less inflammatory response, less likeli-

hood of postoperative infection, and, presumably, less risk of adhesions^{1,4,5,8,9}. However, adhesions have been described in laparoscopic approaches, especially in long duration surgeries, with high insufflation pressures. There is still, therefore, the need to use adhesion prevention mechanisms, for instance in myomectomies^{3,4,10}, in larger areas of peritoneal resection to approach tumors in the retroperitoneum¹, in endometriosis⁹, and in surgery using monopolar energy.

The use of experimental studies in animal models is widely disseminated, mainly because clinical studies in humans are very difficult and, also, because it possible to exercise greater control of the factors that cause the etiology of adhesions. Hence, it is necessary to study an experimental animal model of adhesion formation by videolaparoscopy and to verify their frequency.

The objectives of this study were to verify the frequency of postsurgical pelvic adhesion formation, laparoscopically, in an experimental animal model and to expose the histopathological characteristics of the region affected by the adhesion.

Methods

An experimental study was performed using as controlled variables the time of pneumoperitoneum and anesthetic parameters, such as heart rate, oxymetry and capnometry, besides the score of adhesions and anatomopathological analysis. The main outcome was the presence or absence of surgical adhesions.

This study is part of the project approved by the Ethics and Research Committee of the Research and Graduate Group at Clinics Hospital of Porto Alegre.

Eleven healthy, non-pregnant white rabbits (*Oryctolagus cuniculus*), of the New Zealand breed, aged 5 to 7 months, were used. The animals were submitted to a 6-day quarantine period. Rabbits with previous intraabdominal adhesions, confirmed disease, pre or postsurgical gestation and postsurgical hemorrhage were excluded.

In the first phase of the experiment, after general anesthesia with orotracheal intubation, an access to the abdominal cavity was performed to create a pneumoperitoneum, with an open puncture technique introducing a 10.0 mm trocar 1.0 cm below the xyphoid appendix. Initially 1 liter/min of CO₂ gas is introduced until the cavity fills with 1 liter, which is then increased to 3 liters/min, maintaining an intra-abdominal pressure of 10 mmHg. After introducing the zero degree optics (10.0 mm), the abdomen was reviewed, followed by placing two other trochars, under direct visualization, 10.0 mm into the right iliac fossa, and 5.0 mm into the left.

In order to induce adhesion formation, a peritoneal lesion was made on the anterior wall, associated with cauterization of the raw area. The standardized surgery began with the resection of a portion of peritoneum of the anterior abdominal wall, above the vesical fold (2.0 cm) and to the right, measuring 24mm x 12mm, followed by cauterization with monopolar electrocautery at a 30 W potency, using the hook horizontally and at bleeding points, keeping the surgical field clean and not leaving free clots inside the cavity.

At the end of the procedure, the pneumoperitoneum was undone, slowly infiltrating the skin and the abdominal wall at the sites of the ports with bupivacaine (1.0 mg/kg). The planes of muscle, aponeurosis and skin were closed with single plane suture using mononylon 3-0 thread, and a dressing with adhesive.

Postoperatively, care was taken with recovery from anesthesia: the animals were kept in boxes until they were wide awake, before returning to their cages, when they were allowed a free diet.

The second phase of the experiment occurred 21 days after the first intervention, and then the general anesthesia procedure and videolaparoscopy were repeated to verify the presence or absence of adhesions at the site of the previous surgery.

Table 1 shows the classification used as an adhesion score, according to the modified Diamond¹¹, with a sum of each aspect of type, tension and extension of the adhesion.

TABLE 1 - Classification of adhesion score

Score	0	1	2	3	4
Type	absent	Fine without vessels, translucent	Dense, without vessels, translucent	Dense with small vessels*	Dense with large vessels
Tension	0	Essentially independent	Released with traction	Requires dissection	
Extension	0	Less than 25% surface	25-50% surface	50-75% surface	More than 75% surface

Note: * small vessels = vessels with a caliber smaller than the tip of the laparoscopic scissors.
 † large vessels = vessels with a caliber equal to or larger than the tip of the laparoscopic scissors.

