

## Is peritoneal reflection the best anatomical repair landmark in experimental colorectal surgery on rats?<sup>1</sup>

A reflexão peritoneal é o melhor reparo anatômico na cirurgia experimental colorretal em ratos?

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### ABSTRACT

**Purpose:** To validate Peyer's patch as an anatomical repair landmark for colorectal surgery in rats and to measure the collagen content in segments of the colon containing or not containing Peyer's patch. **Methods:** The distance between Peyer's patch and the peritoneal reflection was measured in forty-five Wistar rats. The colon and rectum were resected for quantification of collagen content by means of computer-assisted image analysis in regions of the colon with and without Peyer's patch. **Results:** There was great variation in the distance between Peyer's patch and the peritoneal reflection when the male and female rats were considered as a single group ( $p=0.04$ ). Comparison between the genders showed that the distance between the patch and the peritoneal reflection was greater in female than in male rats ( $p=0.001$ ). The colonic segment containing Peyer's patch was observed to have lower tissue collagen content than the segment in which this structure was not present ( $p=0.02$ ). **Conclusion:** Peyer's patch can be indicated as an anatomical repair landmark, and there is a need to study the healing of colorectal anastomoses in rats based on differing quantities of tissue collagen existing in the colonic wall with or without this structure.

**Key words:** Colon. Collagen. Anastomosis, Surgical. Image Processing, Computer-Assisted. Rats.

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### RESUMO

**Objetivo:** Validar a placa de Peyer como reparo anatômico para a cirurgia colorretal em ratos e mensurar a quantidade de colágeno em segmentos da parede cólica que contém ou não a placa de Peyer. **Métodos:** Foi aferida a distância entre a placa de Peyer e a reflexão peritoneal em 45 ratos Wistar. O cólon e o reto foram ressecados, para a quantificação do colágeno, por meio de análise de imagem assistida por computador, em regiões do cólon que continham ou não a placa de Peyer. **Resultados:** Existe grande variação entre a distância da placa de Peyer e a reflexão peritoneal quando se consideraram os animais de ambos os gêneros como grupo único ( $p=0.04$ ), sendo a distância entre a placa e a reflexão peritoneal maior entre as fêmeas ( $p=0.001$ ). Constatou-se que o segmento cólico que contém a placa de Peyer apresenta conteúdo menor de colágeno quando comparado ao segmento onde a estrutura não estava presente ( $p=0.02$ ). **Conclusão:** A placa de Peyer pode ser indicada como reparo anatômico e no estudo da cicatrização de anastomoses colorretais em ratos, baseado nas diferentes quantidades de colágeno tecidual existente na parede cólica que contém ou não esta estrutura.

**Descritores:** Cólon. Colágeno. Anastomose Cirúrgica. Processamento de Imagem Assistida por Computador. Ratos.

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### Introduction

It is common to use rats as experimental animals in scientific research, especially for studies involving surgical anastomoses. Several authors have used rats to evaluate a variety of characteristics of colonic anastomoses<sup>1,2,3,4</sup>. In these studies, the

peritoneal reflection is the anatomical repair landmark generally adopted for defining the location where the anastomosis will be performed, just as is done with other experimental animals. However, the region corresponding to the rectosigmoidal transition in rats has a singular lymphoid structure called Peyer's patch (PP).

PP is formed by tissue that differs from that of the remainder of the colonic wall since it is predominantly lymphoid tissue. Hence, this difference could lead to unreliability in comparative analyses of the healing of anastomoses between those performed at this location and those performed away from it if the distance to the anatomical repair adopted were to vary. Even if the distances were only to vary by a few millimeters, this difference could lead to anastomoses being constructed in different tissues. This situation is exacerbated by the assumption that histologically different tissues have different quantities of collagen, a protein of fundamental importance for the healing of colorectal anastomoses.

Thus, adopting the peritoneal reflection as a landmark for intracavitary anatomical repairs, especially for studies on the healing of anastomoses constructed in the left colon of rats, could increase the risk of carrying out the procedure on histologically different structures. Because of their different healing dynamics, this difference could give rise to misinterpretation of the results.

So far, there are no studies in the indexed literature comparing the tissue collagen content in anastomoses performed at PP and away from it with the purpose of adopting this structure as an ideal anatomical repair landmark for experimental surgery in rats.

