The role of macrophages in reproductive-related diseases

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ABSTRACT

The study of reproductive immunology includes the role of immunity in reproductive physiology and reproductive-related diseases. Reproductive-related diseases cause low pregnancy rate mainly through ovulation disorders, low-quality sperm production, embryo implantation failure and pregnancy maintenance disorders. Numerous cell types including infiltrating immune cells perform specific functions in the reproductive system. Physiologically macrophages are enriched in the decidua and testes, and macrophages are involved in endometrial receptivity, embryo implantation and spermatogenesis. Pathologically macrophages are associated with alterations of decidual microenvironment in recurrent implantation failure (RIF) and unexplained recurrent miscarriage (uRM), local inflammation in polycystic ovary syndrome (PCOS) and clearance of endometriotic lesions in endometriosis. Although researchers have recently attempted to uncover the pathogenesis and provide effective treatments for the reproductive-related diseases, the specific mechanisms and effective therapies need to be further explored. Here we summarized the latest mechanisms by which macrophages participate in the progression of the reproductive-related diseases, and the promising immune-based treatments. In addition, we discussed decidual macrophage classification and the importance of immune networks in reproduction-related diseases.

1. Introduction

Reproductive-related diseases interrupt the process of successful pregnancy. The implantation of the fertilized egg in the endometrium in a receptive state is the basis for pregnancy. Infertility due to recurrent implantation failure (RIF) and recurrent miscarriage (RM) is often due to an unfavorable endometrial environment that is not conducive to implantation. In addition, the combination of high-quality sperm and mature egg is a prerequisite for the formation of a fertilized egg. Polycystic ovary syndrome (PCOS) and endometriosis interrupt the production of mature eggs, and male infertility hinders sperm production. The key role of macrophage in these diseases has been gradually revealed, and understanding the pathogenesis of these diseases is beneficial for the diagnosis and treatment.

Researchers suggested that pregnancy can be divided into three immune stages corresponding to fetal development: the first stage is the pro-inflammatory stage of embryo implantation and placenta formation; the second stage is the anti-inflammatory stage related to fetal development; the third stage is the second pro-inflammatory stage associated with parturition [1]. This classification emphasizes the importance of immunity in maintaining normal pregnancy. Abundant immune cells infiltrate the decidua in early pregnancy, of which 70% are decidual natural killers (dNK) and 25% are decidual macrophages (dMφ) [1, 2]. Decidual macrophages derived from monocytes are known as uterine tissue-resident macrophages, and multiple cytokines contribute to the development of monocytes to macrophages [3]. Macrophages can be divided into M1 and M2 phenotype according to their activation status. CD86 and CD80 are known M1 markers, CD163 and CD206 are known M2 markers [4]. M1 macrophages are characterized by pro-inflammatory and antibacterial effects; however, M2 macrophages are characterized by anti-inflammatory effects and promote trophoblast proliferation [5]. Macrophages participate in the

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preparation of a receptive endometrium during embryo implantation and the decidualization [6, 7]. The live birth rate is approximately 30% per in vitro fertilization (IVF) cycle, but some patients still fail to achieve embryo implantation after three and more high-quality embryo transfer [8], and suffer from recurrent implantation failure (RIF). The RIF may be due to decreased endometrial receptivity [9]. Recurrent miscarriage (RM) is also due to abnormality in the decidual microenvironment. The main known causes of RM include genetic factors, anatomical abnormalities, thrombophilic disorders and endocrine disorders, but at least 50% of the causes are unknown, namely unexplained recurrent miscarriage (uRM) [10].

Polycystic ovary syndrome (PCOS) is a common endocrinopathy in women of reproductive age, accompanied by hyperandrogenism, insulin resistance, polycystic ovaries and infertility [11]. The activation status of macrophages fluctuated regularly during the different phases of the menstrual cycle in healthy women, but this fluctuation was weakened in PCOS [12], implying that macrophages are potentially related to PCOS. Endometriosis is defined as the implantation of endometrial-like tissue outside the uterine cavity [13]. One study reported that nearly 50% of infertility women suffered endometriosis [14]. In the normal endometrium, a large inflow of macrophages is accompanied by an increase in macrophage-derived cytokines during the secretory and menstrual periods [15]. In addition, macrophages play an important role in the beginning of endometrial shedding by secreting matrix metalloproteinases (MMPs) during menstruation [16], implying macrophages may be involved in the pathogenesis of endometriosis.

Infertility affects about 15% of couples, male infertility accounts for 50% of all infertility cases, and at least 30% of male infertility cases remain unknown, namely male idiopathic infertility [17]. Macrophages are the dominant leukocyte type in the testis [18]. Testicular macrophages are involved in vasculation and morphogenesis during the embryonic period. Macrophages are also associated with the vasculature in the adult testicular interstitium [19, 20], and in mouse models, testicular macrophages were reported to support Leydig cell development [21, 22].

Therefore, this present paper will review the latest mechanisms by which macrophages participate in the occurrence and progression of the reproductive-related diseases, and will review the promising immune-based treatments. In addition, decidual macrophage classification and the importance of immune networks in reproduction-related diseases will be discussed in this paper.

2. The role of macrophages in recurrent implantation failure (RIF)

The definition of RIF varies from center to center, but the cumulative number of embryos transferred and the number of embryo transfer cycles are usually considered [6, 23]. The roles of macrophages in RIF are summarized in Table 1. Abnormal distribution and number of macrophages may be associated with RIF. A study involving 1989 patients with RIF or RM showed that CD163+ macrophages accumulated in endometrial glands in nearly 70% cases [24]. Researchers found that abnormal accumulation of macrophages in the endometrial glands caused by adenomyosis may affect embryo implantation in a case report and a retrospective study [25, 26]. In addition, a recent study reported a significant increase in the number of CD163+ macrophages in RIF patients compared with normal IVF controls [27]. CCL2 levels were increased in decidual stromal cells in early pregnancy, and CCL2 regulated Treg and monocytes/macrophages migration [28, 29]. The CCL2 levels in patients with RIF were detected to be relatively higher than those in controls in a cross-sectional study, but the authors did not explore the mechanism in detail [30]. The CCL2 signal can only be passed via CCR2 [31]. CCL2-CCR2 axis increased the expression of M1-related HIF1A gene on macrophages [34]; thus, we propose a hypothesis that increased CCL2 may over-polarize macrophages toward the M2 phenotype through CCL2-CCR2 axis influencing polarization-related genes. This M1/M2 imbalance disrupts the pro-inflammatory environment at the maternal-fetal interface in early pregnancy and ultimately leads to failure of embryo implantation [1].

Considering the involvement of immune cells in maternal-fetal interface in embryo implantation, intrauterine administration of peripheral blood mononuclear cells (PBMC) prior to embryo transfer becomes a promising treatment (shown in Table 6). For fresh embryo transfer, the improvement in implantation, pregnancy and live birth rate via PBMC immunotherapy has been recognized [35]. For frozen/thawed embryo transfer, the improvement of implantation and pregnancy rate is mainly reported in patients who had at least three or four implantation failures [36]; moreover, the promotion of live birth rate is controversial, and cleavage stage transfer may be more beneficial for live birth delivery [36]. It is currently believed that the therapeutic effect is mainly due to the cytokines (e.g. TNF-α, IL-1β and IFN-γ) released by PBMC. These cytokines maintain the pro-inflammatory microenvironment required for embryo implantation and promote embryo invasion [37], and hormonal pretreatment for PBMC greatly stimulates cytokine release [38]. Intrauterine administration of PBMC helps RIF patients improve implantation, pregnancy and live birth rate (shown in Table 6), but this treatment mechanism was rarely studied. PBMC is a mixture of peripheral blood cells, and mainly consists of monocytes and lymphocytes. The fate of PBMC after entering the uterine cavity and which immune cells and cytokines play a key role are worth investigating.

Table 1. Summary regarding macrophages in recurrent implantation failure (RIF).

| Author | Main contributions | Summary |
|--------|-------------------|---------|
| Russell et al. [24] | 1. A study involving 1989 patients with RIF or RM showed that decidual CD163+ macrophages accumulated in endometrial glands in nearly 70% cases. | Abnormal distribution and number of macrophages may be associated with RIF. |
| Tremellen et al. [25] | 1. Adenomyosis was associated with aggregation of macrophages in the superficial endometrial glands. 2. This aggregation may interfere with embryo implantation | |
| Tremellen et al. [26] | 1. The number of CD163+ macrophages in the gland lumens of the superficial endometrium was increased in adenomyosis patients. 2. Adenomyosis may interfere with embryo implantation through immune mechanisms | |
| Papúchová et al. [27] | 1. The number of decidual CD163+ macrophage was greatly increased in RIF patients compared with normal IVF controls | |
| Namli Kalem et al. [30] | 1. The CCL2 levels in patients with RIF were detected to be relatively higher than those in controls. | |
| Needham et al. [31] | 1. The CCL2 signal can only be passed via CCR2. | |
| Miyamoto et al. [32] | 1. CD276 regulated M2 polarization through CCL2-CCR2 axis in ovarian cancer | |
| Zhang et al. [33] | 1. Kinesin family member 4A regulated M2 polarization through CCL2-CCR2 axis | |
| Sierra-Filardi et al. [34] | 1. Blockade of CCL2-CCR2 axis increased the expression of M1-related HIF1A gene on macrophages. | We propose a hypothesis that increased CCL2 may over-polarize macrophages toward the M2 phenotype through CCL2-CCR2 axis influencing polarization-related genes. |
In summary, abnormal distribution and increased number of M2 macrophages are associated with RIF, but the mechanisms have been inadequately studied. Increased M2 macrophages may disrupt the decidual pro-inflammatory microenvironment during implantation, thus leading to RIF. The therapeutic effect of PBMC on RIF is satisfactory, but the specific mechanisms of treatment have also been inadequately studied.

