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Mechanism of Endometrial Receptivity Affected by Fibroids

Ping Sun[1] 🔘 | Chunyan Zhang[2] | Weisha Wang[2] | Huagang Ma[1]

[1]Center of Reproductive Medicine, Weifang People's Hospital, Weifang, China | [2]Gynecology Department, Shouguang Hospital of Traditional Chinese Medicine, Weifang, China

Correspondence: Huagang Ma (mahuagang@126.com)

ABSTRACT

Fibroids are the most common benign tumors of the female reproductive system. Most patients with fibroids are asymptomatic, but the presence of fibroids can still cause some abnormal clinical symptoms, such as increased menstrual volume, abnormal uterine bleeding, pelvic pain, urinary tract and gastrointestinal tract compression symptoms, etc. The impact of fibroids on pregnancy is worth discussing. At present, it is believed that submucosal myoma and intramural myoma affecting uterine cavity shape affect the pregnancy outcome of patients, while the impact of type III intramural myoma on pregnancy is still controversial. A number of studies have found that in addition to direct contact with the endometrial compression, uterine myoma also affects the endometrial flexibility through other ways. In this review, we summarized the effects of fibroids on endometrial receptivity and discussed in depth the mechanisms of such effects, including secretion of cytokines, changes in endometrial blood flow and angiogenesis, effects on endometrial peristalsis and mechanical stress conduction, changes in uterine microecological environment, and abnormal signal transduction pathways. Understanding the mechanism of endometrial receptivity affected by fibroids is significant for exploring the treatment of fibroids, improving the pregnancy outcome of patients with fibroids and increasing the clinical pregnancy rate.

1 | Introduction

Fibroids are the most common gynecological benign tumors in the reproductive system of women of reproductive age, with an incidence of about 70%, of which about 30% of patients can show clinical symptoms, including increased menstrual volume, anemia, pelvic compression symptoms and pain, dyspareunia, infertility, early abortion, and recurrent abortion [1]. Submucous myoma (SMM) reduces pregnancy rate by directly pressing the endometrium and altering uterine cavity morphology. In recent years, studies have found that even IMM (intramural myoma) does not affect uterine morphology, it may still have adverse effects on the clinical pregnancy rate and live birth rate of patients [2–4]. Fibroids may affect endometrial receptivity through several mechanisms, such as affecting endometrial decidualization process, interfering with endometrial peristalsis,

affecting uterine blood flow and endometrial vascularization, immune factors, changes in signal transduction pathways, and changes in reproductive tract microflora. The purpose of this review is to systematically review the mechanisms related to the effect of fibroids on endometrial receptivity, to clarify the specific mechanisms by which fibroids may reduce the pregnancy rate.

2 | Methods

We used a variety of strategies to search the literature related to fibroids and endometrial receptivity, focusing on mechanisms affecting endometrial receptivity, infertility, and so forth. When searching literature, we did not limit the publication time of literature, PubMed was used for the literature search, and "fibroids, uterine leiomyoma," "endometrial receptivity" and "infertility,

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subfertility" were used for combined or separate search. In addition, we made reference to the relevant references in the literature we read, and comprehensively read the literature results. We summarized the mechanisms by which related fibroids affect endometrial receptivity and then affect embryo implantation, leading to infertility and adverse pregnancy sac outcomes in this review.

3 | Results

3.1 | Origin of Fibroids

In the past decade, the drivers of genetic mutations that cause fibroids are becoming increasingly clear. Whole-genome sequencing of fibroids found that the same variation was observed in some of the individual tumor nodules, suggesting that these nodules share a common origin [5]. The majority of fibroids exhibit highly specific mutations in the MED12 gene, which occurs in 40%–90% of fibroids. MED12 acts as a bridge between transcription factors and basic RNA polymerase II transcription mechanisms. The types of mutations include heterozygous missense mononucleotide variants, heterozygous in-frame deletions/insertion–deletions, mononucleotide variants that affect splicing, heterozygous deletions/insertion–deletions that cross intron–exon 2 boundaries, affecting splicing receptor sites, and so forth. Among them, nearly 70% of fibroids have tumorigenic mutations in MED12 exon 2 [6]. Recently, it was found that mediator kinase subunits are enriched in myometrial stem cells (MMSC), and in addition, chemical inhibition of CDK8/19 in MMSC leads to reduced phosphorylation of stem cell-enriched transcription factors and altered expression of myogenic genes. MED12 exon 2 mutation may provide a selective advantage to myometrium stem cells by disrupting mediator kinase activity and by altering the growth or differentiation trajectory of myometrium stem cells to form myoma stem cells, which in turn seed and sustain monoclonal tumor growth [7–9]. Mutations in fumarate hydratase (FH) on chromosome 1 in band q42 were found in fibroids [10, 11], the mechanism of tumorigenesis induced by FH mutations remains unclear [12]. The most widely studied hypothesis is activation of the hypoxia pathway. FH biallelic deletion leads to intracellular accumulation of fumaric acid. High levels of fumarate modify cysteine residues of KEAP1 (Kelch-like epoxy chloropropane-associated protein 1), leading to the accumulation and activation of NRF2 [13, 14]. Nrf2 has been shown to contribute to the malignant phenotypes of cancer, including aggressive proliferation [15]. Studies have found that NRF2 signaling pathway is the single most significant dysregulated pathway in FH subtype leiomyoma [16].