The objective of the present study was to demonstrate the validity of adopting PP as an anatomical repair landmark in studies on colorectal anastomoses in rats from an analysis of the quantity of collagen and the anatomical position of PP in relation to the peritoneal reflection.

## Methods

This study was conducted in observance of all the procedures laid out by the Research Ethics Committee of São Francisco University and the requirements of the Research Ethics Board of the National Research Ethics Commission (CONEP) of the Ministry of Health (Resolution CNS196/96).

Forty-five Wistar rats (*Rattus Norvegicus Berkenhout*) were used. There were 30 females and 15 males weighing from 250 g and 350 g (mean: 300 g) and with a mean age of three months. They were obtained from the vivarium of São Francisco University in Bragança Paulista, State of São Paulo.

The animals were anesthetized using 2% xylazine hydrochloride and ketamine hydrochloride at a dose of 1 ml/100 g, administered intramuscularly; anesthesia was maintained using inhaled ethyl ether by means of an open mask. After the animals had attained an adequate level of anesthesia, they were restrained on an appropriate surgical table in the dorsal decubitus position. Abdominal depilation was performed, and polyvinyl-pyrrolidone-iodine (PVPI) was used for antisepsis. A caudal longitudinal medial celiotomy of 2 cm in length was then performed, and the cavity was opened up in layers. The uterus was identified in the females (both bicornuate parts) or the epididymis in the males. Under either of these structures, the end portion of the left colon was identified, thus enabling identification of Peyer's patch.

After identifying PP, the distance from its distal portion to the peritoneal reflection was measured. This measurement was done three times by three different observers by means of a pachymeter, and the arithmetic mean was calculated from these measurements. This mean was taken to be the final value for the distance between PP and the peritoneal reflection. After this procedure, the animals were sacrificed by means of inhaling a lethal dose of ethyl ether in a sealed chamber. The portion of the colon that contained PP was resected from the peritoneal reflection to a point two centimeters above the cranial portion of the patch, for histological analysis.

The resected specimen was fixed in 10% formol and embedded in paraffin. Two sections of 4µm in thickness were cut and stained with hematoxylin-eosin (HE) to identify and histologically evaluate the patch. Masson's trichrome was used to identify the tissue collagen.

The tissue collagen was then quantified by means of computer-assisted image analysis<sup>5</sup>. In brief, the collagen content was determined by reading the slides under an optical microscope coupled to a video camera for image capture. This system was coupled to a microcomputer loaded with image analysis software\*, which processed the images obtained, enabling quantification of the collagen in each field selected. The protein quantification was done at a final magnification of 100x in representative areas on and away from Payer's patch. The software allowed the user to determine the collagen content as a percentage over the whole field selected. Three measurements of tissue content were made both on the patch and away from it, and the mean of the three readings was taken as the final measurement of collagen content.

The results obtained were subjected to statistical analysis using a significance level of 5% ( $p < 0.05$ ). Descriptive statistics (mean, median, standard deviation and confidence interval), normality assessment (Kolmogorov-Smirnov) and analysis of variance (test of equality of means and Wilcoxon test) were applied using the SPSS® software, version 13.0 for Windows.

## Results

PP appears as a whitish, slightly thickened oval-shaped area located on the anterior wall of the colon on the side opposite the mesenteric insertion line, cranial to the reflection in the peritoneal cavity (Figure 1).

The distances measured had a normal distribution curve, independent of the gender of the animals (mean=1.23; SD=0.387;  $p=0.04$ ).

There were large variations in the distances measured from the PP to the peritoneal reflection, with a significant difference ( $p < 0.001$ ) in this distance between the male and female animals (Figure 2).

There was a significantly smaller quantity ( $p=0.02$ ) of collagen in PP (Figure 3).

PP occupies a good proportion of the thickness of the colonic wall; it consists mainly of lymphoid tissue. Cranial and caudal to PP, the colonic wall in rats has all of its layers (Figure 4).

