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Abstract

Introduction

Intersphincteric resection (ISR) enables radical sphincter-preserving surgery in a subset of low rectal tumours impinging on the anal sphincter complex (ASC). Excellent anatomical knowledge is essential for optimal ISR. This study describes the role of the longitudinal muscle (LM) in the ASC and implications for ISR and other low rectal and anal pathologies.

Materials and Methods

Six human adult en-bloc cadaveric specimens (three males, three females) were obtained from the University of Leeds GIFT Research Tissue Programme. Paraffin embedded mega blocks containing the ASC were produced and serially sectioned at 250 µm intervals. Whole mount microscopic sections were histologically stained and digitally scanned.

Results

The intersphincteric plane was shown to be potentially very variable. In some places adipose tissue is located between the external anal sphincter (EAS) and internal anal sphincter (IAS), whereas in others the LM interdigitates to obliterate the plane. Elsewhere the LM is (partly) absent with the intersphincteric plane lying on the IAS. The LM gave rise to the formation of the submucosae and

corrugator ani muscles by penetrating the IAS and EAS. In four of six specimens, striated muscle fibres from the EAS curled around the distal IAS reaching the anal submucosa.

Conclusions

The ASC formed a complex structure, varying between individuals with an inconstant LM affecting the potential location of the intersphincteric plane as well as a high degree of intermingling striated and smooth muscle fibres potentially further disrupting the plane. The complexity of identifying the correct pathological staging of low rectal cancer is also demonstrated.

Key words: longitudinal muscle; internal anal sphincter; external anal sphincter; anal sphincter complex; rectal cancer; intersphincteric resection; sphincter-preserving surgery

Introduction

Low rectal cancer is a challenging disease, particularly when the tumour invades the anal sphincter complex (ASC). These tumours are typically removed by an abdominoperineal excision, though patients requiring this type of surgery end up with a permanent colostomy and have a poorer oncological outcome compared to anterior resection (Wang et al., 2015). Damage to the anal sphincters, iatrogenic tumour perforation and incomplete tumour resection are frequently encountered and related to increased local recurrence and decreased survival (den Dulk et al., 2009).

For a select group of patients in whom low rectal tumours impinge on the anal canal or invade the anal submucosa, a total or partial intersphincteric resection (ISR) might be a viable alternative to abdominoperineal excision (APE). Theoretically, ISR is performed in combination with total mesorectal excision and involves transanal division of the low rectum, removal of part or all of the internal anal sphincter (IAS), and restoration of bowel continuity by means of a coloanal anastomosis (Schiessel et al., 1994; Cong et al., 2014). This can be performed either from above or from below by using transanal minimally invasive surgery (Fernandez-Hevia et al., 2015).

Although the functional outcomes may be suboptimal, long-term oncological outcomes after ISR are satisfactory (Schiessel et al., 1994; Yamada et al., 2007; Martin et al., 2012; Koyama et al., 2014). Historically, decision-making for sphincter-saving surgery was dependent on the tumour's distance to the anal verge. Until the 1980's, a distal resection margin of 5 cm was required, after which a 2 cm distal margin was considered adequate. In the last decade, it has been shown that a distal margin of less than 1 cm is oncologically safe in carefully selected patients (Ueno et al., 2004;

Rullier et al., 2005; Kiran et al., 2011). Locally advanced tumours can also be significantly downsized and downstaged by pre-operative chemoradiation, after which ISR may be justified (Koyama et al., 2014). Given these advances, ISR has been more widely accepted in specific cases of low rectal cancer. This approach is potentially attractive in terms of achieving a balance between oncological safety and preserving continence, yet there is an important risk of compromising radical resections (Fernandez-Hevia et al., 2015). The key in rectal cancer surgery is the selection of appropriate operative planes and achievement of a tumour-free circumferential resection margin in order to reduce local recurrences (Quirke et al., 1986).

