

Efferocytosis and infertility: Implications for diagnosis and therapy

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ARTICLE INFO

Keywords:

Phagocytosis
Infertility
Reproductive Physiology
Apoptotic Cell Clearance
Reproductive Diseases

ABSTRACT

Recent research has shed light on the intricate connection between efferocytosis and infertility, revealing its dysregulation as a contributing factor in various reproductive diseases. Despite the multifaceted nature of infertility etiology, the impact of insufficient clearance of apoptotic cells on fertility has emerged as a focal point. Notably, the removal of apoptotic cells through phagocytosis in the female reproductive system has been a subject of extensive investigation in the field of infertility. Additionally, special functions performed by immune system cell types, such as macrophages and Sertoli cells, in the male reproductive system underscore their significance in spermatogenesis and the efferocytosis of apoptotic germ cells. Dysregulation of efferocytosis emerges as a critical factor contributing to reproductive challenges, such as low pregnancy rates, miscarriages, and implantation failures. Moreover, defective efferocytosis can lead to compromised implantation, recurrent miscarriages, and unsuccessful assisted reproductive procedures. This review article aims to provide a comprehensive overview of efferocytosis in the context of infertility. Molecular mechanisms underlying efferocytosis, its relevance in both female and male infertility, and its implications in various reproductive diseases

Abbreviations: AMPK, AMP-activated protein kinase; AnxI, AnnexinI; APR3, Actin nucleation protein 3; BAI1, Brain-specific angiogenesis inhibitor1; CCLs, Chemokine ligands; CCRs, Chemokine receptors; CDs, Cluster of Differentiations; CMKLR1, Chemokine receptor-like 1; CRKII, CT10 Regulator of Kinase II; CRP, C-reactive protein; CSF1, Colony stimulating factor 1; CX3CL1/ Fractalkine, Chemokine C-X3-C motif ligand 1; CX3CR1, Chemokine C-X3-C motif receptor; CXCL8, Chemokine C-X-C motif ligand 8; DHEA, Dehydroepiandrosterone; DOCK180, Dedicator of Cytokines 180; EGF, Epidermal growth factor; ELMO, Engulfment and Cell Motility; EPHA3, Ephrin Type-A receptor 3; EPO, Erythropoietin; ESCs, Endometrial stromal cells; FAK, Focal adhesion kinase; GAS6, Growth arrest-specific 6; GLUTs, Glucose transporters; GM-CSF, Granulocyte-Macrophage Colony-stimulating factor; HIF, Hypoxia-Inducible Factor; ICAM-1, Intercellular adhesion molecule 1; IGF-1, Insulin-like growth factor-1; ILs, Interleukins; JAK, Janus Kinase; LDH, Lactate dehydrogenase; MAPK, Mitogen-activated protein kinase; MCP-1, Monocyte Chemoattractant Protein-1; MFGE8, Milk Fat Globule-EGF Factor 8; MIF, Migration inhibitory factor; MMPs, Matrix metalloproteinases; MTOR, Mechanistic target of rapamycin; NAD, Nicotinamide adenine dinucleotide; NF-κB, Nuclear Factor-Kappa B; NOX4, NADPH Oxidase 4; PCOS, Polycystic ovarian syndrome; PGE2, Prostaglandin E2; PI3K, Phosphoinositide 3-Kinase; PPAR, Peroxisome Proliferator-activated receptor; RA, Retinoic acid biosynthesis; ROS, Reactive oxygen species; RSA, Recurrent spontaneous abortion; S1P, Sphingosin-1 phosphate; SIRP, Signal regulatory protein; SOCS1, Suppressor of cytokine signaling 1; STAT, Signal transducer and activator of transcription; TIMs, T cell immunoglobulin and mucin Domain-containing proteins; TLRs, Toll-like receptors; TNF, Tumor necrosis factor; TSP-1, Thrombospondin 1; USP2, Ubiquitin-specific protease 2; VEGF, Vascular endothelial growth factor.

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<https://doi.org/10.1016/j.jri.2024.104413>

Received 30 September 2024; Received in revised form 15 November 2024; Accepted 28 November 2024

Available online 29 November 2024

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are elucidated. The elucidation of the intricate relationship between efferocytosis and infertility not only facilitates diagnosis but also paves the way for targeted therapeutic interventions.

1. Introduction

The study of efferocytosis in reproductive physiology and pregnancy-related diseases that try to find the reasons of low pregnancy rate caused by ovulation conditions, poor sperm quality, miscarriage, embryo implantation failure, and pregnancy preservation is necessary to discover solutions for the treatment of male and female infertility. Over the last few years, there has been research to understanding the connection between efferocytosis and infertility (Wu et al., 2023, 2022). The process in which phagocytes eliminate apoptotic cells, efferocytosis is considered as a way to protect tissue homeostasis and reduce inflammation (Kawano and Nagata, 2018).

New studies show that the dysregulation of efferocytosis has played a role in many diseases, including reproductive diseases such as infertility (Wu et al., 2023, 2022). Even though, several factors play a character in the etiology of infertility, the effect of insufficient clearance of apoptotic cells on fertility has been noticed. One of the important stages of the process is the removal of apoptotic cells through phagocytosis in the female reproductive system, which has been studied in the field of infertility. Moreover, several cell types of immune system, such as macrophages and sertoli cells performing special functions in the male reproductive system. Sertoli cells and macrophages are present in high concentrations in the testes and involved in spermatogenesis and the removal of germ cells that have undergone programmed cell death. They are significant in germ cells progression and efferocytosis of apoptotic germ cell. During the menstrual cycle, the endometrium changes to prepare for potential embryo implantation. During this cycle, the tissue integrity is maintained by removing apoptotic cells, and a defect in this process, which refers to impaired efferocytosis, leads to defective implantation, repeated miscarriages, and implantation failure with assisted reproductive procedures (Sheng et al., 2019; Shi et al., 2023; Yefimova et al., 2018; Zhang et al., 2022).

Therefore, understanding the mechanism and pathways related to efferocytosis and its subsequent impact on fertility is significant. Considering the therapeutic potential in efferocytosis pathways will shed light on those struggling with infertility. Furthermore, discovering the relationship between efferocytosis and infertility leads to facilitating diagnosis and therapeutic interventions. In this review article, we aim to provide an overview of efferocytosis in the context of infertility. Here, the general molecular mechanisms involved in this process and its mechanism in female and male infertility and some reproductive diseases are described.

