Contributions to myometrium study in uterine–tubal junction

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Abstract
Complex coordinated contractions of the tubal musculature are thought to be important for movement of the ovum from the distal end to the proximal end of the tube, while at the same time aiding in the movement of sperm from the proximal end to the distal end of the oviduct. Studies of the anatomy and the physiology of the uterine–tubal junction (UTJ), with a main concern for the muscular pattern and the secretory activity of this part of the tube, in relation with the hormonal and nervous control, can play an important role in understanding and impairment of infertility. Related to these aspects, the purpose of our research is mainly to identify the determining factors in maintaining a morphological and structural balance between contractile items and extracellular matrix items within uterine myometrium, particularly uterine–tubal junction. For this reason, the present study includes analyses of anatomical sections within uterus and uterine tubes extracted from patients during therapeutic surgeries. Thirty anatomical pieces from patients aged 23–37-year-old (uterus and tubes) were used to perform microanatomical sections, 10 pieces for each pathology identified, namely leiomyofibroma, leiomyofibroma with pregnancy, ectopic pregnancies. At the same time, the study proposes a statistic and mathematic analysis of the cases with uterine–tubal junction pathology in Oltenia region within a period of 10 years. The novelty of the study is represented by the identification of certain continuity ratios between myocyte fascicles and average tunica of arteries and arterioles, which explains the existence of “vascular units” determined by myometrial structures. Moreover, the stereo-distribution of “vascular myometrial units” explains the existence of a plexiform cavernous network, which enables many authors to describe numerous barely individualized myometrial tunicas. These units are rich in orthosympathetic vegetative innervations that might be responsible for the unfavorable evolution of circulatory myometrial system in postpartum. Considering all these observations, it is highly necessary to reconsider the structure of the uterine wall and, particularly of uterine–tubal junction.

Keywords: uterine tubal junction, uterine–tubal junction, tunica muscularis, stratum vasculosum, myometrial vascular units, microscopy.

Introduction
From an anatomical point of view, the classic approaches describe the myometrium of the uterus, which consists of bundles of unstriped muscular fibers, disposed in layers, intermixed with areolar tissue, blood vessels, lymphatic vessels, and nerves [1, 2]. There are three layers: external, middle, and internal. The external and middle layers constitute the proper muscular coat, while the inner layer is greatly hypertrophied muscularis mucosae. During pregnancy the muscular tissue becomes more prominently developed, the fibers being greatly enlarged [3]. The external layer, placed beneath the peritoneum, consists of fibers which pass transversely across the fundus, and, converging at each lateral angle of the uterus, are continued on to the uterine tube, the round ligament, and the ligament of the ovary: some passing at each side into the broad ligament, and others running backward from the cervix into the sacrouterine ligaments [2]. The middle layer of fibers presents no regularity in its arrangement, being disposed longitudinally, obliquely, and transversely. It contains more blood vessels either than of the other two layers. The internal or deep layer consists of circular fibers arranged in the form of two hollow cones, the apices of which surround the orifices of the uterine tubes, their bases intermingling with one another on the middle of the body of the uterus [4]. At the internal orifice, these circular fibers form a distinct sphincter. As the uterine tube (oviduct) opens into the uterine horn through the uterine ostium (ostium uterinum tubae) this marks the site of the utero–tubal junction. The part of the oviduct that crosses the uterine wall, called the pars interstitialis or intramural segment, has 1–2 cm long and constitutes the uterine–tubal junction. This section extends through the wall of the uterus and the ostium opens within the uterine cavity. The wall of the oviduct has the same basic components as the wall of the uterus [5–7].

Despite the fact that all these aspects are well known, the study of the myometrium structure in the utero–tubal junction is still a controversial problem, especially in humans [8, 9]. Moreover, from the pathological point of view, an international study conducted...
by Chandra A et al. [9], showed that in 2002, 12% of women aged 15–44-year-old (7.3 million women) had impaired fecundity. Recent studies indicate that the pathology of the uterine–tubal junction of different causes is responsible for this: chronic infections and their sequelae, salpingitis isthmica nodosa, polyps, iatrogenic lesions (mainly following sterilization procedures), congenital malformations, ectopic pregnancies.

