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Surgical removal of intramural fibroids improves the TNF-α induced inflammatory events in endometrium

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Abstract. – OBJECTIVE: To investigate whether surgical removal of intramural fibroids makes any changes in the expression of endometrial tumor necrosis factor alpha (TNF-α) and nuclear factor kappa B (NF-κB).

PATIENTS AND METHODS: 20 patients who had no pathology other than intramural fibroid as a cause of subfertility were included in the study. 6 patients who planned tubal ligation were considered as the control group. Type 3 or 4 fibroid diagnosis was made according to the revised International Federation of Gynecology and Obstetrics (FIGO). At the end of the diagnostic and confirmatory tests, 8 patients were diagnosed with type 3 and 12 patients with type 4 fibroids. While the patients in the fibroid group went to myomectomy, the patients in the control group went to tubal ligation. Before the myomectomy, endometrial sampling was performed with a pipelle cannula under anesthesia. The same procedure was repeated three months after the treatment. The same endometrial sampling procedure was also performed on the patients in the control group. TNF-alpha and NF-κB levels were measured with enzyme-linked immunosorbent assay (ELISA) in endometrial samples taken before and three months after myomectomy.

RESULTS: Pre-myomectomy TNF-α levels of the patients in the type 3 fibroid group were significantly higher than the control group (5.10±1.30 *vs.* 2.50±0.11, $p<0.01$). Similarly, the pre-myomectomy TNF-α levels of the patients in the type 4 fibroid group were significantly higher than the control group (4.73±1.76 *vs.* 2.50±0.11, $p<0.01$). There was no significant difference between pre-myomectomy endometrial TNF-α levels of patients in type 3 and 4 fibroid groups (5.10±1.30 *vs.* 4.73±1.76, $p>0.05$). Removal of type 3 fibroids by myomectomy significantly decreased TNF-α levels (5.10±1.30 *vs.* 2.20±0.44, $p<0.03$). Similarly, removal of type 4 fibroids by myomectomy significantly decreased TNF-α levels (4.73±1.76 *vs.* 2.60±0.30, $p<0.04$). Pre-myomectomy NF-κB levels of the patients in the type 3 fibroid group were significantly higher than the control group (2.42±0.39 *vs.* 1.09±0.60, $p<0.02$). Similarly, the pre-myomectomy NF-κB levels of the patients in the type 4 fibroid group were significantly higher than the control group (2.04±0.50 *vs.* 1.09±0.60, $p<0.01$). There was no significant difference between the pre-myomectomy endometrial NF-κB levels of the patients in the type 3 and 4 fibroid groups (2.42±0.39 *vs.* 2.04±0.50 $p>0.05$). Removal of type 3 fibroids by myomectomy significantly decreased NF-κB levels (2.42±0.39 *vs.* 1.02±0.33, $p<0.01$). Similarly, removal of type 4 fibroids by myomectomy significantly decreased NF-κB levels (2.04±0.50 *vs.* 0.97±0.02, $p<0.02$).

CONCLUSIONS: Surgical removal of type 3 or type 4 fibroids contributes positively to receptivity by down-regulating endometrial TNF-α and NF-ƙB.

Key Words:
Fibroid, FIGO, Myomectomy, NF-κB, TNF-α.

Introduction

Although a definite cause-effect relationship cannot be demonstrated between fibroids and subfertility, there is a strong relationship between the location of the fibroid and the fertility outcome. Submucosal fibroids constitute the most prominent group with subfertility effect. Fibroids in this location cause subfertility and early pregnancy loss by disrupting sperm-oocyte interaction mainly by mechanical effect. In addition, the endometrium extending over the submucosal fibroid is defective in terms of receptivity gene expression. Globally reduced expression of essential receptivity genes in patients with submucous fibroids has long been known[1]. Moreover, fibroids in this location cause insufficient decidualization and subfertility, as they increase endometrial proinflammatory cytokine synthesis[2].