Data were retrieved from PubMed, Scopus, and Web of Science to collect related articles on efferocytosis, infertility, immune modulation, and macrophage activity. The search phrase was a combination of search terms, including but not limited to: (efferocytosis) OR (efferocytotic), "efferocytosis in infertility", "immune regulation in reproductive health", "macrophage polarization in PCOS", "estrogen and progesterone effects on macrophages", "Efferocytosis and implantation", "Inflammation and infertility", and the publication years were limited to 1998–2023. Moreover, articles written in English and Only studies with full-text access were selected to maintain consistency and accessibility in interpretation.

2. Definition and overview

Professional and non-professional phagocytes remove apoptotic cells to stop the release of intracellular antigens and cytotoxic substances into the environment. By a process called efferocytosis. It involves the detection, binding, internalization or engulfment, and destruction of apoptotic cells and differs from other types of phagocytosis. In order to

preserve tissue homeostasis, dying cells release chemical signals that guide phagocytes to the sites of death and control the immune response (Gheibi Hayat et al., 2019). Efferocytosis is essential for immune system balance during homeostasis, tissue repair, and the reduction of inflammation. Numerous inflammatory illnesses have been shown to be associated with defective efferocytosis in their etiology (Ge et al., 2022). Defects in this process can result in different disorders, including autoimmune illnesses, inflammation of the airways, atherosclerosis, cancer, transplant rejection and reproductive conditions (Tajbakhsh et al., 2019, 2021a, 2021b; Vafadar et al., 2023).

3. Molecular mechanism of efferocytosis

Efferocytosis is a multi-step process (Fig. 1). First, identification of the prey (apoptotic cells) through the predator (phagocytes) with the release of chemoattractants, which are the same as "Find-me" signals, and are secreted by apoptotic cells. This step is a highly regulated process that involves the activation of caspase-dependent apoptosis. Second, the pleasure of eating delicious prey actually begins with the connection of some ligands called "Eat-me" signals to their receptors, the next steps include internalization and engulfment, and as a result, the surface of dying cells exhibit an increased expression of "Eat-me" signals, and soluble "Find-me" signals are released to facilitate phagocytic uptake. Finally, the phagocyte creates a series of responses in relation to the corpses of apoptotic cells. Actually, the recognition of these signals by phagocytes leads to the rearrangement of their cytoskeleton and engulfment of apoptotic cells (Gheibi Hayat et al., 2019; Ravichandran, 2010). The description of the events of this process is given in Table 1.

"Find-me" signals include the following: The first, adenosine triphosphate and uridine triphosphate, which act through binding to purinoreceptor-2 on the surface of phagocytes (Elliott et al., 2009). The second, some mediators of oxidative stress, which is conceivably a physiological mechanism before cell death for phagocytes (Kirkham, 2007). The third, lysophosphatidylcholine released from apoptotic cells by phospholipase A2, which plays a role in recruiting macrophages by producing chemoattractants that collect with G2 receptors (Peter et al., 2008). Fourth, sphingosin-1 phosphate (S1P) works by increasing the expression of erythropoietin and activating the peroxisome proliferator-activated receptor-gamma (PPAR- γ) pathway and the EPO-EPO receptor and finally increasing Mer Tyrosine Kinase (MerTK), Gas6, milk fat globule-EGF-factor 8 (MFGE8), and CD36 (Gude et al., 2008). Moreover, Fractalkine (CX3CL1) by binding to phagocytes through its CX3CR1 receptor causes their attraction to apoptotic cells and on the other hand increases the expression of MFGE8 (Truman et al., 2008).

When receiving the apoptotic message, phosphatidylserine (PtdSer) is transferred from the inner surface of the plasma membrane to the outer surface as a result of the activation of caspases and the inactivation of flippases. Furthermore, in non-apoptotic cells, scramblases and caspases are simultaneously activated, leading to the transfer of PtdSer to the outer surface. The connection of PtdSer to the surface of phagocytes is done through receptors whose distribution is not the same in all phagocytes, and each phagocyte may have a special set of these receptors. These receptors include T-cell immunoglobulin mucin (TIM-1), TIM-4, brain-specific angiogenesis inhibitor (BAI1), stabilin-2, and the receptor for final glycation products, which TIM-4 is of special importance (Fadok et al., 1998; Li, 2012).

There are two receptor binding sites in bridging molecules, which are connected to the phagocyte from one side and to the apoptotic cell from the other side, thus establishing a bridge between the two. The most

well-known of these molecules is MFGE8, which binds to PtdSer of apoptotic cells on one side and to $\alpha v\beta 3/\alpha v\beta 5$ integrins in macrophages on the other side, thus facilitating the connection. Following this connection, its downstream signals are activated with the help of the Rac1 and CRKII-DOCK180-ELMO complex and cause changes in the cytoskeleton, and finally, the apoptotic cells are cleared by macrophages (Albert et al., 2000). Moreover, MFGE8 increases the expression of anti-inflammatory cytokines from STAT3-SOCS3 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), MAPK signaling pathways. Another bridging molecule is AnnexinI (AnxI), which is located inside the membrane in a normal state similar to PtdSer. However, it is transferred to the outer surface of the cell membrane during the death signal (Yoon et al., 2000). The last one is Gas6 and protein S, which bind to a receptor from the TAM family (Tyro3, AXL and Mer) called MerTK. Activation of MerTK by Gas6 is in a Ca^{2+} -dependent manner, which is performed even in the absence of PtdSer. While protein S requires oligomerization on the PtdSer-rich membrane to activate TAM receptors. While MerTK can connect to both of these bridging molecules, other receptors of this family can only connect to one of them. Also, macrophages have the ability to recognize PtdSer through ProS due to the high expression of MerTK. Indeed, MerTK signaling is highly anti-inflammatory and enhances the expression level of suppressor of cytokine signaling 1 (SOCS1) and SOCS3, which inhibits cytokine-induced JAK/STAT signaling and reduces the expression of inflammatory cytokines. This leads to the polarization of macrophages

towards anti-inflammatory and M2-like states (Lemke, 2013).

