

# Rectal Prolapse: A Morphological Analysis of the Rectal Wall

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## Abstract

**Background:** The etiopathogenetic mechanism of recto-anal prolapse disease with obstructed defecation syndrome (ODS) remains unclear. According to the diagnostic-instrumental approach, the pathology is described as the outcome of a mechanical phenomenon, but studies using a histomorphological approach remain meager. This study aims to identify structural and morphological features of the rectal wall to clarify the etiopathogenetic causes of the disease and to hypothesize new therapeutic indications.

**Methods:** This is a morphological, prospective observational study. A morphological and morphometric analysis of rectal wall incisional biopsies was performed. The specimens were collected from 20 patients with rectal-anal prolapse grade III/IV and ODS after the stapled trans-anal rectal resection (STARR) surgical technique.

**Results:** Histochemical staining of pathological samples confirmed the inflammation of the mucosal layer, detection of histiocytes with foamy cytoplasm with likely mucinous or lipid content, fibrosis of the submucosa, loss of structural integrity, and thickening of the muscularis mucosae (Welch's  $t$ -test:  $p = 2.8459 \times 10^{-7}$ ). There was no significant difference in the mucosal layer thickness compared to controls (Welch's  $t$ -test:  $p = 0.2689$ ).

**Conclusion:** Patients with rectal prolapse and ODS present inflammation of the rectal wall, and the muscularis mucosae shows a loss of mucosal layer consistency, consistent with the genesis of mucosal prolapse. Evidence of our histomorphological features could support the unitary theory of rectal prolapse. The correlation between these frameworks and clinical symptoms should be validated in a larger study.

**Keywords:** Etiopathogenetic mechanism; Histochemical staining; Morphological Features; Rectal prolapse; Stapled trans-anal rectal resection surgical technique (STARR)

## INTRODUCTION

Internal rectal prolapse (IRP) is a benign condition characterized by protrusion of the rectal wall through the recto-anal canal and is clinically associated with a set of signs and symptoms related to obstructed defecation syndrome (ODS).<sup>1,2</sup> The clinical presentation of ODS is related to the inability to satisfactorily empty the rectal ampulla during defecation.<sup>3</sup> According to the new Roma IV criteria, ODS is a functional gastrointestinal disease. There are many different symptoms;<sup>1,4</sup> the most frequent manifestations reported by patients are sense of incomplete evacuation (45%), intense efforts for defecation and digital maneuvers for stool extraction (34%), perineal support (34%), long times in the toilet (33%), faecal evacuation of small volume (23%), hard stools (23%), abdominal bloating (23%), and use of laxatives (16%).<sup>5</sup> However, ODS is not a pathognomonic clinical finding in all patients with IRP. In most cases, a simple physical examination with anoscopy is sufficient for diagnosing prolapse.

The IRP represents the early stage of rectal-anal prolapse, a condition of intussusception of the rectal mucosa within the rectal canal, detectable on anoscopy and that occurs mainly during the effort required to evacuate feces. In the advanced stage, prolapse is evident on perineal examination and can occur spontaneously,<sup>6</sup> for example, in any condition capable of increasing intra-abdominal pressure, even slightly, by reproducing the Valsalva maneuver.<sup>7,8</sup> Although benign, this stage of the disease significantly affects the patient's quality of life.

However, epidemiological data remain difficult to delineate. The true incidence of the disease within the general population is uncertain for two reasons: first, many individuals perceive the symptoms as a normal consequence of aging; second, a substantial proportion of patients endure these manifestations without seeking medical consultation. These factors contribute significantly to the persistently high rates of underdiagnosis and to delays in diagnosis, often resulting in identification only at more advanced stages of the disease. The results include chronic progression of the pathology, an exacerbation of symptoms, and the adoption of unsuitable therapeutic strategies. Some studies have estimated

an incidence rate of 2.5 new cases per 100,000 inhabitants, but these data are likely underestimated.<sup>9,10</sup> However, the distribution trend of the pathology in the population is clearer: 90% of the patients affected by rectal-anal prolapse are female, over 50 years of age, and have at least one vaginal birth. Instead, in the male population, the disease manifests itself at a younger age, between 20 and 40 years old. Finally, an increase in the incidence of rectal-anal prolapse in adults has been observed along with the progressive increase in the average age of the population.<sup>11</sup> Too many patients self-medicate their symptoms, resulting in high economic burdens and disease progression, which can reduce their quality of life.<sup>12</sup> In our clinical experience, we observed that many patients with chronic IRP and high-grade ODS candidates for surgical treatment resort over time to the use of anxiolytics or, worse, antidepressant drugs.

The initial pathophysiological condition of IRP and subsequent recto-anal prolapse can therefore constitute a single pathological condition of an evolutionary nature, with clinical manifestations attributable to ODS.<sup>7,8</sup> Risk factors related to rectal-anal prolapse have been identified in the literature.<sup>9</sup> These include age, multiparity, vaginal birth, previous surgery with pelvic involvement, chronic defecation disorders, concomitant neurological and/or psychiatric disorders, and anatomical anomalies of the pelvic floor. Although these risk factors may affect the etiology of rectal-anal prolapse, they cannot comprehensively explain the etiopathogenetic mechanism of the disease. In fact, it is commonly accepted that recto-anal prolapse can result from mechanical stretching, which can damage the rectal wall; however, the etiology of the disease remains unclear and requires further investigation. In addition, in the scientific literature, there is a prevalence of studies on the pathophysiology of recto-anal prolapse, especially through diagnostic-instrumental methods,<sup>8,10</sup> while only a minority have focused on morphological features and biomarkers.<sup>3,13-15</sup>

Therefore, we conducted a morphological and morphometric investigation of rectal wall biopsies from patients with rectal-anal prolapse to gain new insights into this topic. The most current theory in the literature, which aims to illustrate the genesis of the pathology, is the

unitary theory of recto-anal prolapse by Longo.<sup>16,17</sup> However, it should be considered that this theory describes the pathophysiology of rectal-anal prolapse as a mechanical phenomenon without adequately exploring other potential conditions underlying the genesis of a rectal wall prolapse.

In our study, we proposed analyzing histological features of the rectal wall in patients with IRP and ODS to identify features that could suggest new etiopathogenetic hypotheses and possible therapeutic strategies. Until now, surgery has been the only definitive treatment for this disease, with a significant impact on the resolution or improvement of symptoms related to ODS.<sup>1,2,10</sup>