For leiomyomas lacking MED12 mutations and FH mutations, whole-genome sequencing revealed complex chromosomal rearrangements (CCRs) in these subgroups of leiomyomas. These CCRs are similar to chromothripsis, in which one or more chromosomes break into pieces in a single event and are randomly spliced together [5]. Fibroids may be formed through those CCR events that create tumor-promoting genetic changes, which can impair control of cell-cycle checkpoints and repair of DNA double-strand breaks, such as translocations of the HMGA2 and RAD51B loci [5]. It was found that the expression of HMGA2 in chromosomal rearrangements of 12q15 fibroids was significantly higher than that in karyotypically normal uterine leiomyomas or in myometrium [16, 17]. The proto-oncogene pleomorphic adenoma gene 1 (PLAG1) as one of the most uniquely up-regulated genes in leiomyomas with HMGA2 or HMGA1 aberrations. PLAG1 encodes for a transcription factor whose ectopic expression can trigger the development of several benign mesenchymal tumors, HMGA2 and HMGA1 promote tumorigenesis through the activation of PLAG1 [18]. COL4A5/COL4A6 deletion is a rare subtype that accounts for about 2% of fibroids. Alterations in the COL4A5–COL4A6 locus recurred in fibroids and also arose through chromothripsis [8].

3.2 | Receptivity of Endometrium

The endometrium consists of two regions: functionalis and basalis. In the functionalis region, functional glands extend vertically to the uterine cavity, where the functional cavity cell layer is arranged on the surface of the endometrium. The functional region responds to hormones and undergoes dynamic remodeling of cell morphology and function during the menstrual cycle. The basal glands contain epithelial stem/progenitor cells that are required to regenerate functional glands after menstruation [19]. Stromal cells, fibroblasts, perivascular (PV), and endothelial cells provide support and structural integrity, including a rich vascular system within the tissue [20]. Successful embryo implantation requires the endometrium of the acceptable embryo to be synchronized and functionally coordinated with embryonic development, and the endometrium receptive window period usually occurs 6–10 days after ovulation [21]. After ovulation, the luteum forms and the endometrium begins the process of decidualization. During the secretory phase, decidualized stromal cells specific to the secretory phase (dStromal cells) are described as different cellular states in the early, middle, and late secretory phases and function through different mechanisms [20]. The receptive endometrium is characterized by the appearance of microvilli on the apical surface of luminal epithelial cells, called pinopodes [22, 23]. It was found that there was a close association between the number of microvilli and the endometrial receptivity to the embryo [23, 24]. All of these molecules play an essential role in the communication between the endometrium and the blastocyst.

HOXA is a transcription factor, which is expressed in the endometrium of adult mice and humans, and its expression can be seen in the endometrium proliferation stage, and increases in the secretion stage, reaching its peak in the implantation window period [25, 26]. Studies have found that the endometrial receptivity of thin endometrial mice can be improved by upregulation of HOXA10 expression [27], and the experiments related to endometriosis, low implantation rate, and impaired endometrial receptivity showed decreased HOXA10/HOXA11 expression [26, 28, 29], those results showed that HOXA10/HOXA11 expression was closely related to endometrial receptivity.

Embryo implantation is a complex and precise process, in which a variety of cytokines, growth factors, adhesion factors, transcription factors, and so forth are involved and regulated [19, 30, 31]. Immune cells are essential for endometrial receptivity in embryo implantation and early placental development [32, 33]. In addition, endometrial peristalsis abnormalities, endometrial
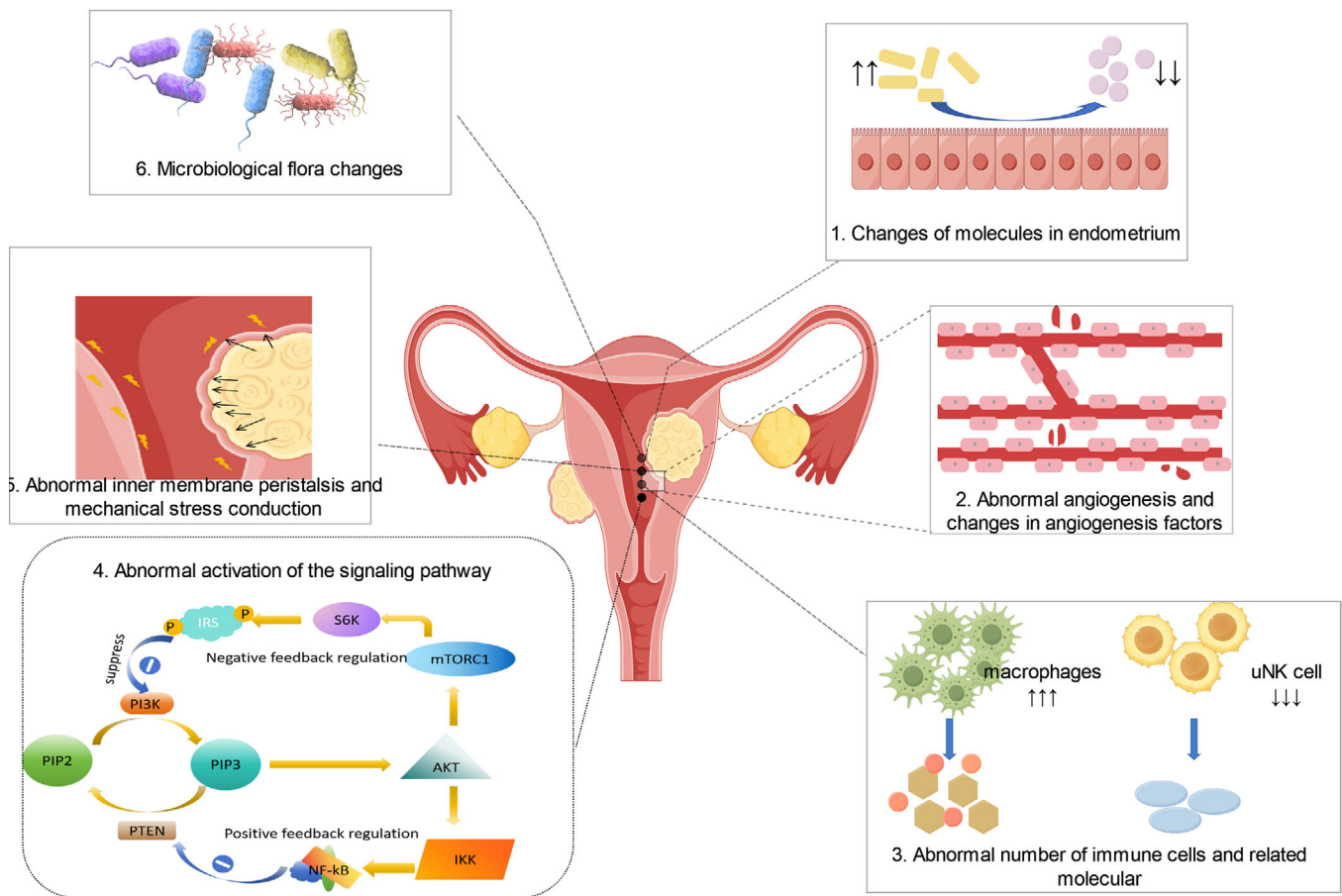
FIGURE 1 | Overview of the mechanism by which fibroids affect endometrial receptivity.