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It is generally accepted that subserous fibroids do not cause subfertility or have little effect on fertility[3]. Controversy continues regarding the subfertility effects of intramural fibroids[3-5]. While some studies[3,4] suggest that intramural fibroids negatively affect fertility, some scholars[6] admit that they have no effect on fertility. In the 2018 revised International Federation of Gynecology and Obstetrics (FIGO), intramural fibroids were reclassified, but the definition remained unchanged[7]. Accordingly, fibroids with total intramural localization and compression on the endometrium were defined as type 3. However, type 3 fibroids are classified as submucous because of their contact with the endometrium. Total intramural localized fibroids that do not come into contact with the endometrium are defined as type 4[6,7]. This new definition has raised the possibility that the subfertility effects of both fibroid types are different. In studies to date, FIGO type 3 and 4 fibroids have been examined under a single heading and their subfertility effects have been defined accordingly. The effects of type 3 fibroids on subfertility are considered the same as submucous fibroids. It has been shown that type 4 fibroids that cannot compress the cavity impair the expression of endometrial receptivity genes[8]. While it has been reported that type 3 fibroids increase endometrial proinflammatory cytokine levels[9], there is no study on whether type 4 fibroids affect cytokine production. In addition, the effect of removal of type 3 or 4 fibroids by myomectomy on proinflammatory cytokine levels has not been investigated.

Tumor Necrosis Factor (TNF) is a cytokine[10] that stimulates systemic inflammation through alpha leukocyte adhesion molecules. In the presence of TNF-α, inflammation is stimulated because the synthesis and release of nuclear factor kappa B (NF-κB), intercellular cell adhesion molecule 1 and vascular cell adhesion molecule 1 are up-regulated[11]. NF-κB is in the form of a homo- or heterodimer and is found in the cytoplasm in a non-functional form due to an inhibitor protein. This dimer is involved in the regulation of cell survival, inflammation and apoptosis[12-14]. It is not known how the endometrial TNF-α and NF-κB pathways are affected by the presence of intramural fibroids. In addition, there is no data on whether myomectomy changes the level of these cytokines. This study was planned to investigate whether surgical removal of FIGO types 3 and 4 fibroids makes any changes in the expression of endometrial TNF-α and NF-κB.

Patients and Methods

Patients Selection and Grouping

20 patients who had no pathology other than intramural fibroid as a cause of subfertility were included in the study. 6 patients who planned tubal ligation were considered as the control group. Patients in the control group were selected from fertile patients with regular cycles and at least 3 children. Type 3 or 4 fibroid diagnosis was made according to the revised FIGO. Type 4 fibroid diagnosis was made by transvaginal sonography. Similarly, the preliminary diagnosis of type 3 fibroid was made by ultrasonography. Cases with a prediagnosis of type 3 fibroid were evaluated by saline infusion sonography or office hysteroscopi, and it was confirmed whether there was compression on the endometrium. At the end of the diagnostic and confirmatory tests, 8 patients were diagnosed with type 3 and 12 patients with type 4 fibroids. Magnetic resonance imaging (MRI) was not required in the differential diagnosis. While the patients in the fibroid group went to myomectomy in the window of implantation, the patients in the control group went to tubal ligation. Mid-luteal phase was determined by ultrasonographic confirmation of ovulation or by measuring serum progesterone levels. The mid-luteal phase was considered 7 to 9 days after ovulation. Patients went for either laparoscopic or abdominal myomectomy. Fibroids were successfully removed in all patients. Patients requiring simultaneous hysteroscopy were excluded from the study. TNF-α and NF-κB levels were measured in endometrial samples taken before and three months after myomectomy. Being diagnosed with type 3 or type 4 fibroid, having a histopathological diagnosis of fibroid after myomectomy, not using hormonal medication, or using an intrauterine device before and after myomectomy were considered as inclusion criteria. Patients with concurrent fibroids at other locations, presence of multiple fibroids, endometrial polyps, Asherman's syndrome, hydroslapinx, endometriosis, and a history of previous myomectomy were not included in the study. Those with a previous history of miscarriage or early pregnancy termination were also excluded from the study. Routine laboratory and radiological tests were requested from all participants. Those with pathology in hysterosalpingography or semen analysis were excluded from the study. This study was conducted after obtaining local ethical approval from Gözde Akademi Hospital and patient consent.

Endometrial Sampling

Before the myomectomy session, endometrial sampling was performed with a pipelle cannula under anesthesia. The same procedure was repeated three months after the treatment. Endometrial samples were washed with sterile saline solution at least twice, and blood and clots were removed. The obtained clean endometrium tissue was placed in RNA stabilization buffer and stored at -20°C until analysis. The same endometrial sampling procedure was also performed on the patients in the control group.