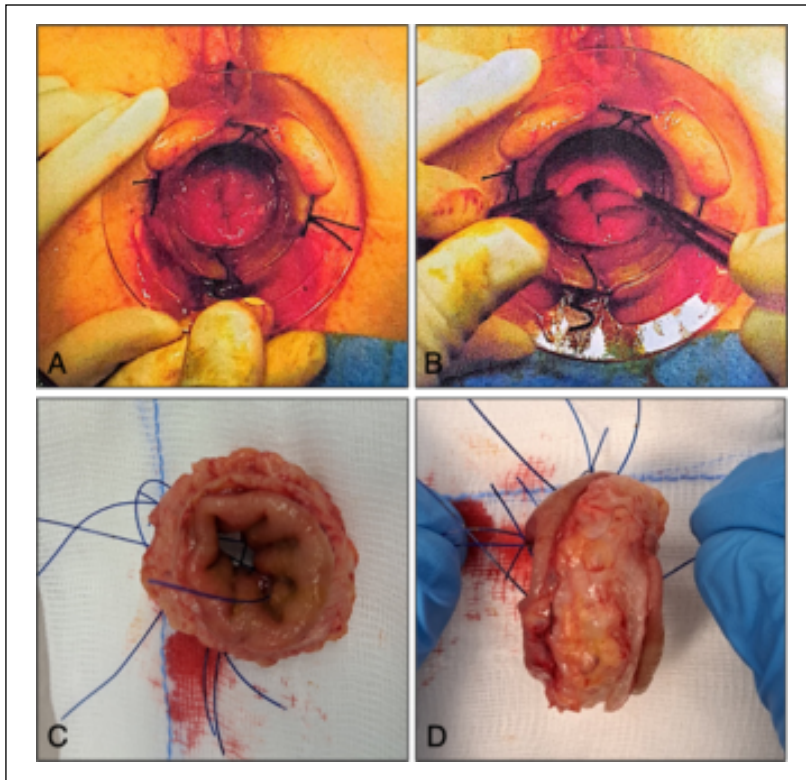
## MATERIALS AND METHODS

This study is a morphological, prospective observational study. Biopsy incisions of the human prolapsed rectal wall were collected from patients with internal rectal-anal prolapse and ODS after stapled trans-anal rectal resection (STARR) surgical technique. The collection of all these samples represented the pathological cases group. Instead, the control group was composed of non-prolapse rectal wall biopsies surgically collected from patients affected by other colon-proctology diseases.

All biopsies were subjected to morphological investigation using light microscopy and to morphometric measurements of the muscularis mucosae and mucosal layer. For each biopsy, two histological slices were prepared for two different histochemical staining methods: hematoxylin and eosin (H&E) and Masson's trichrome stainings. Mayer mucicarmine staining was also performed.

## Subjects

In this study, 40 pathological samples were prospectively collected from patients who underwent STARR surgery (Figure 1A and 1B). For all enrolled patients, the diagnosis of the disease was internal rectal-anal prolapse with ODS. The inclusion criteria were major age, advanced stage rectal prolapse (grade III or IV in the Oxford Classification of prolapse rectal wall, according to defecography exams when available),<sup>9,10</sup> moderate/severe symptoms (according to Wexner score or Altomare ODS score),<sup>18</sup> and rectal prolapse resection with the STARR surgical technique.



**Figure 1. Surgical resection of prolapsed rectal wall. (A) The CAD was placed in the anal canal and fixed with sutures. Evidence of canal obstruction due to prolapse is appreciated. (B) Evaluation of prolapse symmetry. (C) Rectal wall resection obtained during the STARR surgical technique. Superior view. (D) Lateral view.**

The exclusion criteria included previous proctological surgeries, reoperation for recurrence, chronic intake of anti-inflammatory drugs (cortisone or immunosuppressants), hemorrhoids complicated by bleeding, other concurrent proctology pathology (e.g. anal fissure), and tissue inclusion not sufficient for morphological analysis and minor age.

Control samples were collected from patients affected by colon-rectum neoplasia in the early stage, resectable without neoadjuvant therapy, and with up-front surgery indication. The anatomical level of sampling was the high rectum, close to the rectosigmoid junction. To exclude confounders and ensure comparability of the baseline between control and pathological samples, we applied the following exclusion criteria: exclude neoadjuvant radiotherapy or chemotherapy, concurrent proctology disease, and obesity with a body mass index (BMI) > 30. The sampling site was at least 3 cm from the neoplasia, oriented toward the circular suture on the opposite side of the cancer lesion. Ideally, for our team, this strategy represents an easy and

feasible method to obtain appropriate non-prolapse control samples.

Based on these criteria, we selected 20 pathological samples from the initial 40 available and 7 control cases (left hemicolectomy or anterior rectal resection for cancer). For each sample, clinical and morphological evaluations were performed. The clinical data studied included the patient age, sex, BMI, smoking status, and number of pregnancies (Table 1).

Clinical-anamnestic data showed that our case series consisted of young and adult patients, with 5 patients aged > 65 years, of whom only 1 was over 80

years old. When we analyzed the distribution of the pathology by age, we observed that individuals with prolapse aged around 40 years were predominantly male, whereas in females, the prevalence was observed in age intervals greater than 50 years. Ten out of 11 females had at least one vaginal birth prior to surgery. There were 7 smokers out of 20 total cases, with an average number of cigarettes of 15.6 cigarettes/day. The average BMI was at the lower end of the range that defined subjects as “overweight” (BMI 24.9–29.9).

### Clinical examination

Clinical examination of the recto-anal canal was the first essential medical investigation performed for each patient affected by symptoms attributable to ODS. We followed the diagnostic approach outlined by the STARR Pioneers.<sup>19</sup> ODS may arise from dysfunctional and/or organic causes; therefore, it is essential to explore the recto-anal canal to exclude any possible organic lesions. During the proctological examination, our patients were examined in the left lateral decubitus position (or Sims position). For differential diagnosis, particular attention was paid to external inspection of the perineum, perineal skin, and the sacro-coccygeal region. The most frequently encountered findings were prolapsed hemorrhoids, anal fissures, fistulous orifices, swellings related to hemorrhoid thrombosis, or suppurative pathology. In general, clinical findings during digital rectal examination and anoscopy were sufficient to diagnose IRP,<sup>20</sup> and radiological examinations were not necessary for clinical work-up. It was not always possible to perform imaging studies for each patient due to their cost and availability, but it was mandatory in cases of multiorgan prolapse, such as rectocele and colpocele. X-ray defecography was

**Table 1. Clinical data of 20 patients enrolled in the study**

Sex	n	Mean age (year)	Mean BMI	Smokers	Vaginal birth
Females	11	58.91 ± 12.88	24.78 ± 2.65	4	10
Males	9	55.11 ± 16.07	25.46 ± 2.89	3	-
Total	20	57.20 ± 14.53	25.09 ± 2.78	7	10

Abbreviation: BMI: Body Mass Index.

performed in 20 patients (50%, Oxford Grade IV in 15, Grade III in 5), and magnetic resonance defecography was performed as a second-level examination in 4 patients (10%, Oxford Grade IV in all).

The most common sign of internal rectal-anal prolapse during anoscopy examination was a protrusion of the rectal mucosa through the anal sphincter with the appearance of concentric rings ("stacked coins").<sup>21</sup>

The digital rectal examination allowed us to evaluate the tone of the anal sphincter (hypotonic or hypertonic), the function of the external sphincter by assessing the force of the patient's voluntary contraction, and the evaluation of extra-rectal structures, particularly the pelvic floor. For us, it was important to clinically evaluate the perineum because up to one-third of the patients with rectal prolapse could have a concomitant pelvic floor disorder. In the case of rectal-anal prolapse, digital rectal examination revealed a pathological anus with a low anal sphincter tone.

### Surgical treatment

The STARR technique is a minimally invasive surgical approach for resecting IRP in patients with symptomatic progression attributable to ODS.<sup>22</sup> Resection was performed using the transanal approach without the need for abdominal or perineal access and with minimal blood loss (Figure 1C and 1D). The circular stapler performed a full-thickness rectal-anal wall resection.

