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Uterine fibroid pseudocapsule studied by transmission electron microscopy

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ABSTRACT

Objective: The fibroid pseudocapsule is a structure which surrounds the uterine fibroid, separates it from the uterine tissue and contains a vascular network rich in neurotransmitters like a neurovascular bundle. The authors examined the composition of the fibroid pseudocapsule using electron microscopy.
Study design: Twenty non-pregnant patients were submitted to laparoscopic myomectomy by the intracapsular method and samples of the removed pseudocapsules were analyzed using transmission electron microscopy.
Results: At the ultrastructural level the pseudocapsule cells have the features of smooth muscle cells similar to the myometrium. So, the pseudocapsules are part of the myometrium which compresses the leiomyoma.
Conclusion: This ultrastructural feature suggests that when removing fibroids their pseudocapsules should be preserved. This study confirms preliminary evidence that pseudocapsules contain neuropeptides together with their related fibers, as a neurovascular bundle. The surgeon's behavior should be directed to carefully control and spare this muscular surrounding tissue during fibroid excision, in order to preserve the myometrium as much as possible.

1. Introduction

Uterine fibroids (UF) are the most frequent benign tumors of the female reproductive tract, involving 25% of all women. They arise from the muscle cells of the uterus and may be single or multiple, and although they are mostly benign, they are often clinically symptomatic, leading to menstrual irregularities. Because of their size larger UF can compress any of the surrounding organs, leading to urinary and digestive symptoms or sexual disturbances, and they sometimes affect fertility, especially when the cavity of the uterus is distorted [1]. Several methods are available to remove uterine fibroids and relieve the symptoms they cause. Conservative surgery can be offered as well as medical therapy [2] or various novel radiologic interventions [3]. Myomectomy is the most common conservative treatment and it can be performed by classical laparotomy or through endoscopy, a less traumatic method which minimizes complications [4].

The literature shows the possibility of performing myomectomy by removing the fibroid inside its surrounding structure, the pseudocapsule [5]. This is done by stretching and extracting the fibroid pseudocapsule directly from the surrounding fibromuscular structure, breaking up the fibrous bridges [6]. In 2009 a review was published on the pseudocapsule and fibroid histopathology with regard to the outcome after surgical myomectomy or endoscopy [7]. Unfortunately, there are few data on the ultrastructure of the pseudocapsule and its possible involvement in uterine fibroid development, growth and removal, as in post-myomectomy uterine healing. In the present study the authors analyzed the ultrastructural features of the fibroid pseudocapsule using transmission electron microscopy in order to describe the

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fine ultrastructural details of the pseudocapsule and its possible connections to the physiology of the myometrium.

2. Materials and methods

From January to December 2009 20 women, with a mean age of 36.7, underwent single intracapsular endoscopic myomectomy in two university-affiliated hospitals. None had undergone a previous caesarean section. All patients signed an informed consent prior to being included in this study, which was approved by the local ethics committee. The selected patients underwent myomectomy for the following indications: pelvic pain, menorrhagia, and rapid growth of the fibroid which was confirmed by ultrasound. Exclusion criteria were: previous uterine surgery, pre-surgical treatment with GnRH analogues, history of gynecological malignancy, suspected adenomyosis and primary infertility. Preoperative GnRH-analogue treatment was an exclusion criterion due to the reported increased risk of fibroid recurrence, a possible delay in the diagnosis of leiomyosarcoma, and risk of massive hemorrhage from degeneration, since GnRH-analogue treatment decreases the size of the fibroid and causes confluent nodular hyaline degeneration and hydropic degeneration necrosis [8]. In the study design, all fibroids were selected through standardized transvaginal ultrasound performed by expert clinicians: all patients had subserous and/or intramural fibroids, and the transvaginal ultrasound documented the fibroid pseudocapsule (Fig. 1). The fibroid diameters were between 5 and 8 cm; the 4–9 cm limit was selected by the surgeons to avoid prolonged operation times and uterine trauma by removing smaller fibroids. To give homogeneity to the intracapsular endoscopic myomectomy, the authors excluded pedunculated, cervical and intra-ligamentary fibroids.

