Intraoperative Optical Diagnostics of Uterine Microcirculation during Myomectomy

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Abstract. Previous studies have shown that intraoperative optical diagnostics can be used to solve fundamental and applied problems in gynecology. Our article presents results from experimental *in vivo* intraoperative measurements of endogenous fluorescence and blood perfusion in various sections of the uterus, myometrium, endometrium, myomas, and pseudocapsule in female patients undergoing myomectomy. We found that myomas have different blood supply and fluorescence level at wavelength 365 nm depending on their growth intensity and size, most likely due to collagen accumulation in the extracellular matrix and changes in the vascular architecture. The possibility of laser Doppler flowmetry to assess endometrial perfusion is also demonstrated for investigating the effect of myomas of different types on endometrial tissue blood supply. The results obtained can be used to provide a better understanding of pathological processes in uterine leiomyoma, thus helping physicians to choose treatment strategies.

Keywords: optical methods; fluorescence spectroscopy; laser Doppler flowmetry; gynecology; fibroids; myomectomy.

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1 Introduction

Uterine leiomyoma (LM) ranks second among gynecological pathologies and is the most common benign tumor in both women of reproductive age and perimenopausal women. The incidence of the disease is reported to reach up to 70-80% of woman, depending on the cohort studied and the methods of diagnosis [1–3]. Normally, the vascular network of the uterus has an organized structure, and the myometrium has a large number of capillaries to ensure adequate blood supply during pregnancy [4, 5]. LM is characterized by overgrowth of myometrial smooth muscle cells containing extracellular matrix components (fibronectin, collagen, proteoglycan) [6]. During its growth, the extracellular matrix forms a pseudocapsule around the myoma, which separates it from the normal myometrium [7].

Laparoscopic myomectomy is a surgical treatment for symptomatic LM. Despite uterine LM being one of the most well-studied uterine diseases, and laparoscopic myomectomy being a recognized treatment method, a number of unresolved questions remain. The issues of surgical tactics, including the need to preserve the pseudocapsule when removing the myoma during the laparoscopic intracapsular myomectomy [8], and the development of substantiated medical grounds for myomectomy in women with intramural myomas who are planning pregnancy, remain important [9]. The application of optical technologies, which are currently being successfully introduced in minimally invasive surgery [10–13], may help to solve some fundamental and applied problems in gynecology.

Minimally invasive myomectomy techniques aim to remove LM while preserving healthy uterus. Postoperative vascularization of the myometrium is

crucial for the regeneration of damaged tissue, and the myoma pseudocapsule is hypothesized to play an important role in this process. The removal of the pseudocapsule during myomectomy can lead to a decrease in neuropeptides and nerve fibers, and hence, to poor physiological healing of the myometrium with increased fibrosis due to hypoxia and ischemia [14].

To date, little data have been accumulated on instrumental study of the blood supply in the fibrovascular network surrounding LMs. A. Malvasi et al. examined the structure of a pseudocapsule using electron microscopy and concluded that this anatomical structure contains a neurotransmitter-rich vascular network like that of a neurovascular bundle [15]. Standard transvaginal ultrasound mapping of myomas has demonstrated increased blood flow in the pseudocapsule, which is described as a surrounding ring containing clearly visualized vessels [16, 17]. Additional information about the state of blood microcirculation in the pseudocapsule and surrounding tissues can provide new knowledge about the organization of blood supply in this structure.

The search for new objective criteria for performing myomectomies in patients planning pregnancy is of current interest in practical gynecology. The necessity of surgical treatment of intramural LMs in patients with infertility who are planning pregnancy using assisted reproductive technologies based solely on LM size exceeding 4 cm in the absence of clinical manifestations is a controversial issue [9]. A promising direction in surgical treatment for women of reproductive age is the development of new minimally invasive methods to assess the impact of LM on reproductive function. One indicator of the functional state of the endometrium is whether or not it has an adequate blood supply. Among the instrumental diagnostic methods to study blood flow, there are existing results from Doppler studies of both endometrial and sub-endometrial blood flow in patients with intramural LMs. In particular, 3D power Doppler parameters were observed to be similar in patients with and without small intramural LMs [18].