The surgical procedure introduced by Longo<sup>16,23</sup> has established itself in recent years as a potential therapeutic solution for ODS symptoms in patients with rectal anal prolapse.<sup>19,24</sup> The STARR surgical technique is the main operative treatment we have performed in our hospital for internal rectal prolapse for the last five years. In our experience, we had low rates of postoperative complications and recurrence of rectal prolapse, with good outcomes and short hospitalization.

### Samples preparation for light microscopy

Each sample was prepared in two steps: surgical biopsy and laboratory microscopy. Surgical biopsy was performed in the surgery room during rectal wall resection with the STARR technique. We cut a lozenge of rectal tis-

sue from the resected piece of the rectal wall obtained after the application of a single circular mechanical stapler (TST-36 Touchstone International Medical Science Co., Ltd., China). The biopsies were obtained from the resected piece of the rectum. According to the lozenge's length, we could cut it into no more than three small pieces. Macroscopic evaluation of the biopsy site and cutting the lozenge were performed quickly to preserve tissue histology as much as possible (maximum 5 min for all procedures). Rectal wall samples were fixed in 4% formalin (Bio-Optica, Italy), and biopsy sizes ranged from 1 cm<sup>3</sup> to 4 cm<sup>3</sup>. Microscopy was performed on each anatomical sample after fixation. The average fixation time was 72 h, calculated from an average sample size of 1–2 cm<sup>3</sup>. After fixation, optimal dehydration in alcohol (Bio-Optica) and clearing in Bio-Clear (Bio-Optica) were performed, followed by embedding in paraffin.

For histological preparations, the samples were cut into 4 µm sections using a microtome (RM 2265, Leica, Germany). The sections were stained with H&E (Bio-Optica) and Masson's trichrome (Bio-Optica) staining solutions, mounted in Bio-Mount (Bio-Optica), and observed under a ZEISS Axioskop 40 light microscope (Carl Zeiss, Germany) equipped with a Coolsnap video camera (Photometrics, United States of America [USA]).

### Histological and morphometric analysis

Histological and morphometric analyses were carried out, taking into consideration the following aspects:

- Intra-epithelial lymphocytic infiltration;
- Intra-glandular inflammatory thickening of the rectal mucosa compared to the basal physiological condition;
- Thickening, sloughing, loss of integrity, and interrupted continuity of the muscularis mucosae;
- Arteriolar vascular density in the submucosa;
- Degree of fibrosis of the submucosa.

Morphometric measurements and analysis of rectal wall thickness were performed according to the method established in our research laboratory. Analyses were conducted on control and pathological samples. The MetaMorph 6.1r6 software (Universal Imaging Inc.,

USA) was used for measurements and analysis after calibration for use with a 20× magnification objective. For each sample, morphometric measurements focused on the mucosal layers and the muscularis mucosae layers. For both histological structures, five measurements were performed in different fields at the same magnification. The unit of measurement was micrometers. Finally, we obtained the arithmetic mean with standard deviation for each histological structure in the control and pathological samples.

### Statistical analyses

Quantitative data are represented by morphometric measurements focused on the variables "muscularis mucosae thickness" and "mucosal layer thickness." Two separate data groups were established: the arithmetic means of the muscularis mucosae from pathological and control samples, and the arithmetic means of the mucosal layer from the same samples. Finally, the measurements of the two histological structures in both samples were compared using the per-patient mean values and Welch's *t*-test.

### Ethics approval statement

The study protocol was reviewed and approved by the Ethics Committee of the G. d'Annunzio University of Chieti-Pescara and of the Institute of Pierangeli Hospital in Pescara (approval no.: Prot. 0477970/23, 24/11/2023 – Studio STARR - C.Et.R.A. Pescara). Samples were collected after obtaining informed written consent from the patients and in accordance with the Helsinki Declaration and its amendments or comparable ethical standards. All patients gave their informed consent prior to their inclusion in the study.

## RESULTS

### Surgery

None of the patients who underwent surgical rectal resection with the STARR technique experienced any intraoperative or postoperative complications. The average postoperative hospital recovery was three days. In our experience, we had low rates of postoperative complications, with good outcomes, short hospitalization, improvement of symptoms related to ODS, and low rates of recurrence of rectal prolapse.



## Histological and morphological results

Morphological analysis of the control samples did not show any significant intra-epithelial lymphocytic infiltration or inter-glandular phlogistic thickening (Figure 2B and 2C). All control cases showed homogeneous inter-glandular lymphocyte distributions ascribable to normal chronic basal inflammation typical of the rectum (Figure 2A).

Morphological analysis of the pathological specimens revealed increased inter-glandular inflammation in the mucosal layer. This qualitative finding showed an increased inflammation of the rectal wall across all examined specimens (Figure 3A), though with heterogeneous distribution patterns: in some cases, the entire mucosal layer was involved (Figure 3B), whereas in others the involvement was limited to the lamina propria or to the epithelial layer (Figure 3C). Intra-epithelial lymphocytic infiltrates were observed in correspondence with areas of the mucosa with major inter-glandular lymphocyte infiltration in the same microscopic field (Figure 4A and 4B). Samples in which increased inflammatory thickening involved the lamina propria, inflammation extended into the underlying muscularis mucosae, penetrating at sites of fragmentation, and resulting in subsequent infiltration of the submucosa (Figure 4D).

Morphological analysis of the muscularis mucosae in pathological samples revealed varying degrees of delamination, hypertrophy, and disruption of its continuity (Figure 3D and 3E). Conversely, the muscularis mucosae layers in the control cases presented a well-defined and compact structure without significant signs of interruption or detachment (Figures 2B and 5A). The demarcation between the muscularis mucosae and the underlying submucosa was clearly defined (Figures 2E and 5B).

Morphometric analysis demonstrated thickening of the muscularis mucosae layer compared with control samples. The term “loss of structural integrity” was used to identify a severe qualitative degree of structural decay and loss of anatomical continuity of the muscularis mucosae in all pathological cases (Figure 6A). In these samples, in addition to the different degrees of hypertrophy, abnormal organization of smooth muscle bundles was observed (Figure 6B). They appeared to be organized into multiple

groups, isolated from each other, and localized within submucosal fibrosis (Figures 6B and 7A). In fact, morphological analysis of the submucosal layers in all pathological cases showed fibrosis and, in general, increased vascular density compared with controls (Figures 6C and 7B). Fibrosis was confirmed by distinct areas of increased aniline blue intensity reaction from Masson’s trichrome staining. Aniline blue reagent is selective for collagen and allows for the observation of anomalous collagen organization as filamentous structures, thickened, and variably colored in shades of blue (mild, moderate, or severe fibrosis). The general increase in vascular density has been demonstrated in several histological sections, where vessels of different diameters and distributions were observed: medium-caliber arterial vessels in the deeper portions of the submucosa, and small-caliber arterial vessels or arterioles close to the muscularis mucosae (Figures 4C and 7B). However, no fibrosis or variations in vascular density were observed in the control samples (Figures 2D and 5C). The submucosal layer appeared well represented by loose connective tissue, with occasional spots of collagen thickening.

In some pathological samples, significant adipocyte accumulation in the submucosal layer was also observed (Figure 7C and 7D).