The patients underwent a standardized endoscopic operation without injection of vasoconstrictors into the myometrium [6,8]: the visceral peritoneum was incised in the midline longitudinal plane using monopolar scissors or a crochet needle electrode, proceeding in depth into the myometrium to reach the right plane of cleavage. Once the pseudocapsule was identified, it was well exposed by an atraumatic clamp or by an irrigator cannula to provide an optimal panoramic view of the pseudocapsule. The pseudocapsule was incised longitudinally using monopolar scissors or a hook electrode at low voltage (30 watt) to expose the fibroid surface. The fibroid was hooked using a screw or Collins laparoscopic forceps to perform the traction necessary for its gentle enucleation (Fig. 2) supported by an irrigator cannula. Hemostasis of small vessels was achieved using a low voltage bipolar clamp or a hook electrode or monopolar scissors, always at no more than 30 watts, to free the base of the fibroid and the connective bridges from the pseudocapsule. In such a way, complete minimally traumatic fibroid removal was accomplished with minimal blood loss and fibroid pseudocapsule preservation.

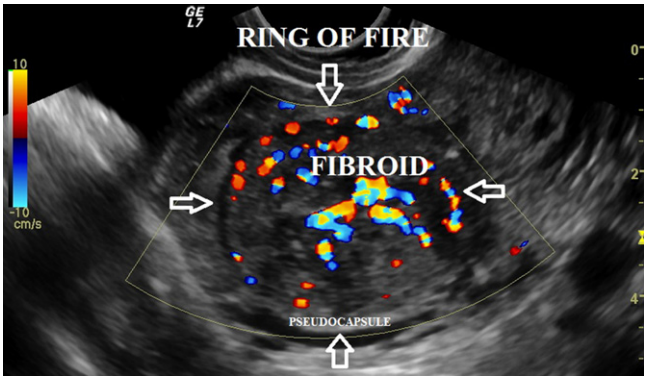

Fig. 1. Transvaginal sagittal sonography showing an intramural myoma, with arrows highlighting the hyperechogenic white fibroid pseudocapsule; the Eco-color-Doppler highlights the "ring of fire" peripheral to the fibroid, which requires gentle hemostasis by low wattage diathermy coagulation.

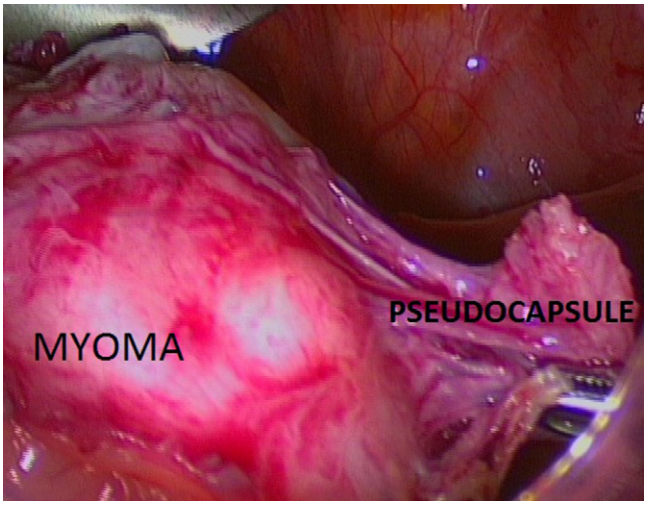

Fig. 2. A laparoscopic image showing fibroid pseudocapsule during myoma stretching after hysterotomy.