3. The role of macrophages in unexplained recurrent miscarriage (uRM)

WHO defines three or more consecutive miscarriages before the 20 weeks of gestation as recurrent miscarriage (RM) [39]. At least 50% of the causes of recurrent miscarriage are unknown, namely unexplained recurrent miscarriage (uRM) [10]. The roles of macrophages in uRM are summarized in Table 2. In the decidua of uRM patients, researchers have commonly observed an abnormal increase in M1 dMφ or an abnormal decrease in M2 dMφ. The excessively pro-inflammatory environment resulting from the M1/M2 imbalance is responsible for uRM. PD-1 is a co-inhibitory molecule that belongs to the CD28 superfamily, and is expressed on B cells, T cells, NK cells, macrophages, activated monocytes and dendritic cells. PD-L1 is the ligand of PD-1 [40]. PD-1/PD-L1 axis was involved in macrophage polarization and trophoblast invasion. PD-1 expressed on dMφ and PD-L1 expressed on trophoblasts were reduced in uRM patients. PD-1 deficiency induced macrophage polarization into M1 phenotype [41]. PD-L1 deficiency inhibited trophoblast invasion through ERK/MMP pathway [42]. Indoleamine 2, 3-dioxygenase (IDO), an intracellular cytoplasmic enzyme, transfers tryptophan (Trp) to N-formyl kynurenine [43]. IDO-exploded dMφ is significantly lower in uRM patients than in normal control, and IDO+dMφ displayed M2 phenotype [44]. The expression of IDO was also reduced in the decidua of RM patients [45]. In a miscarriage mouse model, IDO supplementation reduced miscarriage rate by suppressing the inflammatory response [46]. Researchers have reported that IDO was a protective factor for early.

### Table 2. Summary regarding macrophages in unexplained recurrent miscarriage (uRM).

| Author            | Main contributions                                                                 | Summary                                                                                                                            |
|-------------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| Zhang et al. [41] | 1. PD-1 expressed on dMφ and PD-L1 expressed on trophoblasts were reduced in uRM patients.  
2. PD-1 deficiency induced macrophage polarization into M1 phenotype. | The imbalance of macrophage M1/M2 ratio has been observed in abortions. The excessively pro-inflammatory environment resulting from the M1/M2 imbalance and abnormal trophoblast function regulated by macrophages are responsible for uRM. |
| Chen et al. [42]  | 1. PD-L1 deficiency inhibited trophoblast invasion through ERK/MMP pathway.         |                                                                                                                                 |
| Huang et al. [44] | 1. IDO-exploded dMφ is significantly lower in uRM patients than in normal control,  
2. IDO+dMφ displayed M2 phenotype.                                                                                                                                 |
| Wei et al. [45]   | 1. The expression of IDO was also reduced in the decidua of RM patients.            |                                                                                                                                 |
| Cheng et al. [46] | 1. In a miscarriage mouse model, IDO supplementation reduced macrophage polarization rate by suppressing the inflammatory response |                                                                                                                                 |
| Goto et al. [49]  | 1. Reduced dMφ-derived cathepsin E has been observed in uRM patients. 
2. Live birth rate was reduced in cathepsin E-/-/- mouse. 
3. Cathepsin E disrupted M1/M2 imbalance. |                                                                                                                                 |
| Sheng et al. [50] | 1. dMφ-derived IL-33 was reduced in uRM patients. 
2. dMφ-derived IL-33 promoted M2 bias at the maternal-fetal interface |                                                                                                                                 |
| Yao et al. [51]   | 1. Histone deacetylases (HDACs) are histone modification enzymes. 
2. HDAC8 expressed on dMφ was decreased in uRM patients. 
3. Decreased HDAC8 resulted in a decrease of CD163 expressed on dMφ. 
4. Decreased HDAC8 promoted Mφ apoptosis via ERK signaling pathway. |                                                                                                                                 |
| Meng et al. [52]  | 1. Receptor activator of nuclear factor κB ligand (RANKL), which is secreted by human embryonic trophoblasts and maternal decidual stromal cells, induced M2 polarization toward the M2 phenotype. 
2. Impaired expression of RANKL caused dMφ abnormal polarization in vivo and increased the fetal loss rate in a mouse model. |                                                                                                                                 |
| Kolben et al. [53] | 1. Peroxisome proliferator-activated receptor γ (PPARγ), a nuclear receptor expressed in trophoblasts and dMφ, is associated with M2 polarization. 
2. In RM PPARγ-exploded dMφ was significantly reduced, reduced PPARγ-exploded dMφ may lead to an inflammatory response against the fetus. |                                                                                                                                 |
| Ding et al. [54]  | 1. CD86-exploded dMφ was increased in uRM patients. 
2. Elevated Fas ligand (FasL) expressed on CD86-exploded dMφ was also observed. 
3. The elevated FasL was a potential cause for RM by mediating trophoblast apoptosis. |                                                                                                                                 |
| Wang et al. [55]  | 1. Ubiquitin-specific protease 2a (USP2a) improved trophoblast migration and invasion via PI3K/Akt/GSK3β signaling pathway. 
2. TGF-β secreted by M2 dMφ enhanced the USP2a expression on trophoblasts. |                                                                                                                                 |
| Hao et al. [56]   | 1. dMφ was associated with decreased nitric oxide (NO) in RM decidua. 
2. Protein L-arginine methyltransferase 3 (PRMT3) expressed on dMφ was increased in RM patients. 
3. Asymmetrical dimethylarginine (ADMA), a protein degradation product mediated by PRMT3, abnormally accumulated in decidua. 
4. ADMA as endogenous nitric oxide synthase inhibitor decreased NO production in decidua. In addition, increased PRMT3 expressed on dMφ promoted trophoblast apoptosis. |                                                                                                                                 |
| Bruno et al. [57] | 1. LMWH increased the expression of CD206 and HLA-DR in macrophages, increased CCL20 secretion and reduced CCL2 and CCL22 secretion. | The potential therapeutic strategies via regulating M1/M2 balance bring hope for uRM patients. |
| Cui et al. [58]   | 1. M1 dMφ was increased in uRM patients, and Rev-erba was reduced in M1 dMφ. |                                                                                                                                 |
| Cui et al. [59]   | 1. SR9009, an agonist of Rev-erba reduced M1-like polarization via PI3K/Akt signaling pathway. |                                                                                                                                 |
| Li et al. [60]    | 1. MSCs inhibited fetal loss in a mouse model. 
2. MSCs inhibited CD4+ T cell proliferation and the M2 phenotype in a tumor necrosis factor-stimulating gene 6 dependent manner |                                                                                                                                 |
pregnancy, and that IDO and IDO⁺ dMφ was reduced in uRM decidua [44, 45], but researchers have also reported that IDO was elevated in uRM villi, and there is no significant difference in the expression of IDO in control decidua and in RM decidua [47]; thus, more studies are needed to prove the role of IDO in uRM. Cathepsin E is an intracellular aspartic protease belonging to the pepsin family [48]. Reduced dMφ-derived cathepsin E has been observed in uRM patients, and live birth rate was reduced in cathepsin E⁻/⁻ mouse, cathepsin E disrupted M1/M2 imbalance [49]. dMφ-derived IL-33 was reduced in uRM patients, and dMφ-derived IL-33 promoted M2 bias at the maternal-fetal interface [50]. Histone deacetylases (HDACs) are histone modification enzymes, HDAC8 expressed on dMφ was decreased in uRM patients [51]. Decreased HDAC8 resulted in a decrease of CD163 expressed on dMφ, and decreased HDAC8 promoted dMφ apoptosis via ERK signaling pathway [51]. Receptor activator of nuclear factor NK-κB ligand (RANKL), which is secreted by human embryonic trophoblasts and maternal decidual stromal cells, induced dMφ toward the M2 phenotype. Impaired expression of RANKL caused dMφ abnormal polarization in vivo and increased the fetal loss rate in a mouse model [52]. Peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor expressed in trophoblasts and dMφ. This receptor activation is associated with M2 polarization. PPARγ⁺ dMφ was significantly reduced in uRM patients, and reduced PPARγ⁺ dMφ may lead to an inflammatory response against the fetus [53]. The role of macrophages in the pathogenesis of uRM is shown in Figure 1.

Macrophages can also be involved in uRM by affecting trophoblast functions. CD86⁺ dMφ was increased in uRM patients, and elevated Fas ligand (FasL) expressed on CD86⁺ dMφ was also observed. The elevated FasL was a potential cause for RM by mediating trophoblast apoptosis [54]. Ubiquitin-specific protease 2a (USP2a) improved trophoblast migration and invasion via PI3K/Akt/GSK3β/β-catenin signaling pathway. TGF-β secreted by M2 dMφ disrupted USP2a expression on trophoblasts [55]. dMφ was associated with decreased nitric oxide (NO) in RM decidua. Protein L-arginine methyltransferase 3 (PRMT3) expressed on dMφ was increased in RM patients; thus, asymmetrical dimethylarginine (ADMA), a protein degradation product mediated by PRMT3, abnormally accumulated in decidua of RM patients. Eventually ADMA as an endogenous nitric oxide synthase inhibitor decreased NO production in decidua. In addition, increased PRMT3 expressed on dMφ promoted trophoblast apoptosis [56].

Based on the M1/M2 imbalance, potential therapeutic strategies are increasingly being investigated. Low molecular weight heparin (LMWH) is often used to treat uRM. LMWH affected macrophage polarization and the cytokine profile. LMWH increased the expression of CD206 and HLA-DR in macrophages, increased CCL20 secretion and reduced CCL2 and CCL22 secretion [57]. M1 dMφ was increased in uRM patients, and Rev-erbα, a significant clock gene, was reduced in M1 dMφ. SR9009, an agonist of Rev-erbα reduced M1-like polarization via PI3K/Akt signaling pathway; thus, SR9009 is a potential therapeutic strategy [58, 59]. Mesenchymal stem cells (MSCs) have been considered as a possibility for the treatment of unexplained recurrent miscarriage. dMφ are involved in unexplained recurrent miscarriage pathogenesis through polarization imbalance and inducing trophoblast dysfunction. In patients with unexplained recurrent miscarriage, decidual macrophages have a tendency to polarize toward M1, and M2 polarization is suppressed. The increased M1 macrophages promote the release of pro-inflammatory cytokines, creating an excessively pro-inflammatory maternal-fetal microenvironment (A). In addition, decidual macrophages make it difficult to maintain pregnancy by promoting trophoblast apoptosis and inhibiting trophoblast migration and invasion (B).

