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Inflammation and reproductive function in women with polycystic ovary syndrome†

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Abstract

Polycystic ovary syndrome (PCOS) is one of the most frequent endocrinopathies, affecting 5–10% of women of reproductive age, and is characterized by the presence of ovarian cysts, oligo, or anovulation, and clinical or biochemical hyperandrogenism. Metabolic abnormalities such as hyperinsulinemia, insulin resistance, cardiovascular complications, dyslipidemia, and obesity are frequently present in PCOS women. Several key pathogenic pathways overlap between these metabolic abnormalities, notably chronic inflammation. The observation that this mechanism was shared led to the hypothesis that a chronic inflammatory state could contribute to the pathogenesis of PCOS. Moreover, while physiological inflammation is an essential feature of reproductive events such as ovulation, menstruation, implantation, and labor at term, the establishment of chronic inflammation may be a pivotal feature of the observed reproductive dysfunctions in PCOS women. Taken together, the present work aims to review the available evidence about inflammatory mediators and related mechanisms in women with PCOS, with an emphasis on reproductive function.

Key words: inflammation, PCOS, infertility, ovary, uterus.

Polycystic ovary syndrome phenotypes and relation to metabolic features and inflammation

Polycystic ovary syndrome (PCOS) is a common, multifactorial, and complex endocrine disorder, and the heterogeneity of signs and symptoms in women with this syndrome presents a challenge for its diagnosis [1]. For that reason, different diagnostic criteria have been applied in various studies and at different times and are in continuous debate [2]. The most widely used criteria, known as the Rotterdam criteria, involve the presence of at least two of the following three features (after exclusion of secondary causes): (1) oligo-ovulation leading to oligomenorrhea, or anovulation leading to amenorrhea, (2) hyperandrogenism: clinical (hirsutism, male pattern alopecia, acne) and/or biochemical, (3) polycystic ovarian morphology (PCOM) on ultrasound. Furthermore, the most recent effort in the diagnosis of PCOS came in 2012 at the National Institutes of Health workshop [3], and there was a general consensus to propose the following categories of PCOS: androgen excess (AE) plus ovulatory dysfunction (OD); AE plus PCOM; OD plus PCOM; and AE plus OD plus PCOM.

Besides these categorizations, the etiology of PCOS is largely unknown, and genetic and environmental factors, along with prenatal and peripubertal life events, such as obesity and endocrine factors, appear to exert a major role [4] (FIGURE 1). Having this in mind, adopting a diagnostic criterion highlights the possibility that PCOS phenotypes differ in terms of insulin resistance (IR), obesity, and long-term metabolic and reproductive risks [1]. In this sense, a current issue is the high number of studies not reporting/covering specific PCOS phenotypes, thus leading to controversies in the relationship between obesity, IR, and PCOS. Obesity is frequently

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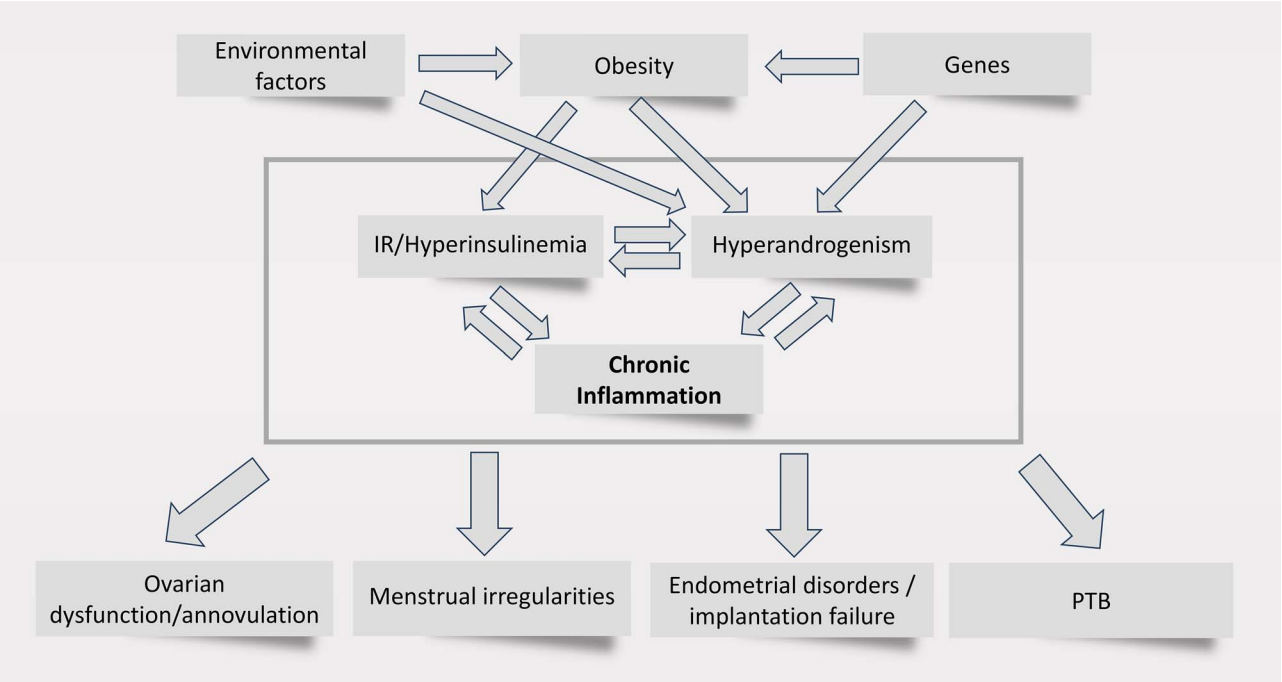