microbial flora disorders, endometrial blood flow changes, and so forth may lead to endometrial receptivity impairment.

3.3 | Effect of Fibroids on Endometrial Receptivity

According to the available research, fertilities are decreased in females with submucosal fibroids [34], and myomectomy prior to in vitro fertilization is recommended to improve reproductive outcomes. Subserosal fibroids do not affect fertility outcomes, and removal does not confer benefits. As for IMM, its effect on patient reproductive outcomes remains controversial. Extensive researches have reported that IMMs even non-cavity distorting IMMs effect the reproductive outcomes [35–37]. A multitude of mechanisms that may influence reproductive outcomes were proposed, for example, effects on implantation, synchronicity disruption, sperm transport, uterine contractility, and endometrial changes (Figure 1). Here, we elucidate the mechanism by which fibroids affect endometrial receptivity.

3.3.1 | Changes of Molecules in Endometrium

There is a consensus that SMM can reduce the implantation rate and clinical pregnancy rate. SMM may interfere with embryo implantation by changing the uterine cavity morphology and endometrial peristalsis, and large fibroids may even hinder sperm passage through the cervix and Ostium fallopian tube. However,

impaired fertility can also be observed in women with small submucosal fibroids and IMMs that do not change the uterine morphology, thus considering that leiomyoma paracrine signaling may lead to myoma-related infertility by affecting endometrial receptivity. Key regulators of implantation, such as homeobox-A10 (HOXA10), HOXA11, and leukemia inhibitory factor (LIF), are known to have defective endometrial receptivity leading to infertility [38]. In patients with SMM infertility, HOXA10 and HOXA11 were reduced, and the results of a study by Rackow et al. showed that global endometrial HOXA10 expression was reduced in SMM and not confined to the endometrial portion covering the fibroids, suggesting that implantation defective can be mediated by signal diffusion [39]. Bone morphogenetic protein-2 (BMP-2) is an important multifunctional growth factor associated with embryo implantation and regulates the expression of HOXA and LIF [40]. BMP-2 usually binds to BMPR in endometrial stromal cells (ESCs) to promote endometrial decidualization. The disappearance of BMP-2 in mouse endometrium will lead to decidualization and embryo implantation failure. Studies show that the transforming growth factor-β 3 (TGF-β 3) has a weak affinity for BMPR. In the presence of leiomyomas, excess TGF-β 3 binds to BMPR, resulting in inhibition of BMPR 1B and BMPR 2 in ESC, resulting in a lack of response to BMP. Neutralization of TGF-β prevented the reduction of BMPR expression and restored endometrial BMP-responsive [38] (Figure 2). In addition, endometrial TGF-β expression is influenced by estrogen and progesterone. A previous study reported that TGF-β expression is stimulated by progesterone, but recent studies confirmed that
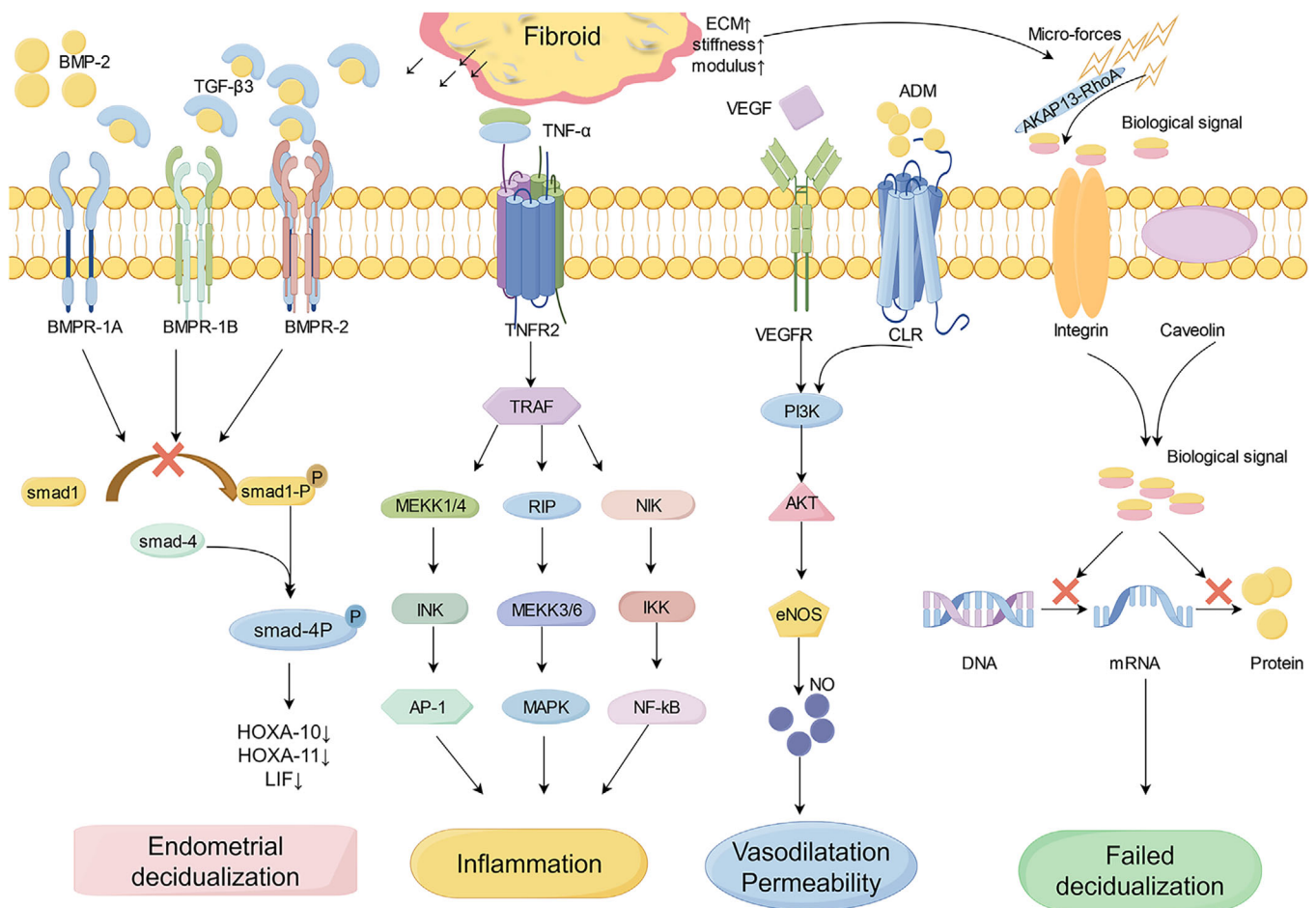
FIGURE 2 | Transforming growth factor-beta3 (TGFβ3) produced by fibroid reduces bone morphogenetic protein receptor (BMPR) expression and interfere with bone morphogenetic protein-2 (BMP-2) binding to receptors results in decreased expression of homeobox-A10、11 and leukemia inhibitory factor (HOXA10, HOXA11, LIF), thus affecting endometrial decidualization and endometrial receptivity. Tumor necrosis factor-alpha (TNF-α) responds to the immune response in three ways, abnormal expression of TNF-α may lead to implantation failure and infertility. Vascular endothelial cell growth factor (VEGF) and adrenomedullin (ADM) are both angiogenic factors that cause abnormal angiogenesis through the PI3K-AKT signaling pathway. The mechanical stress generated by fibroids is transformed into biological signals that interfere with gene expression, resulting in abnormal endometrial decidualization.