\* NIS-Elements – Nikon Inc., Japan

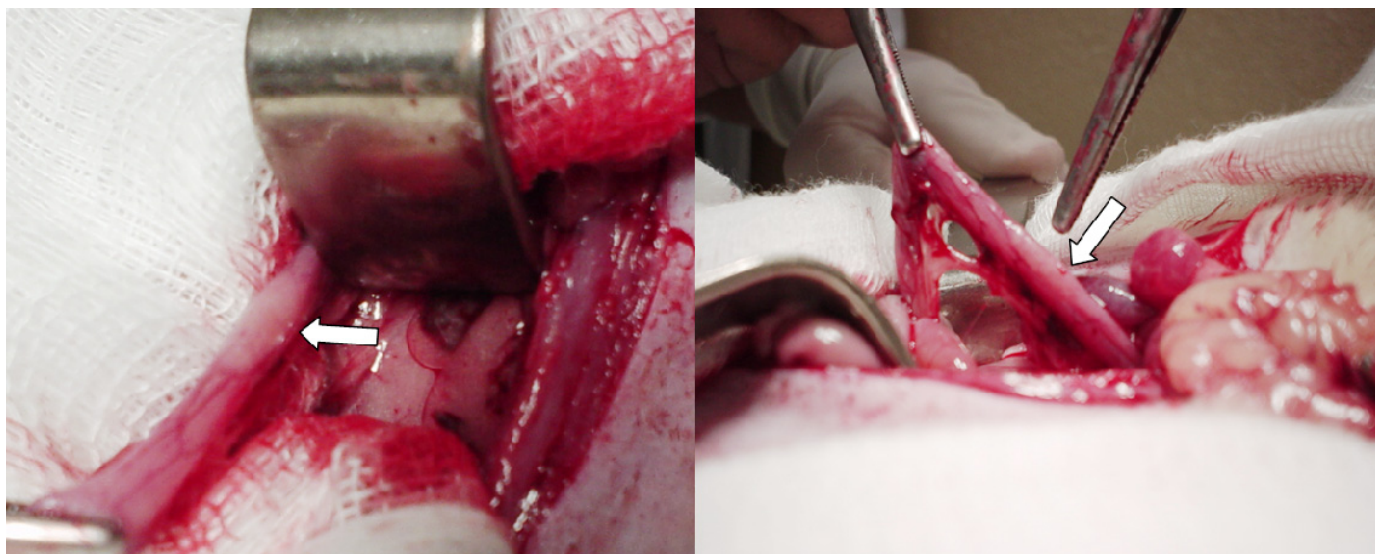


FIGURE 1 - Peyer's patch: front and side view (arrows)