Figure 1. Proposed mechanisms of interactions between IR, hyperandrogenism and chronic inflammation in PCOS, and relation to reproductive outcomes. Environmental and genetic factors may predispose to obesity. These risk factors in turn favor the establishment of hyperandrogenism and IR/hyperinsulinemia. Cross-talk between IR and hyperandrogenism may recreate a welcoming environment to chronic inflammation. Finally, the combination and cross-talk of chronic inflammation, IR and hyperandrogenism may drive many of the pathological reproductive outcomes in PCOS.

observed in PCOS women, and Lim et al. [5] showed that obesity significantly worsened most of metabolic and reproductive outcomes when compared to normal weight women with PCOS. Regardless, both obese and lean women with PCOS are at increased metabolic risk [6]. IR seems to be a central player in this matter, and despite it is not considered for the diagnostic criteria, the majority of lean and overweight women with PCOS have a form of IR intrinsic to PCOS [1]. The compensatory hyperinsulinemia in response to IR seems to drive many of the phenotypic features of PCOS. Hyperinsulinemia promotes ovarian hyperandrogenism, which is present in the majority of women with PCOS [1]. And together, high insulin and androgen levels can disrupt follicle maturation; this is followed by menstrual irregularity, anovulatory subfertility, and accumulation of immature follicles and subsequent ovarian polycystosis [7].

It is important to point out that PCOS is considered a low-grade inflammatory disorder, independently of the presence of obesity [8], although obesity can exacerbate both metabolic and reproductive outcomes [5]. In women with PCOS, chronic inflammation can mediate the long-term cardiometabolic complications and comorbidities [6]. Moreover, the fact that differential expression of inflammatory genes was found in nonobese women with PCOS poses new etiological considerations for the involvement of inflammation in the onset of PCOS [9]. Particularly, chronic inflammation and its interaction with IR and hyperandrogenism in PCOS women continue to be investigated. A current hypothesis is that, in women with PCOS, the cross talk between IR, hyperandrogenism, and chronic inflammation creates a pathological environment that fosters the development of further cardiometabolic disturbances [10]. The increase in multiple markers of inflammation such as tumor necrosis factor-alpha (TNFA) and C-reactive protein (CRP) in addition to increased oxidative stress and endothelial dysfunction is the evidence that PCOS is commonly coupled with low-grade systemic inflammation [11]. Oxidative stress and inflammation markers are also positively correlated with androgen levels in PCOS [12], although the precise interactions between oxidative stress, IR, and inflammation remain to be fully elucidated in PCOS women [13]. Of particular interest for this review is our view that there is a need of studies specifically designed to assess the impact of inflammation on reproductive outcomes in women with PCOS.

Main circulating markers of inflammation in PCOS

An extensive body of literature has established inflammatory molecules as biomarkers of PCOS [11]. The first description of this relationship was put forth by Gonzalez et al. [14], who found increased concentrations of the cytokine TNFA, a well-known inflammatory mediator, in the serum of lean women with PCOS. In another pivotal study, Kelly et al. [15] found PCOS correlated with elevated serum levels of CRP, an observation that was verified by a large-scale meta-analysis [8]. CRP is an acute-phase protein of hepatic origin, whose circulating concentrations rise in response to inflammation [16], and is regulated by pro-inflammatory cytokines such as TNFA and interleukin (IL)-6 [17]. Further, in women with PCOS, the levels of CRP, TNFA, and IL-6 correlate with IR, body weight, and fatty mass [18]. Overall, the systemic inflammatory condition in PCOS women is maintained by the continuous release of cytokines, endothelial factors, acute phase proteins, and adipokines (see Table 1). However, as mentioned in the previous section, taking into account the high incidence of obesity in PCOS women, precautions exist when trying to dissect the systemic inflammatory profile owing to PCOS from the

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Table 1. Inflammation-related risk factors in serum of PCOS.