Samples were taken using scissors from the surface of the fibroid pseudocapsule as soon as hemostasis was achieved. Twenty pseudocapsule specimens of approximately 5 mm in depth, which included full thickness of the surrounding myometrium, were collected and processed for transmission electron microscopy (TEM). The specimens were fixed in 2% glutaraldehyde/2% paraformaldehyde in 0.1 M phosphate buffer for 3 h at 4 °C, postfixed in 1% osmium tetroxide in the same buffer solution, dehydrated in graded alcohols, and embedded in an Epon-Araldite mixture. For each specimen 5–10 random semi-thin sections (2 μm) were obtained with a MICROM HM 355 microtome (ZEISS, Oberkochen, Germany) and stained with toluidine blue. Thin sections were obtained using an MTX ultramicrotome (RMC, Tucson, AZ, USA), stained with lead citrate and examined with a CM10 transmission electron microscope (Philips, Eindhoven, the Netherlands).

3. Results

In semi-thin sections the cells of the fibroid pseudocapsule are fusiform with a centrally located nucleus (Fig. 3). Inflammatory

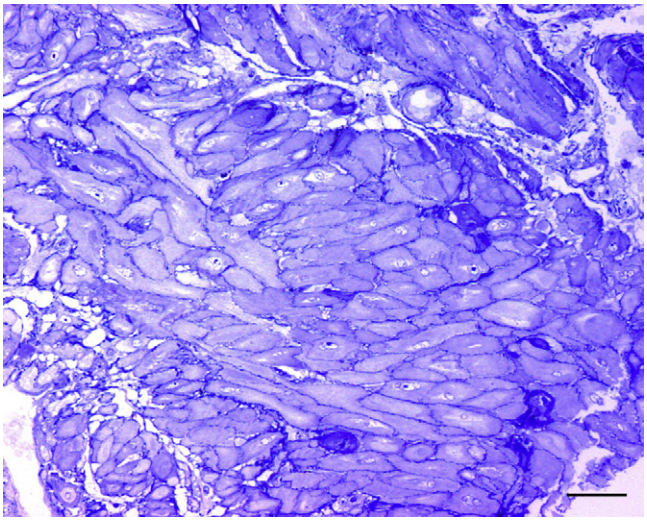

Fig. 3. Toluidine blue-stained semi-thin section of the uterine fibroid pseudocapsule. Scale bar = 25 μm.

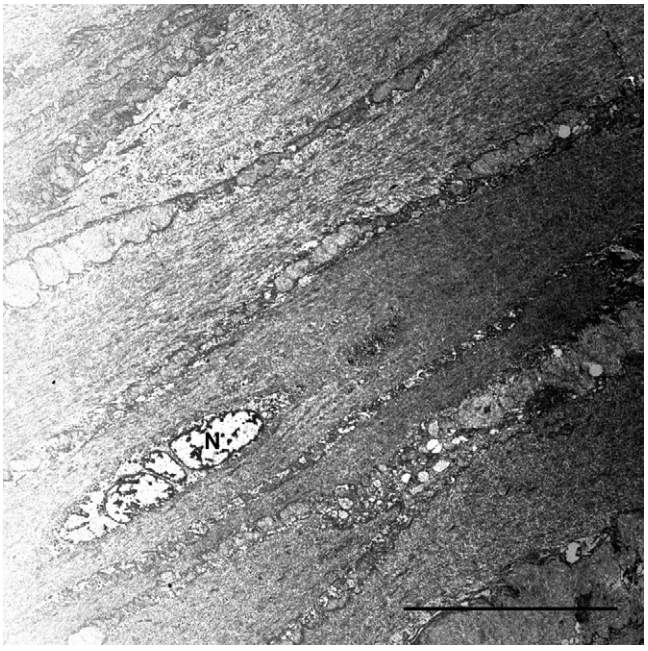

Fig. 4. Smooth muscle cells in longitudinal section. A cell shows the folded nucleus (N). Scale bar = 8 μm.