Related to both mentioned aspects the objectives of the present research consisted in identification of answers to some questions related to the structural anatomy of the myometrium in the uterine–tubal junction, mainly represented by:

* which are the predominant structures within the uterine wall that allow the morpho-functional definition of the uterus: the smooth muscles (the muscular system), extracellular matrix (the conjunctive system), blood vessels (the vascular system), muscles and vessels (myovascular system)?
* what is and how does the structures differentiation at the level of myometrium, particularly in uterine–tubal junction show?;
* which are the temporal and spatial ratios of structural, vascular, muscular items and those of extracellular matrix in ortho- and pathomorphogenesis?;
* how can structural variability of myometrial benign tumors be explained: leymymoma (“typical”, “cellular” or “proliferous”, “atypical” cells, “clear” cells, epitheloid cells, “plexiform” cells), leiomyofibroma and/or fibroma?;
* how can opposite phenomena possibly co-exist in time and space: apoptosis of contractile structures and genesis of intramyometrial circulatory system items (angiogenesis and vasculogenesis)?

## Material and Methods

Clinical casuistic was provided from the archives of Obstetrics-Gynecology Department of the No. 1 Emergency Hospital, Craiova, and from the archives of the “Filantropia” County Hospital in Craiova. During 1996–2007, 11,592 cases were registered in the Pathological Anatomy Laboratory from both health units, forming the basis of a retrospective study. The registered patients were aged 20–70-year-old, suffering of different utero–tubal pathologies requiring surgery.

From this group we selected a number of 30 cases of women aged 23–37-year-old undergoing hysterectomy. The gynecological diseases involving surgery and taken into consideration in our study were: leiomyofibroma, leiomyofibroma with pregnancy, ectopic pregnancies. The study was made with the permission of the local ethics committees and written and informed consent of the patients.

Aiming to analyze microanatomical sections of the UJT, there were selected 10 cases for each above-mentioned pathology, respectively 30 anatomical pieces (uterus and uterine tubes).

As mentioned the material studied within this research consists of uterus and uterine tubes collected during therapeutic surgeries. The collected pieces were prepared for micro- and macroscopy both in the Laboratory of Pathological Anatomy and Cytodiagnosis of the “Filantropia” County Hospital in Craiova, and in the Laboratory of Structural Anatomy in the Department of Human Anatomy of the University of Medicine and Pharmacy of Craiova.

Sagittal sections were performed at the level of uterine–tubal junction, the collected material having been fixed in neutral formalin, alcoholic lead nitrate-formalin, calcium formalin and ethanol chlorhydrate. Classic colorations such as Hematoxylin–Eosin, van Gieson, Cajal–Nonidez method, Toluidine Blue, Resorcinol–Fuchsin (Weigert), PAS reaction (McManus) were used. The myometrium structure was analyzed and evidence of pathological abnormality was recorded using standard histological criteria. The sections were examined by microscopy and there were captured digital images from a 3-CCD color camera Sony and within an image analysis program using a Matrox–Comet acquisition system and a Pentium MMX/233 Hz system. Low magnification images of the whole specimen were obtained by direct scanning of the sections (Nikon). Analyzed items included muscular cells, extracellular matrix and histo-topographic reports, among which “myometrial vascular units”, vascular and nervous structures at this level.

The results obtained by the mentioned methods used for the study of functional structures of myometrium in uterine–tubal region were qualitatively evaluated in an international marking system of affinity degree of structural elements against the reactives used: (-) – lack of affinity; (+) – low affinity; (++) – high affinity (Table 1).

### Table 1 – Qualitative marking system of the results obtained by methods used in studying functional structures of myometrium in uterine–tubal region

<table>
<thead>
<tr>
<th>No.</th>
<th>Method</th>
<th>Focused structure</th>
<th>Color</th>
<th>Name of affinity or reaction</th>
<th>Affinity degree</th>
<th>Marking system</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>vG</td>
<td>Collagenous fibers</td>
<td>Light-red</td>
<td>Picrofustinophilia</td>
<td>Absent</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High</td>
<td>++</td>
</tr>
<tr>
<td>2.</td>
<td>IAG</td>
<td>Reticuline fibers</td>
<td>Dark-black</td>
<td>Argyrophilia</td>
<td>Absent</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High</td>
<td>++</td>
</tr>
<tr>
<td>3.</td>
<td>IAN</td>
<td>Sympathetic post-ganglionar fibers</td>
<td>Light-brown</td>
<td>Argyrophilia</td>
<td>Absent</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parasympathetic fibers</td>
<td>Brown</td>
<td></td>
<td>Low</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neurofibriles and nervous endings</td>
<td>Black</td>
<td></td>
<td>High</td>
<td>++</td>
</tr>
</tbody>
</table>
Results

Statistical and mathematical analysis of uterine–tubal junction pathology

Analyzing the cases selected for the study of uterine–tubal junction on age groups, we determined that a number of 1822 cases were registered within the groups aged 20–29-year-old (918 cases) and aged 30–39-year-old (904 cases) (Table 2).

