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Review Article

Effects of immune cells and cytokines on the endometrial immune microenvironment in polycystic ovary syndrome

Zhenhong Ye [a,b,c,d,e], Jie Zhao [a,b,c,d,e,**,1], Rong Li [a,b,c,d,e,*,1]

[a] Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, 100191, China
[b] National Clinical Research Center for Obstetrics and Gynecology, Beijing, 100191, China
[c] Key Laboratory of Assisted Reproduction, Ministry of Education, Beijing, 100191, China
[d] Beijing Key Laboratory of Reproductive Endocrinology and Assisted Reproductive Technology, Beijing, 100191, China
[e] Research Units of Comprehensive Diagnosis and Treatment of Oocyte Maturation Arrest, Chinese Academy of Medical Sciences, Beijing, 100191, China

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder in women of reproductive age, and its pathogenesis is still unclear. More and more studies have shown that PCOS patients were often accompanied by obesity, insulin resistance and chronic, low-grade inflammation, which were closely related to the endometrial dysfunction. Besides, obesity and insulin resistance also exacerbate inflammatory responses. Therefore, this review provides a summary of the effects of immune cells and inflammatory cytokines on the immune microenvironment of PCOS endometrium, and the impacts of obesity and insulin resistance on endometrial inflammatory homeostasis and possible drug interventions.

1. Introduction

Polycystic ovary syndrome (PCOS) is one of the common endocrine and metabolic disorders and the leading cause of anovulatory infertility in reproductive-aged women. The prevalence of PCOS in Chinese Han women is 7.8%.[1] According to the Rotterdam diagnostic criteria, PCOS was diagnosed when two of the following three features are met: hyperandrogenism, oligo-ovulation and polycystic ovarian morphology.[2] Study has found that women with PCOS were 8–10 times more likely to need assisted reproductive technology (ART) than women without PCOS.[3] PCOS women obtained more oocytes after controlled ovarian stimulation, but the poor quality of oocytes led to lower rates of fertilization, cleavage and implantation, and an increased risk of miscarriage and early natural pregnancy loss.[4] However, study have found that women with PCOS who have spontaneous ovulation have a 5-fold increased risk of adverse pregnancy outcomes compared with non-PCOS women.[5] Therefore, in addition to ovulation disorders, other factors may contribute to the outcome of PCOS assisted reproduction. The expression of some marker molecules related to endometrial receptivity were significantly changed during the implantation window of PCOS,[6,7] suggesting that abnormal endometrial function may be another important factor of female subfertility and adverse reproductive outcomes.

Embryo implantation induced immune activation and balance reconstitution in the endometrium. Disruption of inflammatory homeostasis was a sign of impaired implantation.[8] During decidualization, innate immune cells, especially macrophages, dendritic cells (DCs), and uterine natural killer (uNK) cells promoted the trophoblast invasion by modulating immune responses, growth factors and adaptation of the uterine vasculature.[8–10] In addition, the homeostasis of inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), IL-1β, and interferon γ (IFN-γ) were also involved in embryo implantation

* Corresponding author. Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, 100191, China.
** Corresponding author. Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, 100191, China.
E-mail addresses: 2358044941@qq.com (J. Zhao), roseli001@sina.com (R. Li).

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[1] These authors are corresponding authors

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and trophoblast invasion.[11] A meta-analysis showed that plasma C-reactive protein (CRP) levels were significantly elevated in PCOS.[12] In addition, PCOS also associated with the increased the levels of many cytokines, such as IL-18, IL-6, chemokine (C–C motif) ligand 3 (CCL3), monocyte chemoattractant protein 1 (MCP-1),[13–16] macrophage migration inhibitory factor (MIF) and white blood cell (WBC) count.[17] The elevated level of MIF was positively correlated with luteinizing hormone and free testosterone, and the risk of PCOS increased 1.385-fold in PCOS.[18] Animal experiments have found that androgen could induced local inflammatory responses in the ovary by activating NF-κB and NLRP3 signaling pathways.[19,20] Besides, dihydrotestosterone (DHT)-treated rats had increased levels of serum inflammatory cytokines (TNF-α and IL-1β), and aggregation of ovarian inflammatory cells.[21] Androgen was an important regulator of decidualization and involved in regulating endometrial repair and proliferation.[22] And androgen receptor was abundantly expressed in endometrial stromal cells.[22] These results suggest that androgen may mediate the development of chronic low-grade inflammation in PCOS endometrium.