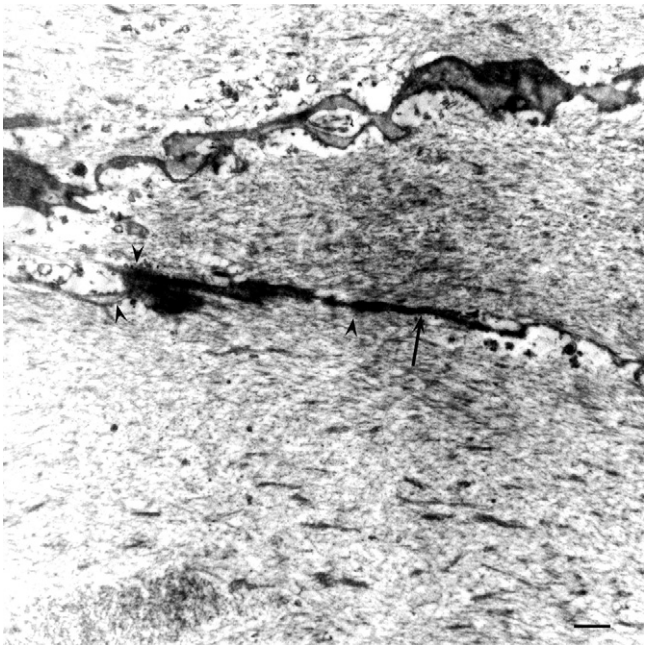

Fig. 6. A junction contact (arrow) between two smooth muscle cells is evident. The micropinocytotic vesicles (arrowheads) are also shown. Scale bar = 0.4 μm.

infiltration was not detected in any section. At the ultrastructural level the cells have the features of the uterine muscle cells. They show nuclei with rounded end, often folded (concertina fashion), notched or with many invaginations (Fig. 4). The cytoplasm is packed with thin (about 6 nm thick) filaments; between the filaments fusiform dense bodies are interspersed (Fig. 5). There are few organelles like mitochondria, glycogen particles and sparse elements of endoplasmic reticulum. Abundant micropinocytotic vesicles are also present (Fig. 6). The cells are connected by junction contacts (Fig. 6) and surrounded by a thin but distinct basal lamina (Fig. 7). For comparison, the ultrastructural aspect of the myometrial smooth muscle cells is shown in Fig. 8.

4. Comments

Since the authors started to study the fibroid pseudocapsule, they have published studies which highlighted the importance of this structure surrounding the fibroids. Many experts assume the presence of fibroid pseudocapsule during myomectomy, even if probably not many surgeons know its structure and

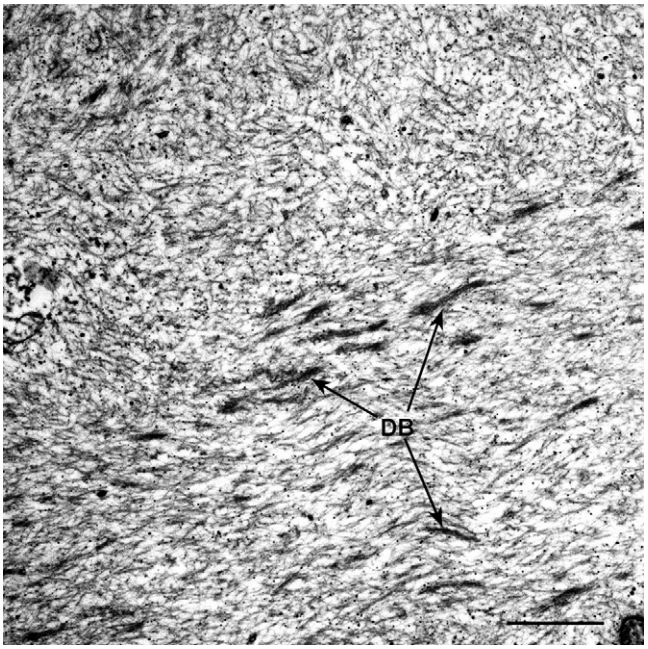

Fig. 5. High magnification of a smooth muscle cell which shows numerous thin filaments and dense bodies (DB) in the cytoplasm. The black dots represent glycogen particles. Scale bar = 1 μm.