Since, unlike apoptotic cells, living cells do not have “Eat-me” signals, phagocytes and macrophages have the ability to distinguish between these two cell types. CD47 (via SIRP α on phagocyte) (Takimoto et al., 2019), CD31 (Brown et al., 2002), and CD300a are among the most important ligands of the don’t Eat-me signals that prevent engulfment and ultimately efferocytosis (Voss et al., 2015).

4. Efferocytosis in female infertility

Infertility affects many couples worldwide, with female infertility accounting for a significant portion of cases (Smith and Noordin, 2006). Recent research has shown that dysregulation of efferocytosis, the process of clearing apoptotic cells, may play a crucial role in the development of female infertility. Efferocytosis is responsible for maintaining tissue homeostasis and immune regulation, making it a vital process to understand in the context of reproductive health (Zhang et al., 2022).

The uterine endometrium is integral to the preservation of mucosal immunity and serves a protective function against pathogens during physiological phenomena such as the menstrual cycle, implantation, and labor. Macrophages, constituting approximately 10 % of leukocytes in the human uterus and similarly 10 % of total uterine cells in murine models, are pivotal in orchestrating these inflammatory responses. These macrophages exhibit an M2-like phenotype, characterized by elevated levels of the anti-inflammatory cytokine IL-10 and the presence

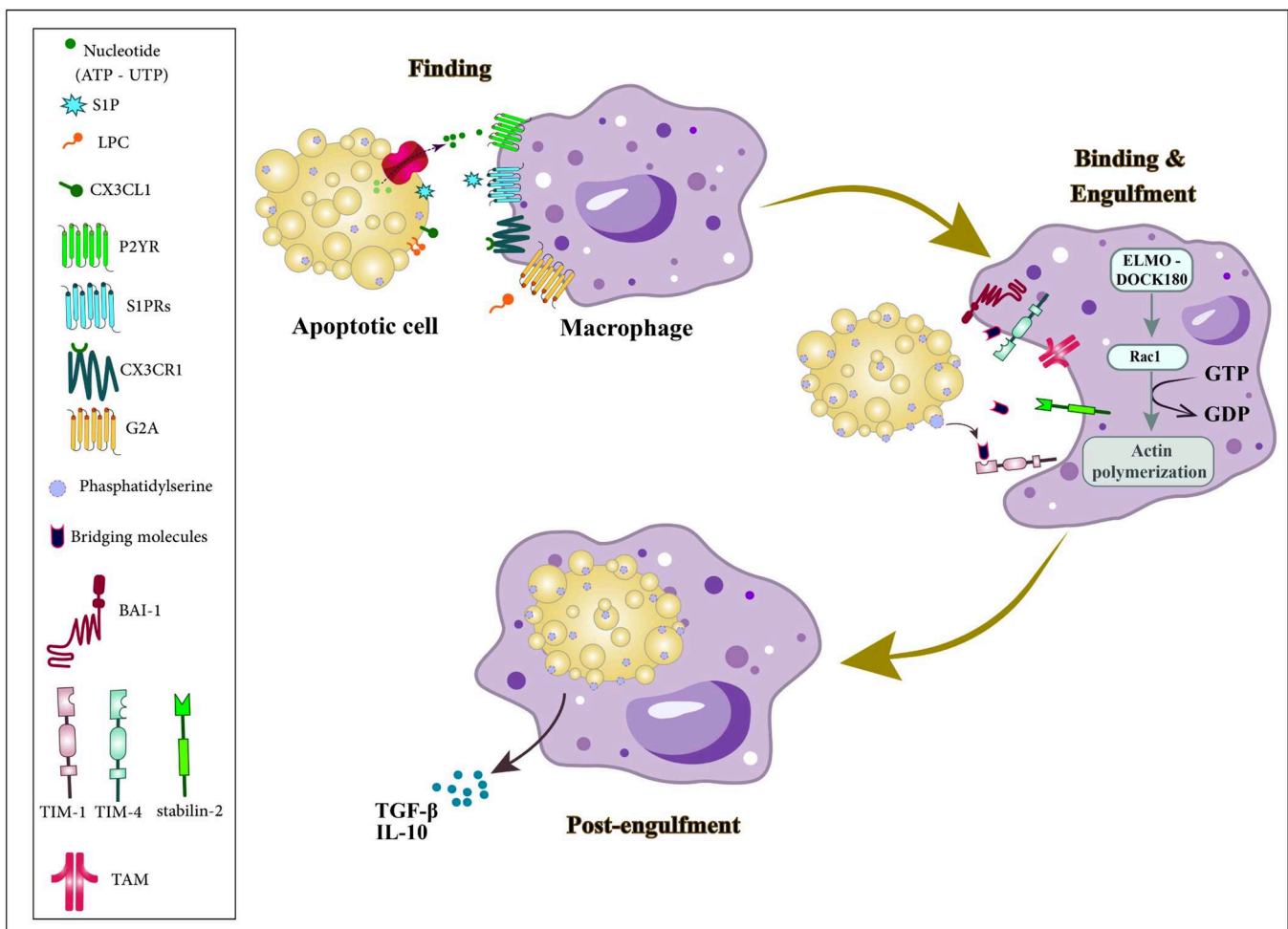


Fig. 1. Different stages of efferocytosis. Macrophages are professional cells in this process, which identify apoptotic cells through find-me signals and connect to them. These signals include a number of ligands on the surface of the apoptotic cell and receptors on the surface of the macrophage cell. Eat-me signals, the most important of which is PtdSer, are triggered, which causes the apoptotic cell to enter the macrophage, which is known as engulfment. Then, there are post engulfment and digestion stages during which anti-inflammatory cytokines are produced.