Optical diagnostic methods provide great opportunities for the study of blood microcirculation and metabolic processes in biological tissues. The method of laser Doppler flowmetry (LDF) is based on measuring the Doppler shift in the spectrum of the reflected laser signal scattered by moving red blood cells. This technology allows one to carry out a dynamic quantitative assessment of perfusion in the microvascular bed of various tissues. In gynecology LDF is recognized as a highly sensitive method for measuring localized endometrial perfusion [19–23]. The use of wavelet transforms to analyze these LDF signals offers broad diagnostic opportunities. This approach helps to evaluate the contribution of individual microvascular bed components to the modulation of blood flow in various physiological and pathological processes [24, 25].

Fluorescence spectroscopy (FS) is based on the excitation of fluorescence in biological tissues by UV or visible light, with subsequent registration of the emitted radiation on a spectrometer (after passing through appropriate filters). FS is widely used to assess the condition of biological tissues in medicine, including gynecology [26, 27]. The most prominent endogenous fluorophores in biological systems are NADH, flavins, collagen and elastin, porphyrin, etc.

Total fluorescence emission contains a lot of diagnostically important information, since it represents the sum of contributions of fluorophore autofluorescence.

In the present study, we used optical techniques of FS and LDF in two separate examinations to demonstrate intraoperative assessments of perfusion and metabolic characteristics of myometrium, myoma, pseudocapsule and endometrium. The first examination was focused on the study of myoma perfusion in women with rapidly growing large-sized LMs and with slowly growing LMs below 5 cm. The second examination evaluated the effect of intramural and intramural-submucosal LMs on endometrial perfusion and metabolic characteristics.

2 Methods and Materials

2.1 The System for Intraoperative Assessment of Perfusion-Metabolic Changes in Biological Tissues

Measurements were performed using a specially designed fiber-optic system (Fig. 1), which implements both FS and LDF methods and includes measurement channels of the diagnostic complex “LAKK-M” (SPE “LAZMA” Ltd, Russia) and a laparoscopic fiber-optic probe (the main units were designed together with SPE “LAZMA” Ltd, Russia) [10]. The probe has a 3 mm diameter rigid part, which provides control and fixation at the analysis point.

To record optical signals, the laparoscopic fiber-optic probe was inserted into the pelvic cavity through the instrumental channel.

In the LDF channel, tissues were probed by laser radiation and subsequent registration of dynamic light scattering at a wavelength of 1064 nm. The LDF 3.2.0.441 program (SPE “LAZMA” Ltd.) was used for frequency analysis of the registered signals. This program implements a continuous wavelet transform using a complex Morlet wavelet as an analyzing wavelet.

In the FS channel, radiation sources with wavelengths of 365 nm and 450 nm, respectively, can be used to excite endogenous fluorescence of biomarkers. Fluorescence spectra across the range of 350–820 nm are then recorded by the spectrometer.

2.2 Study Protocol

The pilot experimental studies were carried out at the Research Institute of Obstetrics, Gynecology and Reproductology named after D.O. Ott (St. Petersburg, Russia), and involved a total of 18 female patients.