In addition to reproductive disorders, PCOS women were often accompanied by metabolic abnormalities. Mendelian randomization analysis reflected a causal relationship between body mass index (BMI)/insulin resistance (IR) and PCOS.[23] PCOS women with high BMI and IR have an increased risk of spontaneous abortion when receiving ART.[24] Hence, obesity and IR may influence the endometrial immune microenvironment in PCOS. Therefore, in this review, we focused on the characteristics of endometrial immune microenvironment in PCOS, in order to provide ideas for a more in-depth understanding of the pathogenesis of PCOS.

2. Changes of immune cell components in endometrium of PCOS

It has been indicated that 30%–40% of all decidual cells are leukocytes during early pregnancy, upon embryo implantation, many immune cells were recruited to the implantation site for establishing specific maternal tolerance toward the semi-allogeneic fetus. The decidual immune cells consist of 65%–70% uNK cells, 10%–20% macrophages, 2%–4% DCs and the rest were T cells.[25] A recent study demonstrated that the number of immune cells in the endometrium of normal-weight PCOS women was different from the normal-weight controls, as shown by elevated percentage of $CD68^+$ and CD163+ M2 macrophages, CD1a + immature dendritic cells, $CD83^+$ mature dendritic cells (DCs) and $CD8^+$ T cells.[26] Changes in the percentage of lymphocyte subsets was also observed in late secretory endometrium of PCOS women, showing lower percentages of CD56+/CD16-and CD56bright/CD16-cells whereas higher percentages of $CD3^+$ cells.[27] Besides, the number of T regulatory cells (Tregs) was reduced in the peripheral blood of women with PCOS.[28] The number of Tregs increased peaking in endometrium at ovulation.[29] Then, the decidual Tregs elevated in the second trimester and decline before birth.[30] What's more, Treg could release the inflammatory factors transforming growth factor-β (TGF-β), IL-10 and CTLA4, which mediated the activation of anti-inflammatory and tolerogenic phenotype of M2 macrophages and DCs, as well as inhibited the cytolytic activity of uNK cells in decidualization.[31,32]All changes above in PCOS would be break the dynamically immune balance in microenviroment of maternal-fetal interface, which may disadvantage to the implantation of embryo or the maintenance of pregnancy by insufficient trophoblast invasion, defective decidual vascular remodeling and inadequate maternal tolerance.[33] However, more research is still needed to elucidate the effect and mechanism of immune cells on the local microenvironment of the endometrium.

3. Changes of cytokines in endometrium of PCOS

Study found that normal-weight women with PCOS developed the pathological endometrial inflammation, which manifested by increased expression of endometrial NF-κB p65 (Rel A). The elevated expression level of Rel A was positively correlated with serum total testosterone levels in PCOS.[34] Besides, activation of NF-κB signaling pathway inhibited the differentiation of macrophages to M2[35], promoted the release of inflammatory cytokines[36] and trophoblast apoptosis,[37] thereby affecting embryo implantation. In the proliferative endometrium of PCOS, the expression of proinflammatory factor IL-18 was increased (Table 1).[38] Study demonstrated that activation of endometrial inflammatory responses in the proliferative phase of PCOS may be caused by toll-like receptor 4 (TLR4) – interferon regulatory factor 7 (IRF-7)- NF-κB signaling, and this effect could be inhibited by metformin (Table 1).[39] In addition, an endometrial proteomics study of PCOS women at proliferative stage indicated that the level of haptoglobin (Hp) was significantly decreased while apolipoprotein A1 (ApoA1) was increased in PCOS endometrium.[40] This result indicated that chronic inflammatory activation mediated by differential expression of anti-inflammatory factors might be involved in PCOS endometrial dysfunction.[40]

In the secretory endometrium, circulating levels of TNF-α and IFN-γ were significantly increased in ovulatory PCOS women and positively correlated with the expressions of CD44 and osteopontin (OPN) in the endometrium, markers of endometrial receptivity.[41] Previous studies have revealed that chemokine ligand 10 (CXCL10), IL-15 and IL-18 participated in the activation of uNK cells during decidualization.[42,43] The mRNA and protein expression levels of CXCL10, IL-15 and IL-18 in the secretory endometrium of the PCOS were lower than the normal

Table 1
Summary of inflammatory cytokines expression and main function in the endometrium of PCOS.