of M2 cell membrane markers, namely CD163 and CD206. The regulation of this macrophage population is likely influenced by the sex hormones estrogen and progesterone, as indicated by variations in leukocyte populations throughout the menstrual cycle. Preliminary investigations in murine subjects demonstrated that ovariectomy, which inhibits the cyclical synthesis of estrogen and progesterone, results in a reduction of macrophage populations within the uterus. Nevertheless, administration of estrogen and progesterone reinstated macrophage populations, thereby underscoring the involvement of these hormones in the modulation of macrophage activity. The estrogen-dominant follicular phase of the menstrual cycle is marked by the proliferation and thickening of the endometrial lining. In the absence of gestation, a decline in progesterone levels triggers an inflammatory cascade that culminates in the disintegration of the functional layer of the endometrium. Estrogen, for example, interacts with estrogen receptor β (ER β) present on macrophages, thereby augmenting their recruitment *via* the upregulation of CCL2, a chemokine that plays a crucial role in macrophage migration. Likewise, progesterone exerts comparable effects by fostering an M2 anti-inflammatory phenotype, which facilitates the preparation of the endometrium for the process of embryo implantation. The uterus's macrophage counts decreases after ovariectomies, which stop the production of these hormones; nevertheless, macrophage counts are restored by progesterone and estrogen injections. This emphasizes how important progesterone and estrogen are for controlling uterine macrophages (Chambers et al., 2021).

During the menstrual cycle, the ovaries undergo cyclic changes involving the growth and rupture of follicles, which release eggs for potential fertilization. Efferocytosis helps regulate follicular dynamics and tissue remodeling necessary for successful reproductive outcomes. It ensures the efficient clearance of dying or damaged cells, preventing the accumulation of cellular debris that could trigger inflammation and disrupt normal tissue function (Trzeciak et al., 2021). Also, dysregulation in the production or release of these find-me signals may impair the recruitment of phagocytes, hindering effective efferocytosis (Zhang et al., 2022).

In the context of female infertility, emerging evidence suggests that a defect in efferocytosis may be responsible for certain reproductive issues. One study indicates that an imbalance in the IL-33/ST2-AXL-efferocytosis axis can lead to pregnancy loss by inducing metabolic reprogramming of decidual macrophages. This imbalance disrupts the

normal efferocytic process, hindering the clearance of apoptotic cells and potentially causing infertility (Sheng et al., 2022).

5. Efferocytosis and sepsis

Unlike women who peritoneal cavity is penetrated by the free end of the fallopian tubes, men possess a peritoneal cavity that stretches from the inferior portion of the diaphragm to the base of the pelvis; this enclosure is sealed and therefore impervious. Relative to men, women face an elevated susceptibility as the infection permeates the entire peritoneal cavity. Furthermore, it appears that women are more adversely impacted in relation to the discourse on sepsis (Dulin and Akers, 2003; Gunyeli et al., 2019).

Moreover, efferocytosis has been found to be inhibited in sepsis, a condition that can contribute to female infertility due to its role in oxidative stress and inflammation in the female urogenital system (Ojo et al., 2023). The exact mechanisms underlying this inhibition are not yet fully understood. However, it is known that the blockade of apoptotic cell efferocytosis by phagocytes exacerbates sepsis. Understanding these mechanisms and regulations of efferocytosis is crucial for identifying potential therapeutic targets to improve reproductive health (Elliott et al., 2017).

One potential modality for upregulating efferocytosis is through the BAI1-ELMO1-Dock180-Rac1 pathway. Most of the phosphatidylserine recognition receptors are like the seven-transmembrane receptor BAI1, which activates the complex ELMO-DOCK that acts as a GTPase Rac, guanine exchange factor, which ultimately changes the cytoskeleton by inducing the production of anti-inflammatory mediators in the body. During phagocytosis, apoptotic bodies reduce inflammation. Additional research is needed to explore the role of efferocytosis in female infertility and to identify therapeutic targets for improving reproductive outcomes (Penberthy and Ravichandran, 2016).

According to performed studies, immune dysregulation is connected with the development of female reproductive system disorders, including ruptured ectopic pregnancy, preeclampsia, and recurrent spontaneous abortion (RSA) (Penberthy and Ravichandran, 2016).

6. Efferocytosis and polycystic ovarian syndrome (PCOS)

The axis comprising the hypothalamus, pituitary gland, and ovaries

Table 1
Signaling molecules in efferocytosis.

Signal type	Molecule or signal	Function	Ref
“Find-me”	Nucleotides (ATP & UTP)	Bind to purinoreceptor–2 on the surface of phagocytes.	(Elliott et al., 2009)
	Oxidative stress	Oxidative stress mediators by employing monocytes to cells at risk of death.	(Kirkham, 2007)
	Lysophosphatidylcholine (LPC)	In macrophage: Connect with phagocyte cell through the G2 accumulation receptor.	(Peter et al., 2008)
	Sphingosine–1-phosphate (S1P)	Increase in the expression of erythropoietin in macrophages. The activation of PPAR- γ pathway occurs <i>via</i> the EPO-EPO receptor axis, increasing MerTK, MFGE8, Gas6, CD36.	(Gude et al., 2008)
	Fractalkine (CX3CL1)	Bind to its receptor CX3CR1, increase in MFGE8 expression.	(Truman et al., 2008)
“Eat-me”	PtdSer	Bind to different phagocyte surface receptors (e.g.TIM–4).	(Fadok et al., 1998; Li, 2012)
Bridging molecules	MFGE8	Binding to apoptotic cell PtdSer and integrin of macrophages and using the complex CRKII-DOCK180-ELMO and Rac1 to clear apoptotic cells.	(Albert et al., 2000)
	Anxl	At the time of cell death, by transferring to the outer layer of the membrane, it disturbs the balance of the membrane.	(Yoon et al., 2000)
“Don’t-Eat-me” (in healthy cell)	Gas6 & Protein S	Bind to MerTK.	(Lemke, 2013)
	CD47	Prevent engulfment and efferocytosis.	(Takimoto et al., 2019)
	CD31 CD300a		(Brown et al., 2002) (Voss et al., 2015)