Hence, excellent anatomical knowledge of the ASC and the degree of variation within individuals is of paramount importance to understand the planes that may be achievable to optimise results from ISR. Studies reporting on the technical aspects of ISR pay little attention to the anatomy of the intersphincteric plane (Schiessel et al., 1994; Akagi et al., 2014). Dissection in this plane should enable separation of the IAS and external anal sphincter (EAS). The longitudinal muscle (LM) forms the longitudinal layer of the smooth muscular component of the ASC and is located between the IAS and EAS. As its anatomy has been mainly studied in the context of benign anal pathology and its function in continence (Morgan and Thompson, 1956; Lunniss and Phillips, 1992; Macchi et al., 2008) potentially important details from a surgical and oncological perspective have not been described. It has remained unclear how the intersphincteric plane can be precisely located during surgery. Moreover, contradicting descriptions of the ASC raise the suspicion that inter-individual differences are present. Although previously suggested (Rociu et al., 2000), a thorough two-dimensional histological analysis of the entire ASC is needed to further examine this concept.

We studied the ASC in whole mount microscopic sections from en-bloc cadaveric specimens. In particular, the anatomy of the LM and the presence of inter-individual differences were studied, and implications for ISR described.

Materials and Methods

Adult cadaveric specimens

Six human adult specimens were obtained through the University of Leeds GIFT Research Tissue Programme (www.gift.leeds.ac.uk) from consented whole-body donors. Ethical approval was granted by the Northern and Yorkshire Regional Ethics Committee, Jarrow, UK (unique reference number 11/H0903/6). The donor bodies belonged to three males aged 68, 89 and 99 years, and three females aged 63, 64 and 74 years, respectively. All female donors had a history of childbirth. All donors were free of macroscopic pelvic pathology at post mortem examination. The specimens were retrieved during tissue donation autopsies performed at St. James's University Hospital, Leeds in the prone jack-knife position according to the technique of extralevator APE as described by Holm et al (2007). The specimens were essentially pelvic exenterations and comprised en-bloc resection of the soft-tissues in the bony pelvis with the anal canal, ASC and rectum up to the recto-sigmoid junction. All specimens were processed in an identical way and fixed in 8% formaldehyde solution for seven days prior to transverse sectioning at 1 cm intervals. The slices were photographed and dissected to fit in Super Mega Cassettes measuring 74.8 x 52.5 x 16.5 mm (CellPath, Powys, UK). The tissues underwent an extended tissue processing cycle in a Leica ASP200 tissue processor as follows: 1 hour (h) in 70% ethanol, 2 h in 80% ethanol, 2 h in 90% ethanol, 3 h in 95 % ethanol, 12 h in 100% ethanol (repeated three times), 12 h in xylene, 24 h in xylene (repeated twice), 24 h in paraffin. All tissues were embedded in paraffin mega blocks.

Histological staining

The mega blocks were sectioned at 5 µm intervals through the full thickness of the block. Whole mount microscopic sections were collected in three different ways to determine the optimal intersectional distance to analyse the ASC. In one male and one female specimen, every 10th section was collected and stained with haematoxylin and eosin (H&E). Additional sections were collected and kept for further stains. In a further male and female specimen, every 48th, 49th and 50th section was collected and stained with H&E and Masson's trichrome (MT) with the third section kept for additional stains. In the final male and female specimens, every 50th section was taken and alternately stained with H&E and MT. In this way, two cases (one male and one female specimen) with a cross-sectional interval of 50 µm and four cases (two male and two female specimens) series with a cross-sectional interval of 250 µm were created. The additional sections were stained with MT, Millers' elastin (Everett and Miller, 1974) (ME) and picrosirius red (PR) to demonstrate elastic fibres and collagen.

Image processing

All glass slides were digitally scanned with an Aperio XT slide scanner (Aperio, San Diego, California, USA). These were viewed using Aperio ImageScope version 10.2.2.2319. Images were created in Adobe Photoshop.