Inflammatory cytokines	Trend	Main function	Reference
IL-1β	↑	Involved in the inflammatory response	Amjadi et al., 2022
IL-2	↑	Involved in the inflammatory response	Liu et al., 2022
IL-6	↑	Involved in the inflammatory response	Liu et al., 2022
		Induced cytokine production and recruitment of macrophages and megakaryocytes	Piltonen et al., 2013
		Inhibited insulin signaling	Oróstica et al., 2020
IL-8	↑	Promoted a proinflammatory response and lineage cell differentiation	Piltonen et al., 2013
IL-11	↑	Involved in the inflammatory response	Amjadi et al., 2022
IL-15	↑	Involved in the inflammatory response	Liu et al., 2022; Amjadi et al., 2022
	↓	Impaired uNK cells recruitment	Matteo et al., 2010
IL-18	↑	Involved in the inflammatory response	Long et al., 2017
	↓	Impaired uNK cells recruitment	Matteo et al., 2010
CCL2	↑	Induced migration of macrophages	Piltonen et al., 2013
CXCL10	↓	Impaired uNK cells recruitment	Matteo et al., 2010
TNF-α	↑	Involved in the inflammatory response	Hu et al., 2021; Amjadi et al., 2022
IFN-α	↑	Involved in the inflammatory response	Hu et al., 2021
IFN-γ	↑	Involved in the inflammatory response	Hu et al., 2021
TGF-β	↑	Involved in the inflammatory response	Amjadi et al., 2022

↑ up-regulation; ↓ down-regulation.
IL, interleukin; CCL2, C–C motif chemokine ligand 2; CXCL10, chemokine ligand 10; TNF-α, tumor necrosis factor α; IFN, interferon γ; TGF-β, transforming growth factor-β; uNK, uterine natural killer. ↑ up-regulation; ↓ down-regulation.

controls (Table 1).[27] Besides, the blunted endometrial decidualization in PCOS women possible mediated by the enhanced secretion of inflammatory cytokines in endometrial stromal fibroblasts, including IL-6, IL-8, MCP1, CCL5 and granulocyte-macrophage colony-stimulating factor (GM-CSF), matrix metalloproteinase 2 (MMP2) and MMP3.[44] Thus, aberrant activation of these pro-inflammatory factors disrupted the immune homeostasis of the endometrium, which may ultimately lead to disturbed embryo implantation.

4. Effects of obesity on the endometrium of PCOS

A meta-analysis included different regions and ethnicities showed that a pooled estimated prevalence of obesity in women with PCOS was 49%.[45] Study found that the WBC counts positively correlated with testosterone and BMI in PCOS.[17] MCP-1 and CCL3 were positively related to central fat mass.[14] In addition, BMI and IR were the main predictors of elevated CRP levels in PCOS.[17] Moreover, some studies have also shown that obesity and IR were the main factors contributing to the elevation of WBC counts in women with PCOS, rather than hyperandrogenism.[46,47] Therefore, we speculate that endometrial inflammation in PCOS is not only regulated by androgen, but may also be associated with obesity and IR. The percentage and number of $CD68^+$ macrophages were elevated in the endometrium of overweight women with PCOS.[26,48] Besides, the elevated IL-6 level was also detected in the proliferative endometrium of PCOS with obese.[49] Consistently, overweight/obese PCOS proliferating endometrial stromal fibroblasts were observed the up-regulated gene expression of a variety of inflammatory factors, including complement component 4A and 4B (C4A/B), C–C motif chemokine ligand 2 (CCL2), intercellular adhesion molecule 1 (ICAM1), α-induced protein (TNFAIP), while only the expression of IL-8 and ICAM1were up-regulated in PCOS endometrial mesenchymal stem cells.[50] The expression of IL-18 was elevated in the proliferative endometrium of overweight women with PCOS, which was not associated with IR.[38] Overweight women with PCOS also manifested by increased expression of endometrial Rel A. In addition, the expression of TNF-α receptor 2 (TNFR2) was enriched in the secretory phase endometrium of obese PCOS women, which might be related to the increased nuclear content of NF-κB in the endometrium.[48] Therefore, obesity is one of the important factors leading to the activation of endometrial inflammatory response in PCOS.

5. Effects of insulin resistance on the endometrium of PCOS

Previous study has exhibited that an estimated 50%–70% of PCOS patients have IR.[2] Compared with controls, PCOS women have a 3-fold increased risk of developing gestational diabetes and a 2-fold increased risk of premature delivery.[51] On the one hand, hyperglycemic state due to IR could enhance the inflammatory response and hyperandrogenemia.[16] And the increase of BMI directly aggravated the degree of IR in PCOS.[52] Consistently, obese women with PCOS have higher levels of IL-18 and it is inversely correlated with insulin sensitivity.[53] In addition, the percentage of $CD56^+$ NK, CD163+ M2 macrophages and expression of NF- κB in endometrium were significantly positively associated with IR in PCOS.[26,34] Insulin treatment of endometrial stromal cell lines could activate NF-κB, which led to the increased expression of inflammatory factors such as TNF-α and IL-6 in vivo.[54] Besides, endometrial glucose transporter 4 (GLUT4) expression was significantly reduced in PCOS patients with IR, which inhibited the glucose uptake and induced IR.[55] On the other hand, increased levels of inflammatory factors may exacerbate abnormal insulin signaling in PCOS.[49] In vitro, TNF-α treatment of endometrial cells could increase the phosphorylation of insulin receptor substrate-1 (IRS1) -S270, while IL-6 reduced the phosphorylation of IRS1-Y612.[49] But TNF-α and IL-6 inhibited the insulin signaling through different mechanisms. TNF-α was able to promoted S6K and JNK activation and AKT-S473 phosphorylation levels, while IL-6 decreased AKT-S473 phosphorylation levels.[49] In addition, TNF-α also reduced the expression of GLUT4 in endometrial cells, which caused IR in local endometrium.[54]