Endometrial Tissue TNF-α and NF-κB Analysis with ELISA

NF-κB and TNF-α concentrations in endometrial samples were measured quantitatively by enzyme-linked immunosorbent assay (ELISA) using human NF-κB and TNF-α ESLISA kits (Cusabio Biotech Co., Ltd., WUHAN, CHINA). The detection range of the NF-κB kit was 0.3 to 20 ng/mL and the minimum measurable level was 0.078 ng/mL). The intra- and inter-assay coefficients of variation were <8% and <10%, respectively. The detection range of the TNF-α kit was 7.8 to 500 pg/mL and the minimum measurable level was 1.95 pg/mL. The intra- and inter-assay coefficients of variation were <8% and <10%, respectively. After the endometrial tissues were dissolved, they were washed with phosphate-buffered saline and turned into pure endometrium tissue and its wet weight was measured. Endometrial samples were homogenized in 1 mL of phosphate-buffered saline and then incubated overnight. Samples were subjected to repeated freezing and thawing in order for the cell membranes to rupture and TNF-α and NF-κB to come out. Then the obtained homogenates were centrifuged at 3,500 rpm for 10 minutes. TNF-α and NF-κB levels were studied in the supernatants obtained by ELISA method. TNF-α and NF-κB concentrations measured by ELISA were divided by the weight of endometrial tissue and presented as ng/mg endometrial tissue or pg/mg endometrial tissue.

Statistical Analysis

Statistical analysis was performed by the use of Statistical Package for the Social Sciences for Windows version 21 SPSS (IBM Corp., Armonk, NY, USA). Normality of the distribution was assessed by using Kolmogrov Smirnov test. Student's *t*-test for normally distributed variables and Mann-Whitney U test for abnormal variables were used. While continuous variables were presented as mean ± standard deviation, categorical variables were expressed in percentages. A p-value of <0.05 was considered statistically significant.

Results

Age, hormone levels on the third day of the cycle, infertility duration and parity of the patients in type 3 and type 4 fibroid groups were recorded as similar. There was no difference between the fibroid sizes of the patients in the type 3 and 4 groups (2.16±0.34 cm *vs.* 2.01±0.29 cm, $p<0.21$). The age and parity of the patients in the control group were significantly higher than in both fibroid groups.

Pre-myomectomy TNF-α levels of the patients in the type 3 fibroid group were significantly higher than the control group (5.10±1.30 *vs.* 2.50±0.11, $p<0.01$). Similarly, the pre-myomectomy TNF-α levels of the patients in the type 4 fibroid group were significantly higher than the control group (4.73±1.76 *vs.* 2.50±0.11, $p<0.01$). There was no significant difference between pre-myomectomy endometrial TNF-α levels of patients in type 3 and 4 fibroid groups (5.10±1.30 *vs.* 4.73±1.76, $p>0.05$). Removal of type 3 fibroids by myomectomy significantly decreased TNF-α levels (5.10±1.30 *vs.* 2.20±0.44, $p<0.03$). Similarly, removal of type 4 fibroids by myomectomy significantly decreased TNF-α levels (4.73±1.76 *vs.* 2.60±0.30, $p<0.04$). TNF-α levels of both groups after myomectomy and the values of the control group were recorded as similar. Myomectomy reduced TNF-α levels to those of healthy controls. TNF-α levels were similar before and after ligation of the patients in the control group (Table I and Figure 1).

Pre-myomectomy NF-κB levels of the patients in the type 3 fibroid group were significantly higher than the control group (2.42±0.39 *vs.* 1.09±0.60, $p<0.02$). Similarly, the pre-myomectomy NF-κB levels of the patients in the type 4 fibroid group were significantly higher than the control group (2.04±0.50 *vs.* 1.09±0.60, $p<0.01$). There was no significant difference between the pre-myomectomy endometrial NF-κB levels of the patients in the type 3 and 4 fibroid groups (2.42±0.39 *vs.* 2.04±0.50 $p>0.05$). Removal of type 3 fibroids by myomectomy significantly decreased NF-κB levels (2.42±0.39 *vs.* 1.02±0.33,

Table I. Comparison of changes in endometrial TNF-α and NF-κB levels before and after myomectomy.