progesterone directly inhibited the endometrial expression of TGF-β 2/3 [41]. These findings suggest that TGF-β is a paracrine mediator of leiomyoma-associated infertility and pregnancy loss and that pharmacologic therapies designed to reduce TGF-β levels in the endometrium or to inhibit TGF-β signaling may be a strategy to improve endometrial receptivity and increase pregnancy rate in patients.

3.3.2 | Endometrial Blood Flow and Angiogenic Factors

It is believed that increased endometrial blood flow is beneficial to embryo implantation and in-vitro fertilization (IVF) outcomes [42, 43]. However, studies have found that with the change of menstrual cycle sex hormone levels, there is a specific vascular balance environment in the endometrium, and any increase or decrease in blood flow will lead to endometrial dysfunctional [44]. Fibroids may lead to abnormal intimal blood flow by causing abnormal endometrial angiogenesis and vascular maturation. A study included 182 patients with IMMs, endometrial volume,

and blood flow index measured with 3D Doppler ultrasound, the results found that endometrial vessels in uterine fibroid (>4 cm) group increased significantly [45]. Moon et al. found that the resistance index (RI) and pulsatile index (PI) of the lower endometrial artery in patients with affected uterine cavity fibroids were significantly higher than that of patients with normal uterine cavity, and the clinical pregnancy rate and embryo implantation rate in this group were significantly reduced [46]. Compared with patients without fibroids, patients with in fibroid group had significantly increased endometrial venous pool, indicating increased vascular diameter, indicating impaired vascular maturity, and increased vascular fragility and permeability [47].

The presence of fibroids upregulated the endometrial angiogenic factors expression, for example, adrenomedullin (ADM) and vascular endothelial cell growth factor (VEGF). VEGF is the major initiator of angiogenesis, ADM could combine with calcitonin receptor-like receptor (CLR), regulating the creation of nitric oxide (NO), and then stimulating the angiogenic [48]. This indicates abnormal endometrial angiogenesis in patients, and may involve disturbances in vascular maturation, leading

to an increased vascular fragility [49]. Endometrial nitric oxide synthase (eNOS) is the major isomer of human endometrial NO and an important angiogenic factor involved in vasodilation and endothelial cell permeability which is induced by VEGF. Although eNOS significantly affects sustained pregnancy and maintaining uterine quiescence during implantation, endometrial eNOS levels were significantly increased in patients with fibroids [50]. The overexpression of eNOS can induce apoptosis or impair the endometrium and its function, supporting the hypothesis that fibroids can impair implantation or increase the risk of abortion [49, 51] (Figure 2). In addition, the Extracellular matrix (ECM) of fibroids contains a lot of basic fibroblast growth factor (bFGF), an angiogenic growth factor that is highly mitogenic to capillary endothelial cells in vitro and can induce angiogenesis in vivo. It has been proposed that fibroids are a reservoir of bFGF, which may affect the endometrial vasculature through paracrine and local endocrine effects [52]. In patients with fibroid and abnormal uterine bleeding, ESC bFGF-R1 expression is disturbed in the early luteal phase, consistent with embryonic attachment and implantation time, so dysregulation of the bFGF receptor/ligand system is implicated in female infertility with leiomyoma [53]. In conclusion, the findings suggest that fibroids may affect intima tolerance by affecting the generation of angiogenic factors and endometrial blood flow, but many factors are only confirmed in a single study, and more studies are needed to verify these conclusions.