Figure 1. Macrophage involvement in the pathogenesis of unexplained recurrent miscarriage. dMφ are involved in unexplained recurrent miscarriage pathogenesis through polarization imbalance and inducing trophoblast dysfunction. In patients with unexplained recurrent miscarriage, decidual macrophages have a tendency to polarize toward M1, and M2 polarization is suppressed. The increased M1 macrophages promote the release of pro-inflammatory cytokines, creating an excessively pro-inflammatory maternal-fetal microenvironment (A). In addition, decidual macrophages make it difficult to maintain pregnancy by promoting trophoblast apoptosis and inhibiting trophoblast migration and invasion (B).
Various immune diseases. MSCs prevented fetal loss in a mouse model, and MSCs inhibited CD4+ T cell proliferation and reprogrammed M1 phenotype toward the M2 phenotype in a tumor necrosis factor-stimulating gene 6 dependent manner [60]. MSCs ability to balance the decidual immune microenvironment offers hope for the treatment of uRM.

In summary, the excessively pro-inflammatory microenvironment resulting from the M1/M2 imbalance (Figure 1A) and abnormal trophoblast function regulated by dMφ (Figure 1B) are responsible for uRM. Therapeutic strategy to regulate M1/M2 balance may offer new hope for patients.

4. The role of macrophages in polycystic ovary syndrome (PCOS)

The hypothalamus-pituitary gland-ovaries axis is known to control ovulation. Spleen may also be involved in ovulation and may be a direct source of leukocytes for the ovary during the ovulation cycle [61]. Macrophages that differentiated from splenic monocytes are capable of migrating to the ovary, suggesting that the spleen may act as the ovarian immune cell bank [62]. Bilateral superior ovarian nerve sections in rats affected splenic macrophage apoptosis and regulated the ovarian steroid secretion, suggesting that sympathetic nerve may regulate macrophage activity [63].

PCOS is a disease characterized by excessive androgen levels and polycystic ovaries, and the range of serum testosterone is 45–150 ng/dL (2–5 nmol/L) in women with PCOS [64]. The roles of macrophages in PCOS are summarized in Table 3. Ovary and peripheral blood were in a low-grade inflammatory condition in PCOS patients. Serum C-reactive protein (CRP), lymphocytes and monocytes were significantly higher, and the number of macrophages and lymphocytes was also increased in PCOS ovarian tissues [65]. Splenic macrophages were associated with hyperandrogenism in PCOS. Cytokines secreted by splenic macrophages from PCOS mouse stimulated androstenedione production by granulosa cells and inhibited estradiol production, but the exact cytokines are unknown [66]. Excessive androgen inhibited the macrophage viability and increased macrophage apoptosis [67], and promoted pro-inflammatory cytokines expression, such as TNF-α [68]. The increased TNF-α in turn inhibited estradiol production [68]. In addition, TNF-α stimulated proliferation of theca-interstitial cells, possibly contributing to PCOS development [69]. Chemerin is an immunologically active adipokine. Chemerin was elevated systemically in PCOS patients [70, 71]. Chemerin exhibited induction of M1 polarization and inhibition of M2 polarization in other immune-related diseases [72, 73]; however, whether chemerin plays a regulatory role on macrophage polarization in PCOS is unclear.

Ovarian M1 macrophages were increased in androgen-treated rats, and chemerin and chemokine-like receptor 1 (CMKLR1) were increased in ovarian tissues and ovarian macrophages respectively, suggesting that increased chemerin induced CMKLR1+ monocytes recruiting to ovary and these CMKLR1+ monocytes are the source of M1 macrophages. Co-culture assay showed that apoptosis of granulosa cells was dependent on these recruited macrophages [74].

Macrophage migration inhibitory factor (MIF) was the first pro-inflammatory cytokine, and plays an indispensable role in acute and chronic inflammatory conditions and inhibited the ability of macrophages random migration [75, 76]. MIF was increased in ovarian tissues in PCOS rat model, and MIF may participate in PCOS via MAPK signaling pathway [77]. The increased MIF may also participate in PCOS via NF-κB pathway leading to inflammation [78]. Plasma MIF levels were significantly higher in PCOS patients than healthy women [79, 80]; thus, plasma MIF levels may be a potential predictor of PCOS. MIF is involved in pathogenesis of PCOS, and in vitro experiments have demonstrated that MIF inhibited macrophage migration [75, 76], thus; it is worth investigating whether MIF is involved in PCOS by inhibiting macrophage migration. Researchers found that MIF was positively correlated with obesity [79], but it has also been found that MIF was not associated with obesity in PCOS [80]; thus, more researches are needed to prove the relationship between MIF and obesity in PCOS.

Overall, the inflammatory response involving macrophages and macrophage-related cytokines are associated with the progression of PCOS. Rodent models of PCOS have been widely used [81], but genomic differences between humans and animals are objective. The use of human-derived cells and biocompatible biomaterials to construct ovarian organoid may provide a new model for the study of the mechanisms of PCOS [82, 83].

5. The role of macrophages in endometriosis

Endometriosis is defined as the implantation of endometrial-like tissue (‘lesions’) outside the uterine cavity, usually in the pelvic cavity or

| Author          | Main contributions                                                                 | Summary                                                                 |
|-----------------|-------------------------------------------------------------------------------------|------------------------------------------------------------------------|
| Xiong et al. [65] | 1. Serum C-reactive protein (CRP), lymphocytes and monocytes were significantly higher in PCOS patients. 2. The number of macrophages and lymphocytes was also increased in PCOS ovarian tissues.            | The inflammatory response involving macrophages and macrophage-related cytokines are associated with the progression of PCOS. |
| Figueroa et al. [66] | 1. In PCOS mouse model, cytokines secreted by splenic macrophages stimulated androstenedione production by granulosa cells and inhibited estradiol production. |                                                                           |
| Li et al. [67]    | 1. Excessive androgen inhibited the macrophage viability and increased macrophage apoptosis                                               | Chemerin is associated with PCOS, but whether chemerin plays a regulatory role on macrophage polarization in PCOS is unclear. |
| Huang et al. [71] | 1. Serum chemerin was elevated systemically in PCOS patients.                                                                       |                                                                        |
| Ji et al. [72]    | 1. Chemerin promotes the pathogenesis of preeclampsia by inducing M1 macrophage polarization                                           |                                                                        |
| Lin et al. [73]   | 1. Chemerin aggravates colitis by suppressing M2 macrophage polarization.                                                            |                                                                        |
| McCartney et al. [74] | 1. Ovarian M1 macrophages were increased in PCOS rat model. 2. Chemerin was increased in ovarian tissues. 3. Chemokine-like receptor 1 (CMKLR1) was increased in ovarian macrophages. 4. Apoptosis of granulosa cells was dependent on these CMKLR1+ macrophages. |                                                                        |
| Zhou et al. [77] | 1. MIF was increased in ovarian tissues in PCOS rat model. 2. MIF may participate in PCOS via MAPK signaling pathway.               | MIF is involved in PCOS via different signaling pathways, and it is not clear whether MIF affects macrophage migration in PCOS. |
| He et al. [78]    | 1. MIF was increased in ovarian tissues in PCOS rat model. 2. MIF may participate in PCOS via NF-κB pathway.                          |                                                                        |
| González et al. [80] | 1. Plasma MIF levels were significantly higher in PCOS patients.                                                                 |                                                                        |
| Bennett et al. [75] | 1. MIF inhibited the ability of macrophages random migration                                                                        |                                                                        |
ovaries. Endometriosis correlates with chronic pelvic pain, menstrual disorders and infertility [13]. Infertility may be due to an inflammatory microenvironment that is not conducive to conception or due to ovulation disorders caused by ovarian endometriosis [84]. The roles of macrophages in endometriosis are summarized in Table 4. Macrophages are associated with pain caused by endometriosis. The concentration of macrophage-derived insulin-like growth factor-1 (IGF-1) was elevated in peritoneal fluid from endometriosis patients and there was a positive correlation between IGF-1 and pain score, possibly because IGF-1 enhanced nerve sensitization and neurogenesis [85]. CT/CC genotype of miR-146b rs1536309 correlated with the risk of pain in endometriosis, the patients carrying the CT/CC genotype of miR-146b rs1536309 had reduced expression of miR-146b in macrophages, and in this condition inflammation was enhanced [86].

Table 4. Summary regarding macrophages in endometriosis.