Finally, in several pathological samples and in a single control case, we observed histiocytes with cytoplasm likely showing a mucinous/lipid appearance (Figure 7E).<sup>14,25</sup> The cytoplasm of these cells showed a positive reaction with Mayer’s mucicarmine staining (Figure 7F).

## Morphometric results

The average thickness of the mucosal layer of control samples was  $651.08 \pm 85.98 \mu\text{m}$ ; the muscularis mucosae of control samples was  $68.08 \pm 4.88 \mu\text{m}$ ;

the mucosal layer of pathological samples was  $695.71 \pm 93.22 \mu\text{m}$ ; and the muscularis mucosae of pathological samples was  $142.79 \pm 43.50 \mu\text{m}$ .

The analyses of the thickness of muscularis mucosae between pathological and control samples showed a greater thickening in pathological cases (Welch’s *t*-test:  $\text{df}(20) = 7.5449$ ,  $p = 2.8459 \times 10^{-7}$ ). No significant difference in thickness was observed from the comparison of mucosal layer thickness (Welch’s *t*-test:  $\text{df}(11) = 1.1644$ ,  $p = 0.2689$ ; Table 2).

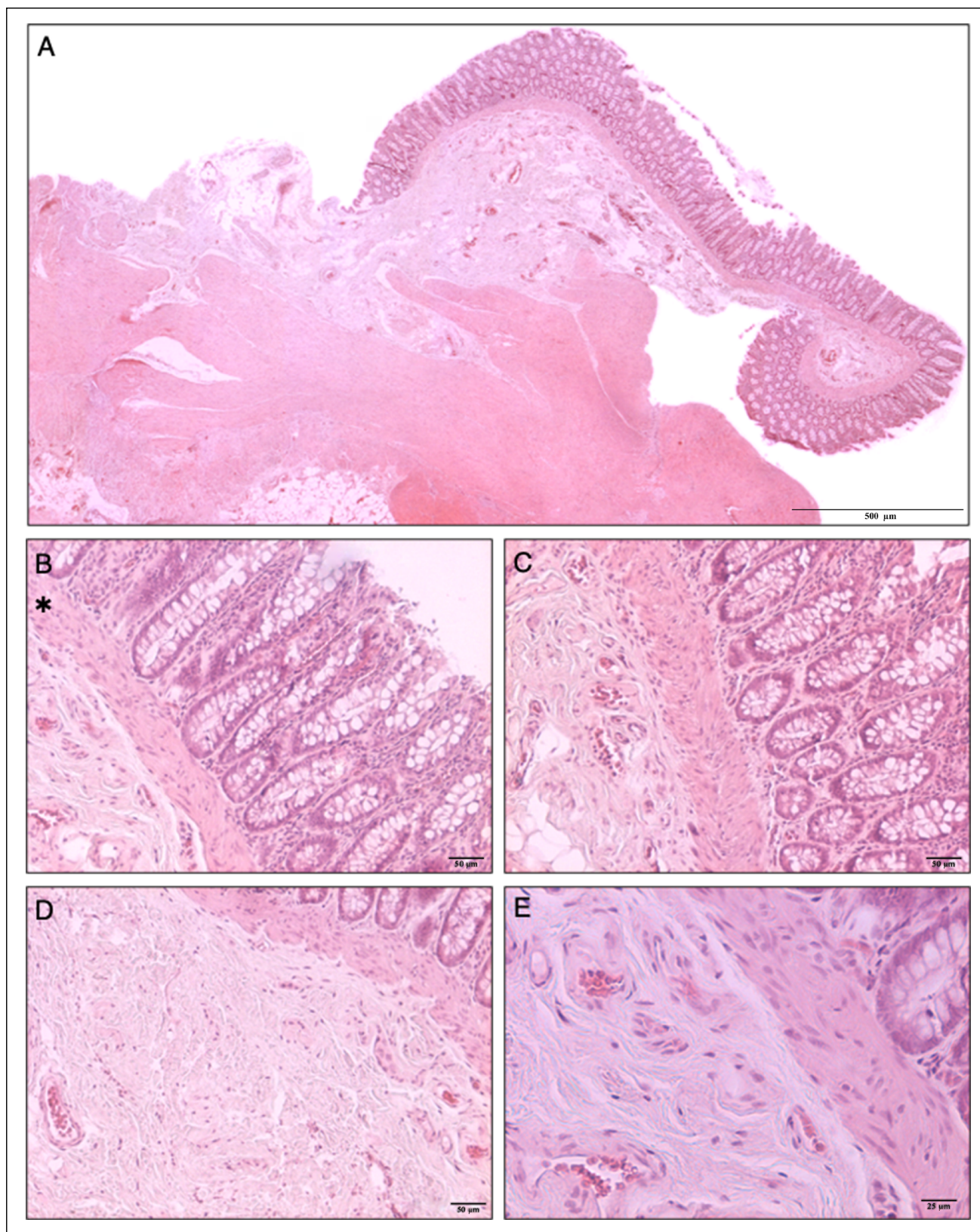
## DISCUSSION

Internal rectal prolapse is a common chronic benign disease; in the initial phase of the disease, patients tend not to consider defecation disorders as problematic. Our series showed that younger patients tended to be male, while those over 55 years of age were female, almost all of whom had a history of vaginal birth. The distribution of this disease is consistent with the expected population-level pattern.<sup>11</sup> The finding of 10 out of 11 females with a history of at least one vaginal birth supports the well-established observation that full-term pregnancy with vaginal delivery is a risk factor for the onset of recto-anal prolapse.<sup>26,27</sup> In particular, 3 of the 10 female patients in our series had precisely reported the onset of recto-anal prolapse within 2–3 months from the date of vaginal birth, with worsening symptoms that were refractory to post-partum pelvic floor exercises.

Cigarette smoking could be a risk factor for the onset of recto-anal prolapse. Moreover, BMI could also suggest a certain correlation between the state of overweight and prolapse disease; if considered globally, the patients in our series were overweight. This aspect may also be related to the histological finding of major adipocyte accumulations in the submucosa (Figure 7C and 7D). This is