3.3.3 | Immune Factors

Endometrial immune cells show significant periodic fluctuation following the menstrual cycle. Abnormal changes in immune cells may affect endometrial decidualization and affect angiogenesis and embryo implantation. Two uterine macrophage (uM) populations (uM1 and uM2) previously identified in the endometrium during pregnancy [20], uM1 expresses pro-inflammatory genes such as IL 1β and EREG, while uM2 expresses anti-inflammatory genes such as HMOX1. At different stages of the menstrual cycle, macrophages in the endometrial specifically expressed role-specific markers. During the proliferative phase, macrophages expressed adhesion and activation markers (CD71, CD69, CD54), suggesting a potential role in the regeneration and proliferation of the endometrial functional layer [54, 55]. In addition, uMs up-regulate TNF (uM1) as well as growth factors such as IGF 1 (uM2) and EREG (uM1) which could stimulate the proliferation and survival of eStromal MMP by binding to their corresponding receptors. Both uM also express immunomodulatory genes (IL 10, LGALS 9, TREM2) that enhance the anti-inflammatory response of the proliferating endometrium. Additionally, macrophages are also involved in the angiogenesis process, with uMs expressing multiple growth factor members of the pro-angiogenesis VEGF family. For example, uM1 express VEGFA and uM2 express VEGFB, TNF61 is expressed in uM1 and OSM 62 and CXCL 8 expressed in both uM types, all of them are vascular remodeling factors [20].

Macrophages begin to increase before menstruation and peak during menstruation, accounting for about 15% of the total number of endometrial leukocytes, in particular, macrophages increased significantly at the implantation site [56]. A significant increase in macrophage number can be observed in the second half of the menstrual cycle, thus considered to play an important role in fertility regulation. In addition, macrophages can secrete LIF which is an important cytokine during embryo implantation [54]. LIF is also considered an effective chemoattractant of macrophages. One study showed that a mouse model with LIF knocked out showed a 50% reduction in the number of macrophages, which led to the failure of embryo implantation [57]. It is therefore concluded that macrophages play a crucial role in fertility and inducing the production of pro-inflammatory cytokines [56]. The study found that the density of macrophages in the endometrial tissue close to the fibroid was significantly higher than that of the endometrial tissue away from the fibroid [58], and the infiltration in patients with muscle layer and endometrium in SMM and IMM was higher than that in patients with subserous fibroid, and the muscle layer and its corresponding macrophages in the endometrium did not depend on the size of the fibroids. Monocyte chemotaxis protein-1 (MCP-1) showed a significant positive correlation with tissue infiltration of macrophages in the endometrium of IMM patients [59]. Therefore, when fibroids exist, the inflammatory reaction change is not only limited to the fibroids itself, but also affect the patients in the endometrial tissue. Direct compression of the endometrial by SMM leads to changes in endometrial ischemia or hypoxia, increased endometrial angiogenesis, increased endometrial vascular activity, and subsequently increased recruitment of inflammatory cells. The disturbance of endometrial inflammatory environment may further affect the abnormal formation of endometrial pro-inflammatory environment, resulting in the disturbance of membrane environment during embryo implantation window, thus affecting the successful implantation of embryos. TNF-α is a type II transmembrane protein which produced by macrophages. TNF-α is involved in tissue homeostasis and systemic inflammation, and is one of the cytokines that cause acute phase reaction [60]. The expression level of TNF-α in fibroid is higher than that in the adjacent myometrium. Moreover, serum TNF-α level is significantly increased in patients with clinically symptomatic fibroids. Studies have shown that compared with normal endometrium, endometrium expression of TNF-α was significantly increased in patients with IMMs [61], and TNF-α was involved in preimplantation development of embryos, loss of immune pregnancy, and regulation of trophoblast invasion. Elevated serum TNF-α levels have been associated with serious pregnancy complications, such as recurrent abortion, premature rupture of membranes, preeclampsia, and intrauterine growth restriction [62]. Abnormal expression of TNF-α may lead to implantation failure and infertility (Figure 2).

In the planting window, uterine natural killer cells (uNK) cells are the main key immune cells to regulate immune tolerance, trophoblast migration and invasion, and spiral artery transformation [63], endometrial uNK cells increase rapidly after ovulation, and further increas in the first trimester. Studies found that the uNK cell is related to the process of decidualization of endometrial, the number of uNK cells began to increase about 3 days after LH peak, so uNK cells increased before endometrial decidualization, especially around the arteries and glands. In the late secretion (about 11–13 days after LH peak), a large number of uNK cells are densely distributed in the inner membrane matrix [64]. Mouse studies found that loss of uNK cells led to normal development and maintenance failure of the decidua and maternal arteries

[65]. A significant increase of uNK cell levels was observed in the endometrium of patients with recurrent abortion and repeated implant failure, indicating that potential disturbances of the immune environment may eventually lead to implantation or placenta formation failure [66]. However, insufficient activation of uNK cells may also lead to recurrent abortion, so moderate and controlled activation of uNK cells is conducive to the pregnancy [32]. A study compared the patients with fibroids and no fibroids different location of the number of endometrial uNK cells and found that away from the location of the endometrium of the uNK cells were significantly less than the control samples, and the number of uNK cells in the endometrial near fibroids were less than away from the location of the endometrium in the proliferative period and after the secretion period [67]. Low levels of IL-11 in fibroid patients may be associated with reduced uNK cell numbers, IL-11 involved in the regulation of trophoblast invasion, and reduced IL-11 may contribute to implantation failure in these women; however, further studies are needed to evaluate this association [58].