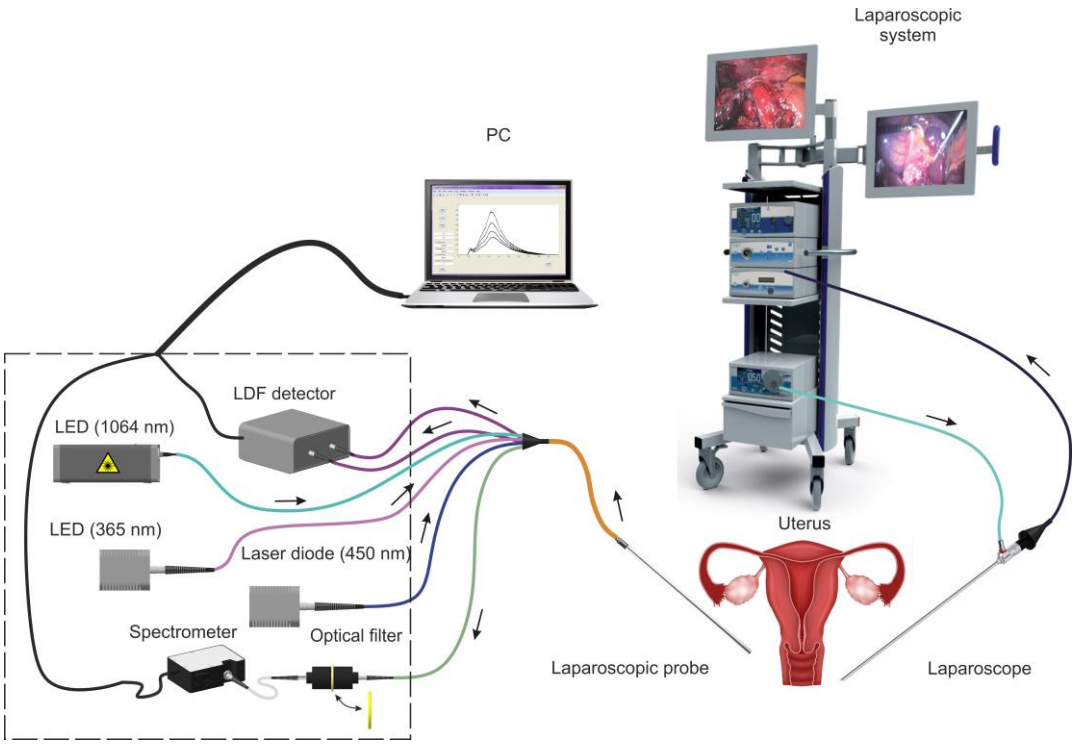


Fig. 1 Schematic of the experimental setup for intraoperative study of perfusion-metabolic changes in biological tissues.

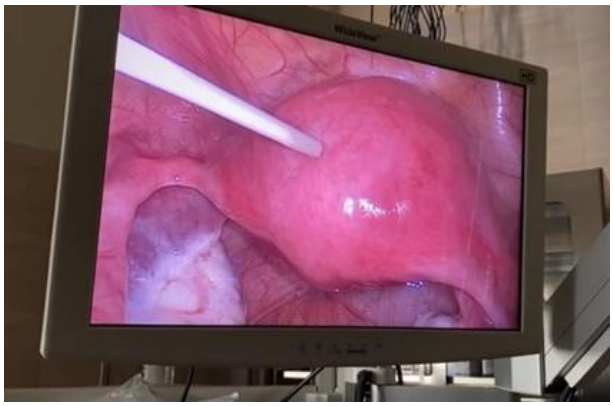


Fig. 2 Monitoring of fiber-optic probe position during intraoperative myometrial perfusion measurement by LDF method.

The studies were performed as an additional diagnostic procedure within a scheduled surgery and were approved by the ethical committee of the Research Institute of Obstetrics, Gynecology and Reproductology named after D. O. Ott (minutes of the meeting No 110, June 10, 2021). The study site was chosen for each woman based on the convenience of the main gynecological surgery.

Participants signed an informed consent indicating their voluntary willingness to participate in the study. LDF and FS signals were recorded in the tissues of the endometrium, myometrium, myoma, and pseudocapsule. The position of the fiber-optic probe was controlled via the monitor on the laparoscopic rack (Fig. 2).

Fig. 3 shows the exemplary records of blood perfusion signal, the corresponding wavelet spectra and fluorescence spectra from the uterine LM.