Therefore, IR may exacerbate the disruption of endometrial immune homeostasis in PCOS.

6. Drug intervention

Many studies have found that some drugs could improve the immune microenvironment of the endometrium in PCOS. Metformin has been widely implicated for ameliorating the insulin sensitivity in PCOS women. Study has shown that PCOS women receiving metformin treatment before and during IVF or intracytoplasmic sperm injection (ICSI) would significantly increase pregnancy and live birth rates,[56,57] suggesting its importance in regulating the endometrial microenvironment. Mechanistically, metformin treatment of endometrial stromal cells in vitro reduced the gene expression of IL-1β and IL-6.[58] What's more, metformin could activate the p38-MAPK inflammatory signaling pathway to reduce the expression of MMP-2, MMP-9 and PGR, thereby inhibiting the decidualization of endometrial stromal cells.[59] However, there were also studies that did not support the effectiveness of metformin in improving pregnancy outcomes.[60] A short acting GLP-1 analog, exenatide attenuated endometrial damage and fibrosis by reducing endometrial oxidative stress and TGF-β levels in diabetic rats.[61] The abnormality of decidualization, angiogenesis and uNK cells were observed in hyperandrogen mouse model.[62] Flutamide (a non-steroidal anti-androgen) treatment could effectively improve above endometrial dysfunction.[62] Flutamide also could enhance lower pulsatility index of the uterine artery in PCOS.[63] Flutamide could restore the decreased HOXA10 expression in testosterone-treated endometrial epithelial cell line, which improved the endometrial receptivity.[64] Furthermore, in vitro treatment of the endometrium of PCOS women with flutamide significantly reduced the activation of NF-κB and the expression of IFN-α and IFN-γ[39]. Therefore, in addition to improving metabolic disorders, reducing androgen level and inducing ovulation,[65,66] flutamide may also improve endometrial function in PCOS. Besides, the increased endometrial, epithelial and stromal thickness, and caspase-3-mediated apoptosis and proliferation were significantly reduced in the PCOS rat model with vitamin D treatment.[67]What's more, Vitamin D could promote the differentiation and maturation of macrophages and DCs, enhance the function of Treg cells, and inhibit NK cell activation and cytotoxicity.[68] However, there was no full clinical evidence that vitamin D improved endometrial receptivity and pregnancy outcomes in PCOS.[67,69] Therefore, more studies are needed to explore and clarify the mechanisms by which these drugs improve the endometrial immune microenvironment in PCOS.

7. Limitations

However, the current research has the following limitations: 1) The causal relationship between endometrial inflammation and endometrial dysfunction in PCOS, as well as the possible molecular mechanisms involved, have not yet been elucidated. 2) The changes in the expression profile of endometrial inflammatory cells and cytokines in different phase require larger samples to conduct a more comprehensive study, which is helpful to reveal the changes in the immune homeostasis of the endometrium under the abnormal hormonal environment of PCOS. 3) Whether androgen can cooperate with obesity and IR to regulate the endometrial immune responses also requires further investigation.

8. Conclusion

In summary, abnormal activation of endometrial inflammatory response may be an important cause of adverse pregnancy outcomes during ART assisted pregnancy in PCOS. There were abnormalities in the number and composition of immune cells in the endometrium of PCOS women, as well as disturbances in the levels of various cytokines, which led to the occurrence of local chronic inflammation in endometrium. The activation of chronic inflammation response was closely related to the

obesity, IR and elevated androgen level. In addition, the drug intervention was expected to improve the endometrium function by impairing the levels of inflammatory cytokines. But more research and clinical data are still needed to explore the corresponding mechanisms and then may provide new ideas for improving outcomes of PCOS assisted reproduction.

Author contributions

Z-HY searched literature and wrote the manuscript. RL and JZ reviewed the manuscript.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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