Then the biopsy was performed at the site of the previous surgery, and euthanasia with thiopental I.V.

The categorical variables are described by absolute frequency and percentage of relative frequency; the quantitative variables, by mean and standard deviation, when their distribution was symmetrical; and the quantitative variables with asymmetrical distribution are described as median, minimum and maximum.

Results

Six (54.5%) cases with adhesion formation were observed (Figure 1), and the median total score of adhesions was 6 (minimum of 3 and maximum of 10), all in the bladder and on the anterior abdominal wall. The mean values and standard deviation of the vital parameters of the animals were heart rate 229.2±12.66 beats per minute; oxymetry 96.85± 1.17% and capnometry 42.89±4.26 mmHg; pneumoperitoneum (time of surgery)16.55±4.18 minutes and 3072.73 ± 253.34 kilograms.



FIGURE 1 - Photograph showing an adhesion at the site of the previous surgery

All of the animals presented an inflammation in the histopathological study of the biopsy, at the site of the previous surgery (Table 2).

TABLE 2 – Histopathological study at the site of surgery

	Absolute value	%
Inflammation	11	100
Granulation*	7	63.6
No collagenization	10	90.9
Granuloma†	9	81.8

Notes: *Granulation= discrete or absence of granulation
 †Granuloma = of the foreign body type

The biopsy presented discrete or absent granulation tissue in 7 (63.6%) cases. Most biopsies (10 – 90.9%) did not present collagenization: Foreign body type granuloma was found in 9 (81.8%) cases.

Discussion

Rats and rabbits have been used to investigate adhesion formation¹⁴. These species are excellent to quantify adhesion formation at a minimum interval of 7 days. Rabbits are an animal model that is favorable for adhesion formation, mainly because they are easily adaptable to videolaparoscopic procedures with conventional equipment, such as nephrectomies and Nissen's operation^{12,13}. The fibrinolytic potential of the peritoneum is variable among animal species, and there is more plasminogen activation in the rabbit than in the rat. Thus, there are less adhesions using rabbits as animal models, than when rats are used.

The models developed in the laboratory animals for adhesion formation range from dryness or abrasion of the intestinal serous surface to injury, removal of the visceral or parietal peritoneum, abrasion or ischemia of the uterine horns¹¹. However, it is difficult to evaluate the different forms of prevention of peritoneal adhesions due to the lack of uniformity of the different induction models and grading techniques that do not allow quantitative classification.

Methods to induce adhesions are so diversified that it is difficult to standardize the evaluation methods, i.e., each author created their own way to quantify adhesions, which makes evaluation more complex. In this study it was decided to evaluate the score using the Diamond modified classification¹¹, since, besides being self-explanatory, it is easy to apply. In this study the adhesions on the abdominal wall of the rabbit were evaluated, and it should be recalled that only the induction points are evaluated. The adhesions that form on the laparotomy incision or the trochar ports are not part of the evaluation.

Since the view of the structures because broader with videolaparoscopy, in this animal model the second videolaparoscopy was essential to reduce the measurement bias, with the animal still alive under conditions in which the vascular network of the adhesions is evaluated.

In this experiment, only the time of pneumoperitoneum was controlled and the intraabdominal pressure was maintained at 10 mmHg. Binda *et al.*¹⁴ reported that the effect of dissection and gas temperature, time of pneumoperitoneum and intraabdominal pressure influence the formation of adhesions during videolaparoscopy. It appears that the mechanism responsible for adhesion induction is peritoneal hypoxia.

The second surgery occurred 21 days later, when most of the scars were already covered with mesothelium, there was dis-

crete or absence of granulation tissue, accompanied by absence of collagenization in almost all the animals studied. Thus, adhesion formation was measured in a timely manner, with the lesions already healed and lined by mesothelium, and there was no more risk of adhesions.

Thus, by better management of an experimental animal model of adhesion formation by videolaparoscopy, other studies can be performed on the use of methods to prevent the formation of surgical adhesions, and also to test adjuvant substances.

Conclusion

The method used presents a high frequency of intra-abdominal adhesion formation.

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