In order to make a three-dimensional reconstruction, we selected H&E and MT stained sections from one male and one female specimen to scan with a Canoscan 9000F Mark II (Canon, UK) at a resolution of 1200 dpi. The cross-sectional interval of these two datasets was 250 µm. The scanned images were uploaded into customised software and registered using a sequential slice-to-slice

image-based registration approach as described in detail before (Roberts et al., 2012). Both datasets were uploaded in Amira software version 5.3.3 (Amira, Hillsboro, Oregon, USA) and structures of interest were manually segmented. 3D volume rendering and interactive visualization in 3D PDF files was achieved using DeVIDE software (Botha and Post, 2008).

Results

The anal sphincter complex

The upper limit of the ASC appeared at the anorectal junction where the circular and longitudinal layers of the rectal muscularis propria thickened and continued as the smooth muscle component of the ASC. The IAS was formed by the circular smooth muscle fibres and was located most medially, whilst the LM was composed of the longitudinal smooth muscle fibres. The lower limit of the ASC was identified by the most caudal extension of all muscular components surrounding the anal aperture.

The intersphincteric plane was located between the outer surface of the smooth muscular component (e.g. the LM and the IAS) and the inner surface of the striated muscular component (e.g. the EAS). The ASC was closely related to the perineal body (PB). At the level of the perineal membrane, the anterior parts of the LM and IAS thickened and anchored into the PB to create a strong fixation. This part was identified as the upper limit of the intersphincteric plane. There was no anatomical plane recognizable from this point in a cranial direction.

Four epithelial zones were recognized with normal rectal mucosa merging with simple columnar epithelium then stratified columnar epithelium and lastly stratified squamous epithelium. The dentate line marked the transformation of the stratified columnar to squamous epithelium and divided the anal canal into two components, an upper third and lower two thirds. The upper third contained muscularis mucosae separating the columnar epithelium from the anal submucosa. The lower two thirds component lacked muscularis mucosae, hence the connective tissue underlining the squamous epithelium is referred to as subepithelium.

Variations in size were not studied as post-mortem shrinkage and formalin fixation is expected to have affected the specimens in an inconsistent manner. 3D reconstructions were created from which interactive PDF files can be explored online at: http://graphics.tudelft.nl/3danalsphinctercomplex.

The architecture of the external anal sphincter

The architecture of the EAS was very consistent as it appeared as a muscular layer of striated muscle fibres in all specimens. The EAS could be subdivided into a cranial and caudal portion. In cranial direction, the EAS was one-and-the-same structure as the puborectalis muscle. The caudal portion showed anterolateral and posterolateral defects in one male and one female specimen (Fig. 1). At the most caudal end, the muscle fibres turned strongly inwards reaching the subepithelium just below the lower limit of the IAS (Fig. 2). In two male and two female specimens, a bundle of loose striated muscle fibres extended all the way around the distal end of the IAS and ascended within the subepithelium over a short distance. Some loose striated muscle fibres intermingled in a random manner with the LM in the intersphincteric space (Fig. 3). The relationship of the EAS with the coccyx was assessable in one male specimen, in whom the caudal EAS was connected to the coccyx by the anococcygeal ligament.

The architecture of the longitudinal muscle

The LM constituted the longitudinal layer of the smooth muscle component in the ACS. It appeared as a well-developed layer located between the IAS and the EAS. The architecture of the LM was rather complex. In some specimens, the LM was so compact that it was difficult to examine the precise course of the muscle fibres. In other specimens where the LM was less compact, an inner layer of thick bundles of smooth muscle fibres running in longitudinal direction and an outer layer of smooth muscle fibres running in a more circular direction were observed. This outer layer was closely adherent to the EAS. At the upper limit of the ASC, both layers were visible, yet at the caudal part of the ASC only the longitudinal fibres remained.