Abbreviations list:
CRKII: CT10 regulator of kinase II;
DOCK180: Dedicator of Cytokinesis 180;
ELMO: Engulfment and Cell Motility;

is widely recognized for its role in the regulation of ovulation. Moreover, it is possible that the spleen is implicated in ovulation as well, possibly serving as a direct provider of leukocytes to the ovary all over the ovulation cycle (Oakley et al., 2010). The spleen can serve as an ovarian immune cell facility due to macrophages that developed from splenic monocytes can migrate to the ovary (Oakley et al., 2011). According to the studies conducted on bilateral superior nerve sections in rats, it was concluded that sympathetic nerves may regulate the activity of macrophages (Figuerola et al., 2018). Polycystic ovarian syndrome (PCOS) is a prevalent endocrine disorder that impacts females in their reproductive years (Palafox-Gomez et al., 2023). In the following, the role of macrophages, which are the main actors in the process of efferocytosis, is given. In PCOS patients, the peripheral blood and ovary were at low inflammatory levels, while there is a significant high level of serum C-reactive protein (CRP), lymphocytes and monocytes, and also in the ovarian tissues of PCOS patients, the amount of lymphocytes and macrophages increased (Xiong et al., 2011). In the studies conducted on PCOS, it was found that excess androgen in these patients inhibits the viability of macrophages, increases their apoptosis (Figuerola et al., 2012; Li et al., 2021) and induces the expression of pro-inflammatory cytokines, such as TNF- α . This increase of TNF- α inhibits the production of estradiol (Figuerola et al., 2015) and stimulates the proliferation of theca-interstitial cells and subsequently improves the condition of PCOS patients (Spaczynski et al., 1999).

Another protein involved in PCOS is an immunologically active adipokine, Chemerin (Mariani and Roncucci, 2015). Among its many splicing variations is the CMKLR1-binding isoform, which affects inflammatory reactions in PCOS. Although its regulatory role in the polarization of macrophages (M_1 , M_2) in PCOS patients is not clear, it has

been seen that its increase induces the recruitment of chemokine-like receptor 1 (CMKLR1) monocytes to the ovary and these monocytes are the source of macrophages M_1 , and these macrophages M_1 are increased in mouse models (Ji et al., 2022; Lin et al., 2014).

Macrophage migration inhibitory factor (MIF), another involved protein is a pleiotropic protein involved in immune and inflammatory responses, which has a negative effect on the migration of macrophages (Javeed et al., 2008), and in PCOS patients, it acts through MAPK signaling by inducing DHEA (dehydroepiandrosterone) (Zhou et al., 2018) and the nuclear factor (NF)- κ B pathway, and also the increase of MIF causes inflammation and the increase of inflammatory factors (He et al., 2021). And finally, since the plasma level of MIF is higher in women with PCOS syndrome, it was proposed as a potential predictor (Calan et al., 2016) (Fig. 2).

7. Efferocytosis and endometriosis

Endometriosis is a widespread and incapacitating disorder affecting millions of women worldwide. The characteristic of this disease is the presence of endometriosis-like tissue outside the uterine cavity, which leads to chronic pelvic pain, infertility, and other distressing symptoms. Research on endometriosis continues, and it is clear that immune system disorders play an imperative role in its pathogenesis (Hogg et al., 2020). The disease plays a role Among the immune cells, macrophages have emerged as the main players in endometriosis and the process of efferocytosis, regulating inflammation, tissue regeneration and angiogenesis (Vercellini et al., 2014). There is a correlation between higher pain and elevated levels of insulin-like growth factor-1 (IGF-1) in people with endometriosis. Endometrial-like tissues build up in the peritoneal cavity

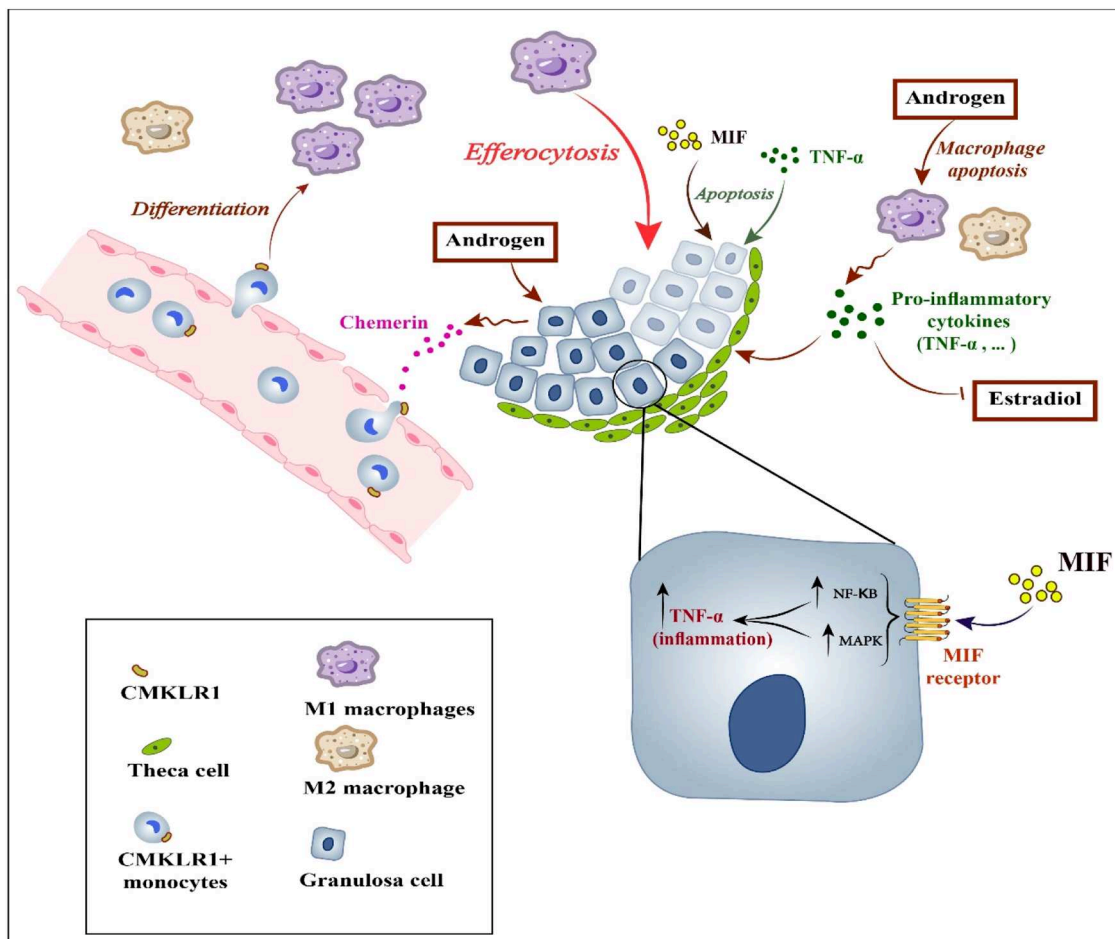


Fig. 2. Summary of the role of efferocytosis in pcos and endometriosis.