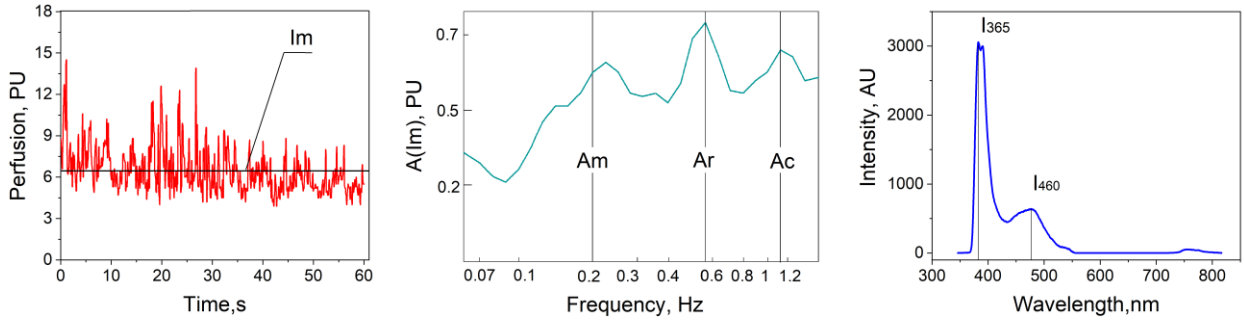
The duration of LDF signal recording was at least 1 min, to enable further selection of 60 s recordings without motion artifacts. Blood microcirculation index (Im, perfusion units, PU) was analyzed during the experimental studies according to the standard technique (Fig. 3a) [11, 25, 28]. Due to the limited duration of LDF recordings, the time-averaged amplitude of blood flow oscillations was estimated by the maximum values in three frequency bands: myogenic (Am) range: 0.047–0.145 Hz, respiratory (Ar) range: 0.2–0.4 Hz, and cardiac (Ac) range: 0.8–2 Hz, respectively (Fig. 3b) [24].

The FS channel was used to record fluorescence spectra excited at a wavelength of 365 nm. Parameters for analysis were the maximum fluorescence amplitudes (I_{365}) in the range of 450–470 nm, normalized to the intensity of backscattered excitation radiation (I_{460}), respectively ($I_{460norm}$), associated with NADH and collagen fluorophores (Fig. 3c) [29].

2.3 Examinations

2.3.1 Examination 1. Study of Perfusion and Metabolic Changes in Myometrium, Myoma and Pseudocapsule in Women with Different Sizes and Intensities of Myoma Growth Rate.

The study involved 8 women of reproductive age with uterine LM diagnosed by pelvic ultrasonography. Pregnant women, peri- and postmenopausal women (cessation of menstruation for > 1 year), women with endometriosis, adenomyosis or other pelvic neoplasms,



(a) (b) (c)

Fig. 3 Exemplary blood perfusion record (a), the corresponding wavelet spectra (b), and fluorescence spectra (c) from the uterine LM.

and patients with pelvic inflammatory diseases were excluded. Two groups were defined based on the medical history: group 1 – women with LMs of large size (6–8 cm) and having rapid growth (more than 4 weeks of conditional pregnancy per year); group 2 – patients with smaller LMs (4–5 cm), abnormal uterine bleeding, growth dynamics not exceeding 1 cm per year. LDF and FS signals were recorded in myometrium, myoma, and pseudocapsule tissues.

2.3.2 Examination 2. Effects of Intramural and Intramural-Submucosal Myomas on Endometrial Perfusion

Twenty patients with myomas of different types were examined. Two groups were identified, based on the location of myomas: group I – 10 patients with one or several intramural-submucosal LMs not exceeding 5 cm in size, type 2–3 according to FIGO (The International Federation of Gynecology and Obstetrics) uterine myoma classification; group II – 10 patients with intramural, intramural-subserosal LMs up to 5 cm in diameter, type 5–6 (FIGO). Exclusion criteria were hypergonadotropic ovarian insufficiency, chronic endometritis, and prior history of endometrial hyperproliferative disease. The studies were performed on the 8–11 days of the menstrual cycle. LDF and FS signals were recorded only in endometrial tissue.

2.4 Statistical Analysis

Statistical analysis was performed using Origin Pro software (OriginLab Corporation). Hemodynamic parameters and fluorescence intensities are shown as the Me(Q1–Q3), where Me is the median, Q1 is the first quartile, Q3 is the third quartile. Given the limited sample size, the nonparametric Mann-Whitney *U*-test was chosen for checking the reliability of statistical differences in the results. The *p*-value of less than 0.05 was considered significant.

3 Results and Discussion

3.1 Examination 1. Study of Perfusion and Metabolic Changes in Myometrium, Myoma and Pseudocapsule in Women with Different Sizes and Intensity of Myoma Growth Rate.