Furthermore, we observed that the LM was composed of merely smooth muscle fibres (Fig. 4). Several, but very small, loose bundles of striated muscle fibres were detected in the LM. It was not possible to determine either the origin or course of the loose striated muscle fibres. At the anorectal junction, strands from the LM extended in anterolateral direction as the recto-urethralis muscle to create a strong fixation of the anorectum to puborectalis (Fig. 5). There was also variability in the thickness of the LM and in the presence of adjacent fat separating it from the EAS creating a potentially variable intersphincteric plane. In the male aged 89 years, anterior parts were absent, whilst in the male aged 68 years, posterolateral parts of the LM were missing (Fig. 6). Table 1 summarizes the different locations of the actual intersphincteric space in all specimens. Caudally, the longitudinal smooth muscles fibres extended as finger-like extensions and penetrated through the IAS and EAS at the level of the anal transition zone. Those fibres penetrating the IAS anchored in the subepithelium to form the submucosae ani muscle which surrounded the superior hemorrhoidal plexus. The fibres piercing the EAS formed the corrugator ani muscle that approached the peri-anal skin and ischiorectal fossa (Fig. 7). There was a degree of variability in the length to

which the LM consisted of thick muscle bundles before it turned into fibro-elastic septa. At some points, the LM and IAS were strongly related forming a single structure with no plane between them (Fig. 8). In addition, it was noted that multiple blood vessels, numerous nerves and adipose tissue were related to the LM. Blood vessels and nerves were not confined to run on either lateral or medial sides of the LM but crossed the LM and the IAS (Fig. 6). Towards the anal aperture the amount of adipose tissue increased, and Pacinian corpuscles were seen revealing the presence of mechanoreceptors.

Specimen	**Location of true intersphincteric space**
Male, 68 years old	Posterior and lateral, at the level of the inferior hemorrhoidal plexus
Male, 89 years old	Anterior, below the inferior hemorrhoidal plexus
Male, 99 years old	Posterior and lateral, at the level of the inferior hemorrhoidal plexus
Female, 63 years old	No actual space, consistently a very close relation of the EAS, LM and IAS
Female, 74 years old	Posterolateral and anterolateral, below the inferior hemorrhoidal plexus
Female, 82 years old	Anterolateral, below the inferior hemorrhoidal plexus

Table 1. Different locations of true intersphincteric space in the six specimens.

Internal anal sphincter

The IAS was a direct continuation of the circular layer of the rectal muscularis propria and formed the circular layer of the smooth muscle component of the ASC. It appeared as a very consistent and well-defined circular smooth muscle layer located directly under the submucosae ani and

subepithelium. The IAS did not exceed the lower limit of the EAS and fibro-elastic septa from the LM. Along the length of the ASC, the IAS was crossed by small blood vessels and nerves (Fig. 4).

Discussion

In ISR for early-stage low rectal cancer invading the ASC, excellent understanding of the anatomy is needed to identify the surgical planes that can be achieved to provide oncological safety and obtain good functional results. There is a need to enhance the anatomical knowledge of the ASC as the tumour's lower edge is no longer a limit for sphincter-preserving surgery. A distal resection margin of 1 cm does not adversely influence oncological outcomes when patients are carefully selected (Rullier et al., 2005; Kiran et al., 2011). Patients should be selected based on the tumour's histological characteristics in order to limit the chance of intramural distal tumour spread and subsequent tumour involvement of the distal margin (Ueno et al., 2004). Based on these insights, more patients may receive sphincter-preserving surgery.

Until now, detailed descriptions of the ASC in the context of such an oncological approach are lacking. By using a range of histological stains in whole mount microscopic sections it was possible to accurately study the intricate anatomy of the ASC. This study reveals the significant, and previously underappreciated, role of the LM in the formation of the ASC and potential difficulties when generating the intersphincteric plane.