of endometriosis patients due to peritoneal macrophages' impaired phagocytic capacity (Forster et al., 2019). Increased PGE2 levels prevent macrophages from expressing CD36 and annexin A2, which reduces phagocytosis (Chuang et al., 2010; Wu et al., 2002). Additionally, the TSP1-CD47-SIRP α axis facilitates the growth of endometriosis. While elevated TSP-1 levels in the peritoneal fluid prevented endothelial cell death, elevated CD47 binding to SIRP α on peritoneal macrophages impaired their ability to engulf cells and promoted a pro-inflammatory phenotype (Liu et al., 2020; Xie et al., 2019). The peritoneal fluid's heme buildup weakened the phagocytic capacity, and tissues that resembled endometria expressed higher levels of CD200.

Increased peritoneal macrophage and plasma levels of CD200 may hinder macrophage phagocytosis and possibly facilitate the immunological escape of endometriosis lesions, indicating the possibility of using plasma CD200 as a diagnostic tool (Weng et al., 2020; Clark et al., 2018). M2 macrophages become polarized upon exposure to exosomes and homogenates derived from ectopic endometrial stromal cells (ESCs). CCL2 then recruits these macrophages via NF- κ B (Gou et al., 2019) and estrogen receptor β (ER β) signaling.

Furthermore, NF- κ B plays a critical role in controlling inflammatory reactions; its dysregulation can lead to persistent inflammation and disrupt efferocytosis. Infertility can result from NF- κ B activation in macrophages, especially in reproductive tissues, which can upset tissue homeostasis. Likewise, MAPK signalling has a role in immune cell response and activation, which is necessary for the regulated removal of apoptotic cells from reproductive organs (Ge et al., 2022).

Sphingosine 1-phosphate (S1P) and IL-17A, two substances that are abnormally high in peritoneal fluid, can cause M2 polarization, which in turn can promote the proliferation of ectopic ESCs, angiogenesis, endometrial stromal cell invasion, and lymphangiogenesis (Miller et al., 2020; Ono et al., 2021).

On the other hand, the development of endometriosis is influenced by macrophages. Drugs that target macrophage function have potential as therapeutics. MCP-1 stimulates macrophage recruitment, and blocking focal adhesion kinase (FAK) reduces MCP-1 secretion (Nagai et al., 2020). Niclosamide is a drug that inhibits the growth of endometriosis-like lesions by targeting STAT3 and NF- κ B signaling, as well as M2 macrophage promotion of ectopic ESC viability (Prather et al., 2016). In mice with endometriosis, EPHA3 expression is decreased, but mTOR expression is elevated. EPHA3 and mTOR are promising therapeutic targets for endometriosis, but more research is needed to determine the precise molecules involved in the mTOR signaling pathway (Xu et al., 2019). Moreover, endometriosis development is aided by galectin-3, but it is inhibited by galectin-3 inhibitors through the suppression of angiogenesis and M2 macrophage enrichment. When an infection is present, M1 macrophages can take the role of M2 macrophages, and their secretions aid in the clearing of lesions (de Mattos et al., 2019). Here in this review focuses on the molecular pathways underlying macrophage involvement in endometriosis, with an emphasis on their recruitment, activation, and functional contributions. In the discussion of macrophage recruitment, one of the important factors of chemotaxis is that in endometriotic lesions, there is an upregulation of certain chemokines, including as CCL2, CCL5, and CXCL8, which facilitates the recruitment of macrophages. Macrophage trafficking is regulated by the interaction of ligands in the endometriotic microenvironment with chemokine receptors, such as CCR2 and CCR5 (D'Império Lima and Bortoluci, 2015; Angsana et al., 2016; Thiruchelvam et al., 2016).

The next important factor is adhesion molecules that the adherence and transmigration of macrophages across the endothelium into the peritoneal cavity and endometriotic lesions are facilitated by integrins, selectins, and ICAM-1 (Wiesolek et al., 2020).

Another important factor in this path, as mentioned above, is macrophage activation, which includes other important factors, such as polarization, Toll-like receptors (TLRs) and Hypoxia-inducible factor (HIF)-1 α . Macrophages have a range of functional phenotypes, including

pro-inflammatory M1 and anti-inflammatory M2 polarization states. The equilibrium between these states is upset in endometriosis, favouring an M2-dominant phenotype that feeds the growth of pro-fibrotic and angiogenic microenvironments (Angsana et al., 2016). Extracellular matrix and debris, which are components of endometriotic tissue, activate TLRs, causing the generation of pro-inflammatory cytokines and exacerbating the chronic inflammatory response associated with endometriosis. In endometriotic lesions, hypoxia stimulates HIF-1 α expression, which in turn encourages M2 polarization, angiogenesis, and macrophage tissue remodeling.

Finally, macrophages are involved in the development of endometriosis which in fact, TNF- α and IL-1 β , two pro-inflammatory cytokines that support local inflammation and are a substantial source of pain in endometriosis, are produced by macrophages. Moreover, in angiogenesis discussion, vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs) are two examples of macrophage-derived substances that promote the development of new blood vessels within endometriotic lesions, hence aiding in the survival and proliferation of ectopic endometrial tissue. In addition, macrophages contribute to the development of fibrosis by stimulating the differentiation of fibroblasts into myofibroblasts and facilitating the accumulation of extracellular matrix proteins (Sharkey et al., 2000; Zhang and Nothnick, 2005).