Inflammation-related risk factor	Finding in serum of PCOS. References included
Adiponectin	Decreased levels in PCOS [10], IR-PCOS [19, 20], and obese PCOS women [21] comparing to appropriate controls
CRP	Increased plasma levels in PCOS women comparing to controls [22, 23]. Increased levels in obese PCOS women comparing to lean control patients [24]. A systematic review revealed increased plasma levels in PCOS women [8]
E-selectin	Increased plasma levels in PCOS [22], non-IR-PCOS [25], and IR-PCOS [25] comparing to appropriate controls
IFNg	Increased levels in obese nonhyperandrogenic PCOS women comparing to obese and nonobese controls [26]
IL-1b	Increased plasma levels in PCOS women comparing to appropriate controls [27]
IL-1R	Increased plasma levels in PCOS women comparing to controls [28]
IL-6	Increased plasma levels in PCOS [27–30], non-IR-PCOS [25], and IR-PCOS [25] comparing to appropriate controls [29]
IL-7	Decreased plasma levels in PCOS comparing to appropriate controls [27]
IL-8	Increased plasma levels in PCOS women comparing to controls [30]
IL-10	Decreased levels in PCOS women comparing to controls [30]
IL-17	Increased plasma levels in PCOS women comparing to appropriate controls [28, 31]. Increased levels in obese PCOS women without hyperandrogenism, comparing to obese and nonobese controls [26]
IL-18	Increased plasma levels in PCOS [32, 33], IR-PCOS [33], and obese PCOS [33] women comparing to appropriate controls. Increased levels in obese nonhyperandrogenic PCOS women comparing to obese and nonobese controls [26]
Leptin	Increased fasting levels in PCOS women comparing to controls [34]
MCP1	Increased levels in PCOS women comparing to normal control women [23, 35] or women with endometriosis or unexplained infertility [36]
MIF	Increased levels in PCOS women comparing to control women [24, 35]
MMP2	Increased levels in PCOS women comparing to controls [37]
MMP8	Increased levels in PCOS women comparing to controls [38]
MMP9	Increased levels in PCOS women comparing to controls [39]
MPO	Increased plasma levels in IR-PCOS and non-IR-PCOS women comparing to controls [25]
ROS production	Increased total and mitochondrial ROS production by leukocytes from IR-PCOS and non-IR-PCOS versus controls [25, 30, 40]
sICAM-1	Increased plasma levels in PCOS [22], non-IR-PCOS [25], and IR-PCOS [25] comparing to appropriate controls
sVCAM-1	Increased plasma levels in PCOS and IR-PCOS [25] comparing to appropriate controls
TIMP1	Increased levels in PCOS women comparing to controls [37]
TNFA	Increased plasma levels in PCOS [27, 29, 30], non-IR-PCOS [25], and IR-PCOS [25] comparing to appropriate controls [29]
Visfatin	A systematic analysis identified increased levels of Visfatin and an association with PCOS [41]

IFNg, interferon gamma; MCP1, monocyte chemoattractant protein-1; MIF, macrophage migration inhibitory factor; MPO, myeloperoxidase; ROS, reactive oxygen species; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; TIMP1, tissue inhibitor matrix metalloproteinase 1.

obesity-related systemic inflammation. In the following sections, we will expand the role of inflammation in the reproductive tissues of women with PCOS.

Physiological and PCOS-related inflammation in the ovary

Physiological inflammation in the ovary

In the ovaries, sex steroids and gonadotropins are largely responsible for the dynamics of changes in the ovarian follicular development, ovulation, and corpus luteum formation. These changes involve inflammation as an essential feature, both in folliculogenesis and ovulation [42]. Since the 1970s, it was known that inhibition of the synthesis of the inflammatory mediator prostaglandins (PGs) blocked ovulation in rats [43], and the same effect was observed in other mammals, including human [44]. Later, many of the molecules responsible for inducing the inflammatory cascade, including PGs, cytokines, and leukotrienes, have been found in the ovary [45]. Just before ovulation, a rise in gonadotropins induces an acute inflammatory reaction [46], leading to increased protease activity in the granulosa and theca layers of follicles and consequent degradation of the extracellular matrix (ECM) of the connective tissue in the ovary [47]. Cytokines such as TNFA and IL-1 might be involved in the process, by increasing levels of inflammatory PGE and PGF2 [48], which in turn act via PG receptors to upregulate essential genes responsible for cumulus expansion, regulation of vascular permeability, and degradation of ECM. These result in increasingly intrafollicular pressure, with the subsequent rupture of the ovarian surface epithelium (OSE) and release of the cumulus–oocyte complex from the ovary [49].