Dissection in the intersphincteric plane should enable removal of the anal canal together with the entire smooth muscle component of the ASC, e.g. the IAS and LM, whilst preserving the EAS (Akagi et al., 1989; Spanos, 2012). Surgeons must realise that the generation of this plane depends mostly on the anatomy and might be located on the IAS rather than the LM. In some places the LM is (partly) lacking and the area between the IAS and EAS is (mainly) filled with adipose tissue, whereas in other places the LM takes up the complete area between the IAS and EAS**,** obliterating

the intersphincteric space. In this perspective, we believe that the usage of the term intersphincteric space is misleading. There is no true space**,** as adipose tissue is present when parts of the LM are lacking. The intersphincteric plane is a potential plane rather than an actual plane, wherefore the term intersphincteric space should be avoided to reduce anatomical confusion. As the amount of adipose tissue between the IAS and EAS generally increases towards the anal aperture, surgeons may prefer to start an ISR by perineal dissection.

In this study, we have revealed two unique features of the LM that potentially complicate the generation of the intersphincteric plane. Firstly, the extent to which the LM fills the area between the EAS and the IAS is likely to vary. Macchi et al. (2008) described the LM to be more densely compact at the anterior and posterior sides. On the contrary, we could not determine a specific site in which the LM was more compact, but encountered incomplete parts to be randomly located anteriorly, posteriorly and laterally. In one female specimen, the LM was so compact that it was hard to distinguish it from the IAS on a microscopic level. This might be an inter-individual variability and/ or explained by age-related atrophy, yet more specimens need to be studied to further analyse this. Our specimens were representative of the age of bowel cancer presentations and thus of the types of anatomy surgeons would experience. These are important observations as the intersphincteric plane might be irregular and unpredictable, and surgeons might create planes on the surface of the IAS reducing the clearance achieved close to superficial low-lying tumours. Secondly, the LM traverses major structures throughout the whole ASC challenging the surgeon to identify the smooth muscle and properly separate it from the striated sphincter layers. Thirdly, it creates opportunities for the spread of tumour or infection through the IAS and into deeper structures.

This work supports the presence of inter-individual differences in the ASC, as has been previously reported (Oh and Kark, 1972; Rociu et al., 2000), and stresses the need to further examine this across a population and in the context of other pathology affecting the ASC, e.g. obstetric injury at child birth. Even though only six en-bloc cadaveric specimens were studied in detail, inconstancies were observed in the caudal EAS and the upper part of the IAS. All female specimens had a history of childbirth, which might explain the muscular defects in the caudal EAS. The defects in the male specimen could be iatrogenic (because of the specimen retrieval and tissue processing) or explained by atrophy. To rule out the effect of age-related degeneration on variability, specimens from younger individuals should be studied, including nulliparous females. However, our specimens represented a realistic age group that is encountered daily by rectal cancer surgeons. Age- and sex-related variations of the LM and EAS have been described previously using endoanal magnetic resonance imaging (Rociu et al., 2000), but not confirmed microscopically.

Furthermore, this study demonstrates the potential complexity of identifying the correct pathological staging of low rectal tumours involving the ASC. Primary tumour staging in colorectal cancer is based on penetration through specific anatomical structures, whereas in anal cancer this is based on size. We have shown the inconsistency of the LM and the intermingling of striated and smooth muscle fibres as they traverse planes. Thus, muscle type, i.e. smooth or striated, cannot be used alone without some understanding of the entire ASC and the extent of tumour spread that has occurred. Small blood vessels and nerves cross the IAS suggesting that lymphatics do the same. What is the most appropriate modern staging for this complex site**,** especially when radiochemotherapy is frequently used as the major treatment modality? This needs further work in large series. Any new TNM classification system for low rectal tumours impinging on or invading the

ASC should consider these factors when determining its evolution**,** and further work is needed to understand in detail the lymphatic pathways.