8. Efferocytosis in male infertility

About 15 % of couples are affected by infertility and half of all cases of infertility occur in men; moreover, the cause of 30 %-40 % of male infertility patients is unclear, to be precise male idiopathic infertility (Bracke et al., 2018). The dominant leukocyte type in the testis is macrophages, and they constitute the largest population of testicular immune cells (Mass et al., 2016), which are M2 CD163⁺ macrophages (Ponte et al., 2018). Macrophages were confined neighboring undifferentiated spermatogonia, and they express factors which are important to spermatogonial growth and differentiation, such as colony stimulating factor 1 (CSF1) and enzymes of retinoic acid biosynthesis (RA). Macrophage depletion results in undifferentiated spermatogonia and decreases in the number of proliferative spermatogonia respectively (DeFalco et al., 2015). A large amount of 25-Hydroxycholesterol produced by testicular macrophages can be converted to testosterone production by Leydig cells (Lukyanenko et al., 2001). Leydig cells are considered as the main source in testosterone production and macrophages are considered as the main regulators of this steroidogenesis. This close accompanies between macrophages and Leydig cells is very important in developmental process and testicular maturation (Gu et al., 2022). Testicular macrophages, which reside in the testicular interstitial space, assumed to play an important role in immune privilege. Secretion of pro-inflammatory cytokines leads to the suppression of corticosterone generated by testicular macrophages and maintains the immune privilege and immunosuppressive environment in the testis to control the immune response against germ cells and preserve male fertility (Wang et al., 2017). Testicular macrophages participate in the pathogenesis of male idiopathic infertility. Ubiquitin-specific protease 2 (USP2) which controls cytokine production in macrophages, affected spermatogenesis because its expression has been identified in late-elongated sperm and affects sperm function (Lin et al., 2000). The USP2 in mice macrophages involved in sperm activation, mobility and movement, and USP2 preserved GM-CSF expression in testicular macrophages, which is an important factor for sperm mobility (Hashimoto et al., 2021). In the testis of idiopathic infertility cases, there was an increase in the number of CD68 macrophages, and these cells by phagocytosis or by secretion of pro-inflammatory cytokines, such as IL-1 and TNF- α may involve in impaired spermatogenesis and male infertility. Increased number of CD68 macrophages in the testes involved in the regulation of steroid production, Sertoli cell activity and germ cell survival (Frungieri et al., 2002). Increased in catecholaminergic neural elements which is important in the maintenance of testicular homeostasis links to

increased macrophages in human testis could start inflammation, homeostasis dysregulation and idiopathic male infertility (Matzkin et al., 2019). Infertile men with reduced testosterone exhibit elevated levels of estradiol, a risk factor for infertility, that may result in male infertility and in mouse model estradiol induce Leydig cell to express growth arrest-specific 6 (GAS6). GAS6 mediates and bridges phosphatidylserine on Leydig cells to macrophages receptors, such as AXL, which results in engulfment of Leydig cell by testicular macrophages (Yu et al., 2014). Macrophage concentration in semen contributes to semen quality and further success of fertilization. Sperm motility, morphology, cell membrane integrity and cell DNA damage showed negative correlation with macrophages concentration in semen (Chen and Zheng, 2021). Sperminophages are activated macrophages, which involved in sperm phagocytosis (spermiophages), assumed to be a marker of innate immunosystem activation. Sperminophages are involved in sperm features and male infertility. Presence of sperminophage in ejaculated sperm associated with decreased total number of sperms, decreased sperm forward motility and increased number of degenerating sperm (Pelliccione et al., 2009).

9. The role of Sertoli cells in male idiopathic infertility

Apoptosis occurs in 75 % of testicular germ cells, and then they are removed by Sertoli cells. phosphatidylserine resides in the inner side of

membrane in normal cells. Xkr8 is a transmembrane protein and while it is digested by caspase-3 in apoptotic cells, the inner phosphatidylserine exposed to cell surface. Exposed phosphatidylserine on apoptotic germ cells acts as an “Eat-me” signal which then recognized by TIM-4 and Mer, which results in clearance of the germ cells by Sertoli cells. Xkr8 apoptotic germ cells in *Xkr8*-deficient male mice cannot present exposed phosphatidylserine on the surface and cannot be removed by Sertoli cells and as a result, apoptotic cells engulf residual bodies and cellular debris. Xkr8-mediated phosphatidylserine is crucial for clearance of apoptotic cells. In *Xkr8*-deficient, defects in engulfment of apoptotic cells may starve the Sertoli cells and lose its capacity to maintain spermatogenesis (Yamashita et al., 2020). Moreover, it was reported that Sertoli cells spot phosphatidylserine on apoptotic spermatogenic cells by class B scavenger receptor type 1 and this phagocytosis is necessary for sperm production (Nakanishi and Shiratsuchi, 2004).

Efferocytosis of apoptotic germ cells that performs by Sertoli cells has an important role in testis homeostasis. The accumulation of apoptotic germ cells in the seminiferous tubules due to their defective clearance disrupts male infertility. Efferocytosis is a highly dependent-energy consuming procedure. Sertoli cells perform efferocytosis via glucose uptake and cytoskeletal orchestration; moreover, for this function interaction of glucose transporter (Glut)1 and β II-spectrin seems to be necessary. β II-spectrin is a regulator of glucose metabolism and cytoskeletal architecture. Deficiency of β II-spectrin, as a regulator of glucose