Following ovulation, repair of the site is necessary to form a functional corpus luteum. Infiltrating macrophages produce TNFA, which acts in the proliferation and repairing of OSE cells [50].

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Besides TNFA, other cytokines including IL-1 [51], IL-6, and IL-8, nuclear factors such as nuclear factor kappa B (NF-kB) [52], the limiting enzyme of PG synthesis cyclooxygenase 2 (COX2), PGs, leukocytes as well as growth factors participate in these processes [53]. Inflammatory cytokines also enhance the steroidogenic environment in granulosa cells (GCs) and OSE cells. This, in turn, enhances adrenal cortisol levels, which facilitate the repair and counteract the inflammatory response [52]. Although the mechanistic pathway is unknown, the ovarian inflammatory mechanisms may be linked to systemic markers of inflammation, since it was shown that the levels of the inflammatory mediator CRP fluctuate in the menstrual cycle, with a peak near ovulation [54]. Elevated serum CRP concentration was found in women with three ovarian follicular waves rather than the more common, two follicular waves [54]. This study suggests that systemic CRP concentrations may trigger changes in follicular dynamics, reinforcing the hypothesis of a possible link between ovarian inflammation and systemic inflammation.

Inflammation and ovarian function in PCOS

Inflammatory dysregulations are associated with infertility diseases and, therefore, have been shown to affect ovarian function, oocyte quality, and endometrium receptivity [55]. In PCOS women, a chronic pro-inflammatory state along with insulin dysregulations might contribute to the pathogenesis of functional AE/hyperandrogenism through upregulation of the ovarian theca androgen synthesis [56, 57]. We mentioned before the association between circulating CRP and ovarian function. In PCOS women, a seminal study showed increased levels of serum CRP levels, independent of BMI [8]. One possible pathway by which systemic CRP may impact ovarian inflammatory pathways is via receptors in the specific subset of phagocytes located in ovarian tissue [58], which is increased in PCOS ovaries and regulates the reproductive inflammatory response [59]. Particularly on ovarian function in PCOS, most studies come from GCs of follicular fluids from women undergoing in vitro fertilization (IVF). Thus, owing to this limited ovarian tissue availability, the ovarian functional link in PCOS women remains partially elusive. From these studies, it was shown that elevated concentration of ovarian PGs, TNFA, and IL-6 [29, 60] correlate with an abnormal pattern of IL-12 and IL-13 [61]. Also, the upregulation of ILs, TNFA, chemokine (C-C motif) ligand 20 (CCL20), and COX2 in GCs of women with this syndrome was shown [57, 62]. Interestingly, Zhao et al. [63] proposed that WNT family member 5A (WNT5a) act as a pro-inflammatory factor since the upregulated expression of ovarian WNT5a in PCOS increased inflammation and oxidative stress via the phosphatidyl inositol 3-kinase/AKT/NF-kB signaling pathway, which in turn increased ovarian cytokine gene expressions of TNFA, IL-s, chemokines, and CRP. In a similar approach, Adams et al. described the ovarian GC pro-inflammatory pattern in PCOS and proposed that intrafollicular androgens and cytokines may comprise a local regulatory pro-inflammatory loop that regulates GC expression of cytokines, chemokines, and the presence of immune cells, although the molecular pathways remain to be determined. They also proposed that obese PCOS patients can be seen as a distinct ovarian PCOS subtype, since this subtype presented the most pronounced increases in pro-inflammatory and immune-related factors in GCs [57]. Also, the inflammatory profile of GCs may be different to the ovarian stroma, since Schmidt et al. [62] described a distinct pattern of differentially expressed inflammation-related genes in the stromal versus GCs of PCOS women. In addition, Qu et al. showed, in a culture of GCs, that hyperandrogenism induces epigenetic alterations of important nuclear factors, which may act in the regulation of the inflammatory process, such as peroxisome proliferator-activated receptor gamma (PPARG) and nuclear co-repressor [64]. Finally, it was shown that ovaries from PCOS women had an increased number of macrophages and lymphocytes immersed throughout, adding more evidence to the hypothesis of a persistent and chronic ovarian pro-inflammatory state in these women [59]. The evidence presented here supports the presence of a pro-inflammatory persistent condition in women with this syndrome, which may impact on the normal ovarian function, impairing the synthesis and release of sexual hormones, the follicular maturation, and the subsequent ovulation. Table 2 summarizes inflammatory mediators described in PCOS women (see also FIGURE 1).