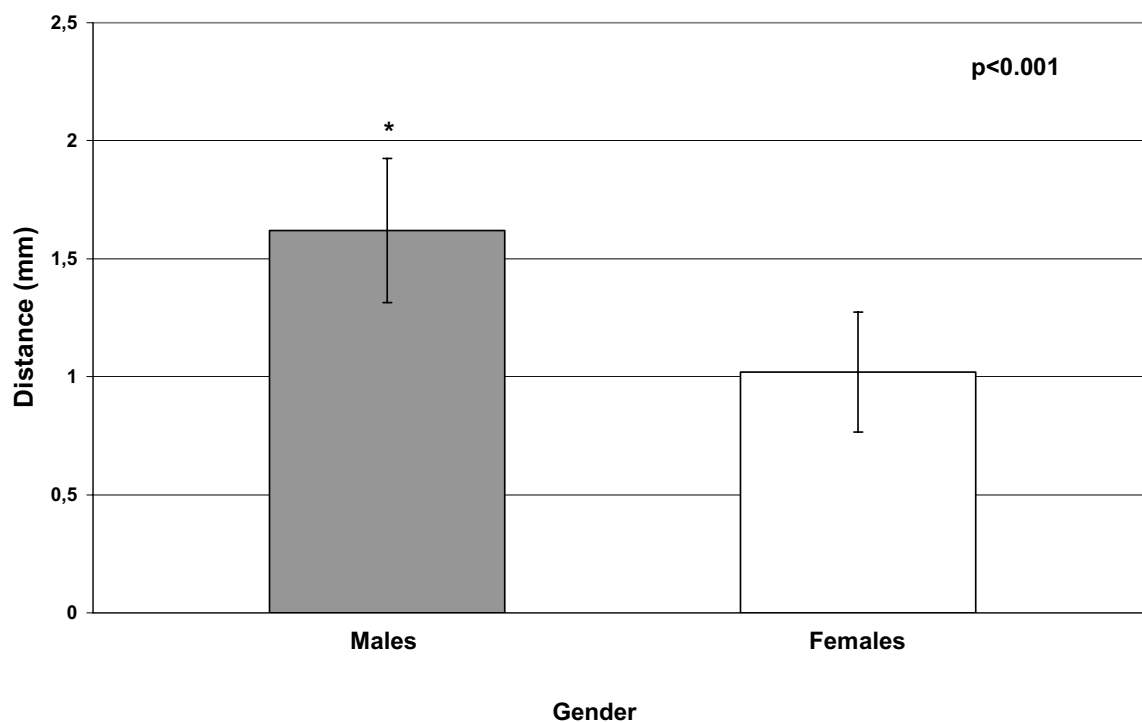
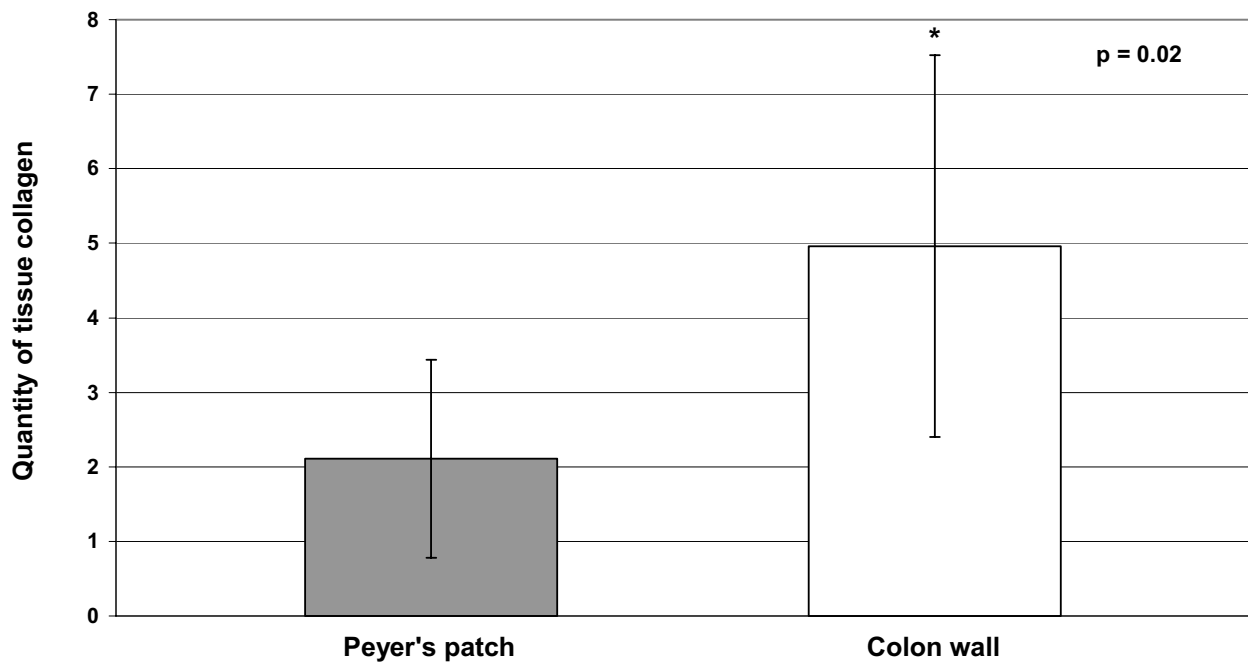
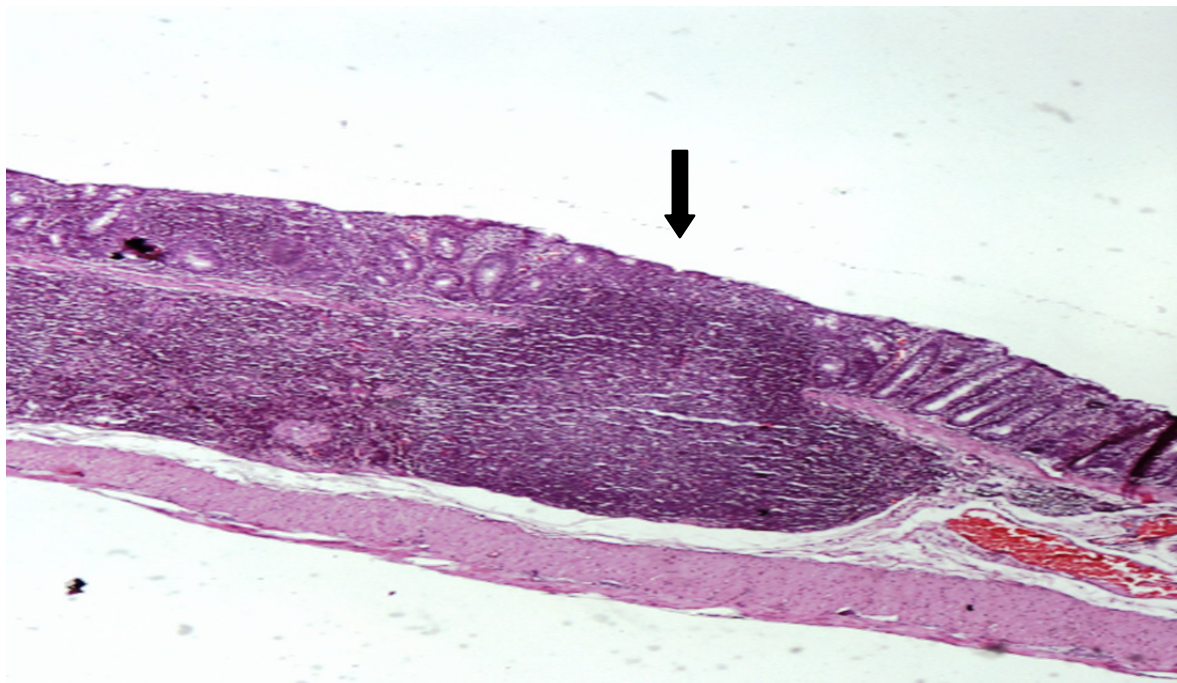


