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# Distribution of Substance P (SP) and Vasoactive Intestinal Peptide (VIP) in pseudocapsules of uterine fibroids

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ABSTRACT

The authors examined the presence of Substance P (SP) and Vasoactive Intestinal Polypeptide (VIP) and their related fibers in the pseudocapsule of uterine fibroids (PUF) and in normal myometrium (NM) during myomectomies in 57 non-pregnant women. 4 samples were removed from the normal myometrium (NM) and from PUF. The samples were sent for histological and immune-fluorescent investigations. SP and VIP values were found non-significantly higher in PUF than in NM: SP values were $10.2 \pm 0.1$ conventional units (C.U.) in PUF at the fundus of the uterus (FU) vs. $8.1 \pm 0.6$ C.U. of NM in the FU ($p > 0.05$), and SP values were $25.1 \pm 0.9$ C.U. in PUF in the uterine body (UB) compared to. $23.2 \pm 1.4$ C.U. of NM in the myometrium of the UB ($p > 0.05$). VIP values were $11.5 \pm 0.9$ C.U. in the PUF in FU compared to $9.8 \pm 1.4$ C.U. of NM in the FU ($p > 0.05$), and VIP values were $33.9 \pm 3.9$ C.U. in the PUF in the UB vs. $32.6 \pm 4.8$ C.U. of the NM in the UB ($p > 0.05$). These findings show that SP and VIP neurofibers are present in the fibroid pseudocapsule, similar to the values in the normal myometrium of a non-pregnant uterus. An intracapsular myoma excision which respects the pseudocapsule permits a physiological healing process of the uterine scar, due to a neurotransmitter sparing at the hysterotomic site. In women planning pregnancy, the myomectomy should be preferably performed respecting the pseudocapsule in order to preserve the neurotransmission.



## 1. Introduction

When the architecture of the myometrium and its surrounding in the presence of fibroids was studied, a kind of pseudocapsule surrounding the fibroid was found. This pseudocapsule separates the fibroid from the normal myometrium.

The fibroid is attached to the pseudocapsule by connective bridges in continuation with the uterine collagen skeleton, but lacks its own vascular pedicle, since a vascular network surrounds the fibroids, so that the excision of a fibroid needs to be done inside the pseudocapsule separating this vascular network [27].

In the uterine structure different neurofibers and neuropeptides exist and among these, two estrogen-regulated neuropeptides, Substance P (SP) and the Vasoactive Intestinal Polypeptide (VIP), both involved in the musculature regulation.

The SP, in the genital tract, has afferent fibers which are involved in pain regulation and efferent fibers which are involved in microvasculature regulation. The SP plays an important role in the cervical ripening and in the perception of pain during labor [29]; in non-pregnant women, different studies described nerve fibers containing immunoreactivity to SP, associated either to cervical vascularization (particularly involved in vasodilatation) in the cervix and in the myometrium (for myoregulation) [7].

The VIP functions as neurotransmitter in the nervous control of the reproductive tract. In the female genital system, the nerve fibers

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containing VIP are found in blood vessels, non-vascular smooth muscles and in the epithelium lining [4,15].

There is a lack of experimental and clinical studies concerning the role of SP and of VIP related fibers in uterine fibroids and in its pseudocapsule.

The authors examined the SP and VIP distribution in the pseudocapsule and in the non-pregnant myometrium adjacent to the fibroids.

## 2. Material and methods

### 2.1. Materials

Between January 2005 and December 2009, 64 women were scheduled for single or multiple myomectomies in affiliated University Hospitals.

The indications for myomectomy were the following symptoms: severe pelvic pain, heavy menorrhagia not responding to conservative treatment or uncontrolled growth as verified by repeated ultrasounds.

Only 57 of the women agreed to sign an informed consent as approved by the local institutional research ethics committee and participated in the study. Prior to the surgery, pregnancy was excluded by a β-HCG test. All these women were Caucasians.

The exclusion criteria were: previous uterine scar (including cesarean section) and post-treatment of GNRH analogs as well as a history of gynecological tumors.

The exclusion criterion for women treated with GNRH analogs was due to the reported increased risk of recurrence and possible delay in the diagnosis of leiomyosarcoma as well as a risk of massive hemorrhage due to difficulties in entering the cleavage plane, and a greater extent of hyalinization [7].