		TNF-α = pg/mg tissue	NF-κB = ng/mg tissue
Type 3 fibroid	Before myomectomy	5.10 ± 1.30	2.42 ± 0.39
	After myomectomy	2.20 ± 0.44	1.02 ± 0.33
		$p < 0.03$	$p < 0.01$
Type 4 fibroid	Before myomectomy	4.73 ± 1.76	2.04 ± 0.50
	After myomectomy	2.60 ± 0.30	0.97 ± 0.02
		$p < 0.04$	$p < 0.02$
Control	Before sterlization	2.50 ± 0.11	1.09 ± 0.60
	After sterilization	2.28 ± 0.58	0.78 ± 0.74
		$p < 0.43$	$p < 0.20$

$p<0.01$). Similarly, removal of type 4 fibroids by myomectomy significantly decreased NF-κB levels (2.04±0.50 *vs.* 0.97±0.02, $p<0.02$). The NF-κB levels of both fibroid groups after myomectomy were found to be similar to those of the control group. Myomectomy reduced NF-κB levels to those of healthy controls. The NF-κB levels of the patients in the control group were similar before and after ligation (Table I and Figure 2).

Discussion

When FIGO updated the fibroid classification in 2018, some changes occurred in the subfertility effects and management of intramural fibroids. The inclusion of type 3 fibroids in the submucous fibroid group in the revised FIGO led to the idea that these fibroids cause subfertility and early pregnancy loss similar to submucous fibroids[7]. It has been reported that type 3 fibroids increase endometrial inflammation through matrix metalloproteinases (MMPs) and negatively affect implantation[9]. Moreover, it has been noted that type 3 fibroids disrupt decidualization by activating lipo- and cyclooxygenease pathways as well as leukocyte accumulation in the endometrium. Its anti-inflammatory and immunomodulatory effect by reducing T cell and uterine natural killer cells in the endometrium is proof that myomectomy plays an important role in the healing of endometrial inflammation[15]. On the other hand, the relationship between type 4 fibroids and subfertility is not very clear. However, some scholars[8] published in the last decade suggest that fibroids in this location also cause subfertility. Type 4 fibroids may cause subfertility by disrupting endometrial architecture and receptivity gene expression with their mechanical effect[2]. It has been suggested that mechanical stress due to the fibroid, even if it is not in direct contact with the endometrium, turns into a biological signal, disrupting feto-meternal communication and causing inad-

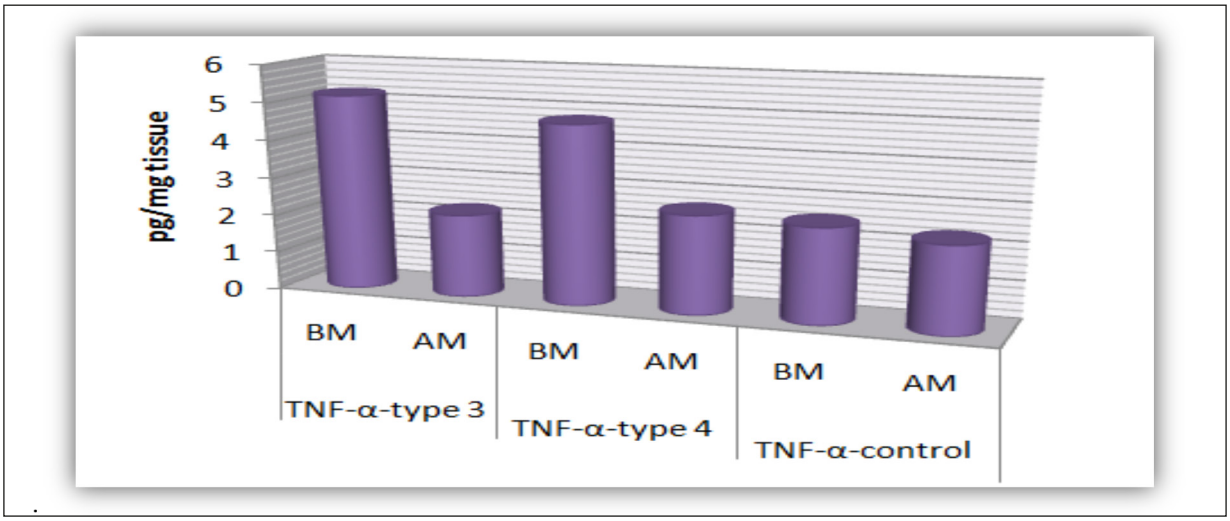

Figure 1. Comparison of endometrial TNF-α levels of type 3 and 4 fibroid patients with the control group.