| Author | Main contributions | Summary |
|--------|-------------------|---------|
| Forster et al. [85] | 1. The macrophage-derived insulin-like growth factor-1 (IGF-1) was elevated in peritoneal fluid from endometriosis patients.  
2. There was a positive correlation between IGF-1 and pain score.  
3. IGF-1 enhanced nerve sensitization and neurogenesis. | Macrophages are associated with pain caused by endometriosis. |
| Zhang et al. [86] | 1. CT/CC genotype of miR-146b rs1536309 correlated with the risk of pain in endometriosis.  
2. The patients carrying the CT/CC genotype of miR-146b rs1536309 had reduced expression of miR-146b in macrophages. | The phagocytic ability of peritoneal macrophages was weakened in patients with endometriosis. The weakened clearance of peritoneal macrophages eventually cause the development of endometriosis. |
| Wu et al. [80] | 1. The concentration of prostaglandin (PG) E₂ (PGE₂) was elevated in peritoneal fluid from patients with endometriosis. | The phagocytic ability of peritoneal macrophages was weakened in patients with endometriosis. The weakened clearance of peritoneal macrophages eventually cause the development of endometriosis. |
| Wu et al. [91] | 1. PGE₂ inhibited the expression of annexin A2 on peritoneal macrophages via the EP₂/EP₄ receptor-dependent signaling pathway.  
2. Decreased annexin A2 was associated with weakened macrophage phagocytosis. | The weakened clearance of peritoneal macrophages was weakened in patients with endometriosis. The weakened clearance of peritoneal macrophages eventually cause the development of endometriosis. |
| Chuang et al. [92] | 1. PGE₂ inhibited the expression of CD36 on peritoneal macrophages.  
2. Decreased CD36 was associated with weakened macrophage phagocytosis. | The weakened clearance of peritoneal macrophages was weakened in patients with endometriosis. The weakened clearance of peritoneal macrophages eventually cause the development of endometriosis. |
| Liu et al. [94] | 1. The expression of CD47 on ectopic endometrial stromal cells (ESCs) was significantly elevated in endometriosis patients.  
2. As CD47 ligands, thrombospondin-1 (TSP1) and signal regulated protein α (SIRPs) were also increased.  
3. The elevated CD47 binding to elevated SIRPα expressed on peritoneal macrophages exhausted macrophage phagocytic ability and improved M2 polarization. | The weakened clearance of peritoneal macrophages was weakened in patients with endometriosis. The weakened clearance of peritoneal macrophages eventually cause the development of endometriosis. |
| Liu et al. [96] | 1. Hemat abnormally accumulated in the peritoneal fluid from patients with endometriosis,  
2. High concentration of hem treatment exacerbated the damage to phagocytic ability. | The weakened clearance of peritoneal macrophages was weakened in patients with endometriosis. The weakened clearance of peritoneal macrophages eventually cause the development of endometriosis. |
| Clark et al. [97] | 1. The expression of CD200 was upregulated in endometrial-like tissues.  
2. Soluble CD200 (sCD200) was also increased in the small veins of endometrial-like tissues. | The weakened clearance of peritoneal macrophages was weakened in patients with endometriosis. The weakened clearance of peritoneal macrophages eventually cause the development of endometriosis. |
| Weng et al. [98] | 1. The expression of CD200 receptor in peritoneal macrophages was also elevated.  
2. The phagocytic ability of macrophages in vitro gradually decreased with the increasing CD200 levels. | The weakened clearance of peritoneal macrophages was weakened in patients with endometriosis. The weakened clearance of peritoneal macrophages eventually cause the development of endometriosis. |
| Nie et al. [100] | 1. Ectopic endometrial homogenates promoted M1 to M2 macrophage polarization. | M2 macrophages were increased in patients with endometriosis, and the increased M2 macrophages are associated with ectopic ESCs proliferation, angiogenesis and lymphangiogenesis |
| Sun et al. [101] | 1. After treatment with Exosomes from endometriosis, the macrophages were polarized towards an M2 phenotype. | M2 macrophages were increased in patients with endometriosis, and the increased M2 macrophages are associated with ectopic ESCs proliferation, angiogenesis and lymphangiogenesis |
| Gou et al. [102] | 1. The ectopic ESCs-derived CCL2 recruiting macrophages through estrogen receptor β (ERβ) and NFκB signaling. | M2 macrophages were increased in patients with endometriosis, and the increased M2 macrophages are associated with ectopic ESCs proliferation, angiogenesis and lymphangiogenesis |
| Huang et al. [103] | 1. The ectopic ESCs-derived exosomal miR-301a-3p enhanced M2 polarization via PTEN-PI3K pathway. | M2 macrophages were increased in patients with endometriosis, and the increased M2 macrophages are associated with ectopic ESCs proliferation, angiogenesis and lymphangiogenesis |
| Sun et al. [104] | 1. The ectopic ESCs-derived extracellular vesicular legumain pseudogene 1 (EV-LGMNP1) was increased in endometriosis patients.  
2. The elevated EV-LGMNP1 induced M2 polarization, and serum increased EV-LGMNP1. | M2 macrophages were increased in patients with endometriosis, and the increased M2 macrophages are associated with ectopic ESCs proliferation, angiogenesis and lymphangiogenesis |
| Miller et al. [105] | 1. IL-17A could induce macrophage recruitment and M2 polarization. | M2 macrophages were increased in patients with endometriosis, and the increased M2 macrophages are associated with ectopic ESCs proliferation, angiogenesis and lymphangiogenesis |
| Ono et al. [106] | 1. The sphingosine 1-phosphate concentration was greatly increased in peritoneal fluid from patients with endometriosis  
2. Sphingosine 1-phosphate induced M2 polarization. | M2 macrophages were increased in patients with endometriosis, and the increased M2 macrophages are associated with ectopic ESCs proliferation, angiogenesis and lymphangiogenesis |
| Itoh et al. [107] | 1. M2 macrophages accelerated ectopic ESCs proliferation through Stat3 activation. | M2 macrophages were increased in patients with endometriosis, and the increased M2 macrophages are associated with ectopic ESCs proliferation, angiogenesis and lymphangiogenesis |
| Ono et al. [108] | 1. M2 macrophages contributed to the angiogenesis in the endometriosis mouse model. | M2 macrophages were increased in patients with endometriosis, and the increased M2 macrophages are associated with ectopic ESCs proliferation, angiogenesis and lymphangiogenesis |
| Chan et al. [109] | 1. Macrophages facilitated the invasion of endometrial stromal cells. | M2 macrophages were increased in patients with endometriosis, and the increased M2 macrophages are associated with ectopic ESCs proliferation, angiogenesis and lymphangiogenesis |
| Hattori et al. [110] | 1. Macrophages-derived VEGF-C and VEGF-D promotes lymphangiogenesis in a VEGFR1-dependent manner. | M2 macrophages were increased in patients with endometriosis, and the increased M2 macrophages are associated with ectopic ESCs proliferation, angiogenesis and lymphangiogenesis |
| Nagai et al. [111] | 1. MCP-1 is an inducer of macrophage recruitment.  
2. Focal adhesion kinase (FAK) inhibitors significantly inhibited the secretion of MCP-1 by ectopic ESCs. | New targets based on the regulation of macrophage function may provide new ideas for the treatment of endometriosis. |
| Sekulovski et al. [113] | 1. M2 macrophages promoted ectopic ESCs viability.  
2. This promotion was also inhibited by niclosamide. | New targets based on the regulation of macrophage function may provide new ideas for the treatment of endometriosis. |
| Xu et al. [114] | 1. EPHA3 promoted macrophage apoptosis and autophagy by inhibiting the mTOR signaling pathway in endometriosis mouse model.  
2. EPHA3 and mTOR may be new therapeutic targets. | New targets based on the regulation of macrophage function may provide new ideas for the treatment of endometriosis. |
| Mattos et al. [120] | 1. vGalectin-3 promoted the development of endometriosis.  
2. Galectin-3 inhibitors reduced endometriosis by inhibiting angiogenesis and the enrichment of M2 macrophages. | New targets based on the regulation of macrophage function may provide new ideas for the treatment of endometriosis. |
Fifty percent of human peritoneal leukocytes are macrophages [87], and peritoneal macrophages are involved in inflammatory and infectious diseases through various immunomodulatory mechanisms [88]. The role of macrophages in the pathogenesis of endometriosis is shown in Figure 2. The phagocytic ability of peritoneal macrophages was weakened in patients with endometriosis; thus, these peritoneal macrophages lacked the ability to remove endometrial-like tissues in the peritoneal cavity [89]. The concentration of prostaglandin (PG) E$_2$ (PGE$_2$) was elevated in peritoneal fluid from patients with endometriosis [90]. PGE$_2$ inhibited the expression of annexin A2 on peritoneal macrophages via the EP$_2$/EP$_4$ receptor-dependent signaling pathway [91], and inhibited the expression of CD36 on peritoneal macrophages [92]. The decreased annexin A2 and CD36 were associated with weakened macrophage phagocytosis. TGF-$\beta$ inhibitor increased the expression of CD36 in vitro; thus, TGF-$\beta$ inhibitor may be a potential treatment [93]. The TSP1-CD47-SIRP$_\alpha$ axis promoted the development of endometriosis through several ways. CD47 is a phagocytic check point protein, the expression of CD47 on ectopic endometrial stromal cells (ESCs) was significantly elevated in endometriosis patients [94]; furthermore, as CD47 ligands, thrombospondin-1 (TSP1) and signal regulated protein $\alpha$ (SIRP$_\alpha$) were also increased [93, 94]. The elevated CD47 binding to elevated SIRP$_\alpha$ expressed on peritoneal macrophages exhausted macrophage phagocytic ability and improved M2 polarization [94], and elevated CD47 reduced the apoptosis of ectopic ESCs [95]. Heme abnormally accumulated in the peritoneal fluid from patients with endometriosis, and the high concentration of heme (30 $\mu$mol/L) treatment exacerbated the damage to phagocytic ability [96]. The expression of CD200 was upregulated in endometrial-like tissues and soluble CD200 (sCD200) was also increased in the small veins of endometrial-like tissues [97]. The expression of CD200 receptor in peritoneal macrophages was also elevated. The phagocytic ability of macrophages in vitro gradually decreased with the increasing CD200 levels, indicating that CD200 damaged the macrophage phagocytosis and may facilitate the immune escape of ectopic lesions [98]. The expression of CD200 in the plasma was also elevated; thus, plasma CD200 may be may be a new diagnostic strategy for endometriosis [98].

M2 macrophages were dominant in endometriotic lesions and peritoneal fluid [99]. The polarization of macrophages toward M2 phenotype was upregulated upon exposure to exosomes and homogenates from ectopic ESCs in vitro, implying that ectopic ESCs were responsible for M2 polarization [100, 101]. The ectopic ESCs-derived CCL2 recruiting macrophages through estrogen receptor $\beta$ (ER$\beta$) and NF-$\kappa$B signaling [102]. The ectopic ESCs-derived exosomal miR-301a-3p enhanced M2 polarization via PTEN-P13K pathway [103]. The ectopic ESCs-derived extracellular vesicular legumain pseudogene 1 (EV-LGMN1) was increased in endometriosis patients. The elevated EV-LGMN1 induced M2 polarization, and serum increased EV-LGMN1 suggested that EV-LGMN1 may have clinical implications [104]. Abnormally elevated components in peritoneal fluid may also be inducers of M2 polarization, such as IL-17A and sphingosine1-phosphate (S1P) [105, 106]. M2 macrophages accelerated ectopic ESCs proliferation through stat3 activation [107], and contributed to the angiogenesis through producing VEGF-A and TGF$\beta$1 [108]. Macrophages facilitated the invasion of endometrial

![Figure 2](image_url)

**Figure 2.** Macrophage involvement in the pathogenesis of endometriosis. The phagocytosis to ESCs is weakened, resulting in the inability to clear proliferating ectopic ESCs (A). In addition, abnormal components in ectopic ESCs and peritoneal fluid promote M2 polarization. M2 macrophages promote angiogenesis and lymphangiogenesis, as well as promote the proliferation and invasion of ectopic ESCs (B). The weakened clearance and promoted growth of ESCs eventually cause the development of endometriosis.
Eubler et al. [135] 1. The cation channel TRPV2 was expressed on CD206
2. Human frozen/thawed
3. Human frozen/thawed

Yu et al. [134] 1. The increased estradiol may be a risk factor for infertility. Estrogen promoted the expression
2. Human fresh oocyte cleavage CRH, 2 days
3. Implantation and pregnancy rate were promoted
4. Makrigiannakis et al. [633]

Lin et al. [129] 1. Ubiquitin-specific protease 2 (USP2) affected spermatogenesis because it is merely expressed
2. in early-elongated sperm
3. in fertility men; however, these two subtypes were not expressed on testicular macrophages
4. in mouse with endometriosis [114]. Xu et al. found that
5. EPHA3 promoted macrophage apoptosis and autophagy by inhibiting the
6. mTOR signaling pathway in mouse model of endometriosis [114]; thus, the
7. mTOR signaling pathway could be a potential therapeutic target for endometriosis.