Table 2 – Distribution of the studied cases on age groups and types of surgeries

<table>
<thead>
<tr>
<th>Year</th>
<th>Ectopic pregnancy</th>
<th>Other surgeries</th>
<th>Total</th>
<th>&lt;20</th>
<th>20–29</th>
<th>30–39</th>
<th>&gt;40</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>165</td>
<td>566</td>
<td>731</td>
<td>4</td>
<td>66</td>
<td>83</td>
<td>12</td>
</tr>
<tr>
<td>1997</td>
<td>153</td>
<td>578</td>
<td>731</td>
<td>4</td>
<td>60</td>
<td>81</td>
<td>8</td>
</tr>
<tr>
<td>1998</td>
<td>169</td>
<td>667</td>
<td>836</td>
<td>1</td>
<td>71</td>
<td>85</td>
<td>12</td>
</tr>
<tr>
<td>1999</td>
<td>161</td>
<td>772</td>
<td>933</td>
<td>0</td>
<td>70</td>
<td>81</td>
<td>10</td>
</tr>
<tr>
<td>2000</td>
<td>161</td>
<td>873</td>
<td>1034</td>
<td>3</td>
<td>75</td>
<td>68</td>
<td>15</td>
</tr>
<tr>
<td>2001</td>
<td>162</td>
<td>896</td>
<td>1058</td>
<td>4</td>
<td>75</td>
<td>83</td>
<td>10</td>
</tr>
<tr>
<td>2002</td>
<td>160</td>
<td>910</td>
<td>1070</td>
<td>3</td>
<td>80</td>
<td>65</td>
<td>12</td>
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<td>2003</td>
<td>158</td>
<td>886</td>
<td>1044</td>
<td>2</td>
<td>78</td>
<td>70</td>
<td>8</td>
</tr>
<tr>
<td>2004</td>
<td>164</td>
<td>838</td>
<td>1002</td>
<td>1</td>
<td>79</td>
<td>73</td>
<td>11</td>
</tr>
<tr>
<td>2005</td>
<td>170</td>
<td>868</td>
<td>1038</td>
<td>0</td>
<td>85</td>
<td>75</td>
<td>10</td>
</tr>
<tr>
<td>2006</td>
<td>171</td>
<td>884</td>
<td>1055</td>
<td>0</td>
<td>90</td>
<td>73</td>
<td>8</td>
</tr>
<tr>
<td>2007</td>
<td>169</td>
<td>891</td>
<td>1060</td>
<td>1</td>
<td>89</td>
<td>67</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>1963</td>
<td>9629</td>
<td>11592</td>
<td>23</td>
<td>918</td>
<td>904</td>
<td>128</td>
</tr>
</tbody>
</table>

The geographical differences in terms of patients’ provenance (urban, rural) were insignificant. We could notice the high frequency of ectopic pregnancies in the casuistics offered by the Emergency County Hospital of Craiova within the studied period; however, this frequency is constant numerically within the entire time (average value of 160 cases per year).

Analysis of ratios between contractile structures and extracellular matrix elements

Our study showed that contractile elements in uterine–tubal junction are organized in fascicles of isolated myocytes through extracellular myometrial matrix elements.

On series sections, stained with Hematoxylin–Eosin, we could easily determine the topography of muscular fibers fascicles sectioned under variable angles: transversal, longitudinal and oblique (Figure 1). Extracellular matrix elements circumscribe contractile structures, transversally and longitudinally sectioned.

Fascicles of muscular cells depicted architectural alterations through the differentiations of structural elements. Hence, we could see clear spaces, margined by myocytes with ovoid nucleus; and at muscular cells periphery elements of extracellular matrix (Figure 1D).

Remodelling of extracellular matrix was realized through a vascular network, which separated fascicles from muscular cells (Figure 1A).

Fascicles of muscular cells sectioned longitudinally, transversally and oblique were in contiguity ratios with the structures of intercommunicating vascular network (Figure 1D).

Dissociation of muscular cell fascicles by extracellular matrix was associated with an angiogenesis process (Figure 1B).

![Figure 1](image-url)
We could easily notice the erase of fascicular structure of contractile elements and the appearance of new capillaries in regions with fascicles of muscular cells (Figures 1 and 2).