The results are presented in Table 1. Group 1 – women with large myomas of 6–8 cm, with rapid growth (more than 4 weeks of conditional pregnancy per year) are designated as "↑↑growth of myoma"; group 2 – patients with myomas of 4–5 cm, with myoma growth not exceeding 1 cm per year – "↑growth of myoma".

Table 1 Hemodynamic parameters and fluorescence intensities of uterine tissues.

Parameter	Group	Im, PU	Am, PU	Ar, PU	Ac, PU	$I_{460norm}$, AU
Myometrium	↑↑growth of myoma	5.1* (3.4–6.8)	0.54* (0.45–0.73)	0.51* (0.32–0.80)	0.56* (0.39–0.83)	0.49* (0.48–0.49)
	↑growth of myoma	9.3 (9.2–9.3)	1.03 (0.90–1.13)	1.21 (1.12–1.60)	1.22 (1.06–1.27)	0.30 (0.27–0.32)
Myoma	↑↑growth of myoma	3.2* (2.3–3.8)	0.47* (0.36–0.58)	0.39* (0.31–0.42)	0.34* (0.27–0.37)	0.60* (0.49–0.61)
	↑growth of myoma	7.0 (6.4–7.8)	0.76 (0.62–1.03)	0.76 (0.69–0.95)	0.75 (0.69–0.99)	0.25 (0.23–0.27)
Pseudocapsule	↑↑growth of myoma	5.8* (4.9–6.0)	0.64 (0.51–0.78)	0.57* (0.41–0.68)	0.52 (0.38–0.66)	–
	↑growth of myoma	7.9 (7.0–8.2)	0.83 (0.65–0.94)	0.84 (0.63–2.41)	0.76 (0.56–0.81)	7.9 (7.0–8.2)

*$p < 0.05$.

Processing of the data showed that myometrial blood microcirculation was a variable parameter in both groups (5.1 [3.4–6.8] PU and 9.3 [9.2–9.3] PU respectively). However, the level of myometrial perfusion was significantly higher in the group with slow–growing myomas. The high value of myometrial blood microcirculation in group 2 and the amplitudes of myogenic (1.03 [0.90–1.13] PU) and cardiac (1.22 [1.06–1.27] PU) oscillations confirms the assumption that the characteristic feature of uterine blood circulation is the vasomotor nature of blood movement through microvessels. At the same time, intermittent blood flow in the uterine capillaries is primarily due to changes in the tone of precapillary sphincters [30]. The results also show that the mean perfusion measured in the pseudocapsule was higher in the group of "↑growth of myoma" than in the comparison group (5.8 [4.9–6.0] PU vs 7.9 [7.0–8.2] PU). A similar pattern was observed in myomas: 3.2 [2.3–3.8] PU and 7.0 [6.4–7.8] PU, respectively.

Thus, opposite to the expectations that higher perfusion would be observed in the group of patients with rapidly growing myoma, we obtained the opposite result. These findings may be explained as follows. In the presence of triggering factors, the increasing size of the LM may cause deformation or even complete compression of the vessels of the pseudocapsule feeding the LM. Unlike the normal vascular network, which matures and stabilizes rapidly, the blood vessels of myomas have structural and functional characteristics. The vascular network of LMs has a chaotic organization, with tortuosity and increased vascular permeability. This means that once LMs reach a certain size, their blood supply is impaired to some extent, resulting in reduced microcirculation [31]. Further studies including Doppler sonography are needed to clarify the true reasons for the higher level of blood microcirculation in the group with slow-growing LMs.

For additional analysis, a portion of the results from Table 1 were grouped together and presented as a bar chart in Fig. 4.

Fig. 4a demonstrates that the pseudocapsule is better supplied with blood than the myomas in both groups, confirming evidence that this structure is the vascular capsule surrounding the LM and providing its nutrition [7, 32]. An interesting histopathological feature of LMs is the difference in structure of their vascular network compared with the adjacent myometrium. Previous studies have shown that LMs have lower vascular area and microvascular density than adjacent myometrium [33–35]. It was long believed that solid tumors require increased microvascular density to support the growth of the tumor mass. In the present study, no statistical differences were found between the blood microcirculation levels of LMs and the adjacent myometrium, but Fig. 4a shows that the perfusion of myomas is generally lower.