Table 5. Summary regarding macrophages in male idiopathic infertility.

| Author          | Main contributions                                                                 | Summary                                                                 |
|-----------------|-------------------------------------------------------------------------------------|------------------------------------------------------------------------|
| DeFalco et al.  | 1. Macrophages were localized near undifferentiated spermatogonia.                 | Testicular macrophages play a pivotal role in spermatogenesis, steroid production, and immune privilege. |
|                 | 2. Macrophages expressed factors related to spermatogonial growth and differentiation. |                                                                        |
|                 | 3. In the absence of macrophages undifferentiated spermatogonia exhibited defect in differentiation, and the number of proliferative spermatogonia was decreased. |                                                                        |
| Lukyanenko et al. | 1. 25-Hydroxycholesterol secreted by testicular macrophages improved the testosterone production by Leydig cells. |                                                                        |
| Wang et al. [128] | 1. Corticosterone produced by testicular macrophages suppressed the secretion of proinflammatory cytokines, maintaining the immune privilege in the testis. | Testicular macrophages participate in the pathogenesis of male idiopathic infertility. |
| Lin et al. [129] | 1. Ubiquitin-specific protease 2 (USP2) affected spermatogenesis because it is merely expressed in late-elongated sperm. |                                                                        |
| Bedard et al. [130] | 2. The USP2/–/– male mouse had severe infertility, the infertility was related to defects in sperm motility. |                                                                        |
| Hashimoto et al. [131] | 3. The USP2 in macrophages facilitated sperm movement and hyperactivation, and USP2 maintained GM-CSF expression in testicular macrophages, GM-CSF was also associated with sperm motility. |                                                                        |
| Frungieri et al. [132] | 1. In the testis of idiopathic infertility, the number of CD68+ macrophages was increased. 2. These macrophages expressed IL-1 and TNF-α. The pro-inflammatory cytokines may damage sperm. |                                                                        |
| Matzkin et al. [133] | 1. The prostaglandin D2 (PGD2) produced by testicular macrophages was increased via β1-and β2-adrenergic receptors (ADRs). 2. The α1- and β3-ARs expressed on testicular macrophages were observed in idiopathic infertility men; however, these two subtypes were not expressed on testicular macrophages in fertility men. |                                                                        |
| Yu et al. [134] | 1. The increased estradiol may be a risk factor for infertility. Estrogen promoted the expression of growth arrest-specific 6 (GAS6) on Leydig cells, and GAS6 bound to the phosphatidylserine exposed on the Leydig cell surface. 2. Macrophage phagocytosis was promoted through GAS6 binding to Axl expressed on macrophages, leading to reduced Leydig cell functions. |                                                                        |
| Eibler et al. [135] | 1. The cation channel TRPV2 was expressed on CD206+ testicular macrophages, and elevated estrogen promoted TRPV2 expression, but whether TRPV2 plays a role in infertility is unknown. |                                                                        |

Table 6. Clinical trials in PBMC immunotherapy.

| Number of RIF patients | Oocyte source | Embryo stage | PBMC pretreatment | Main results | Reference |
|------------------------|---------------|--------------|-------------------|--------------|-----------|
| 35                     | Human fresh oocyte | blastocyst | HCG, 2 days | Implantation rate, pregnancy rate and live birth rate were promoted | Yoshioka et al. [35] |
| 253                    | Human frozen/thawed embryo | Cleavage and blastocyst | No (freshly isolated PBMC) | Implantation and pregnancy rate were promoted in patients who had three or more implantation failures; No effect on live birth rate | Okutsu et al. [145] |
| 45                     | Human fresh oocyte | blastocyst | CRH, 2 days | Pregnancy rate was promoted | Makrigiannakis et al. [146] |
| 54                     | Human fresh oocyte | cleavage | HMG, 3 days | Implantation and pregnancy rate were promoted | Madkour et al. [147] |
| 633                    | Human frozen/thawed embryo | Cleavage and blastocyst | HCG, 2 days | Implantation rate and pregnancy rate were promoted in patients with four or more implantation failures; live birth rate was promoted in patients receiving cleavage stage embryo transfer | Li et al. [36] |
| 26                     | Human fresh oocyte | cleavage | CRH, 2 days | Implantation and pregnancy rate were promoted | Makrigiannakis et al. [148] |
| 250                    | Human frozen/thawed embryo | Cleavage and blastocyst | CRH, 2 or 3 days | Pregnancy rate was promoted in patients with three or more implantation failures | Nobijari et al. [149] |
| 100                    | Human frozen/thawed embryo | Cleavage and blastocyst | HCG, 2 days | Pregnancy and live birth rate were promoted; miscarriage rate was lower | Pourmoghadam et al. [38] |
| 207                    | Human frozen/thawed embryo | Cleavage and blastocyst | HCG, 4 h | No effect on implantation rate and live birth rate | Mei et al. [150] |

RIF: recurrent implantation failure; PBMC: peripheral blood mononuclear cells; HCG: human chorionic gonadotrophin; CRH: corticotropin releasing hormone; HMG: human menopausal gonadotrophin.
The authors attempted to use this mechanism to explain the pathogenesis of endometriosis, but the authors only studied the changes in EPHA3 and mTOR protein levels and did not examine in detail which specific molecules in the mTOR signaling pathway play a role. Previous studies have found that macrophage apoptosis can be inhibited via PI3K/Akt/mTOR pathway [115], AMPK/mTOR/TFE3 pathway [116] and TREM2/mTOR axis [117]; moreover, macrophage apoptosis can be inhibited via Akt/mTOR [118] and AMPK/mTOR pathway [119]. Based on the above discussion, it is worthwhile to investigate which molecules that cause mTOR inhibition are driven by EPHA3 ultimately leading to increased apoptosis and autophagy of macrophages in endometriosis. Galectin-3 promoted the development of endometriosis, galectin-3 inhibitors reduced endometriosis by inhibiting angiogenesis and the enrichment of M2 macrophages [120]. M2 macrophage are dominant in endometriosis patients in the absence of disruption for immune system. In the presence of infection, the number of M1 macrophages increased and replaced the dominance of M2 macrophages [121]. M1 macrophage-derived secretions were beneficial for lesion clearance via polarizing M2 to M1 [122]. LPS-induced macrophages reduced endometriosis-like lesion growth in an IL-10-dependent manner [123]; thus, whether the development of endometriosis can be inhibited by inducing a mild inflammatory response is an interesting question.

Overall, in terms of clinical symptom, macrophages are involved in pain caused by endometriosis. In terms of the mechanism of endometriosis, macrophages play a key role in the weakened clearance (Figure 2A) and promoted growth of ESCs (Figure 2B), and both processes promoted development of endometriosis.

6. The role of macrophages in male idiopathic infertility

Male infertility accounts for approximately 50% of global infertility cases, and 30%–40% of the causes are unclear [17]. Testicular macrophages are the largest immune cell population in the human testis, and the subpopulation of resident macrophages was CD163<sup>+</sup> M2 macrophages [124, 125]. The roles of macrophages in male reproduction physiology and male idiopathic infertility are summarized in Table 5. Testicular macrophages play a pivotal role in spermatogenesis, steroid production and immune privilege. Macrophages were localized near undifferentiated spermatogonia, and these macrophages expressed factors related to spermatogonial growth and differentiation, such as colony stimulating factor 1 (CSF1) [126]. In the absence of macrophages undifferentiated spermatogonia exhibited defect in differentiation, and the number of proliferative spermatogonia was decreased [126]. 25-Hydroxycorticosterone produced by testicular macrophages suppressed the secretion of proinflammatory cytokines, maintaining the immune privilege in the testis [127] Abbreviation and full name cross-reference table was shown in Table 7.

Testicular macrophages participate in the pathogenesis of male idiopathic infertility. Ubiquitin-specific protease 2 (USP2) affected spermatogenesis because it is merely expressed in late-elongated sperm [129]. The USP2<sup>−/−</sup> male mouse had severe infertility, the infertility was related to defects in sperm motility [130]. The USP2 in macrophages facilitated sperm movement and hyperactivation, and USP2 maintained GM-CSF expression in testicular macrophages, GM-CSF was also associated with sperm mobility [131]. In the tests of idiopathic infertility, the number of CD68<sup>+</sup> macrophages was increased, and these macrophages expressed IL-1 and TNF-α. These pro-inflammatory cytokines may damage sperm [132]. Although the number of CD68<sup>+</sup> macrophages was increased in the tests of idiopathic infertility [132], no specific mechanism of abnormal macrophage polarization was found; hence, it is interesting to explore whether abnormal changes in testicular microenvironment resulting from abnormal macrophage polarization cause male infertility.