Figure 2 – A: Fascicles of myocytes sectioned transversally (1) and oblique (2) with structure apoptosis process and surrounded by an extracellular matrix containing numerous arterioles, venules, metaarterioles and capillaries; B: Myocytes with cytoplasm vacuolizations; karyorexis and karyolysis. Condensing extracellular matrix at the periphery of myocyte fascicles in structural apoptosis; C: Structural heterogeneity in a microscopic site of myometrium through the presence of two controversial processes: “structural apoptosis” and angiogenesis which are present both at the level of myometrial vascular unit (1) and myocyte fascicle (2); D: Detail on “myometrial vascular unit” (1). The vascular lumen is separated by endothelium with plate cells (2). Contractile elements are sectioned under variable angles. Extracellular matrix (3) appears condensed at the periphery of myometrial vascular unit. Hematoxylin–Eosin staining: oc. ×7, ob. ×20 – ×140 (A); oc. ×7, ob. ×40 – ×280 (B); oc. ×7, ob. ×20 – ×140 (C); oc. ×7, ob. ×40 – ×280 (D).

Besides this, we also observed intense phenomena of angiogenesis and re-directing of blood irrigation system from the territory occupied by extracellular matrix. The phenomenon of structural apoptosis of contractile units is present as well (Figure 2C).

On the sections colored using van Gieson method we could determine the presence of myocyte fascicles sectioned transversely and oblique, with processes of structure apoptosis and existence of extracellular matrix containing numerous arterioles, veins, meta-arterioles and capillaries (Figure 2). Under examination with objective ×40, myocytes presented cytoplasm vacuolizations; karyorexis and karyolysis; extracellular matrix is condensed at the periphery of myocyte fascicles under “structure apoptosis” (Figure 2B).

On series sections at the level of myometrium in uterine–tubal junction of pregnant uterus, we could also notice a structural heterogeneity of myometrium through the presence of two contrary processes “structure apoptosis” and angiogenesis. These processes happen both at the level of myometrial vascular unit and myocyte fascicle (Figure 2C).

Upon examination of objectives ×20 and ×40, we observed the presence of a process of angiogenesis between extracellular matrix and myocyte fascicles. We could also notice new capillaries formed in continuity ratios with myocytes; fascicles of muscular cells in “structural apoptosis” under the condition of an intense local microcirculation; histo-topographic ratios between fascicles of muscular cells elements of extracellular matrix. In the centre of the image, a metaarteriole with a permeable lumen can be seen; nuclear alterations of myocytes: pyknosis, nuclear vacuolizations, karyolysis, nucleol hypertrophy, stasis in interfascicular space, marking nuclear chromatin (Figure 3).

Figure 3 – A: Angiogenesis process at the limit between extracellular matrix and myocyte fascicles. We can notice new capillaries in progression ratios with myocytes; B: Muscular cell fascicles in „structural apoptosis” under intense local microcirculation conditions; C: Histotopographic ratios between muscular cell fascicles and extracellular matrix elements. We can notice a meta-arteriole with permeable lumen in the centre of the image; D: Nuclear alterations of myocytes: pyknosis (1), nuclear vacuolizations (2), karyolysis (3), delimitation of nuclear chromatin (4), nucleoli hypertrophy (5). Marked stasis in interfascicular space. Hematoxylin–Eosin staining: oc. ×7, ob. ×20 – ×140 (A–C); oc. ×7, ob. ×40 – ×280 (D).
Figure 4 presents the way argyrophile collagenous fibers are grouped in fascicles which, by crossing themselves, form a spatial plexiform network representing the support of myocyte fascicles.

**Discussion**

The human uterus is a pear-shaped organ, the gross morphology of which is determined by the nature of the myometrial smooth muscle coat. This muscle coat undergoes dramatic remodelling during pregnancy in order to accommodate the growing fetus [10, 11]. The uterus would appear to have specific features that are not observed in other adult organs, which allow this degree of remodelling. It has been postulated, for example, that the highly coiled nature of the myometrial arteries is designed to permit expansion of the myometrial smooth muscle as it hypertrophies during pregnancy.

In histological sections, the human myometrium is a continuous layer of smooth muscle cells organized in large interwoven bundles [2]. This pattern is distinct from the circular and longitudinal layering of the myometrial smooth muscle observed in other species such as the mouse, which has a bicornuate uterus. Despite the lack of distinct morphological layering in the human uterus, evidence has emerged of distinct inner and outer zones.

In the non-pregnant uterus, highly specialized contraction waves originate exclusively from the junctional zone and participate in the regulation of diverse reproductive events, such as sperm transport, embryo implantation, and menstrual shedding [12].