All fibroids were diagnosed using standardized transabdominal and transvaginal ultrasound myoma mapping by an expert technician; the fibroids were subserous, intramural, corporal and fundal and the ultrasound data were recorded for postsurgical evaluation.

All the fibroids in this study were single or multiple located in the fundus or the corpus. Pedunculated, cervical and intraligamentary fibroids were excluded as they show a different pseudocapsule and due to the absence of the normal myometrium around the fibroid.

All myomectomies were performed by laparotomy or laparoscopy, depending on their dimension and the surgeons' preference. The diameter of the fibroids was between 5 and 10 cm. All women were given a standard prophylactic antibiotic dosage of cefazolin 2 g I.V. All the operations were performed under general anesthesia with endotracheal intubation.

All surgical procedures were performed by experienced gynecologists or senor residents who master the correct methodology of removing fibroids from within the pseudocapsule as the reported evidence-based data show [26,27].

Fibroids are generally surrounded by vascular network, and the separation of the fibroids needs to occur in the inner aspect of the pseudocapsule, in order to prevent excessive bleeding and postoperative complications such as intra-myometrial hematomas and facilitate a favorable post-operative healing.

The laparotomy or endoscopic myomectomies were performed using a standardized method. The incisions were done longitudinally, preferably in the midline using a monopolar or bipolar coagulation gradually until opening the pseudocapsule enabling to enter the relatively bloodless plan between the pseudocapsule and the fibroid (Fig. 1). Once the surface of the fibroid was reached and its fiber bridges freed, the fibroid was hooked and extracted from its capsule by traction and pushing down the capsule.

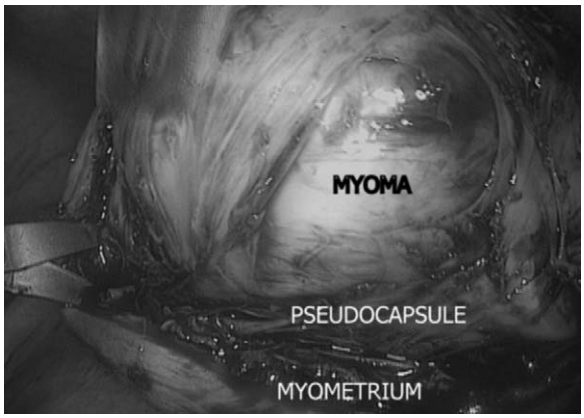


**Fig. 1.** Intraoperative laparoscopic picture of uterine myoma intracapsular removing; surgeons expose by scissors the myoma pseudocapsule and normal myometrium before sampling.

### 2.2. Experimental procedure

Samples were taken by scissors from the surface of the pseudocapsule as soon as good hemostasis was reached, and four samples of approximately 5 mm in depth, which included full thickness of the surrounding myometrium, were collected and sent to the laboratory in a dry-ice container for histological and immunefluorescent studies.

In the laboratory, the samples were washed by immersion in a cold Krebs-Ringer's solution, and examined through immunefluorescent techniques for detection of SP and VIP nerve fibers. Slides were prepared with cryostat to obtain sections of 40 μm. Each section was placed on a cover slide, to which it adhered due to the temperature difference. Each slide was checked for SP, according to the method described by Lorton et al. [19], and for VIP according to the methodology suggested by Gomariz et al. [9].

The analysis of the samples was carried out with a fluorescence Leitz Ortoplan microscope equipped with an epi-illumination system. The light source used was a mercury lamp (HB 100) combined with selective Leitz filters.

### 2.3. Analysis of SP and VIP

The density of SP and of VIP fibers was calculated by quantitative analysis using a Quantimet Leitz image analyzer which measures the following parameters:

1. number of SP and of VIP containing fibers counted in randomly chosen 10 fields;
2. percentage of the total area occupied by those fields;
3. number of observed varicosities;
4. number of crossings or intersections of the nerve fibers
5. the total perimeter of SP and of VIP structures in proportion to an average value (100 for each field).

Five consecutive serial sections were obtained by cryostat microtome, placed on five separate slides and prepared for the detection of each neurotransmitter and were placed in different batches.

In the first batch: primary or secondary antiserum omitted, denatured or previously absorbed by corresponding peptide in excess; in the second batch: primary or secondary antiserum replaced by a non-immune serum; in the third batch: sample previously fixed by immersion in a 4% solution of formaldehyde in PBS which did not preserve the immune-reactive sites; in the fourth batch: these samples were denatured with formaldehyde before or