### Table 7. Abbreviation and full name cross-reference table.

| Abbreviation | Full Name |
|--------------|-----------|
| ADMA         | Asymmetrical dimethylarginine |
| ADrs         | Adrenergic receptors |
| AMPK         | Adenosine monophosphate-activated protein kinase |
| CCL2         | Chemokine (C-C motif) ligand-2 |
| CCL22        | Chemokine (C-C motif) ligand-22 |
| CR2          | Chemokine (C-C motif) receptor-2 |
| CMKLRI1      | Chemokine-like receptor 1 |
| CRP          | C-reactive protein |
| CSF1         | Colony stimulating factor 1 |
| dMpg         | Decidual macrophages |
| dNK          | Decidual natural killer |
| EPHA3        | Erythropoietin-producing hepatocellular carcinoma A3 |
| ERβ          | Estrogen receptor β |
| ESCs         | Endometrial stromal cells |
| EV-LGMNP1     | Extracellular vesicular legumain pseudogene 1 |
| FAK          | Focal adhesion kinase |
| Fn-L         | Fas ligand |
| GAS6         | Growth arrest-specific 6 |
| GM-CSF       | Granulocyte-macrophage colony stimulating factor |
| HDACs        | Histone deacetylases |
| IDO          | Indoleamine 2, 3-dioxygenase |
| IFN-γ        | Interferon-gamma |
| IGF-1        | Insulin-like growth factor-1 |
| IL-10/1β/33 | Interleukin-10/1beta/33 |
| IVF          | In vitro fertilization |
| LMWH         | Low molecular weight heparin |
| LPS          | Lipopolysaccharide |
| MAPK         | Mitogen-activated protein kinase |
| MIF          | Macrophage migration inhibitory factor |
| MMPs         | Matrix metalloproteinases |
| MSCs         | Mesenchymal stem cells |
| mTOR         | Mammalian target of rapamycin |
| NF-κB        | Nuclear factor-kappaB |
| NK           | Natural killer |
| NO           | Nitric oxide |
| PAMM         | Placenta-associated macrophages |
| PMBC         | Peripheral blood mononuclear cells |
| PCOS         | Polycystic ovary syndrome |
| PD-1         | Programmed cell death-1 |
| PD-L1        | Programmed cell death-ligand 1 |
| PGD2         | Prostaglandin D<sub>2</sub> |
| PG12         | Prostaglandin E2 |
| PI3K         | Phosphatidylinositol-3-kinase |
| PPARγ        | Peroxisome proliferator-activated receptor γ |
| PRMT3        | Protein L-arginine methyltransferase 3 |
| RANKL        | Receptor activator of nuclear factor NK-xB ligand |
| RIF          | Recurrent implantation failure |
| RM           | Recurrent miscarriage |
| S1P           | Sphingosine-1-phosphat |
| STPα          | Signal regulated protein α |
| TGF-β         | Transforming growth factor-beta |
| Th1         | T helper type 1 |
| TNF-α        | Tumor necrosis factor-alpha |
| TSPO         | Thrombopondin-1 |
| uBGM         | Unexplained recurrent miscarriage |
| USP2         | Ubiquitin-specific protease 2 |
| USP2a        | Ubiquitin-specific protease 2a |
infertility. The prostaglandin D2 (PGD2) produced by testicular macrophages was increased via β1- and β2-adrenergic receptors (ADRs) [133]. The α1- and β3-ADRs expressed on testicular macrophages were observed in idiopathic infertility men; however, these two subtypes were not expressed on testicular macrophages in fertility men, but the function of α1- and β3-ADRs was unclear [133]. The increased estradiol may be a risk factor for infertility. Estrogen promoted the expression of growth arrest–specific 6 (GAS6) on Leydig cells, and GAS6 bound to the phosphatidylserine exposed on the Leydig cell surface. Macrophage phagocytosis was promoted through GAS6 binding to AXL expressed on macrophages, leading to reduced Leydig cells [134]. The cation channel TRPV2 was expressed on CD206+ testicular macrophages, and elevated estrogen promoted TRPV2 expression, but whether TRPV2 plays a role in infertility is unknown [135].

In a word, macrophages are indispensable in the normal physiological process of male reproduction by regulating spermatogenesis, hormone production and immune tolerance, and are also important in the pathological processes of male infertiltiy.

7. Discussion

The M1/M2 classification of macrophages was referenced to the Th1/Th2 classification of helper T cells [136]. IFN-γ, LPS, TNFα and GM-CSF act as stimuli for M1 polarization (Figure 3A). M1 macrophages highly express CD80 and CD86, and secrete pro-inflammatory cytokines (Figure 3A). IL-4, IL-10, IL-13and TGF-β act as stimuli for M2 polarization, and CD206 and CD163 have been suggested as M2 markers [5, 137] (Figure 3A); however, this simple M1/M2 classification is not perfect. CD163+ macrophages are elevated in tumor microenvironments with Th1 signature, implying that CD163 alone may not be suitable as a marker for M2 [138]. Combination of CD163 and the transcription factor CMAF more accurately identifies M2 macrophages [139]. There is no information that CD169+ macrophages in lymph node and spleen can be classified into M1 or M2 macrophages [137]. The M1/M2 classification is the most commonly used in the study of reproductive-related diseases, but recently researchers have made efforts to break this classification (Figure 3B). Strominger et al. divided dMφ into CD11c+ and CD11c− macrophages (Figure 3B). CD11c+ macrophages were associated with lipid metabolism and inflammation, CD11c− macrophages were associated with extracellular matrix formation and tissue growth. Each of these populations secrete both pro- or anti-inflammatory cytokines; thus, these populations cannot be simply classified into M1 or M2 macrophages [140]. In 2018, Vento-Tormo et al. divided decidual macrophages into ITGAX+ dM1 and ITGAX+ dM2 using single-cell reconstitution at the maternal-fetal interface, and found the gene expression of IL-10, IL-6, IL-1β was significantly higher in ITGAX+ dM1 than in ITGAX+ dM2 [141] (Figure 3B). In 2021, Thomas et al. identified placenta-associated macrophages (PAMM) using single-cell sequencing. PAMM was subdivided into 3 populations: PAMM1a (FOLR2 hi/CD9loCCR2lo/int), PAMM1b (FOLR2 hi/CD9−/CCR2hi) and PAMM2 (HLA-DR hi/FOLR2hi) (Figure 3B). PAMM1a adhered to the surface of the placental villi and had a repairing effect, PAMM1b was monocyte and PAMM2 was similar to dM2 that was described by Vento-Tormo et al. [142]. Whether these new classifications can better categorize macrophage heterogeneity and functions requires further study.

One immune cell is not an island, one immune cell usually needs the help of other immune cells and cytokines to function, and the role of immune network consisting of immune cells and cytokines in disease...
cannot be ignored. The complex immune network has been studied in infection and cancer. For example, in infection, TGF-β secreted by Treg and other immune cells, binds to Treg and builds a bridge between Treg and effector T cells, ultimately leading to the suppression of effector T cell functions [143]. In cancer, IFN-γ secreted by T helper type 1 (Th1) recruits M1 macrophages, and IL-12 secreted by M1 macrophages recruits and activates Th1 cells, and also promotes the maturation of dendritic cells [144]. Although researchers have attempted to explore immune networks in reproductive-related diseases, these studies only revealed the relationship of immune cells in numbers and distribution, and did not investigate the molecular interactions between immune cells or the cytokine communication. For example, the number of decidual CD163⁺ macrophages in RIF patients was greatly higher than those in normal controls, and CD163⁺ macrophages were positively correlated with CD56⁺ dNK [27]. The distribution of CD56⁺ dNK cells is similar to that of CD8⁺ T cells in the endometrium of RIF patients [24]; therefore, in future researchers should pay more attention to the role of immune networks and delve deeper into the interaction mechanisms in reproductive-related diseases.

8. Conclusion

In summary, macrophages are essential for embryo implantation, pregnancy maintenance and spermatogenesis. Macrophage polarization and phagocytosis are involved in the development of endometriosis; however, current researches have not been able to reveal the precise regulatory mechanisms of macrophages in reproductive-related diseases, and there is still a long way to go in the exploration of macrophages. The immune system is a complex regulatory network. Although macrophages are enriched in the decidua and testis, the regulatory role of other immune cells cannot be ignored. In future, studies on reproductive-related diseases should pay more attention to the interregulation between macrophages and other immune cells.

Declarations

Author contribution statement

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Additional information

No additional information is available for this paper.