In Figure 5, we can notice the presence of some nervous branches at the level of extracellular matrix, parallel with myocyte fascicles and perpendicular on the wall of arterioles.

In Figure 5, **A, B**: In extracellular matrix, we can notice the presence of nervous branches with variable trajectories: parallel with myocyte fascicles and perpendicular on arterioles wall. Silver staining on Cajal–Nonidez block: oc. ×7, ob. ×10 – ×70 (A); oc. ×7, ob. ×20 – ×140 (B).

![Image](image-url)
Complex coordinated contractions of the tubar musculature are thought to be important for movement of the ovum from the distal end to the proximal end of the tube, while at the same time aiding in the movement of sperm from the proximal end to the distal end of the oviduct. Studies of the anatomy and the physiology of the uterine–tubal junction with a main concern for the muscular pattern and the secretory activity of this part of the tube, in relation with the hormonal and nervous control can play an important role in understanding and impairment of infertility [18, 19].

Speaking about the pathology of the UTJ that can impair fertility, many authors identify different causes: chronic infections and their sequelae, salpingitis isthmica nodosa, polyps, iatrogenic lesions (mainly following sterilization procedures), congenital malformations [20–22].

Other pathologies might be ectopic pregnancies, 2.5% of all tubal pregnancies, and neoplasms even if not related to infertility. In 2002, 12% of women aged 15–44-year-old (7.3 million women) had impaired fecundity. This number has increased about 2% from the levels seen in 1988 and 1995 [9].

Adrenergic nerves mainly innervate the human oviduct, particularly circular smooth muscles.

The key question regarding a further potential of developing a new type of anti-fertility component is based on the autonomous mechanism neuro-receptor on smooth muscles in oviduct, this mechanism having an essential role in ovum transport. Experimental studies in this field have been limited to laboratory animals due to practical reasons.

In general, the problems aroused in deciding the role of oviduct motility in ovum transportation are considerable [23]. Some of them appear to be of conception nature, others methodological ones. The methods used in registering oviduct motility are criticized and have no scientific support [6].

From the conceptual point of view, it is highly necessary to define the questions and the ways of getting the answers. Methodologically, we have to determine the techniques applied in obtaining these answers.

Recording oviduct motility arises difficult problems due to the varied, tight caliber of lumen, muscle layers, low lumen volume, the lack of a distinct separation of muscular layers, the presence of a high fat quantity, linking tissue and collagen around extern layer and different secretions in oviduct. In addition, it is very difficult to observe and record ovum transportation.

In relationship with these studies, our research demonstrated that the endometrio–myometrial interface constitutes a distinct, hormone-dependent uterine compartment, as termed the junctional zone. Conversely, growing evidence suggests that disruption of the normal endometrio–myometrial interface plays an integral role in diverse reproductive disorders. This study reviews our current understanding of the mechanisms that govern the cyclic changes in the uterine junctional zone and summarizes the evidence implicating the endometrio–myometrial interface in uterine pathological processes.

Conclusions

The balance between contractile structures and extracellular matrix elements at the level of uterine–tubal junction is hormone-reliable and is maintained on the one hand, through reactionary capacities of myocytes and on the other hand, by extracellular matrix elements. Myocytes contribute to the genesis process of “novo” of blood vessels (vasculogenesis) and remodelling of the myocyte fascicles through apoptosis determines the reaction of extracellular matrix translated by the increase of collagen quantity, fibroblasts and intense angiogenesis processes (forming new vessels by blossoming the existent ones).

Reliability of uterine wall under its cavity dilatation due to pregnancy is mainly determined by myometrial vascular components. From this point of view, the uterus, as a whole, is a vasculary system with variable morphology. Continuity ratios between myocyte fascicles and average tunica of arteries and arterioles explain the existence of “vascular units determined by myometrical structures”.

In addition, stereo-distribution of “myometrial vascular units” was observed, explaining the existence of a plexiform network of cavernous type, which has determined many authors to describe numerous myometrical tunicas that could not be individualized. These units have a rich ortho-sympathetic vegetative innervation, which might be responsible for the unfavorable evolution of myometrical circulatory system postpartum.

Taking into consideration all these observations, we have to reconsider the structure of uterine wall and, particularly, of uterine–tubal junction, description of three myometrical layers: extern, middle and intern being utopic, as “stratum vasculosum” described in classical studies as representing the internal layer of myometrium is a morphologic reality in the entire thickness of myometrium. These observations explain the so-called “in vivo connections” which appear in the moment of deliverance.

References

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Received: August 5th, 2009
Accepted: October 20th, 2009