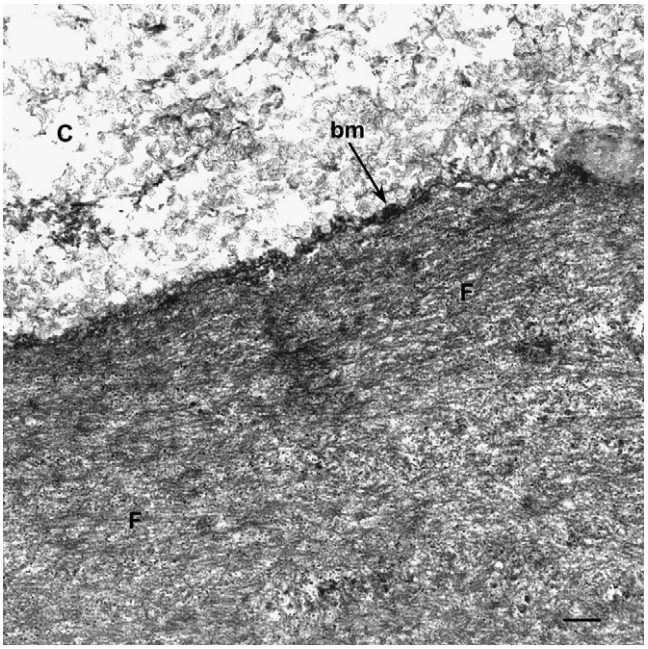

Fig. 7. High magnification of a smooth muscle cell. The cytoplasm is packed with numerous thin filaments (F). A distinct basal membrane (bm) surrounds the cell at the periphery. C, collagen. Scale bar = 0.25 μm.

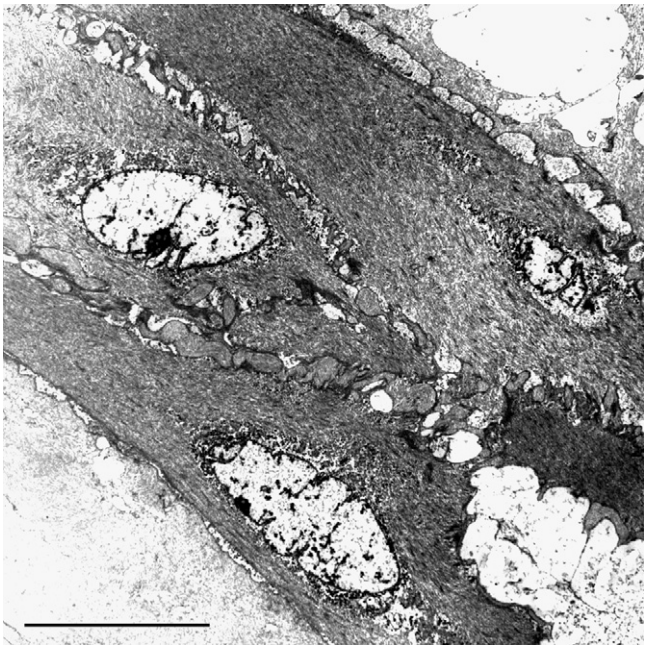

Fig. 8. Smooth muscle cells of myometrium. Scale bar = 10 μm.

functionality. Moreover, scientific information concerning the vascular supply of fibroids is scanty and to some extent controversial, so that some gynecologists even dispute the existence of a pseudocapsule [9–12] or poorly describe the presence of a capsule surrounding fibroids [5]. Myomectomy is generally described as follows: a longitudinal vertical monopolar incision in the most prominent part of the fibroid, a screw inserted into the fibroid, the fibroid enucleated using constant traction combining adequate traction with a tenaculum forceps with counter-traction using a grasper and monopolar coagulation needle or scissors or a bipolar clamp for blunt dissection, and the myometrium sutured in two or three layers depending on the uterine incision. Large-scale studies of good methodological quality are required in order to accurately define a standard laparoscopic myomectomy technique and to precisely assess the advantages or drawbacks of the many variants suggested for each step of the surgical procedure. It is not surprising that the operation procedure relies mainly on the operator's preferences and may therefore vary among surgeons.