### 3.3.4 | Aberrant Activation of the Cell Signaling Pathway

During the process of endometrial decidualization, several cellular pathways are altered, including: WNT/β-catenin pathway, cAMP/PKA pathway, Notch signaling pathway, ERK1/2 pathway, TGF β signaling pathway, BMP2-WNT4 signaling cascade, phosphatidylinositol 3-kinase (PI3K)/AKT pathway, and so on [68, 69]. The alteration of abnormal signaling pathways may affect endometrial decidualization and thus affect the successful implantation of embryos. Since the endometrial epithelium is the first site of contact between maternal and embryonic tissue, significant changes in the adhesion properties of the uterine epithelium are critical to the process of implantation. The study suggests that Wnt-/β-catenin signaling pathway may play an important role in the implantation process of human embryos [70]. Downregulation or deletion of E-cadherin and β-catenin (important intracellular mediators of the Wnt signaling pathway) protein expression during the implantation window may be necessary to enable epithelial cell segregation and blastocyst invasion [71]. Matsuzaki et al. studied the expression of E-cadherin and β-catenin in the endometrium of patients with endometriosis, uterine fibroids, and infertility, and found that in healthy control groups, the expression of E-cadherin in glandular and coelomic epithelium at the proliferative stage, early and middle secretory stage was significantly lower than that in the later secretory stage. However, in patients with fibroid infertility, no matter the coelomic epithelium or glandular epithelium, the expression of E-cadherin was significantly lower than that in the later secretory stage. There were no significant periodic differences in the expression of E-cadherin [72].

Relevant studies have found that IMM may affect endometrial decidualization by affecting PI3K-AKT pathway. Although the signaling mechanisms and pathways of decidual degeneration are still unknown, several lines of evidence point to the PI3K/AKT pathway as the main regulator of decidual cell proliferation and survival [73–75]. Activated Akt (p-Akt) phosphorylates and activates a variety of downstream proteins, and is involved in various cellular functions, including growth, proliferation, differ-entiation, survival, apoptosis, and migration. After the addition of PI3K/Akt signaling pathway inhibitor, the number of embryo implantation was significantly reduced [76, 77]. The expression levels and distribution characteristics of genes related to the PI3K/Akt signaling pathway at implantation sites and interimplantation sites in endometrial suggest that the PI3K/Akt signaling pathway is involved in the early embryo implantation process, especially during the embryo implantation window period, and may promote stromal cell migration [77]. Akt expression in stromal cells was detected only in the functional layer, where tissue remodeling occurs during menstruation or implantation. Therefore, Akt activation may be involved in human endometrial and decidual cell survival and extracellular matrix remodeling [73, 78]. Makker et al. took patients without IMM as the control group to study the expression, cell distribution, and activation status of key signaling components of PI3K/PTEN/Akt pathway in endometrium during the mesocrine phase in IMM infertility patients [79]. It was found that nuclear PTEN staining in endometrial mesenchyme in the experimental group was significantly lower than that in fertile women. The expression of p-PTEN and the ratio of p-PTEN to PTEN increased in the nucleus, suggesting the loss of PTEN biological activity. Although there is no information on pPTEN/PTEN expression in the endometrium, a study of women with tubal infertility showed that pregnancy success was associated with the highest expression levels of PTEN mRNA, suggesting that PTEN is essential for endometrial receptivity and successful implantation [80]. Laguee et al. found that PTEN regulates decidual degeneration and trophoblast invasion, and indicated that PI3K/AKT signaling activity must be suppressed in decidual cells at the maternal/fetal interface to allow these processes on the rails [81]. Loss of PTEN may lead to changes in the function of the uterine glands, resulting in sterility or loss of pregnancy in these mice. In addition, Akt1 mRNA levels and nuclear Akt1 expression were significantly up-regulated in the endometrial stroma of IMM infertile women. Elevated Akt1 expression is associated with reduced fertility in endometriosis patients [82]. In the fibroid group, the expression of Akt2 in the endometrial stroma was significantly up-regulated. Akt2 is a key factor in the PI3K/Akt signaling pathway and is involved in the maintenance of glucose homeostasis. Abnormalities in this process may be represented by implantation failure and poor pregnancy outcomes. It has been reported that alterations in glycolytic metabolism lead to endometrial dysfunction in patients with PCOS [83, 84]. PCOS, endometriosis, and fibroids are estrogen-dependent disorders which associated with infertility [85]. The presence of fibroids leads to changes in the expression of PI3K/PTEN/Akt pathway signaling components in endometrium. The decrease of nuclear PTEN is accompanied by the increase of nuclear phosphorylated PTEN and the subsequent activation of nuclear Akt in the stroma, which may affect endometrial decidualization through regulating endometrial proliferation and apoptosis during implantation, and participate in the infertility mechanism of patients with fibroids (Figure 3).