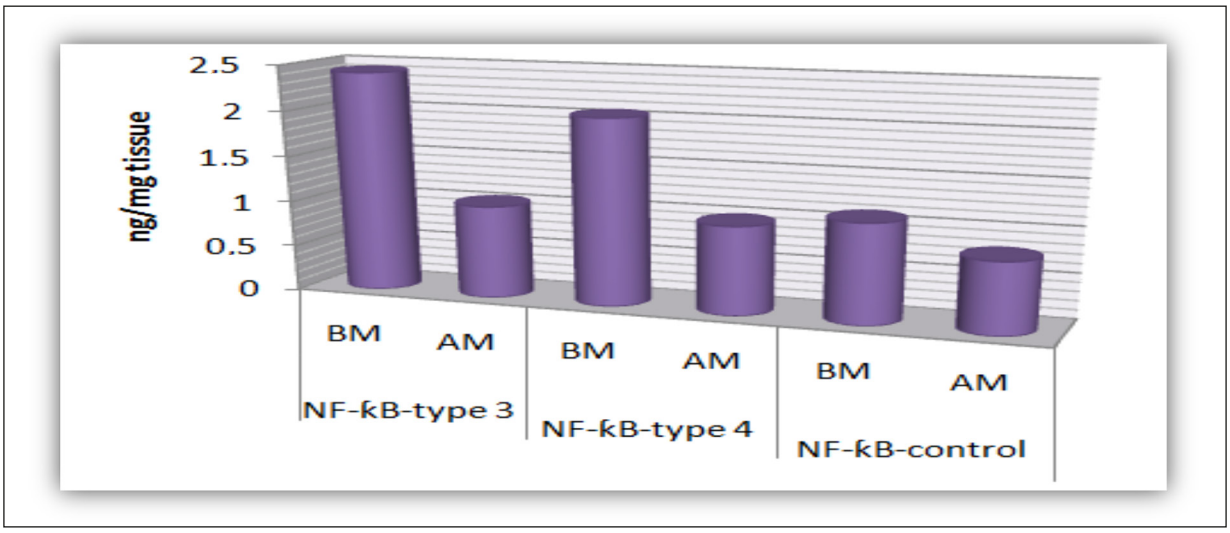

Figure 2. Comparison of endometrial NF-κB levels of type 3 and 4 fibroid patients with the control group.

equate placentation[2,16,17]. Consistent with this, it has been reported that removal of type 4 fibroids by myomectomy leads to a significant increase in the expression of receptivity genes[8].

Our study is of clinical importance in terms of showing for the first time that endometrial NF-κB and TNF-α levels are significantly reduced in the presence of type 3 or type 4 fibroids compared to healthy controls. The return of proinflammatory cytokine synthesis to normal levels with myomectomy suggests that the increase in endometrial inflammation is a fibroid-dependent process. Regardless of contact with the endometrium, both type 3 and type 4 fibroids resulted in increased levels of NF-κB and TNF-α. In this context, we can argue that it is not necessary for a fibroid to be in direct contact with the enometrium in order to affect the endometrium cytokine synthesis. Although contact with the endometrium causes a remarkable increase in NF-κB and TNF-α expression, a similar increase in the synthesis of these cytokines has been found in type 4 fibroids. TNF-α is a cytokine and initiates inflammation by increasing NF-κB expression[11]. NF-κB, whose expression is increased in the presence of fibroids, becomes active in the cytoplasm, reaches the nucleus and stimulates its target genes[12-14]. Inadequate decidualization occurs because pathologically increased NF-κB cannot regulate endoetrial cell proliferation, apoptosis, and inflammation. The decrease in NF-κB and TNF-α levels after myomectomy suggests that these two molecules act in a coordinated manner.