References

[1] G. Mor, P. Aldo, A.B. Alvero, The unique immunological and microbial aspects of pregnancy, Nat. Rev. Immunol. 17 (8) (2017) 469–482.
[2] J.N. Bulmer, D. Pace, A. Ritson, Immunoregulatory cells in the human decidua: morphology, immunohistochemistry and function, Reprod. Nutr. Dev. 28 (6B) (1988) 1599–1613.
[3] R.H. McIntire, K.G. Ganacias, J.S. Hunt, Programming of human monocytes by the uteroplacental environment, Reprod. Sci. 15 (5) (2008) 437–447.
[4] A. Viola, F. Munari, R. Sánchez-Rodríguez, T. Scolaro, A. Castegna, The metabolic signature of macrophage responses, Front. Immunol. 10 (2019) 1462.
[5] Y. Yao, X.H. Xu, J. Jin, Macrophage polarization in physiological and pathological pregnancy, Front. Immunol. 10 (2019) 792.
[6] B.K. Tan, P. Vanderkerckhove, R. Kennedy, S.D. Keey, Investigation and current management of recurrent IVF treatment failure in the UK, BJOG 112 (6) (2005) 773–780.
[7] P.G. Lea, D.A. Clark, Macrophages and migratory cells in endometrium relevant to implantation, Baillieres Clin. Obstet. Gynaecol. 5 (1) (1991) 25–59.
[8] G. Bahadur, R. Homburg, J.E. Bosmans, et al., Observational retrospective study of UK national success, risks and costs for 319,105 IVF/ICSI and 30,669 IUI treatmentcycles, BMJ Open 10 (3) (2020), e025365.
[9] J. Bellver, C. Simon, Implantation failure of endometrial origin: what is new? Curr. Opin. Obstet. Gynecol. 30 (4) (2018) 229–236.
[10] P. Saino, G. Migita, H. Hakuta, A. Okamoto, K. Hata, Analysis of chromosome microstructures in products of conception associated with recurrent miscarriage, Reprod. Biomed. Online 38 (5) (2019) 787–795.
[11] M.O. Goodarzi, D.A. Dumesic, G. Chazenbalk, R. Aziz, Polycystic ovary syndrome: etiology, pathogenesis and diagnosis, Nat. Rev. Endocrinol. 7 (4) (2011) 219–231.
[12] S. Tedesco, M.P. Adorni, N. Ronda, et al., Activation profile of monocyte/macrophages and HIF1α function in healthy women in relation to menstrual cycle and in polycystic ovary syndrome patients, Endocrine 66 (2) (2019) 360–369.
[13] C. Hogg, A.W. Horne, E. Greaves, Endometriosis-associated macrophages: origin, phenotype, and function, Front. Endocrinol. 11 (2020) 7.
[14] C. Meuleman, B. Vandenabeele, S. Fieuws, C. Spiessens, D. Timmerman, T. Dhoooge, High prevalence of endometriosis in infertile women with normal ovulation and nonosmopismospermic partners, Fertil. Steril. 92 (1) (2009) 68–74.
[15] H.O. Critchley, R.W. Kelly, R.M. Brenner, D.T. Baird, The endocrine environment of menstruation-a role for the immune system, Clin. Endocrinol. 55 (6) (2001) 701–710.
[16] A.J. Sharky, K. Day, A. McPherson, et al., Vascular endothelial growth factor expression in human endometrium is regulated by hypoxia, J. Clin. Endocrinol. Metab. 85 (1) (2000) 402–409.
[17] A. Bracke, K. Peeters, U. Punjabi, D. Hoogewijl, S. Dewilde, A search for molecular mechanisms underlying male idiopathic infertility, Reprod. Biomed. Online 36 (3) (2018) 327–339.
[18] E. Mass, I. Ballesteros, M. Farlik, et al., Specification of tissue-resident macrophages during organogenesis, Science 353 (6304) (2016) aaf4238.
[19] T. DeFalco, I. Lhattaachia, J.A. Williams, D.M. Sans, B. Capel, Yolk-sac-derived macrophages regulate fetal testis vascularization and morphogenesis, Proc. Natl. Acad. Sci. U. S. A. 111 (23) (2014) E2834–E2893.
[20] D.A. Hume, D. Halpin, H. Charlton, S. Gordon, The mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigens F4/80: macrophages of endocrine organs, Proc. Natl. Acad. Sci. U. S. A. 81 (13) (1984) 4174–4177.
[21] F. Gaytan, C. Bellido, E. Aguilar, N. van Rooijen, Requirement for testicular macrophages in Leydig cell proliferation and differentiation during prepuberal development in rats, J. Reprod. Fertil. 102 (2) (1994) 393–399.
[22] S. Kern, S.A. Robertson, V.J. Mau, S. Maddocks, Cytokine secretion by macrophages in the rat testis, Biol. Reprod. 53 (6) (1995) 1407–1416.
[23] J. Rinnehart, Recurrent implantation failure: definition, J. Assit. Reprod. Genet. 24 (7) (2007) 284–287.
[24] P. Russell, L. Anderson, D. Lieberman, et al., The distribution of immune cells and macrophages in the endometrium of women with recurrent reproductive failure I: Techniques, J. Reprod. Immunol. 91 (1-2) (2019) 90–102.
[25] K. Tremellen, P. Russell, Adenomyosis is a potential cause of recurrent implantation failure during IVF treatment, Aust. N. Z. J. Obstet. Gynaecol. 51 (3) (2011) 280–283.
[26] K.P. Tremellen, P. Russell, The distribution of immune cells and macrophages in the endometrium of women with recurrent reproductive failure. II: adenomyosis and macrophages, J. Reprod. Immunol. 93 (1) (2012) 58–63.
[27] H. Papachóva, M.H. Saxtorph, T. Hallager, et al., Endometrial HLA-E expression is influenced by genotypes and correlates differently with immune cell infiltration in IVF and recurrent implantation failure patients, Hum. Reprod. (8) (2022) 1816–1834.
[28] X. Huang, V. Cai, M. Ding, B. Zheng, H. Sun, J. Zhou, Human chorionic gonadotropin promotes recruitment of regulatory T cells in endometrium by inducing chemokine CCL2, J. Reprod. Immunol. 137 (2020), 102856.
[29] U. Thiruchelvam, I. Draansfeld, F.T. Saunders, H.O. Critchley, The importance of the macrophage within the human endometrium, J. Leukoc. Biol. 93 (2) (2013) 217–225.
[30] M. Namlı Kalem, N. Akgün, Z. Kalem, B. Bakaırkar, T. Celik, Chemokine (C-C motif) ligand 2 (CCL2) and oxidative stress markers in recurrent pregnancy loss and repeated implantation failure, J. Assit. Reprod. Genet. 34 (11) (2017) 1501–1506.
[31] M. Needham, N. Sturgess, G. Cerillo, et al., Monocyte chemotactic protein-1: receptor interactions and calcium signaling mechanisms, J. Leukoc. Biol. 60 (6) (1996) 793–803.
[32] T. Miyamoto, R. Murakami, J. Hanamishi, et al., B7-H3 suppresses tumor immunity via the CCL2-CR2-M2 macrophage Axis and contributes to ovarian cancer progression, Cancer Immunol. Res. 10 (1) (2022) 56–69.
[33] Y. Zhang, S. Liu, D. Qin, et al., KIf4A mediate the accumulation and reeducation of macrophages during organogenesis, Front. Immunol. 10 (2019) 11686.
[35] S. Yoshioka, H. Fujiwara, T. Nakayama, K. Kosaka, T. Mori, S. Fujii, Intrauterine administration of autologous peripheral blood mononuclear cells promotes implantation rates in women with repeated failure of IVF-embryo transfer. Hum. Reprod. 21 (12) (2006) 3290–3294.

[36] S. Li, J. Wang, Y. Cheng, et al., Intrauterine administration of hCG-activated autologous human peripheral blood mononuclear cells (PBMC) promotes live birth rates in repeated failure of IVF-embryo transfer cycles of patients with repeated implantation failure. J. Reprod. Immunol. 119 (2017) 15–22.

[37] T. Nakayama, H. Fujiwara, M. Maeda, et al., Human peripheral blood mononuclear cells (PBMC) in early pregnancy promote embryo invasion in vitro: HCG enhances the effects of PBMC. Hum. Reprod. 17 (1) (2002) 207–212.

[38] Z. Pourgohar, M.S. Soltani-Zangbar, G. Sheikhansari, et al., Intrauterine administration of autologous hCG-activated peripheral blood mononuclear cells improves pregnancy outcomes in patients with recurrent implantation failure: A double-blind, randomized control trial study. J. Reprod. Immunol. 142 (2020), 103182.

[39] WHO, Recommended definitions, terminology and format for statistical tables related to the peripartum period and use of a new nomenclature for cause of perinatal deaths. Modifications recommended by FIGO as amended October 14, 1976, Acta Obstet. Gynecol. Scand. 56 (3) (1977) 247–253.

[40] C. Zhang, H.M. Rong, T.I. K, Z. Zhi, T.H. Tong, PD-1 deficiency promotes macrophage activation and T helper cell type 1/T-helper cell type 17 response in pneumoerysina pneumonia. Am. J. Respir. Cell Mol. Biol. 62 (2020) 767–782.

[41] Y. Zhang, L. Ma, X. Hu, J. Ji, M. Aro, L. Aiao, The role of the PD-1/PD-L1 axis in macrophage differentiation and function during pregnancy, Hum. Reprod. 34 (1) (2019) 25–36.

[42] M. Chen, N. Gilbert, H. Liu, Reduced expression of PD-L1 in autoimmune thyroiditis attenuates trophoblast invasion through ERK/MMP pathway, Reprod. Biol. Endocrinol. 17 (1) (2019) 86.

[43] F. Heidarzadeh, A. Rezaei, M. Esmailifar, M. Ramazkhanlou, Indoleamine 2,3-dioxygenase: a professional immunomodulator and its potential functions in immune related diseases, Int. Rev. Immunol. 2021 1–8.

[44] H.L. Huang, H.L.Y. Lang, Z.Z. Lai, S.L-Y. Yang, M.Q. Li, D.I.J. Li, Decidual Ido-1 macrophage proliferation and function regulates the apoptosis of trophoblasts, J. Reprod. Immunol. 148 (2021), 103634.

[45] H. Wei, S. Liu, R. Lian, et al., Abnormal expression of indoleamine 2,3-dioxygenase in human recurrent miscarriage. Reprod. Sci. 27 (8) (2020) 1656–1664.

[46] H. Cheng, Y. Huang, D. Hu, et al., Effect of long-acting progestins on pregnancy in mice with recurrent pregnancy loss. Reprod. Sci. 28 (1) (2021) 52–59.

[47] Y. Obayashi, Y. Ozaki, S. Goto, et al., Role of indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase in patients with recurrent miscarriage, Am. J. Reprod. Immunol. 75 (1) (2021) 69–77.

[48] T. Nishioku, K. Hashimoto, K. Yamashita, et al., Involvement of cathepsin E in human recurrent miscarriage, Reprod. Sci. 27 (8) (2020) 1656–1664.

[49] Y.R. Sheng, W.T. Hu, C.Y. Wei, et al., IL-33/ST2 axis affects the polarization and efferocytosis of decidual macrophages in early pregnancy, Am. J. Reprod. Immunol. 86 (3) (2021), e13436.

[50] Y. Zhang, L. Ma, X. Hu, et al., Circadian rhythm-associated Rev-erb suppresses placental trophoblast apoptosis: a potential cause of recurrent pregnancy death. Modulation recommended by FIGO as amended October 14, 1976, Acta Obstet. Gynecol. Scand. 56 (3) (1977) 247–253.

[51] T.M. Kolben, E. Rogatsch, A. Vattai, et al., PPAR gamma-2 mediates altered macrophage polarization and function during pregnancy, Hum. Reprod. 34 (1) (2019) 77–86.

[52] R.Z. Spaczynski, A. Arici, A.J. Duleba, Tumor necrosis factor-alpha stimulates migration inhibitory factor expression in human decidual macrophages, Biol. Reprod. 89 (5) (2013) 155632.

[53] M. Calan, T. Kume, O. Yilmaz, et al., Possible link between luteinizing hormone suppression and poor reproductive outcome, J. Reprod. Immunol. 119 (2017) 15–22.

[54] R. Gonzalez, N.S. Rote, J. Minium, A.L. Weaver, J.P. Kirwan, Elevated circulating levels of macrophage migration inhibitory factor in polycystic ovary syndrome, Cytochrome 51 (3) (2015) 240–244.

[55] S. Yoshioka, H. Fujiwara, T. Nakayama, K. Kosaka, T. Mori, S. Fujii, Intrauterine administration of autologous peripheral blood mononuclear cells improves pregnancy outcomes in patients with recurrent implantation failure. J. Reprod. Immunol. 142 (2020), 103182.

[56] Y. Zhang, L. Ma, X. Hu, et al., Circadian rhythm-associated Rev-erb modulates polarization of decidual macrophage via the PI3K/Akt signaling pathway, Am. J. Reprod. Immunol. 86 (3) (2021), e13436.

[57] Y. Li, D. Zhang, L. Xu, et al., Macrophage contact with proinflammatory macrophages enhances the immunosuppressive effect of mesenchymal stem cells in two different models. Cells. Mol. Immunol. 16 (12) (2019) 908–920.