3.3.5 | Abnormal Inner Membrane Peristalsis and Mechanical Stress Conduction

The direction of peristalsis of the uterus changed significantly with different stages of the menstrual cycle. During
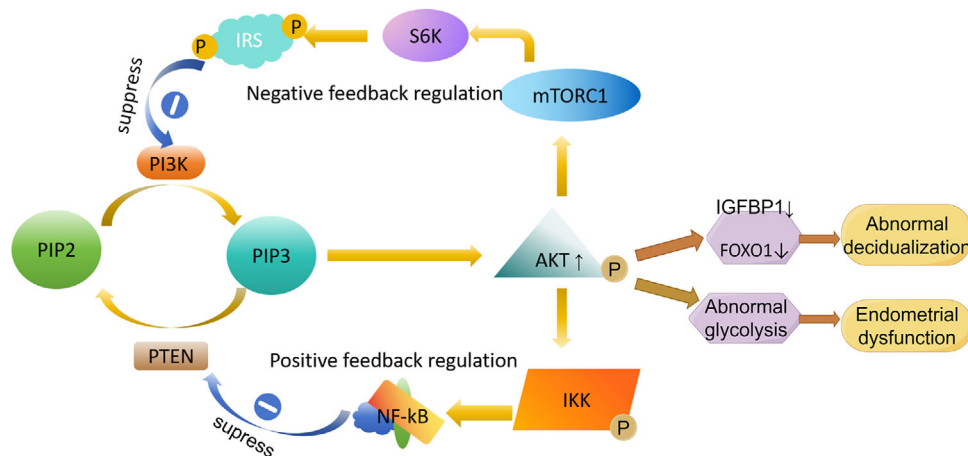
FIGURE 3 | PI3K-AKT signal path: PI3K phosphorylates phosphatidylinositol-4, 5-diphosphate (PIP2) to phosphatidylinositol-3,4, 5-triphosphate (PIP3), and PIP3 activates AKT. Increased phosphorylated AKT reduces the expression of decidation-specific gene insulin-like growth factor binding protein-1 (IGFBP1) and decreases the level of nuclear forkhead transcription factors of the O class (FOXO1). Causes abnormal decidualization of endometrial stromal cells in women with endometriosis. It can also lead to endometrial dysfunction by causing changes in glycolytic metabolism.

menstruation, the uterus peristalsis moved from the base of the uterus to the cervix, while the direction of ovulation was the cervix to the base of the uterus. The frequency of uterine peristalsis decreased significantly after ovulation, and this peristalsis pattern was conducive to activities such as menstrual blood discharge, sperm transport, and embryo implantation [86]. However, the presence of fibroids may affect embryo implantation by affecting the direction, frequency, and even amplitude of peristalsis of the endometrium muscularis junction [87]. Fanchin et al. examined the uterine peristalsis of infertility patients without uterine abnormalities with ultrasound, and the study proved that the frequency of uterine peristalsis on the day of embryo transfer was negatively correlated with pregnancy outcome [88]. Abnormal uterine peristalsis may expel the embryo from the uterine cavity [89]. A previous study of endometrial peristalsis using Cine magnetic resonance imaging (Cine MRI) found abnormal menstrual and midluteal peristalsis in three out of five patients with fibroids [90]. In a study of 51 IMM patients, it was found that 22 patients with high-frequency endometrial peristalsis had no pregnancy, while 34% of patients with low-frequency peristalsis had pregnancy, so a higher midluteal uterine peristalsis frequency may be one of the reasons for infertility in IMM patients [86]. However, the relationship between uterine peristalsis and fibroids is unclear. Since estrogen can induce peristalsis by increasing endometrial oxytocin receptor density, expression of aromatase in fibroids may lead to increased tissue estrogen concentration, which in turn leads to increased endometrial peristalsis [86, 91]. The study found that hysteromyectomy reduced the frequency of abnormal peristalsis in all patients, and 14 of 15 patients with IMM who underwent surgery returned the frequency of peristalsis to the normal range (0 or 1/3 min). In addition, 6 of the 14 patients had a successful pregnancy after hysteromyectomy [92]. Therefore, the presence of fibroids may lead to increased abnormal endometrial peristalsis leading to infertility, and myomectomy may increase the pregnancy rate of patients by restoring the frequency of endometrial peristalsis [93].

The ECM, composed of glycosaminoglycan and interstitial collagen, is the determining factor of the hardness of fibroids, which are 2–4 times harder at different locations than adjacent muscle layers [94, 95]. The hardness produced by each biological cell or the response to mechanical signals is different. Fibroid stem cells are more likely to proliferate and generate mechanical signals at higher hardness. The increased hardness not only makes the mechanical signals stronger but also makes the signal transmission more efficient and faster. The transmission of mechanical signals from myoma to adjacent myometrium and then to endometrium is a solid conduction process, and the transformation of mechanical signals into biochemical signals requires a series of pathways. Rho-GTPases are key enzymes in this pathway. The ECM composition and Rho-GTPases of fibroids are the main factors that determine the efficiency of this pathway. Mechanical signals from fibroids are converted into biochemical signals and transmitted to fibroids, myometrium, and endometrium by transmembrane receptor integrins, cadherins, or caveolins [95]. Signals directly entering fibroids may stimulate fibroid growth and those directly entering the endometrium may impair the receptibility of endometrium [93, 94, 95] (Figure 2). Under physiological conditions, micromechanical forces caused by subendometrial muscle contraction reach the endometrium and activate epithelial sodium channel (ENaC). ENaC activation has a positive effect on receptivity by stimulating the activation of prostaglandin E2. If the mechanical signal binds to the lysophatidic acid (LPA) receptor in the individual cell, the Rho GTPase is activated, and then the actin–myosin complex comes into play, and this whole process produces a positive progression in decidualization [95]. Due to the low ECM content in the early stage of muscle nucleus formation, small strength and small force may have a positive effect on decidualization. However, the continuity of mechanical signals and the effect on decidualization are impaired due to increased ECM and hardness. Because of the different hardness and modulus of fibroids, the influence of different location and size of fibroids on endometrial receptivity is different. The myometrium has an elastic modulus due to its biological properties, and fibroids have a chondroid modulus due to their collagen and glycosaminoglycan content. The chondroid modulus makes the fibroid five times harder than the muscle layer, and the different modulus of the fibroid and muscle layer convert the mechanical signals into

biological signals and transmit them to the adjacent muscle layer and endometrium [94, 96]. Therefore, fibroids in the early stages of development, if the fibroids are not hard enough, may not be enough to produce biological signals. The main factor determining fibroid hardness is not size but matrix composition, and if a fibroid is small and dense, it may produce stronger mechanical stimulation than a larger fibroid with less ECM. In this way, the mechanical signals of small and hard fibroids that do not directly touch the endometrial membrane can also reach the endometrial membrane and affect the endometrial decidualization process.