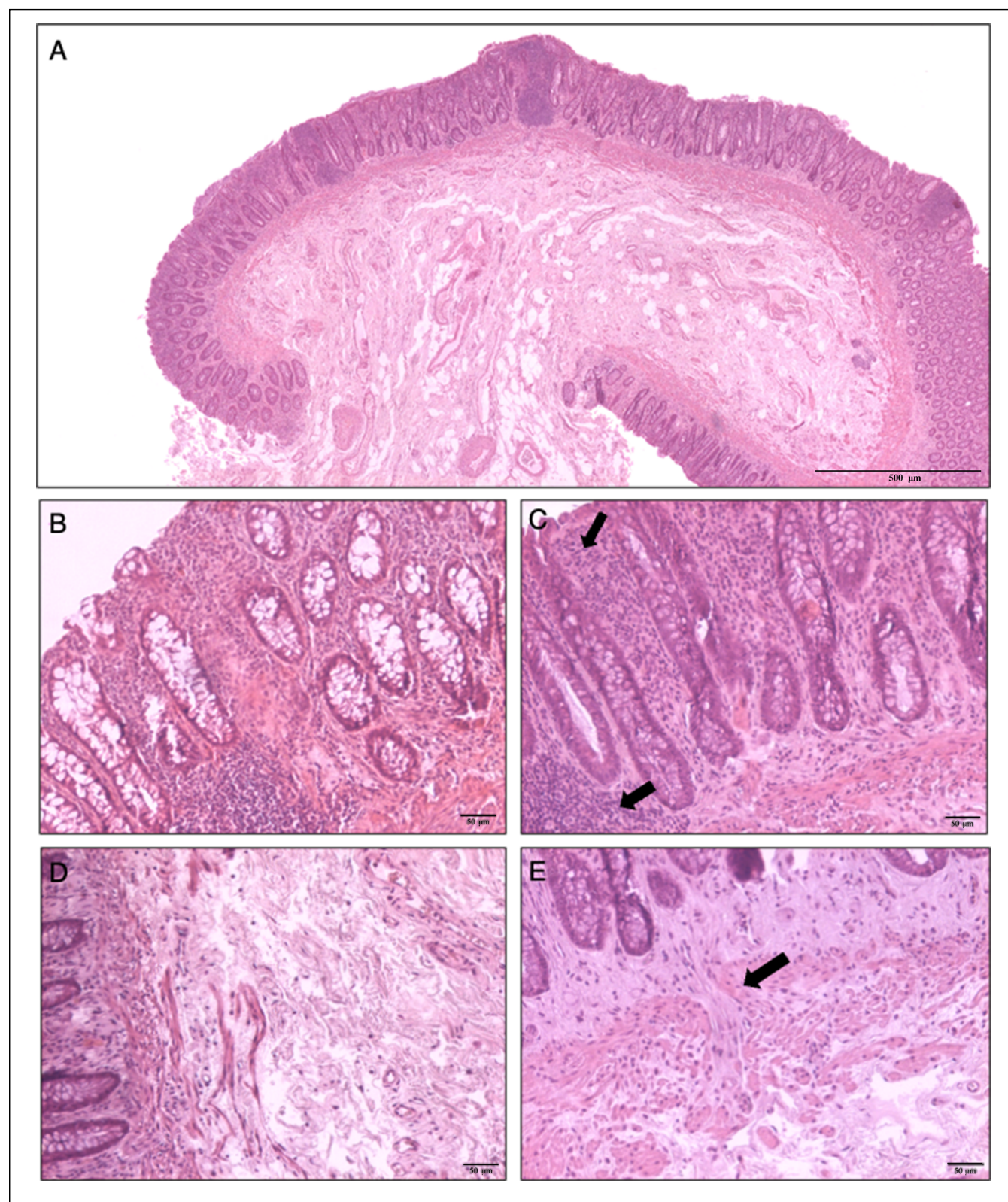
**Table 2. Morphometric analysis results**

Group	Mucosal layer ( $\mu\text{m}$ )	Muscularis mucosae ( $\mu\text{m}$ )
Control	$651.08 \pm 85.98$	$68.08 \pm 4.88$
Pathological	$695.71 \pm 93.22$	$142.79 \pm 43.50$
<i>p</i> -value (Welch’s <i>t</i> -test)	0.2689	$2.8459 \times 10^{-7*}$
Note: $*p < 0.05$ .		



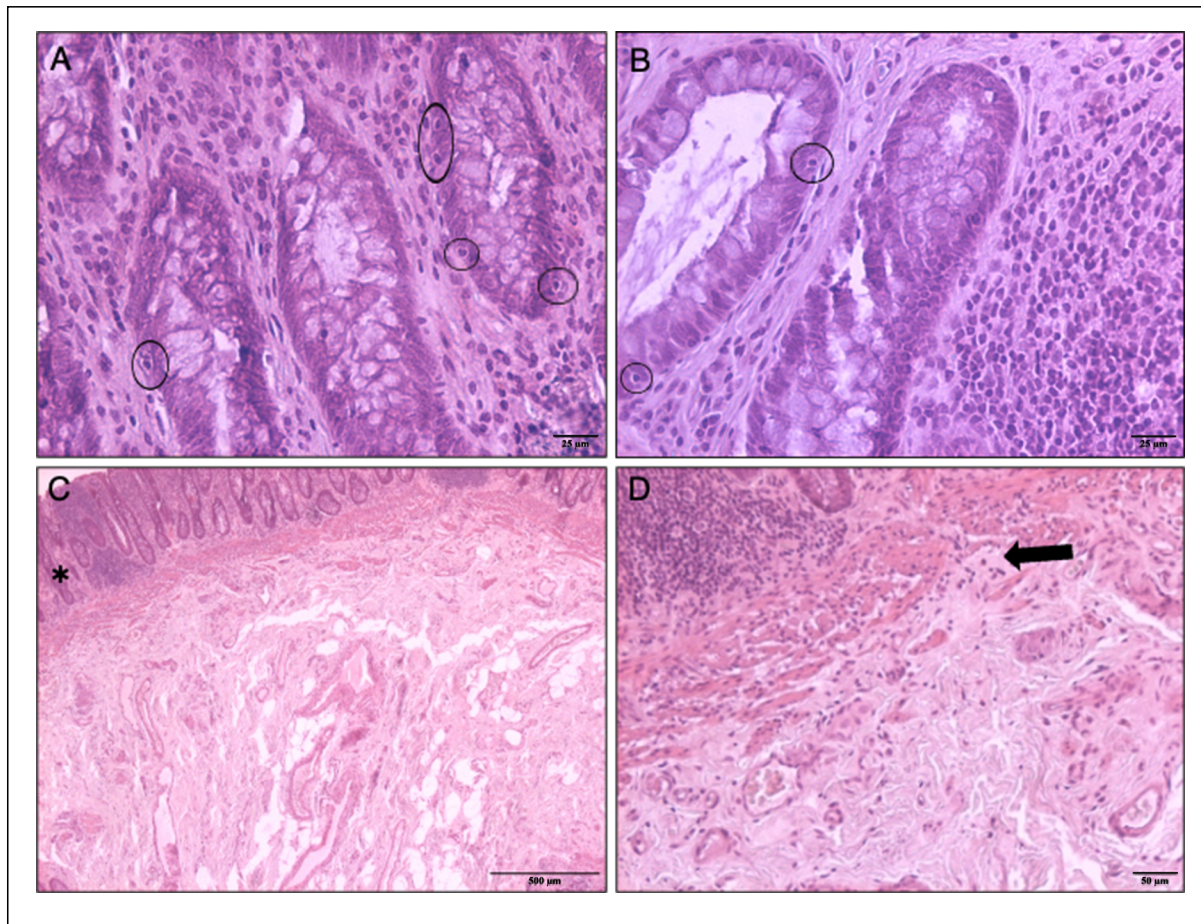
**Figure 2. Control samples of the rectal wall. Hematoxylin and eosin stain. (A) Merge 2D reconstruction, histology of control samples. Scale bar: 500 µm; magnification: 2.5×. (B–C) No evidence of increased inter-glandular phlogistic thickening of the mucosal layer or phlogosis infiltration of muscularis mucosae (\*). Scale bars: 50 µm; magnifications: 10×. (D) The submucosal layer in the control case appears more homogeneous. Scale bar: 50 µm; magnification: 10×. (E) The muscularis mucosae in control cases appears compact and well-defined. Scale bar: 25 µm; magnification: 20×.**





**Figure 3.** Pathological samples of the rectal wall. Hematoxylin and eosin stain. (A) Merge 2D reconstruction, histology of the rectal wall in a patient affected by rectal-anal prolapse. Scale bar: 500 µm; magnification: 2.5×. (B) Zone of increased inter-glandular phlogistic thickening of the mucosal layer of the rectal wall. (C) Different distributions of the increased inter-glandular phlogistic thickening: at the apex and the base of the mucosal layer. (D) The muscularis mucosae is split up, with phlogosis extending into the submucosa and increased vascular density. (E) Hypertrophy, non-laminated, split up, and loss of structural identity of the muscularis mucosae, with evidence of a break point of itself. (B-E) Scale bars: 50 µm; magnifications: 10×.