[58] O.R. Oakley, H. Kim, I. El-Amouri, et al., Peroxivascular leukocyte infiltration in the rat placenta, Endocrinology 150 (1) (2019) 4551–4559.

[59] F. Sugruea, G. Mendoza, D. Cardozo, F. Mohamed, L. Oliveros, M. Forneris, Sympathetic Innervation regulates macrophage activity in rats with polycystic ovarian syndrome, J. Endocrinol. 235 (1) (2023) 45–45.

[60] T.A. Penttila, L. Anttila, A. Torbäck, P. Koskinen, R. Ekkola, K. Ijala, Serum free testosterone in polycystic ovary syndrome measured with a new reference method, Fertil. Steril. 65 (1) (1996) 55–60.

[61] W. Li, X.Y. Liang, X. Li, et al., Decreased USP2a expression inhibits trophoblast invasion in human recurrent miscarriage, Reprod. Sci. 27 (8) (2020) 1656–1664.

[62] S. Yoshioka, H. Fujiwara, T. Nakayama, K. Kosaka, T. Mori, S. Fujii, Intrauterine administration of autologous peripheral blood mononuclear cells improves pregnancy outcomes in patients with recurrent implantation failure. J. Reprod. Immunol. 119 (2017) 15–22.

[63] Y. Wei, S. Liu, R. Lian, et al., Abnormal expression of indoleamine 2,3-dioxygenase in human recurrent miscarriage, Reprod. Sci. 27 (8) (2020) 1656–1664.

[64] Y. Zhang, L. Ma, X. Hu, et al., Circadian rhythm-associated Rev-erb suppresses placental trophoblast apoptosis: a potential cause of recurrent pregnancy death. Modulation recommended by FIGO as amended October 14, 1976, Acta Obstet. Gynecol. Scand. 56 (3) (1977) 247–253.

[65] Z. Pourgohar, M.S. Soltani-Zangbar, G. Sheikhansari, et al., Intrauterine administration of autologous hCG-activated peripheral blood mononuclear cells improves pregnancy outcomes in patients with recurrent implantation failure: A double-blind, randomized control trial study. J. Reprod. Immunol. 142 (2020), 103182.

[66] WHO, Recommended definitions, terminology and format for statistical tables related to the peripartum period and use of a new nomenclature for cause of perinatal deaths. Modifications recommended by FIGO as amended October 14, 1976, Acta Obstet. Gynecol. Scand. 56 (3) (1977) 247–253.

[67] F. Figueroa, R. Davicino, B. Micalizzi, L. Oliveros, M. Forneris, Macrophage secretion modulates the steroidogenesis of polycystic ovary in rats: effect of testosterone on macrophage pro-inflammatory cytokines, Life Sci. 90 (19-20) (2012) 733–739.

[68] Y. Li, L. Li, D. Ouyang, Y. Zhu, T. Yuan, The abnormal expression of kisspeptin regulates pro-inflammatory cytokines, cell viability and apoptosis of macrophages in hyperandrogenism induced by testosterone, Gynecol. Endocrinol. 37 (1) (2021) 72–77.

[69] J. Reprod. Immunol. 148 (2021), 103364.

[70] O.R. Oakley, M.L. Frazer, C. Ko, Pituitary-ovary-spleen axis in ovulation, Trends Endocrinol. Metabol. 22 (9) (2011) 345–352.
[93] Q. Xie, H. He, Y.H. Wu, et al., Eutopic endometrium from patients with endometriosis modulates the expression of CD36 and SR-B1 in peritoneal macrophages, J. Immunol. Res. 45 (5) (2019) 1045-1057.

[94] Y. Liu, M. Li, C. Wei, et al., TSP1-CD47-SIRPα signaling facilitates the development of endometriosis by mediating the survival of ectopic endometrium, Am. J. Reprod. Immunol. 83 (6) (2020) e13266.

[95] J. Li, S. Yuan, Q. Li, et al., Endometriosis-associated immune checkpoint CD47 blocking ameliorates endometriosis, Mol. Hum. Reprod. 28 (5) (2022), gaa010.

[96] Y.Y. Liu, Y.K. Liu, W.T. Hu, et al., Elevated heme impairs macrophage phagocytosis in endometriosis, Reproduction 158 (3) (2019) 257-266.

[97] D.A. Clark, J.M. Dmetrichuk, S. Dhesy-Thind, M.A. Crowther, J.L. Arredondo, A single-cell analysis of macrophage populations reveals that the microenvironment is a major driver of macrophage plasticity in endometriosis, Mol. Reprod. Dev. 90 (2) (2021) 181-193.

[98] L.C. Weng, S.H. Hou, S.T. Lei, H.Y. Peng, M.Q. Li, D. Zhao, Estrogen-regulated CD200 macrophages phagocytose in endometriosis, J. Immunol. 138 (2020), 103996.

[99] C. Deng, L. Zhang, H.K. Yosef, et al., Single-cell Tumor-removal analysis revealing immunometabolism changes in peritoneal fluid in endometriosis, Scand. J. Immunol. 95 (5) (2022), e13093.

[100] M.F. Nie, Q. Xie, Y.H. Wu, et al., Serum and ectopic endometrium from women with endometriosis modulate macrophage M1/M2 polarization via the smad3 pathway, J. Immunol. Res. 2018 (2018), 6285813.

[101] H. Sun, D. Li, M. Yuan, et al., Macrophages alternatively activated by endometriosis-exomes contribute to the development of lesions in mice, Mol. Hum. Reprod. 25 (11) (2019) 5-16.

[102] Y. Gou, X. Li, L. Li, et al., Estrogen receptor-α upregulates CCL2 via NF-κB signaling in endometriotic stromal cells and recruits macrophages to promote the pathogenesis of endometriosis, Hum. Reprod. 34 (4) (2019) 646-658.

[103] Y. Huang, L. Zhu, et al., Endometriotic microRNA-101a-3p mediates macrophage polarization via regulating PTEN-PPIK axis, Biomed. Pharmacother. 147 (2022), 112680.

[104] S.G. Sun, J.J. Guo, X.Y. Qu, et al., The extracellular vesicular pseudogene LGMNP1 induces M2-like macrophage polarization by upregulating LGMN and serves as a novel promising predictive biomarker for ovarian endometriosis recurrence, Hum. Reprod. 37 (3) (2022) 447-465.

[105] J.E. Miller, S.H. Ahn, R.M. Marks, et al., IL-17A modulates peritoneal macrophage recruitment and pathogenesis of endometriosis, Hum. Reprod. 2019, 110107.

[106] Y. Ono, T. Kawakita, O. Yoshino, et al., Sphingosine 1-phosphate (S1P) in the microenvironment regulates the recruitment of macrophages and the progression of endometriosis, Cell. Endocrinol. 486 (2019) 1.

[107] M.E. Matzkin, E. Riviere, S.P. Rossi, et al., β-adrenergic receptors in the up-regulation of COX2 expression and prostaglandin production in testicular macrophages: possible relevance to male idiopathic infertility, Mol. Cell. Endocrinol. 498 (2019), 110545.

[108] W. Yu, H. Zheng, W. Liu, et al., Estrogen promotes Leydig cell engorgement by macrophages in male infertility, J. Clin. Invest. 124 (6) (2019) 2709-2721.

[109] M. Hanzheng, S. Kimura, C. Kanno, et al., Macrophage ubiquitin-specific protease 2 contributes to motility, hyperactivation, capacitation, and in vitro fertilization competency of mouse sperm, Cell Mol. Life Sci. 77 (2020) 519-534.

[110] M.B. Frunieri, R.S. Calandra, L. Lustig, et al., Number, distribution pattern, and identification of macrophages in the testes of infertile men, Fertil. Steril. 78 (2002) 298–306.

[111] M.D.E. Martzkin, E. Riviere, S.P. Rossi, et al., β-adrenergic receptors in the regulation of COX2 expression and prostaglandin production in testicular macrophages: possible relevance to male idiopathic infertility, Mol. Cell. Endocrinol. 498 (2019), 110545.

[112] W. Yu, H. Zheng, W. Liu, et al., Estrogen promotes Leydig cell engorgement by macrophages in male infertility, J. Clin. Invest. 124 (6) (2019) 2709-2721.

[113] K. Eubler, P. Rantakari, H. Gerke, et al., Exploring the ion channel TRPV2 and its role in normal and infertile spermatozoa, Biol. Reprod. 102 (5) (2020) 1011–1022.

[114] B. Burkholder, R.Y. Huang, R. Burgess, et al., Tumor-induced perturbations of cytokines and chemokines in the human endometrium facilitate the development of ectopic endometrial lesions, Cytokine 138 (2021), 105216.

[115] A. Madkour, N. Bouamoud, N. Louanjli, et al., Intrauterine insemination of frozen/thawed embryos in patients with repeated implantation failure, J. Reprod. Immunol. 92 (1-2) (2011) 82–90.

[116] A. Makrigiannakis, M. BenKhali, T. Vrekousis, S. Malhijb, S.N. Kalantaridou, In utero implantation: a new potential treatment option, Eur. J. Clin. Invest. 45 (9) (2015), e13084.

[117] A. Makrigiannakis, M. Ben Khali, T. Vrekousis, S. Malhijb, S.N. Kalantaridou, In utero implantation: a new potential treatment option, Eur. J. Clin. Invest. 45 (9) (2015), e13084.

[118] F.F. Nobili, S.S. Arefi, A. Moini, et al., Endometrium immunomodulation by intrauterine insemination administration of treated peripheral blood mononuclear cells prior to embryo transfer improves clinical outcome for patients with repeated implantation failures, J. Fertil. Investig. 90 (2) (2012) 82-87.

[119] A. Makrigiannakis, T.J. Vrekousis, F. Makrigiannakis, H. Ruan, S.N. Kalantaridou, T. Gorgan, Intrauterine CRH-treated PBMC in repeated implantation failure, Eur. J. Clin. Invest. 49 (9) (2019), e13084.

[120] A. Madkour, N. Bouamoud, N. Louanjli, et al., Intrauterine insemination of cultured peripheral blood mononuclear cells prior to embryo transfer improves clinical outcome for patients with repeated implantation failures, Fertil. Steril. 104 (2021) 105216.