Physiological and PCOS-related inflammation during menstruation

Physiological inflammation and menstruation

After ovulation, the levels of luteal progesterone increases, promoting a series of changes in the endometrium to prepare the tissue for an implanting conceptus, which includes inhibition of cellular proliferation, DNA synthesis, and cellular mitotic activity, and the onset of cellular differentiation [80]. Early inflammatory events are triggered by progesterone during endometrial decidualization, which involves the infiltration of leukocytes, and subsequent modifications of the ECM and vascular permeability. The endometrium is a steroid hormone-dependent tissue, where the growth and remodeling of its cellular components respond to changes in circulating hormones in normal ovulatory cycles [81] and, when pregnancy is absent, is subject to disintegration and remodeling every menstrual cycle. Seven to 10 days post ovulation, the endometrium becomes receptive to embryonic implantation, in the so-called window of implantation. It represents the height of progesterone priming, in which progesterone-induced decidualization of endometrial stromal cells is a key step to allow successful trophoblast invasion [82].

In the absence of conception, endometrial tissue desquamation and menstruation occur [55]. Progesterone withdrawal is central to menstruation events, and molecular inflammatory pathways identified include those associated with NF-kB, PGs, cytokines, chemokines and matrix metalloproteases (MMPs) [55]. Particularly, endometrial PGs have an active role in these endometrial events [83, 84]. Endometrial PG actions result in ischemia and subsequent tissue necrosis and shedding [85]. Endometrial PGF2A and prostacyclin (PGI2) are highest before the onset of menstruation and induce cyclic blood vessel vasoconstriction and vasodilation, respectively [86]. Moreover, the levels of PGF2A and PGE2 during the menstrual cycle are regulated by the catabolic enzyme prostaglandin-15-dehydrogenase (PGDH), which in turn is regulated by progesterone [86]. Following progesterone withdrawal, PGDH expression declines to lead to a rise in the levels of PGs peri-menstrually [87]. Progesterone withdrawal allows the translocation of NF-kB into the nucleus of endometrial cells, which in turn activates transcription of various cytokine genes, COX2 enzymes, and PGF2A production [86]. This effect induces a substantial influx of inflammatory-type leukocytes in response to chemoattractant cytokines and chemokines [86]. Activation of this type of leucocytes favors cellular interactions, which in turn are important for inducing MMPs expression and matrix degradation [55]. This is further enhanced by the action of PGE2 on the blood vessels to induce capillary leakage [86]. Thus,

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Table 2. Inflammation-related risk factors and ovarian function in PCOS.

Inflammation-related risk factor	Ovarian function finding in PCOS. References included
CCL-2/MCP1	Decreased gene expression in ovaries from PCOS women [62]. Aberrant ovarian expression in a prenatal PCOS-model [65]
CCL-20	Increased gene expression in GCs of lean and obese PCOS women [57, 66]
CD45/PTPRC	Decreased expression in ovarian stroma of PCOS women [62]. Increased expression in GCs from PCOS women [57]
COX2	Increased gene expression in GCs of PCOS women [62]. Aberrant expression in a culture of GCs derived from nonobese PCOS women comparing to controls [67]
CXCL-1	Increased gene expression in GCs from obese and normal weight PCOS women [57, 65] versus controls. Differential GC expression associated with oocyte maturation and embryo quality in PCOS women [68]
G-CSF	Decreased protein levels in FFs from PCOS women associated with poor embryo quality [69]
HDAC3	Increased gene expression in GCs from hyperandrogenic PCOS women comparing to nonhyperandrogenic PCOS, and increased expression associated with failed pregnancy [64]
IL-12	Decreased protein levels in FFs from PCOS women, and protein expression correlated with T lymphocyte numbers in FFs [61]
IL-13	Decreased protein levels in FFs from PCOS women, and protein expression correlated with T lymphocyte numbers in FFs [61]
IL-1B	Increased gene expression in GCs of PCOS women [62]. Ovarian expression associated with ovarian function and pathophysiology in PCOS women and models [70]
IL-1R1	Decreased gene expression in ovaries from PCOS women [62]. Ovarian expression associated with ovarian function and pathophysiology in PCOS women and models [70]
IL-6	Increased protein levels [29, 71] and gene [57] expression in FFs of PCOS women
IL-8	Increased gene expression in GCs of PCOS women [57, 62] Decreased expression in ovaries from PCOS women [62]
IL-10	Increased protein levels in FFs of PCOS women [71]
LIF	Decreased protein levels in FFs of PCOS women [72]. LIF levels in FFs may predict IVF outcome [72]. Increased gene expression in GCs of PCOS women [62]
MMP-2	Increased protein levels in FFs and GCs from PCOS women comparing to controls [73]
MMP-9	Increased protein levels in FFs and GCs from PCOS women comparing to controls [73]
NCOR1	Increased gene expression in GCs from hyperandrogenic PCOS women comparing to nonhyperandrogenic PCOS, and increased expression associated with failed pregnancy [64]
NOS2	Decreased expression in ovaries from PCOS women [62]. Increased gene expression in GCs of PCOS women [62]
PGE2	Increased levels and release from FFs of nonobese PCOS women comparing to control [60, 67, 74]
PGF2	Increased levels and release from FFs of nonobese PCOS women comparing to controls [67]
15-d-delta 12,14-PGJ2	Increased levels and release from FFs of nonobese PCOS women comparing to control [67]
PPARG	Decreased gene expression in GCs from hyperandrogenic PCOS women comparing to nonhyperandrogenic PCOS, and decreased expression associated with failed pregnancy [64]. Aberrant expression in GCs from PCOS women and PCOS models [75, 76]
RUNX2	Aberrant expression in FFs and GCs may predict IVF outcome in PCOS women [68, 77]. Decreased gene expression in ovarian stroma from PCOS women [62]
TIMP1	Decreased expression in FFs from PCOS women compared to controls [78]. Decreased gene expression in ovarian stroma from PCOS women [62]
TNFA	Increased gene expression in GCs [57] and protein levels in FFs of PCOS women [29, 71]. Increased FFs levels associated with IVF outcome in PCOS women [69, 79]
WNT5A	Increased gene expression in GCs of PCOS women. WNT5A upregulation in GCs increased inflammation-related genes and ROS species [63]