**Figure 4. Morphology of pathological samples' rectal wall. Hematoxylin and eosin stain. (A) Evidence of intra-epithelial lymphocytic infiltration, and (B) that in correspondence with the areas of major inter-glandular lymphocyte thickening. Scale bars: 25 µm; magnifications: 20×. (C) A full-thickness increase in interglandular inflammation of the mucosal layer can be observed (\*). Evidence of increased vascular density in the submucosal layer close to the muscularis mucosae. Scale bar: 500 µm; magnification: 2.5×. (D) Evidence of fragmentation and structural anomalies of the muscularis mucosae with loss of continuity. At the site of interruption, the invagination of the lamina propria into the submucosa is observed, with transposition of inflammatory cells beyond the muscularis mucosae. Scale bar: 50 µm; magnification: 10×.**

particularly evident in samples from a patient with a BMI of 30.03 (first-degree obesity). According to the unitary theory of recto-anal prolapse, prolapse should result solely from a mechanical traction mechanism of the rectal mucosa caused by the transit of fecal material and worsened by a global increase in intra-luminal pressure during defecation efforts. This can lead to progressive traction of the rectal wall, beginning with initial prolapse of the mucosal layer and progressing to complete extrusion. However, this model did not consider histological investigations.

Our experimental study of the morphological features of pathological and control samples revealed significant structural differences. The pathological samples showed inflammation compared with the control. Several microscopic fields showed marked inter-glandular

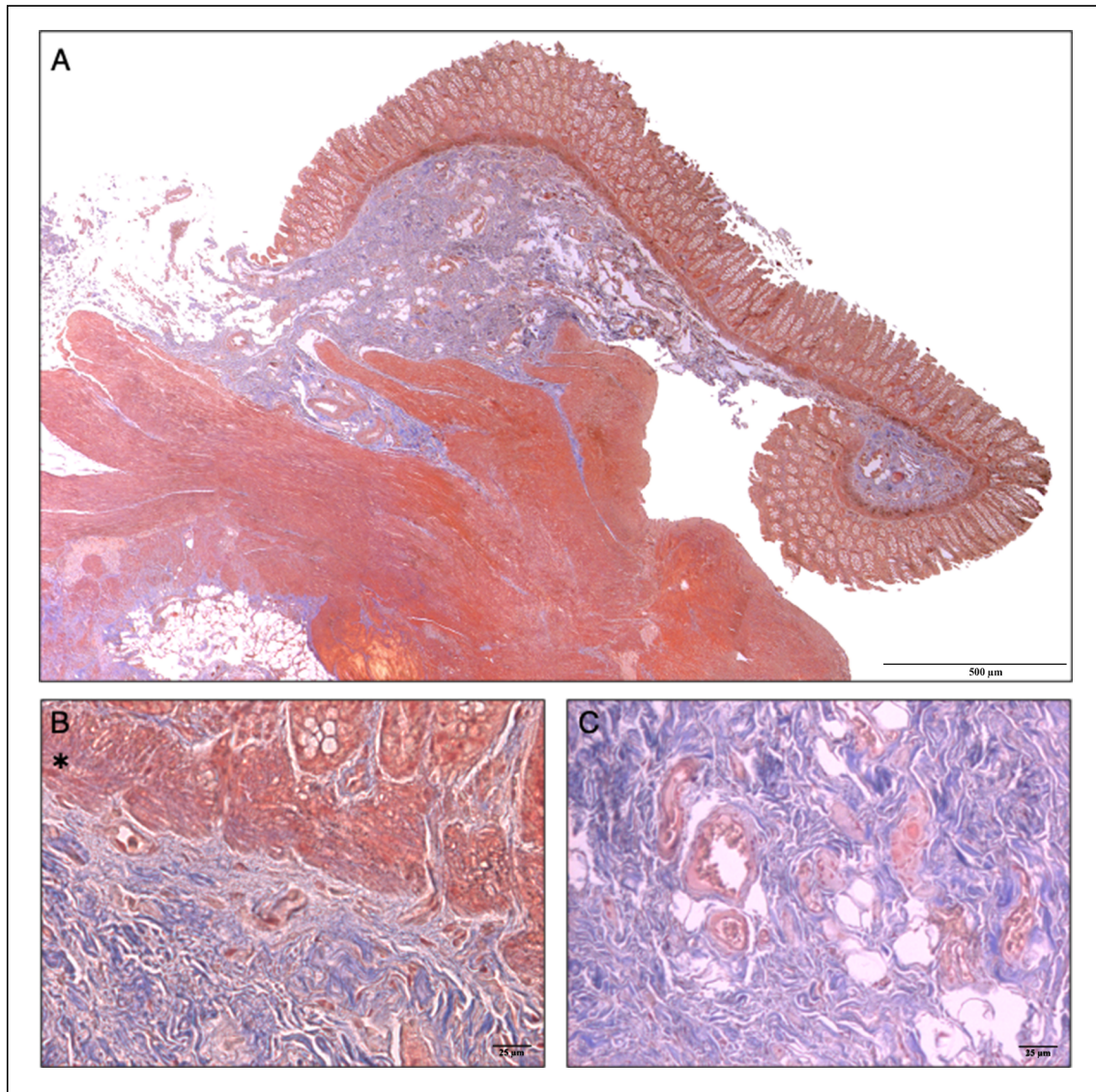
inflammatory thickening of the mucosal layer compared to the baseline condition. Intra-epithelial lymphocytic infiltrates were also observed. These signs were present within the same microscopic fields of multiple samples. Their presence was supported by histiocytes with foamy cytoplasm—likely containing mucinous or lipid material—along with fibrosis and increased vascular density in the submucosal layer. Taken together, these findings allowed us to qualitatively conclude that the prolapsed rectal wall was inflamed. Phlogistic thickenings were often observed near the intestinal lumen, suggesting a potential interaction with luminal irritants. This evidence also suggests that there may be a primary irritant trigger at the mucosal level, for example, reflected in alterations in the microbiota<sup>28,29</sup> This hypothesis could suggest the pathophys-

iological chronic intestinal inflammation capable of generating structural and permeability alterations of the intestinal mucosal layer.<sup>30,31</sup> Therefore, even if the claim is speculative, we cannot completely exclude that microbiota alterations, such as the prolonged permanence of the feces in contact with the mucosa, could have a role in structural alteration of the colon-rectal wall.