Anatomical teaching of the ASC is mainly based on the studies of Morgan (1936, 1950), and Morgan and Thompson (1956) who described the ASC from the perspective of anal fistula surgery. They reported on a trilaminar arrangement of the EAS encompassing a deep, superficial and subcutaneous part (Garavoglia et al., 1993; Al-Ali et al., 2009). Shafik (1975) extensively studied the anatomy of the anal sphincter complex and published important work on the external anal sphincter and its subsequent role in anal continence, describing it as a “tri-loop structure” consisting of a series of U-shaped loops which are distinguishable as a ‘top’, ‘intermediate’ and ‘base’ loop. According to Shafik’s descriptions, no concentric circular muscle bundles could be detected at any level of the external sphincter except in the base loop. Our results confirm the findings of Fritsch et al. (2002) with the EAS consisting of a caudal part traversed by the LM and a cranial part that is deficient anteriorly. Shafik studied also the LM and described three muscle bundles (medial, intermediate and lateral) which were separated by fascial septa forming a ‘central tendon’ in a caudal direction. This so-called central tendon gave rise to multiple small fibrous septa penetrating the IAS to give rise to the corrugator ani muscle (Shafik, 1975). Our study did confirm the formation of the corrugator ani muscle by decussating fibres from the LM, but we could not identify the three muscle bundles and fascial septa. Contrasting earlier reports (Macchi et al., 2008; Steele et al., 2011), the LM in our English specimens consisted of merely longitudinal smooth muscle fibres rather than a mixture of smooth and striated muscle fibres. Kim et al. (2015) obtained similar results in Japanese specimens. Previous descriptions of the LM might be caused by misinterpretations of

the traversing fibres**,** but most studies analysed specific parts of the ASC in isolation without integrating the entire ASC.

Additionally, the current study helps to better understand the role of the LM in benign anal pathology. By integrating the EAS and IAS, the LM minimizes deterioration of sphincter function after surgical division and prevents haemorrhoidal and rectal prolapses (Haas and Fox, 1977). The LM divides adjacent tissues into subspaces, which may cause septation of thrombosed external haemorrhoids (Lunnis and Phillips, 1992). Also, LM fibres form potential routes for fistulae extension. Inter- , trans-, supra- and extrasphincteric fistulae can be identified based on their location and relation to the muscles in the ASC (Stein, 2003). The submucosae ani muscle might reinforce the anal submucosa and support the superior hemorrhoidal plexus. Failure of this suspensory mechanism and anatomical weakness caused by fibres from the LM crossing the planes may be the key to understanding such pathology.

Although microscopic sections reveal the anatomy of the anal sphincter complex in detail, this study has several limitations. Due to the distance between the transverse sections, it was not possible to examine the exact course of the different muscular layers and bundles. Longitudinal sections might have been helpful in determining this. Also, macroscopic dissections are needed to further examine the delicate interfaces of the different muscular layers and their mutual relationship. The tissue processing, serial sectioning and staining of the specimens was a very time-consuming process, which limited us to study only six specimens. This number is too small to study the aetiology of muscular defects, such as age or parturition. We preferred to study six specimens in depth rather than more specimens in less detail. Microscopic analysis of the six specimens revealed that there is

variability in the anatomy of the ASC, which has a great significance for surgeons operating in this anatomical region.

In conclusion, the LM plays a dominant role in the ASC and presents surgical challenges when generating the intersphincteric plane in ISR. The high degree of complexity is reflected by the intermingling of longitudinal, circular smooth and striated muscle fibres and their penetration of major structures in the ASC plus the presence of inter-individual differences. Future studies should focus on revealing the lymphatic, neural and vascular pathways related to the ASC to better understand the spread of low rectal and anal cancer and the variability of the ASC across populations subdivided by age, sex, obstetric trauma and preoperative treatment.

Conflict of interest

We declare no conflicts of interest.

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Legends

Figure 1

This figure shows defects of the caudal portion of the EAS in a male (windows a and b) and female ASC (windows c and d). The line in detail window b shows an anterolateral defect of the EAS. The line in detail window d shows a posterolateral defect of the EAS. The star in overview windows a and c marks the part of the caudal EAS connected to the anococcygeal ligament. MT: Masson's trichrome, HE: haematoxylin and eosin, A: anterior, P: posterior, AC: anal canal, EAS: external anal sphincter, LM: longitudinal muscle, IAS: internal anal sphincter. Scale bar overview 8 mm; detail 2 mm.