CCL-2/MCP1, chemokine (C-C motif) ligand 2/monocyte chemoattractant protein 1; CCL-20, chemokine (C-C motif) ligand 20; CD45/PTPRC, cluster of differentiation 45 antigen/protein tyrosine phosphatase, receptor type, C; COX2, cyclooxygenase 2; CXCL-1, chemokine (C-X-C motif) ligand 1; GC, granulosa cell; G-CSF, granulocyte colony-stimulating factor; FF, follicular fluid; HDAC3, histone deacetylase 3; NCOR1, nuclear receptor co-repressor 1; NOS2, nitric oxide synthase 2; PGE2, prostaglandin E2; PGF2, prostaglandin F2; PGJ2, prostaglandin J2; ROS, reactive oxygen species; RUNX2, Runt-related TF 2; TIMP1, tissue inhibitor matrix metalloproteinase 1; WNT5A, Wnt family member 5A.

when pregnancy is absent, the endometrium is cyclically exposed to an inflammatory-like mechanism [88].

Inflammation and menstruation in PCOS women

PCOS is one of the most common causes of menstrual irregularities, and it can present with amenorrhea and dysfunctional uterine bleeding [89]. Since anovulatory or oligo-anovulatory PCOS women present chronic estrogen exposure or an abnormal capacity to synthesize progesterone, they are in a state of chronic unopposed estrogen action and do not undergo regular progesterone withdrawal endometrial bleeding [80]. The lack of progesterone cyclicity may play an important role, not only in menstrual irregularities but also in gonadotropin and androgen secretion in these women [80]. In that sense, elevated endometrial AR expression was found in women with PCOS [90]. In these women, there is a relatively constant circulating level of estradiol (E2) similar to the early follicular phase, due in part to increased peripheral conversion of androstenedione to estrone and insulin downregulation of sex hormone-binding globulin