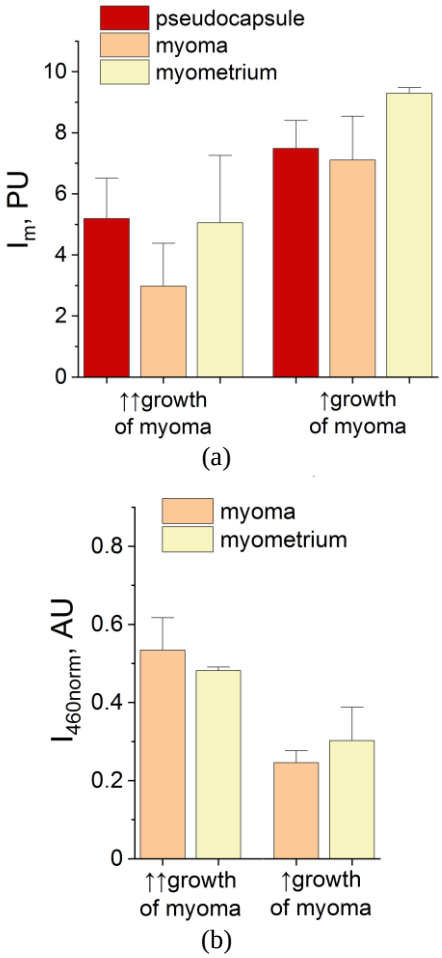


Fig. 4 Indices of microcirculation (a) and fluorescence intensity (b) of uterine tissues in the compared groups.

Of particular interest is the result that blood microcirculation in the pseudocapsule of rapidly growing LMs ("↑↑growth of myoma" group) significantly exceeds the blood supply of the myoma itself (5.8 [4.9–6.0] PU vs 3.2 [2.3–3.8] PU). When analyzing FS channel data, we noticed that $I_{460norm}$ fluorescence signal increased in rapidly growing large LMs along with decreased perfusion compared to the surrounding myometrium (0.60 [0.49–0.61] AU vs 0.49 [0.48–0.49] AU) (Fig. 4b). We associate this observation with the fact that myomas in the rapid growth group probably continue to enlarge as a result of both increased myocyte proliferation and synthesis and deposition of extracellular matrix [36]. Collagen from the extracellular matrix can be present to varying degrees in tumors of the compared groups. It is known that at the late stages of development, collagen can account for 10–50% of tumor mass of myomas, which exceeds the content of collagenous component in myometrium [37, 38]. In addition, there is an observation that insufficient blood supply to the tumor itself develops at this stage and this eventually leads to interstitial ischemia [36]. This also corresponds to the greater decrease in perfusion in the myoma compared to the surrounding pseudocapsule in the group with large LMs and rapid growth.

Table 2 Hemodynamic parameters and endometrial fluorescence intensities depending on myoma type.

Parameter	Im, PU	Am, PU	Ar, PU	Ac, PU	$I_{460norm}$, AU
I group, type 2–3 (FIGO)	3.6* [2.0–5.3]	0.3 [0.3–0.4]	0.2 [0.2–0.4]	0.4 [0.2–0.6]	0.45 [0.29–0.47]
II group, type 5–6 (FIGO)	7.6 [6.0–8.6]	0.4 [0.3–0.7]	0.3 [0.2–0.5]	0.5 [0.3–0.9]	0.26 [0.23–0.34]

*$p < 0.05$.

Improvements in our knowledge of uterine tissue blood microcirculation can play an important role in understanding the role of the pseudocapsule as a distinct anatomical and surgical structure, as well as in understanding tumor growth processes, which will further help to determine the correct treatment tactics for patients with uterine LM.

3.2 Examination 2. Effects of Intramural and Intramural–Submucosal Myomas on Endometrial Perfusion

The results obtained by optical diagnostic methods are summarized in Table 2.