There are two important references in the literature that support our morphological features.<sup>3,14</sup> The papers in question define different degrees of fibrosis and nervous degeneration in resected rectal walls<sup>3</sup> and the description of foamy cells.<sup>14</sup>

The study by Bruscianno *et al.*<sup>3</sup> supports our evidence of rectal wall fibrosis in pathological samples, stating that rectal prolapse is an irreversible disease due to fibrosis. For this reason, the suc-





**Figure 5. Control samples of the rectal wall. Masson's trichrome stain. (A) Merge 2D reconstruction, histology of control samples. Scale bar: 500µm; magnification: 2.5×. (B) Normal aspect of the muscularis mucosae (\*). (C) Homogeneous appearance of the submucosal layer. (B-C) Scale bars: 25 µm; magnifications: 20×.**

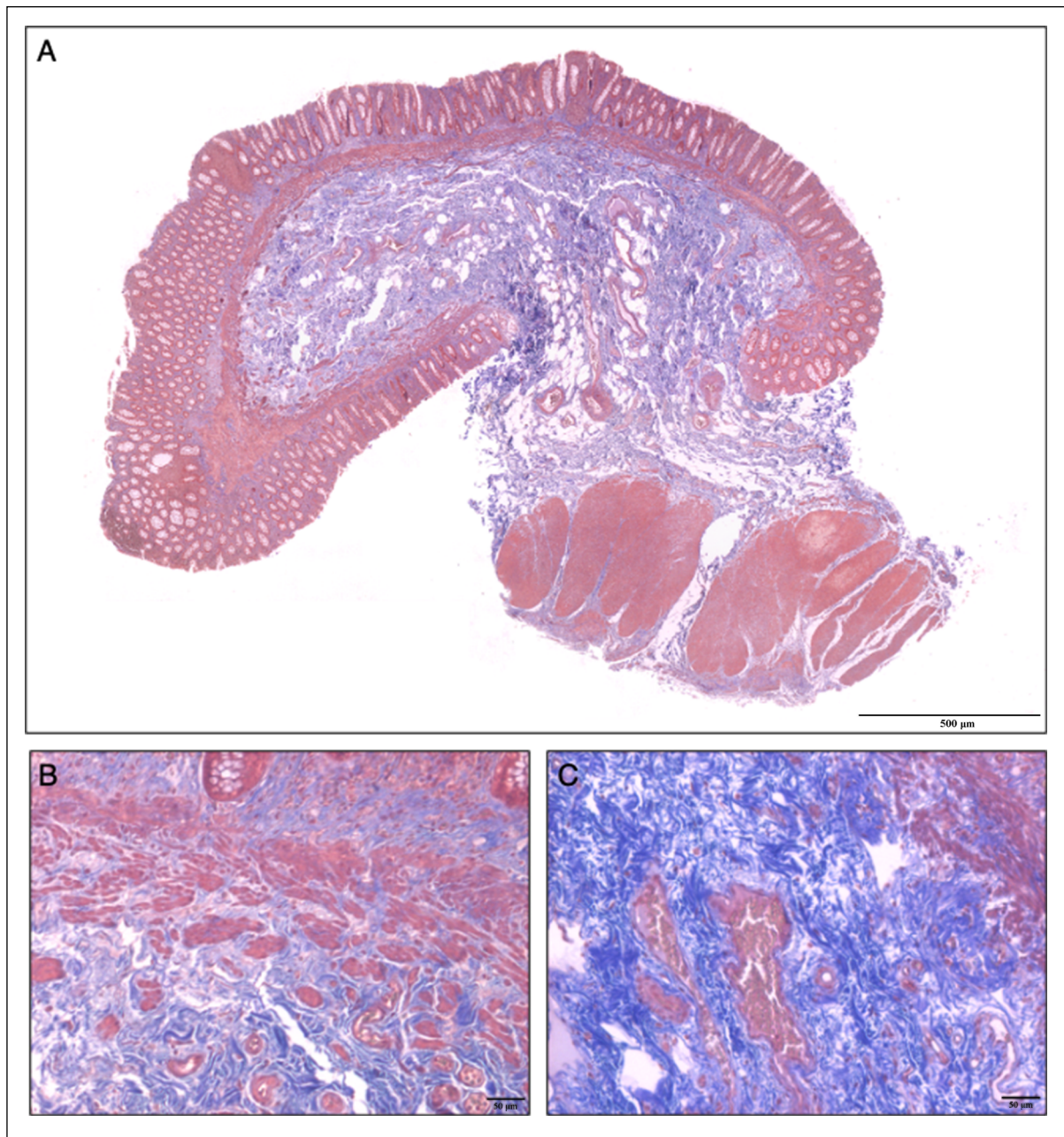
cess of the STARR surgical technique lies not only in the resolution of ODS symptoms but also in the removal of an anatomical segment that has irreversibly lost its functionality.

Our study agrees with the scientific evidence by Bejarano *et al.*,<sup>14</sup> who described fibrosis and atrophy of the lamina propria in the rectal wall, in accordance with the histiocytes with foamy cytoplasm observed in the same pathological sample. These cells were mainly localized in the lamina propria of the mucosal layer, with a prevalent arrangement along the muscularis mucosae, and showed a positive reaction with Mayer's mucicarmino staining. How-

ever, as a secondary finding, the presence of histiocytes in a single control case warrants mention. As a hypothesis, their mild and non-preponderant presence in the lamina propria, compared with the pathological cases, could suggest a correlation with the neoplasia diagnosed in the patient from whom the control case was recovered. These cellular elements at the level of the rectal mucosa, in addition to being signs of inflammation and varying degrees of submucosal fibrosis, could play a role or correlate with neoplastic pathologies of the colorectum.<sup>14,25</sup> In addition, similar histiocytes with foamy cytoplasm were described in ductal adenocarci-

noma pancreatic cancer tissue,<sup>32</sup> upon which ultrastructural characterization with transmission electron microscopy was performed. The contents of these histiocytes and their findings in different tissues suggest an interesting possible role in gastrointestinal diseases that needs further investigation.

The most innovative contribution of our study was the substantial structural difference in the muscularis mucosae between the pathological and control groups. Based on the evident anomalies in the muscularis mucosae, we predicted lower morphometric measurements in pathological samples than in controls. However, the results were