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[80]. As a result, systemic free E2 and testosterone are elevated. Chronic estrogen exposure leads to a thickening of the endometrium with irregular, unpredictable shedding, and bleeding [80]. Moreover, there is evidence of the existence of progesterone resistance (decreased responsiveness of target tissues to bioavailable progesterone) in PCOS endometrium, which alters the expression of several progesterone-regulated genes [91]. This, in turn, could explain the lack of progesterone-induced changes in the endometrium in these women. Further evidence of the causal hypotheses comes from endometriosis, in which there is a loss of progesterone signaling in eutopic and ectopic endometrial tissues [92]. Maternal and neonatal "preconditioning" was postulated, where progesterone resistance is established in the newborn [93]. Another hypothesis points out the link between progesterone resistance and inflammation. Increased levels of pro-inflammatory cytokines, and particularly increased NF-kB expression, downregulate progesterone receptor (PR) expression and progesterone action, thereby establishing a chronic endometrial pro-inflammatory state [93]. In PCOS women, the lack of progesterone withdrawal alters menstrual inflammatory mechanisms through cytokine and PGs production, with the consequent absence of the influx of leukocytes into the endometrium and altering the production of chemokines and MMPs, disrupting the physiological tissue degradation [81]. In that sense, Piltonen et al. showed that different endometrial cell populations in PCOS women present different levels of inflammatory genes, with dysregulation of some inflammatory genes such as chemokine ligand 2 (CCL2), IL-6, and TNFA-induced protein 6 (TNAIFP6) in epithelial endometrial cells. Conversely, they found upregulation of inflammatory genes CCL2, intercellular adhesion molecule (ICAM1), and TNFA-induced protein 3 (TNFAIP3) in endometrial stromal fibroblasts [94]. Dysregulations in the physiological and timely secretion of inflammatory cytokines, prostanoids, and angiogenic/permeability factors may exert key roles in menstrual dysfunctions observed in PCOS women. This hypothesis is reinforced considering that menstrual dysfunction disorders were associated with the deregulation of local inflammatory mediators [81]. See Table 3 for a summary of uterine PCOS (also see FIGURE 1).

Physiological and PCOS-related inflammation in the implantation process

Physiological implantation and placentation

There is a controlled balance between pro- and anti-inflammatory mechanisms in the establishment of a receptive endometrium ("window of implantation") and embryo-endometrium communication; any deviation of this balance is detrimental to the successful implantation and pregnancy outcome [112]. During this receptive period, the human endometrium is primed for blastocyst attachment, as it has reached a particular morphological and functional state [112]. If implantation occurs, the decidualization of endometrial cells begins during the secretory phase and increases throughout the endometrium [82]. An increased number of molecular mediators have been identified so far, which are involved in the initiation of early fetal-maternal interactions, including adhesion molecules, immune cells, cytokines, and growth and transcription factors [113].

IL-6 family members (including leukemia inhibitory factor (LIF), IL-11, and IL-6), IL-1, and TNFA, in particular, have emerged as candidate genes responsible for activation and regulation of the pro-inflammatory cascade at the fetal-maternal interface [114]. Receptors for these cytokines are localized at endometrial implantation sites and expressed by several cell types [114]. These early pro-inflammatory cytokines activate the expression of other cytokines, as well as chemokines, COX enzymes, and PGs [115], and may play important roles regulating implantation efficiency [116]. Chemokines such as CCL4, CCL7, and CCL13 recruit distinct leukocyte subpopulations such as macrophages, T cells, and uterine natural killer cells present in the decidua, especially at the sites of trophoblast invasion, which interact with the allogeneic placenta [114]. Particularly, T cell-derived cytokines may act in the regulation of fetal allograft survival. Th1-type cytokines promote allograft rejection, whereas the production at the fetal-maternal interface of Th2-type cytokines such as IL4 and IL10 inhibits the Th1 responses and improves fetal survival [117]. Progesterone may play a major role in regulating (Th1/Th2)-type cytokine balance [118], through the progesterone-induced blocking factor (PIBF), a mediator that exerts substantial anti-abortive activities [119]. PIBF inhibits arachidonic acid liberation—that is converted into PGs by COX enzymes—and modulates the profile of cytokine secretion resulting in an increase in the production of noninflammatory ILs associated with Th2 responses (e.g., IL-3, IL-4, and IL-10) and a reduction in the production of inflammatory cytokines associated with Th1 responses (e.g., interferon-δ, TNFA, and IL-2) [119]. PGs also have an important role during implantation of the blastocyst and decidualization [120], influencing the luteal function (luteotrophic/antiluteolytic signals) [83]. PGs are elevated in areas of increased endometrial vascular permeability associated with the initiation of implantation [121]. Pharmacological inhibition of PGs delays or inhibits the localized increase in vascular permeability and implantation [122], and mice with COX2 ablated have multiple reproduction abnormalities including retarded decidualization [123]. Although the type(s) and roles of PGs and receptors involved in human embryo implantation are not fully understood [120], PGF2A may act as a pro-inflammatory and vasoconstrictor uterine PG that initiates the luteolytic process [124] in the absence of pregnancy, whereas PGE2 exerts luteotrophic action [49] leading to maintenance of corpus luteum function.