It is for these reasons that the authors investigated the ultrastructure of the pseudocapsule, showing that at the electron microscopy level the pseudocapsule cells have features similar to uterine muscle cells. Hence, the pseudocapsule is probably part of the myometrium compressing the fibroid, being a different structure. Anatomically, fibroids arise from the myometrium and typically are comprised of fascicles of smooth muscle cells with abundant pink cytoplasm and uniform spindle-shaped nuclei. Fibroids show a monoclonal proliferation of uterine muscle cells with some mitoses, minimal atypical nuclei, and an absence of coagulative necrosis and pseudocyst formation. Approximately 40–50% of fibroids have karyotypic abnormalities, particularly involving chromosomes 6, 7, 12, and 14. Within a fibroid, all cells are identical and a monoclonal origin has been confirmed. Different karyotypes are noted in fibrotic uteri, suggesting that every single fibroid is an individual biological event [13].

Revisiting the difference between fibroids and pseudocapsules and the endocrine-anatomical status of both, for possible pseudocapsule co-involvement in uterine leiomyomatosis, Stewart et al. showed 50% more collagen in fibroids than the corresponding myometrium, with a specific up-regulation and increase of collagen types I and III, the major components of the extracellular matrix, distinguishing fibroids from myometrium [14]. The extracellular matrix is a non-cellular entity among the cells increased in fibroids, which contributes to their fibrous texture. Thus, leiomyomas could be considered as a fibrotic process containing an abundant amount of extracellular matrix, while on the contrary the myoma pseudocapsule is more elastic due to the lower content of its extracellular matrix, which enables adaptation to the fibroid growth. Moreover, fibroids exhibit a low mitotic index but still can undergo rapid growth and, conversely, a rapid decrease in size when GnRH agonists (GnRH-a) are used [1,2,15].

Estrogen (E) and progesterone (P) appear to influence fibroid growth and have also been reported to affect peptide synthesis of the extracellular matrix in pregnant uteri [13–15]. Fibroids also over-express E and P receptors compared to myometrium and are therefore more sensitive to the effects of these [15]. The pseudocapsule should be hence differentiated from the fibroid in its response to GnRH stimulation. In fact, the fibroid's biological behavior changes according to its estrogenic environment: when the estrogen level is low, the fibroids' size and hyperechogenicity are reduced, while the pseudocapsule US detection becomes more evident [16].

These data should be linked to the published neuroendocrinology of the pseudocapsule [17–19] and confirm the anatomical differences between the fibroid and its pseudocapsule. Malvasi et al. evaluated the distribution of the neuropeptides Substance P (SP) and vasoactive intestinal peptide (VIP) in the pseudocapsule of uterine fibroids, showing that neurofibers are present in the pseudocapsule as well as in the normal myometrium of non-pregnant uteri. The authors suggested removing fibroids while respecting the pseudocapsule, as a neurovascular bundle rich in neurofibers, which is necessary for correct subsequent muscular healing and myometrial functioning, particularly in future pregnancies. The neurovascular bundle concept of the pseudocapsule was confirmed in further investigation [18,19]. Mettler et al. compared the pseudocapsule vascular network, which is rich with neurotransmitters, to the neurovascular bundle surrounding the prostate. Urologists make efforts to preserve the neurovascular bundle, located outside the prostatic capsule, to avoid complications after anatomic radical prostatectomy: they must strike a balance between achieving cancer control and preservation of the neurovascular bundle by endoscopic tailored micro-surgery [18]. Similarly, myomectomy should be performed using an intracapsular method, preserving the pseudocapsule and leaving the fibrovascular network surrounding fibroid intact. This surgical tailored minimally invasive method [6,8] reduces bleeding and trauma and, consequently, has a favorable impact on the healing of the uterus and its successive functionality, since the uterus is fully innervated. Each myomectomy site could lead to uterine neurovascular and muscular damage.