FIGURE 2 - Comparison between distances from Peyer's patch to the peritoneal reflection



**FIGURE 3** – Collagen content, comparing colonic tissue with and without Peyer's patch



**FIGURE 4** – Peyer's patch. Microscopic view. Longitudinal section of the rat colon wall. Exuberant lymphoid tissue (Peyer's patch) partially replaces the mucosa and narrows the muscularis externa layer (arrow). (HE; 40x).

## Discussion

Rats are among the experimental animals most used within scientific settings, and they occupy this prominent position because of characteristics that make them close to the ideal for such studies. Little space is needed to house them; procreation occurs in large numbers and rapidly, ensuring almost identical lineages with animals that are genetically and physiologically similar. The management and maintenance costs are small compared with those for other animals; they are easy to obtain and handle, and are resistant animals<sup>5</sup>. In addition to these characteristics, it has been accepted since the study by Herrmann in 1964<sup>6</sup> that the fecal microflora of rats is very similar to that of humans. Moreover, from a histological point of view, the layers of the intestinal wall in these animals present important similarities to the layers in the human colon<sup>7,8</sup>. Thus, the rat model for studies on healing of the colon is adequate since it demonstrates structural and microbiological characteristics that are common to the human colon<sup>6,7,8</sup>.

Several studies have used rats in evaluations of intestinal anastomoses. A variety of anatomical repairs have been considered, and these most commonly use the peritoneal reflection and anal margin as landmarks<sup>1,2,3,4,8,9,10,11,12,13</sup>. A single study that compared the resistance to bursting pressure with the quantity of hydroxyproline in colorectal anastomoses adopted PP as the anatomical landmark for constructing the anastomosis, but made no reference to the quantity of collagen in the PP, and only used this structure to determine the site for the colonic sutures<sup>14</sup>.

Lymphoid tissues are found routinely in several animal species, ordinarily in the ileum. PP is found in sheep, calves, swine and horses in the ileum, while in the appendix in rabbits, and bursa in chickens<sup>15</sup>. Recent studies have indicated an interest in PP as an immune response mediator<sup>15,16,17</sup>, without giving importance to the distinct characteristics of the colonic tissue in evaluating the healing processes that take place on this structure. In the indexed literature, one single study makes mention of the collagen in this region<sup>18</sup>.