Unfortunately, we cannot compare the endometrial blood flow values given in Table 2 with those measured by LDF in previous studies [19, 20], owing to the differences in probes used and study protocols.

Processing of the obtained data showed a statistically significant decrease of the microcirculation index in the endometrial tissues in the group of patients with type 2–3 LMs (FIGO) of the uterine cavity (3.6 [2.0–5.3] PU vs 7.6 [6.0–8.6] PU). There is a tendency towards decreased oscillation amplitudes across all bands of endometrial microcirculatory flow regulation, however this did not reach statistical significance. Although the LDF method is semi–quantitative and does not give absolute values for blood flow, it can be used to perform a qualitative assessment of endometrial microhemodynamics.

When analyzing FS data, we noticed an increase in the $I_{460norm}$ signal in group I patients compared to group II patients: 0.45 [0.29–0.47] AU vs 0.26 [0.23–0.34] AU. It is known that in the proliferative phase of the menstrual cycle (during which all patients were studied), type VI collagen is abundantly present in the endometrium [39]. We suggest that the increased $I_{460norm}$ signal may be related to abnormal collagen accumulation in the extracellular matrix or to the accumulation of NADH, associated with circulatory insufficiency and the initial stages of hypoxia [40].

Women with unexplained infertility are known to have impaired endometrial perfusion [41], implying that an adequate blood supply to the endometrium is required for implantation. Our results suggest that endometrial blood perfusion in patients with LMs of the uterine cavity is reduced and may be the cause of impaired receptivity. Meanwhile, intramural LMs retain high endometrial tissue perfusion. Thus, optical technologies are promising diagnostic methods for the analysis of endometrial blood supply.

To date, there are conflicting reports that the intramural localization of LM negatively affects fertility. Physicians should have clear criteria to perform myomectomy and to predict the effectiveness of surgical treatment for uterine LMs. Surgery significantly increases the risk of myoma recurrence and has complications associated with uterine scarring. Additional diagnostic information may help in choosing a treatment strategy for LMs, including non–surgical options [9].

4 Conclusion and Limitations

The described experimental studies confirm the possibility of intraoperative tissue diagnostics through optical methods of FS and LDF during myomectomy. The introduction of these methods into endoscopic gynecological surgery seems promising, extending the capabilities of standard laparoscopy by allowing the surgeon to obtain additional information about perfusion and metabolic characteristics of uterine tissues.

These technologies still have some limitations. They are spectroscopic methods providing single–point measurements, which creates a potential source of error. However, this measurement uncertainty can be minimized by standardizing the location at which signals are registered, and by performing multiple measurements to gain average values.

An important limitation of the presented work is the small number of patients included in each sample, especially in Examination 1. The study requires further data collection in order to form clinical guidelines. The article aims to demonstrate the potential of multimodal optical diagnostics during endoscopic procedures in gynecology.

Another limitation of the study was the lack of a control group. The composition of the control group was limited by a number of problems, including ethical ones: (1) it is impossible to perform laparoscopy in completely healthy patients without any medical grounds; (2) comparison with conditionally healthy myometrium in patients undergoing diagnostic laparoscopy for infertility can also be inaccurate, as most of them may be diagnosed with pathological changes in the endometrium, hormonal disorders, or inflammatory diseases of the pelvic organs; (3) if there are no visible myomas, we cannot be sure that the patient does not have adenomyosis, as there are currently no non–invasive diagnostic methods that provide an accurate diagnosis.

The research described is a pilot study. The data obtained during this study indicate the need for its extension. We suggest that further collection of data on the blood supply to the pseudocapsule may provide additional evidence to justify preserving this structure and preferably performing a laparoscopic intracapsular myomectomy. In our work, we also demonstrated that type 5–6 (FIGO) myomas do not affect endometrial microcirculation in the way that type 2–3 (FIGO) myomas do. A subsequent study will look more closely at patients with type 5–6 (FIGO) myomas checked by Doppler sonography. The practical value will lie in the development of more valid medical grounds for myomectomy in this LM localization.

Disclosures

All authors declare that there is no conflict of interests in this paper.

Acknowledgements

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