When intramural fibroids are collected in a pool without any classification, besides their mechanical effects, they cause an increase in the endometrial level of inflammatory cytokines and a decrease in the expression of receptivity genes, thus paving the way for subfertility/infertility[6,18]. In this study, we evaluated the expression of TNF-α and NF-κB, an extracellular signal-activated proinflammatory transcription factor, in the presence of intramural fibroid and after myomectomy. In both type 3 and type 4 fibroids, pre-myomectomy endometrial TNF-α and NF-κB levels were significantly higher than in healthy fertile controls without fibroids. Compared to type 4 fibroids, type 3 fibroids had higher endomerial NF-κB and TNF-α levels before myemectomy. Although type 3 fibroids are in contact with the endometrium, lack of direct contac of tip 4 fibroids with endometrium may explain the difference in NF-κB and TNF-α expression between the two groups. Since the contact of the type 3 fibroid with the endometrium in a large area will lead to a global vascular remodeling and inflammatory cell accumulation in the endometrium, the synthesis and release of TNF-α and NF-κB may be increased[17]. Myomectomy caused a significant decrease in endometrial TNF-α and NF-κB levels in both groups. This finding is important evidence that surgical removal of type 3 or 4 fibroids improves the impaired inflammatory response regardless of the type of fibroids and their attachment to the endometrium. While fibroid-related endometrial inflammatory changes negatively affect

receptivity, no connection could be established between endometrial cancer formation and inflammatory changes[19,20].

In order to have a reproductive tarctus pathology to cause an inflammatory change in the endometrium, it does not have to be in direct contact with the endometrium. An increase in endometrial TNF-α and NF-κB levels has also been reported in patients with chronic inflammatory etiology such as endometrioma, hydrosalpinx and Polycystic ovary syndrome (PCOS) that do not have a direct connection with the endometrium[13,14]. Treatment of the present pathology with salpingectomy, endometrioma resection, or laparoscopic ovarian drilling returned inflammatory cytokines expression to normal. For all these reasons, subfertility in the presence of type 3 and 4 fibroids may be due to increased endometrial TNF-α and NF-κB levels. The decrease in inflammatory cytokine level after myomectomy suggests that the increase in fibroid-related endometrial inflammation is a fibroid-dependent reversible process.

Conclusions

The limited number of cases and the failure to evaluate simultaneous receptivity gene expression is an important limitation. However, we can draw clear conclusions from this study as we evaluated TNF-α and NF-κB levels before and after myomectomy and compared them with healthy controls. Significant decrease in endometrial TNF-α and NF-κB levels after myomectomy suggests that the molecular changes detected in the endometrium in the presence of type 3 or 4 fibroids are a reversible process that can be corrected by surgery.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

None.

Informed Consent

Informed consent obtained from all participants. Data regarding any of the subjects in the study has not been previously published unless specified.

Ethics Committee Approval

This study was conducted after obtaining Local Ethical approval from Gözde Akademi Hospital.