Figure 2

This figure shows that at the very distal end of the anal sphincter complex, striated muscle fibres from the external anal sphincter (EAS) reach the subepitelium (Sub.E) below the level of the internal anal sphincter. In this figure, the internal anal sphincter is not visible, but the adipose tissue indicated by the star in window a indicates the area just below the caudal end of the internal anal sphincter. The arrow in detail window b shows the striated muscle fibres from the EAS. A: anal canal. MT: Masson's trichrome. Scale bar overview 7 mm; detail 800 µm.

Figure 3

This figure shows the intermingling of striated muscle fibres of the external anal sphincter (EAS) in a male ASC. Striated fibres are found in the subepithelium (Sub.E; arrow in detail window d) and randomly in the intersphincteric space intermingled with the longitudinal muscle (LM; arrows in detail windows b and c). The star in detail window c shows “true” intersphincteric space. MT: Masson’s trichrome, HE: haematoxylin and eosin, A: anterior, P: posterior, AC: anal canal, IAS: internal anal sphincter, B: bulbospongiosus muscle, STP: superficial transversus perineii muscle, E: epithelial lining of the anal canal. Scale bar overview 7 mm; detail 800 µm.

Figure 4

This figure shows that the longitudinal muscle (LM) is composed of smooth muscle fibres. The upper arrow in detail window b demonstrates the inner layer of longitudinal muscle fibres whereas the lower arrow shows the outer layer of more circular muscle fibres. Note the very close relation of the LM to the internal anal sphincter (IAS) and the levator ani muscle (LAM). SMA: smooth-muscle actin. R: rectum. Scale bar overview 7 mm; detail 800 µm.

Figure 5

The longitudinal muscle (LM) is formed by the outer layer of the muscularis propria of the rectum. A small smooth muscular layer extends from the LM covering the medial parts of the levator ani muscle (LAM) to create a strong fixation of the anorectum. The arrows in detail window b show a smooth muscular layer running onto the inner part of the LAM which is connected to the longitudinal muscular layer of the rectum (lowest arrow). ME: Miller’s elastin, A: anterior, P: posterior, AC: anal canal, LAM: levator ani muscle, LM: longitudinal muscle, IAS: internal anal sphincter, P: prostate (caudal part). Scale bar overview 6 mm; detail 2 mm.

Figure 6

This figure demonstrates the variability in the extent that the LM occupies the intersphincteric space. Window a shows a male ASC in which the LM is incomplete posterolaterally (arrows). Window b shows a different male ASC in which the LM is incomplete anteriorly (arrow). MT: Masson's trichrome, A: anterior, P: posterior, AC: anal canal, EAS: external anal sphincter, LM: longitudinal muscle, IAS: internal anal sphincter, B: bulbospongiosus muscle. Scale bar 6 mm.

Figure 7

This figure shows the close relation between the longitudinal muscle (LM) and internal anal sphincter (IAS). Note that blood vessels penetrate the IAS (arrow in detail window b). Note in detail window b the penetration of LM-fibres through the IAS. ME: Miller's elastin, A: anterior, P: posterior, EAS: external anal sphincter, LM: longitudinal muscle, IAS: internal anal sphincter. Scale bar overview 8 mm; detail 2 mm.

Figure 8

Below the dentate line, fibres of the longitudinal muscle (LM) penetrate the internal anal sphincter (IAS) to form the submucosae ani muscle in the subepithelium (arrows in detail window b indicate the LM bundles containing elastin (black)). Fibro-elastic fibres of the LM penetrate also the caudal external anal sphincter (EAS) as is shown by the arrows in detail window c. The star in detail window b marks the squamous epithelium of the anal canal. ME: Miller's elastin, A: anterior, P: posterior, PB: perineal body. Scale bar overview 8 mm; detail window b 1 mm; detail window c 2 mm.

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