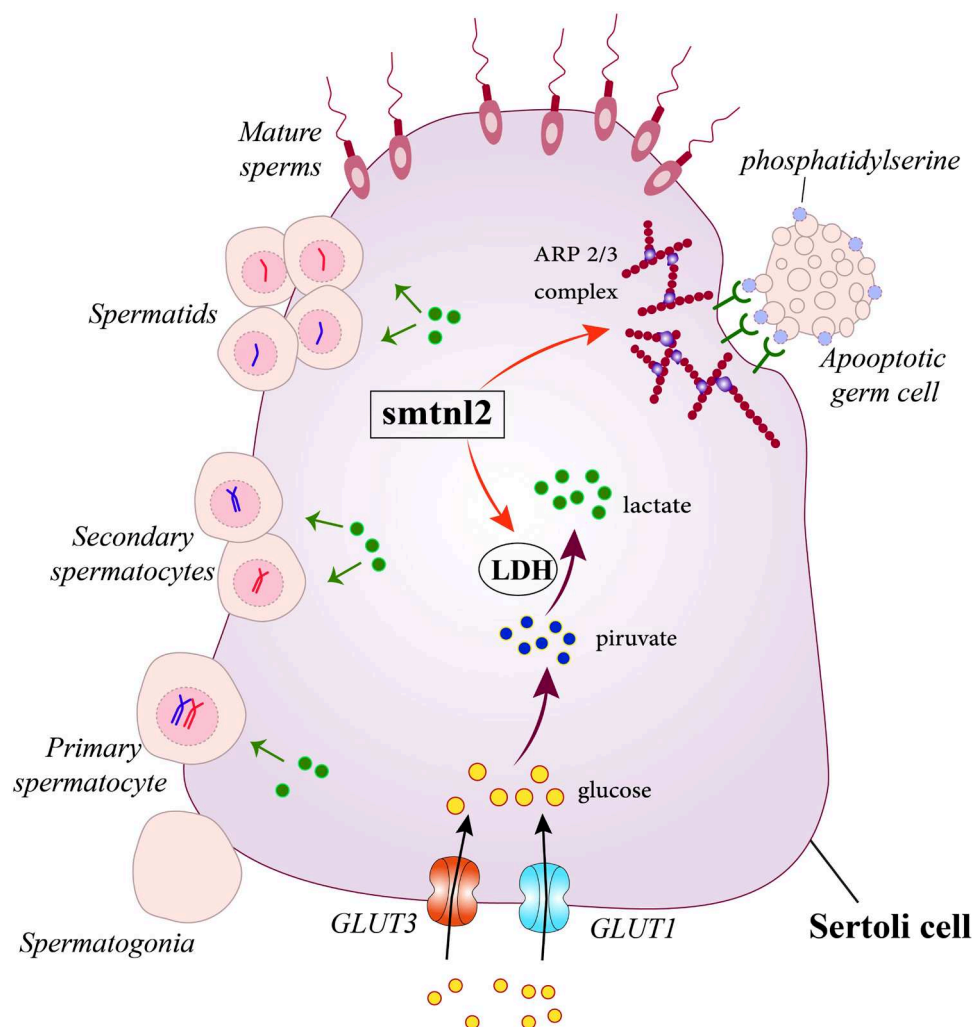


Fig. 3. Interactions of Sertoli cell and germ cell play an important role in the spermatogenesis. Sertoli cells nurse germ cells and provide structural support; moreover, Sertoli cells regulate the development of germ cells by regulating metabolism and signaling pathways. Smtnl2 is a central molecule which regulates lactate production via LDH. Smtnl2 regulates actin polymerization via actin nucleation protein APR3 and efferocytosis of apoptotic cells.

metabolism and cytoskeleton structure, leads to dysfunction of Sertoli cells in glucose uptake and cytoskeleton formation that results in defective efferocytosis (Wu et al., 2023).

Another protein which has a role in efferocytosis is Smoothelin-like 2 (Smtnl2). In Sertoli cells Smtnl2 involves in organization of cytoskeleton in efferocytosis of apoptotic germ cells and increased expression of Smtnl2 results in increased efferocytosis and Smtnl2 deficiency ends in defective efferocytosis by breakdown of cytoskeleton via change in glucose metabolism and also via change in actin nucleation protein 3 (APR3). The expression of lactate dehydrogenase is controlled by Smtnl2, which in turn regulates glucose metabolism (Fig. 3) (Wu et al., 2022).

Germ cells need high energy level, and it should be provided by Sertoli cells. Sertoli cells uptake glucose through GLUTs and metabolize it to lactate, which is then transported to germ cells. In Insufficient glucose resource, Sertoli cells increases GLUTs expression to preserve lactate production by activating AMP-activated protein kinase (AMPK), PI3K and P38 mitogen-activated protein kinase (MAPK)-dependent pathways (Wu et al., 2022).

Lactate is the main source of energy in germ cells and any alteration in energy metabolism in testis might relate to disturb of spermiogenesis. It is transported and then used by germ cells and changed to pyruvate. Lactate oxidation to pyruvate would increase NADH, which is a substrate for NAD(P)H oxidase4 (NOX4). Reactive oxygen species (ROS) which is a product of NOX4 activity, may act as a regulator for signal transduction pathways and gene expressions of Akt and p38-MAPK (Galardo et al., 2014).

10. Conclusion

In conclusion, efferocytosis—the process by which phagocytes clear apoptotic cells—is essential for maintaining tissue homeostasis and reducing inflammation. This review highlights the significance of efferocytosis in reproductive physiology and its connection to infertility across both genders. Dysregulation of this process has been linked to various infertility issues, including implantation failures and repeated miscarriages in females, and impaired spermatogenesis in males. In females, effective efferocytosis during the menstrual cycle is crucial for successful embryo implantation, with defects potentially leading to miscarriages. In males, Sertoli cells and macrophages play significant roles in the removal of apoptotic germ cells and the maintenance of spermatogenesis. Macrophages, in particular, are involved in the growth and differentiation of spermatogonia and express factors, such as CSF1 and pro-inflammatory cytokines, which can negatively affect sperm quality when dysregulated. Research suggests that immune dysregulation, related to the pathogenesis of conditions like preeclampsia, further complicates reproductive health. Impaired efferocytosis can hinder the clearance of apoptotic cells, contributing to reproductive challenges in both males and females. Understanding the molecular mechanisms regulating efferocytosis is vital for identifying potential treatments and interventions for infertility.

This review underscores the importance of efferocytosis and the roles of macrophages and Sertoli cells in idiopathic male infertility while emphasizing the need for further research to fully elucidate the mechanisms involved in efferocytosis and its implications for both male and female infertility. Developing effective therapeutic interventions will depend on a deeper understanding of these processes.

Funding

None.

CRediT authorship contribution statement

Sayed Mohammad Gheibihayat: Writing – review & editing, Writing – original draft, Visualization, Validation, Project

administration, Methodology, Investigation, Conceptualization. **Amir Tajbaksh:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Nasim Naseri:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Noorodin karami:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Soroosh Shahryarhesami:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Sareh Bakhshandeh Bavarsad:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

None.

Author contributions

SM.G. conceived the study and designed the study. S.B., S.S., N.K., AT., and N.N prepared the first draft of the manuscript. N.N. designed the figures. All authors critically revised the first draft and approved the final version.

Data availability statement

Data will be made available on request.

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