References

1) Rackow BW, Taylor HS. Submucosal uterine leiomyomas have a global effect on molecular determinants of endometrial receptivity. Fertil Steril 2010; 93: 2027-2034.
2) Celik O, Koc O, Yurci A, Ersahin A, Celik N, Tanilir F. Receptivity-based uterine fibroid surgery: an updated systematic review of the evidence. Clin Exp Obstet Gynecol 2022; 49: 114-125.
3) Pritts EA, Parker WH, Olive DL. Fibroids and infertility: an updated systematic review of the evidence. Fertil Steril 2009; 91: 1215-23.
4) Sunkara SK, Khairy M, El-Toukhy T, Khalaf Y, Coomarasamy A. The effect of intramural fibroids without uterine cavity involvement on the outcome of IVF treatment: a systematic review and meta-analysis. Hum Reprod 2010; 25: 418-429.
5) Metwally M, Raybould G, Cheong YC, Horne AW. Surgical treatment of fibroids for subfertility. Cochrane Database Syst Rev 2020;1:CD003857.
6) Munro MG, Critchley HOD, Broder MS, Fraser IS. FIGO classification system (PALM-COEIN) for causes of abnormal uterine bleeding in nongravid women of reproductive age. Int J Gynaecol Obstet 2011; 113: 3-13.
7) Munro MG, Critchley HOD, Fraser IS. The two FIGO systems for normal and abnormal uterine bleeding symptoms and classification of causes of abnormal uterine bleeding in the reproductive years: 2018 revisions. Int J Gynaecol Obstet 2018; 143: 393-408.
8) Unlu C, Celik O, Celik N, Otlu B. Expression of Endometrial Receptivity Genes Increase after Myomectomy of Intramural Leiomyomas not Distorting the Endometrial Cavity. Reprod Sci 2016; 23: 31-41.
9. Governini L, Marrocco C, Semplici B, Pavone V, Belmonte G, Luisi S, Petraglia F, Luddi A, Piomboni P. Extracellular matrix remodeling and inflammatory pathway in human endometrium: insights from uterine leiomyomas. Fertil Steril 2021; 116: 1404-1414.
10 Lu ZY, Chen WC, Li YH, Li L, Zhang H, Pang Y, Xiao ZF, Xiao HW, Xiao Y. TNF-α enhances vascular cell adhesion molecule-1 expression in human bone marrow mesenchymal stem cells via the NF-kB, ERK and JNK signaling pathways. Mol Med Rep 2016; 14: 643-648.
11) Jia Z, Nallasamy P, Liu D, Shah H, Li JZ, Chitrakar R, Si H, McCormick J, Zhu H, Zhen W, Li Y. Luteolin protects against vascular inflammation in mice and TNF-alpha-induced monocyte adhesion to endothelial cells via suppressing IKBα/NF-κB signaling pathway. J Nutr Biochem. J Nutr Biochem 2015; 26: 293-302.
12) Celik O, Ersahin A, Acet M, Celik N, Baykus Y, Deniz R, Ozerol E, Ozerol I. Disulfiram, as a candidate NF-κB and proteasome inhibitor, prevents endometriotic implant growing in a rat model of

endometriosis. Eur Rev Med Pharmacol Sci 2016; 20: 4380-4389.

13) Celik O, Celik E, Turkcuoglu I, Yilmaz E, Ulas M, Simsek Y, Karaer A, Celik N, Aydin NE, Ozerol I, Unlu C. Surgical removal of endometrioma decreases the NF-kB1 (p50/105) and NF-kB p65 (Rel A) expression in the eutopic endometrium during the implantation window. Reprod Sci 2013; 20: 762-770.
14) Fan B, Dun SH, Gu JQ, Guo Y, Ikuyama S. Pycnogenol attenuates the release of proinflammatory cytokines and expression of perilipin 2 in lipopolysaccharide- stimulated microglia in part via inhibition of NF-(B and AP-1 activation. PLoS One 2015; 10: 1-20 e0137837.
15) Wang X, Qin J, Chen J, Wang L, Chen W, Tang L. The effect of high-intensity focused ultrasound treatment on immune function in patients with uterine fibroids. Int J Hyperthermia 2013; 29: 225-233.
16) Sinclair DC, Mastroyannis A, Taylor HS. Leiomyoma simultaneously impair endometrial BMP-2-mediated decidualization and anticoagulant expression through secretion of TGF-β3. J Clin Endocrinol Metab 2011; 96: 412-421.
17) Navarro A, Bariani MV, Yang Q, Al-Hendy A. Understanding the Impact of Uterine Fibroids on Human Endometrium Function. Front Cell Dev Biol 2021; 9: 633180.
18) Kitaya K, Yasuo T. Leukocyte density and composition in human cycling endometrium with uterine fibroids. Hum Immunol 2010; 71: 158-163.
19) Capozzi VA, Rosati A, Rumolo V, Ferrari F, Gullo G, Karaman E, Karaaslan O, HacioĞlu L. Novelties of ultrasound imaging for endometrial cancer preoperative workup. Minerva Med 2021; 112: 3-11.
20) Gullo G, Etrusco A, Cucinella G, Perino A, Chiantera V, Laganà AS, Tomaiuolo R, Vitagliano A, Giampaolino P, Noventa M, Andrisani A, Buzzaccarini G. Fertility-Sparing Approach in Women Affected by Stage I and Low-Grade Endometrial Carcinoma: An Updated Overview. Int J Mol Sci 2021; 22: 11825.