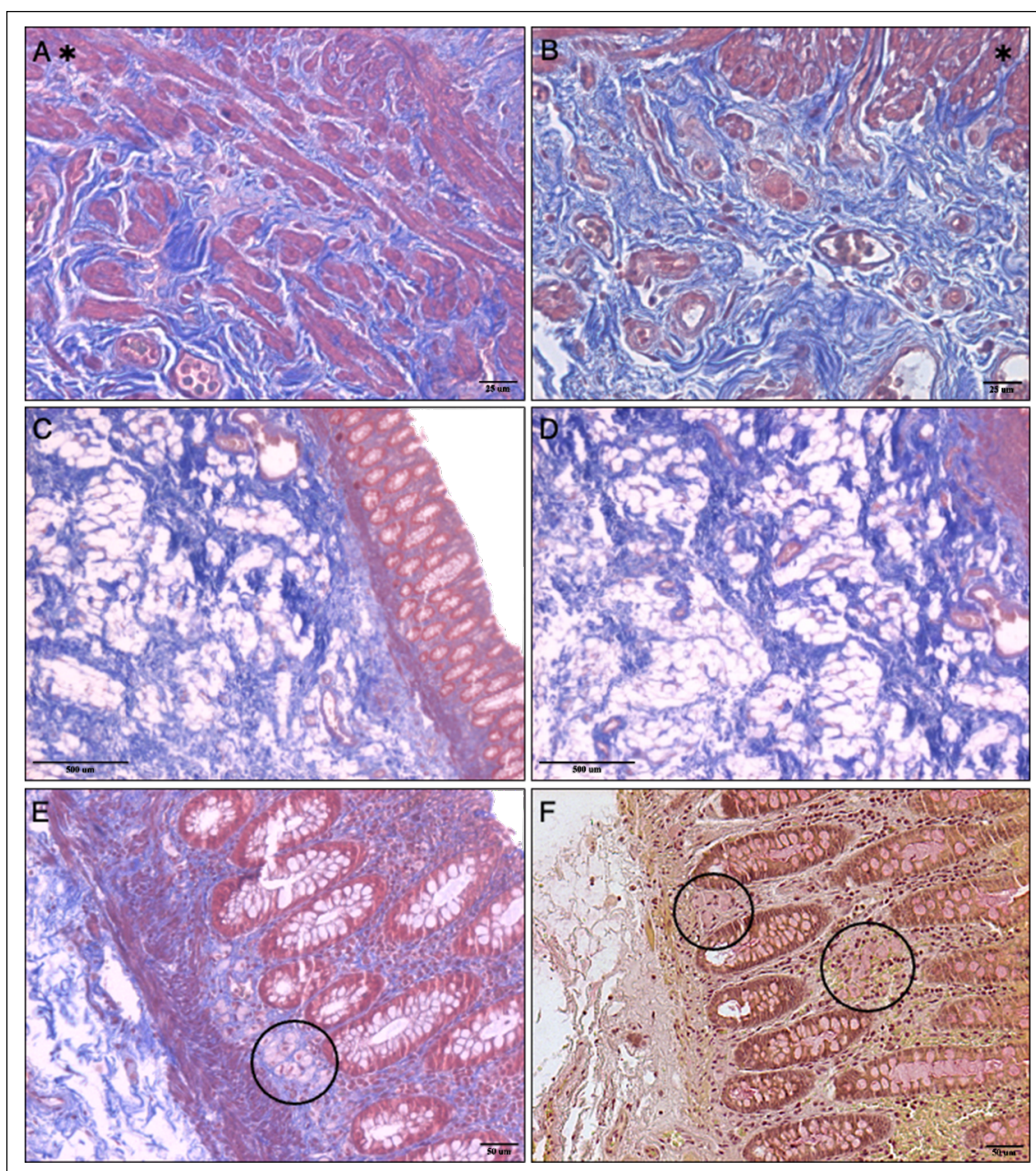
**Figure 6. Pathological samples of the rectal wall. Masson's trichrome stain. (A) Merge 2D reconstruction, histology of a pathological case. Scale bar: 500 µm; magnification: 2.5×. (B) Major evidence of hypertrophy, non-laminated, split up, and loss of structural identity of the muscularis mucosae with increased vascular thickening of the submucosal layer, in particular close to the muscularis mucosae itself. (C) Qualitatively different degrees of collagen thickening of the submucosal layer, highlighted by variations in aniline blue coloration. (B–C) Scale bars: 50 µm; magnifications: 10×.**

different. In fact, the muscularis mucosae of pathological samples showed, in its entire extension, areas of greater hypertrophy, others of marked thinning, and still others of interruption. Thus, we hypothesized that morphometric thickening may be a compensatory response to the areas of greatest deterioration in the muscularis mucosae, resulting in a succession of atrophy and thickening. This rectal wall structure plays a role, to a certain extent, in the trophism of the

mucosal layer, glandular secretion, and intestinal peristalsis. Any structural alterations could directly affect the motility and consistency of the mucosal layer. Based on our results, we hypothesized that alterations in the muscularis mucosae may lead to a loss of consistency in the mucosal layer. This weakening could impair its structural support. It may also, from a morphological perspective, contribute to the initial development of recto-anal prolapse. This early

stage is represented by mucosa prolapse. Therefore, considering the unequivocal influence of mechanical wall stress on the genesis of prolapse, starting with an initial extrusion of the mucosal layer,<sup>7,8</sup> we hypothesized that mucosal prolapse could be due to structural anomalies of the muscularis mucosae. Since this structure contributes to maintaining the integrity and trophism of the mucosal layer, its alteration could account for the initial phase of prolapse genesis.





**Figure 7. Morphology of pathological samples' rectal wall. Masson's trichrome stain. (A) Increased vascular density close to the muscularis mucosae (\*) and associated fibrosis can be observed. Structural anomalies of the muscularis mucosae. (B) Fibrosis and blood vessels increase close to the muscularis mucosae (\*). (C-D) Evidence of increased adipocyte storage in the submucosal layer. (E) Evidence of cellular elements interposed at the level of the lamina propria between the overlying glandular cells and the underlying muscularis mucosae. These cells are described as histiocytes with foamy cytoplasm with likely mucinous or lipid content, and (F) their cytoplasm is positive for the Mayer's mucicarmine stain. (A-B) Scale bars: 25 µm, magnifications: 20x; (C-D) Scale bars: 500 µm, magnifications: 2.5x; (E-F) Scale bars: 50 µm, magnifications: 10x.**

Instead, progressive involvement of the entire rectal wall during the advanced stages of the disease would be better justified by a mechanical phenomenon. Although it may be difficult to establish whether the pathological features of the muscularis mucosae represented a *primum movens* in the genesis of the disease, we advanced the unitary theory

of the recto-anal prolapse model with morphological evidence.

## CONCLUSION

Prolapsed rectal wall specimens showed irreversible structural damage, and the benefit of the STARR surgical technique lies not only in the resolution of ODS symptoms but also in the

removal of an anatomical segment that has lost its functionality. Morphological and morphometric analysis of prolapsed rectal wall specimens proved inflammation of the rectal wall, fibrosis, loss of structural identity, and thickening of the muscularis mucosae. Gut dysbiosis could represent an initial inflammatory trigger at the mucosal level. It may not necessarily be linked to mechanical stress due

to defecation disorders. This condition could induce primary structural alterations of the intestine. Histiocytes with foamy cytoplasm showed a positive reaction with Mayer's mucicarmine staining. The contents of these histiocytes could differ and not be limited to mucin or lipids. Further investigations are needed to clarify their potential role in rectal and other gastrointestinal diseases.

The study found structural abnormalities in the rectal muscularis mucosae in STARR surgical specimens from patients with rectal prolapse. The muscularis mucosae showed structural alterations that could have a direct effect on the trophism, motility, and consistency of the mucosal layer, which could, in turn, result, as a hypothesis, in a loss of integrity of the mucosal layer. The significance of this in the occurrence and development of rectal prolapse remains to be further studied. The pathological features of rectal prolapse samples support the model of the unitary theory of recto-anal prolapse.

#### AUTHORS' DISCLOSURE

This research received no external funding. All authors read and approved the final manuscript. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the G. d'Annunzio University of Chieti-Pescara and of the Institute of Pierangeli Hospital in Pescara. Informed consent was obtained from all subjects involved in the study. Data supporting the findings of this study are available from the corresponding author upon request.

#### CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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