PP is located at the level of the rectosigmoidal transition in rats, and it was first described in Brazil in 1947 as a lymphoid structure in the end region of the intestine in rats<sup>19</sup>. It is found anterior to the epididymis in males or anterior to the uterus in females. It is easily located macroscopically, since it is always more cranial than the reflection in the peritoneal cavity. Hence, this arrangement allows for more easily obtainable measurements of the intracavitary distances, in comparison with those of the peritoneal reflection, which is located in a deeper region of the pelvis. Thus, adopting PP as the anatomical landmark rather than the peritoneal reflection makes it possible to reduce the length of the surgical incision and the duration of intracavitary procedures, because of the ease of identifying PP and its more cranial location in the peritoneal cavity. Adoption of this anatomical repair makes it possible to decrease the time taken to close the abdominal wall and avoids prolongation of the anesthesia, thereby ensuring the survival of animals subjected to extensive and complex surgical procedures that are not always well tolerated.

In humans, PP is mostly located in the submucosa of the colon<sup>20</sup>. On the other hand, histological analysis under an optical microscope demonstrates that, in rats, the lymphoid tissue in PP is exuberant and partially replaces the mucosa and narrows the muscularis externa. Examination of the colon of rats under a

microscope readily shows that PP occupies a good proportion of the thickness of the colonic wall. At the site of the patch, the thickness of the colonic wall is seen to consist mainly of lymphoid tissue with rare fibroblasts. Cranial and caudal to the PP, the colonic wall in rats has all its layers arranged in the way that is similar to what is seen in humans. This arrangement points towards the observation that even if experimental anastomoses were invariably constructed in the PP and if the patch was kept a fixed distance from the peritoneal reflection (which was not confirmed by the present study), it would still be important to consider the results obtained with caution, since PPs in rats and in humans are different structures.

Although the PP has a constant anatomical relationship to the colon, there is variation in the distance from the patch to the peritoneal reflection. This variation occurs from one animal to another, and particularly in relation to gender. There is significant variation in the distance from the patch to the peritoneal reflection both in male and in female rats. This observation reinforces the importance of experimental studies evaluating the healing of anastomoses in the left colon, especially studies using animals of only a single gender. Studies have habitually used animals of male gender<sup>1-4,6-8,10-13,16,18</sup>, even though there is no restriction on using females, purely from the point of view of location of the PP<sup>5,14,21</sup>.

The present study found differences in comparing the collagen content in the colonic segments with PP or away from PP. Lower collagen content was found in the colon segment containing PP than in the segment without this structure. In this way, similar structures are not being analyzed when the peritoneal reflection is used as an anatomical landmark in case the anastomosis has been made over the PP. Studies that evaluate healing may make interpretations on anastomoses that were performed in locations with different quantities of collagen and consequently with different healing dynamics, if the peritoneal reflection is adopted as the anatomical landmark for performing these anastomoses. The resistance strength of the anastomosis depends both on the collagen content and on the quality of the collagen around the suture. These details depend not only on differences in the collagen content found in the PP, but also in its subtypes<sup>18</sup>. Both aspects determine the resistance strength of the anastomosis. Ohtsuka *et al.*<sup>18</sup> found differences in the tissue expression of the collagen type in the region of PP, such that the subendothelial and subepithelial basal lamina presented type IV collagen. Thus, not only the quantity but also the subtypes of collagen may give rise to bias in interpreting the results obtained when anastomoses performed on PP and away from it are compared.

Through adopting PP as an anatomical landmark in experimental anastomoses performed on the distal colon of rats, the possibility of comparing anastomoses constructed in locations with different functions, quantities and subtypes of collagen content, and thus with different healing dynamics, is ruled out. Adopting this technique has the possible additional advantage of reducing the duration of the surgery and the time under anesthesia, consequently making it possible for the animals to have greater tolerance of complex and prolonged procedures. It also ensures that the results obtained can be extrapolated in order to seek improvements in anastomoses of the colon in clinical practice.

The results from the present study allow us to state that

setting the distance measured from the PP in order to perform experimental colorectal anastomoses in rats may avoid misinterpretations of the results obtained. This use of PP as an anatomical repair landmark reduces the risks of anastomoses performed on colonic tissue in terms of differences in compositional, histological, biochemical and functional characteristics<sup>16,18,19,20</sup>. Uniformity of the tissue chosen ensures precision in studies relating to the healing of anastomoses and their resistance to pressure and tension tests, and in biochemical and histological studies that qualitatively and quantitatively analyze the collagen content in surgical anastomoses. PP should especially be considered in studies on the healing of distal colorectal anastomoses.

### Conclusion

Peyer's patch can be adopted as an anatomical repair in experimental surgery in rats.

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