3.3.6 | Microbiological Flora Changes

The microbiota of the human body plays an important role in affecting the health status of the body. In the past, the uterine cavity was thought to be a sterile environment, but now it is found that there is a unique microbiome in the female reproductive tract, which accounts for about 9% of the total bacteria in the female body [97]. Researchers have found differences between endometrial and vaginal microbial communities in terms of microflora classification and relative abundance, and further study found that lactobacillus is the most representative bacterial genus in endometrial samples [98, 99]. A large-scale study by Chen et al. evaluated the microbiome composition characteristics of 110 women at six sites in the female reproductive tract. The research demonstrated that lactobacillus is the main taxa of the vaginal flora. However, this gradually disappears as the sample enters the endometrium and upper reproductive tract. The degree of correlation between lower and upper reproductive tract microbiota varies among different patient populations, possibly reflecting health and disease phenotypes [100–102]. The results of a number of studies on infertility in people treated with ART suggest the possibility that lactobacillus is the dominant microflora in the endometrial [103–105]. However, subsequent studies did not confirm the lactobacillus advantage originally assumed. Higher diversity appears to be associated with better outcomes after IVF surgery [106, 107]. The results of the current study suggest that the composition of uterine microflora is unclear and needs to be further investigated. It is worth noting that contamination during sampling and environmental contamination received (including kit reagents, surgery, and laboratory environment) should be excluded during the study [108].

The endometrial microbiome may predict reproductive outcomes, some evidence linking the endometrial microbiota to early miscarriage. However, whether there is a causal relationship between the need's further clarification [101]. The endometrial microbiome may have a symbiotic relationship with the endometrium and its local immune mediators, promoting implantation and regulating immune tolerance [109]. The mother's immune system is crucial to the success of early pregnancy, immune-mediated trophoblast invasion and spiral artery remodeling are the keys to success. Liu et al. compared the microbiota characteristics of the endometrial lavage fluid in patients with recurrent miscarriages (RM) with healthy patients and found that β-diversity was significantly higher in the endometrial lavage fluid samples, but β-diversity was not found in tissue biopsies from patients who experienced RM [110]. In addition, concomitant inflammatory changes were observed

in these patients, although implantation is an inflammatory event, excessive inflammation outside the implantation window is associated with reproductive dysfunction. In a study of patients with endometritis, it was found that the relative abundance of lactobacillus was significantly reduced in patients with endometritis [111]. If the content of lactobacillus in endometrial is less than 90%, it is not conducive to reproductive outcomes and can be determined as an ecological imbalance, >90% lactobacillus content was significantly associated with better implantation results and sustained pregnancy [112]. Relevant studies have also shown that CE is associated with a significant decrease in α diversity within the endometrial microbiota and an increase in the expression of pro-inflammatory genes associated with apoptotic pathways, including IFN-α and tumor necrosis factor-α [113, 114]. Endometriosis, ovarian or endometrial cancer, and polycystic ovary syndrome have been shown to affect the microbiota within the female reproductive tract [115]. In addition, there may be cross-regulation between the microbiota and the uterine immune system. Emerging ideas suggest that the host immune system and the microbiota maintain a symbiotic relationship, and the microbiota can affect the host immune system function. However, the nature of the interaction between the microbiota and immune cells in the uterus is unclear. The symbiotic relationship between the uterine microbiota and the innate and adaptive immune systems may play an important role in maintaining a balanced inflammatory environment, as this mild bacterial stimulation can induce the formation of a microenvironment conducive to embryo implantation. On the other hand, the regulatory stimulation of the immune system by the microbiome helps build tolerance to semen [56]. The presence of fibroids may affect the vagino-uterine microbiome because fibroids may cause inflammation and changes in local nutrients, such as increased menstrual bleeding and prolonged periods, which can affect the vagino-uterine microbiome. Winters et al. found different distribution of microflora in vaginal and endometrial samples of patients with fibroids. The endometrial samples were dominated by acinetobacter, pseudomonas, syncytiaceae, and pipiobacterium, rather than lactobacillus [116], Mao et al. found that the cervical and vaginal microbiome of patients with fibroids increased in firmicutes. In the difference analysis of relative abundance, the abundance of Clostridium erysipelosus, myxospira, and fingeroldeira increased, while the abundance of erysipelothrix and spolactobacter decreased. The presence of fibroids may lead to the imbalance of vaginal and cervical microecology [117]. However, there are few relevant studies on patients with fibroids, and further studies are needed to prove the relationship between changes in intrauterine microecology and pregnancy outcome, as well as the impact of fibroids on intrauterine microecology.

4 | Conclusion

In summary, the most direct reason why fibroids affect endometrial function is that direct compression on endometrium and changes uterine cavity shape, thus affecting endometrial peristalsis, resulting in endometrial angiogenesis and abnormal blood flow. In addition, myoma without uterine compression can also change endometrial receptivity by producing excessive growth factors and cytokines [38, 40]. In addition, fibroids interfere with endometrial receptivity by affecting endometrial angiogenesis,

causing inflammatory environment, abnormal mechanical stress conduction, abnormal protein kinase expression during signal transduction pathways, and abnormal endometrial microbial environment. However, it is far enough to simply understand the mechanism of the effect of fibroids on endometrium. More researches are needed to discover and demonstrate the effects of fibroids on the endometrium and other possible molecular mechanisms. Our review provides a reference for clinicians to understand the effects of fibroids on endometrial receptivity, and provides a basis for future solutions to problems such as fibroids affecting embryo implantation and causing abortion.

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