The pseudocapsule vessels were also studied by a preliminary three-dimensional mathematical model which showed an increase vascular tortuosity, disarray, an abnormal branching and the presence of "cul-de-sac" vessels [19]. These features were similar to the characteristics of malignant neoplastic tissue vessels present in malignant tumors. It could be linked either to the rare but possible necrosis of the fibroid, or degeneration of smooth muscle cells by angiogenic growth factors. It was not possible in such a study to explain how the pseudocapsule vasculature network could be produced, and the authors suggest further studies to determine the role of the myoma pseudocapsule and the neurovascular bundle on the formation, growth, and pathophysiological consequences of fibroids, including pain, infertility, and the reproductive outcomes, since relationship between fibroids and infertility remains a critical and unresolved question [19].

Another study investigated the presence of collagen IV and laminin in the pseudocapsule of fibroids by immunochemical staining for collagen IV, immunohistochemical location of vascular membrane-bound laminin and quantitative analysis of their images [20]. Collagen IV and laminin are related to loss of the basal membrane and to tissue aging: increase in the collagen IV is linked to the increase of amorphous substance, including the glycosaminoglycans and the glycoproteins. The results revealed a laminin reduction and an increase of collagen IV with increasing diameter of fibroids and the blood vessels of the pseudocapsules. Therefore the authors suggest removing the fibroids while sparing the pseudocapsule, either in women planning pregnancy or before the fibroid size reaches voluminous dimensions with compression of the surrounding tissues [20].

All these studies support the surgical concept of carefully protecting the pseudocapsule during fibroid removal, avoiding destructive measures when coagulation with at high wattage is used. This rationale of tailored muscular fiber dissection, as defined by Mettler et al. [18], was also suggested in another publication on postoperative adhesions after fibroid removal. Adhesion formation is a significant problem after myomectomy, and adhesions are associated with decreased fertility, bowel obstruction and chronic pelvic pain. Tinelli et al. reported their surgical experience with intracapsular laparoscopic and laparotomic fibroid removal with or without anti-adhesion barriers: outcomes were checked by a non-systematic second look, concerning the presence and severity of adhesions. There was a higher rate of adhesions in laparotomy without barrier (28.1%) compared to laparoscopy with no barrier (22.6%) [21]. This study indicates that a combination of intracapsular laparoscopic myomectomy, which respects the pseudocapsule, together with an absorbable adhesion barrier, significantly reduces the incidence of postsurgical adhesions, along with other advantages such as short hospitalization, decreased need for postoperative analgesia, less intraoperative blood loss and a good outcomes in subsequent pregnancy.

The aim of this study was to add an anatomo-pathological rationale in order to identify the correct procedures when fibroids are being removed, and to standardize this procedure which might influence reproduction, labor and delivery. This study could contribute to the increased information concerning pseudocapsules and their underlying fibroids, allowing differentiation of their structures.

5. Conclusion

Transmission electron microscopy shows that the presence of muscle cells in the fibroid pseudocapsule is similar to myometrium and differs from fibroids. This ultrastructural feature supports the concept that fibroids should be removed while sparing their pseudocapsules, which are a functional part of the myometrial muscular fibers. This study supports preliminary evidence that the pseudocapsule contains neuropeptides together with their related fibers, as a neurovascular bundle of the utmost importance for optimal healing of the uterine musculature. These observations support the necessity of preserving the pseudocapsule when performing myomectomy. The significance of the pseudocapsule should be further investigated with studies in other groups of patients such as postmenopausal women, in order to obtain more information concerning the ultrastructural pattern of the pseudocapsule in different groups.

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