Inflammation dysfunction during implantation and early pregnancy in PCOS women

Proliferation, migration, and invasion of trophoblastic cells into the maternal endometrium are essential steps, and failure of any of these due to endometrial dysfunction in women with PCOS may contribute to fertility dysfunction [94, 95]. Infertility in PCOS women mostly derives from chronic anovulation and implantation failure, leading to complications in achieving pregnancy in women with this disorder [80]. Ovulation is generally successful after medical induction, but the implantation rate remains lower and the early pregnancy loss rate is augmented in PCOS women [125]. The abnormal endometrial milieu in PCOS women may contribute to adverse pregnancy outcomes through hormonal, metabolic, and inflammatory mechanisms [125]. The endometrium of PCOS women presents an altered pro-inflammatory cytokine profile, chemokine, and MMPs compared to healthy women [80, 95]. In this sense, increased TNFA levels in the follicular fluids of women with PCOS correlated with poor quality oocytes and reduced rates of fertilization, embryonic development, and pregnancy outcome [69]. Elevated IL-6 is frequently found in altered cytokine profile characteristics of unexplained infertility, recurrent miscarriage, preeclampsia, and preterm delivery [126]. Piltonen [95] et al. also found, in isolated endometrial stromal fibroblasts from PCOS women, altered levels of

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Table 3. Inflammation-related risk factors in uterus/endometrium of PCOS.

Inflammation-related risk factor	Uterine/endometrial finding in PCOS. References included
CCL2/MCP1	Increased secretion by endometrial fibroblasts was associated with abnormal decidualization [95]. Aberrant expression in the endometrium of a combined PCOS+Insulin model [96]. Increased expression in different cell populations from proliferative-phase endometrium of obese PCOS women, versus obese controls [96]
CCL5	Increased secretion by endometrial fibroblasts was associated with abnormal decidualization [95]. Aberrant expression in the endometrium of a combined PCOS+Insulin model [96]
COX2	Reduced expression in endometrium from patients with recurrent implantation failure (including PCOS patients) [84]. Aberrant expression in the uterus at implantation sites, in PCOS models [97, 98]
cPLA2a	Reduced expression of cPLA2a in endometrium from patients with recurrent implantation failure (including PCOS patients) [84]
E-Cadherin	Increased expression in secretory-phase endometrium of PCOS, versus control patients [99]
GM-CSF	Increased secretion by endometrial fibroblasts was associated with abnormal decidualization [95]
Hif1A	Decreased gene and protein expression in endometrium of PCOS women, comparing to control patients [100]
HOXA-10	Decreased expression in midsecretory or proliferative-phase endometrium of PCOS women, versus control [101–103]. Increased endometrial expression in PCOS women after laparoscopic ovarian drilling [103]
HOXA-11	Decreased expression in midsecretory or proliferative-phase endometrium of PCOS women, versus control [1042, 103]. Increased endometrial expression in PCOS women after laparoscopic ovarian drilling [103]
HSPB1	Identified as possible proteomic biomarkers for PTB in PCOS [104, 105]
ICAM-1	Lower expression in proliferative-phase endometrium of PCOS women versus control patients [99]. Increased expression in different cell populations from proliferative-phase endometrium of obese PCOS women, versus obese controls [94]
IL-6	Increased secretion by endometrial fibroblasts was associated with abnormal decidualization [95]. Increased expression in different cell populations from proliferative-phase endometrium of obese PCOS women, versus obese controls [94]. Increased uterine gene expression in a PCOS-model [106]
IL-8	Increased expression in different cell populations from proliferative-phase endometrium of obese PCOS women, versus obese controls [94]. Increased secretions by endometrial fibroblasts were associated with abnormal decidualization [94, 95]
LIF	Decreased expression in midsecretory or proliferative-phase endometrium of PCOS women, versus control [102]. Lower expression in PCOS secretory endometrium versus control patients, from a DNA microarray analysis [91]
L-Selectin	Lower immunoexpression in secretory-phase endometrium of PCOS women, versus control patients [99]. Increased immunoexpression in endometrium of women with PCOS or endometriosis [107]
MMP2	Increased secretion in endometrial fibroblasts associated with abnormal decidualization [95]. Increased uterine gene expression in a PCOS model [106]
MMP3	Increased secretion in endometrial fibroblasts associated with abnormal decidualization [95]. Increased uterine gene expression in a PCOS model [106]
NF-kB	Increased expression in endometrium of obese PCOS women [108]
PGE2	Decreased uterine secretion in PCOS model [109]
PGF2	Increased uterine secretion in a PCOS-model [98, 110]
PIBF	Decreased expression in the uterus at implantation sites, in a PCOS model [98]
S100P	Lower expression in PCOS secretory endometrium versus control patients, from a DNA microarray analysis [91]. Lower expression in the endometrium of obese PCOS women with no luteal phase [111]
sPLA2a	Increased expression in endometrium from patients with recurrent implantation failure (including PCOS patients) [84]
TNFA	Increased uterine gene expression in a PCOS model [106]. Increased expression in endometrium of obese PCOS women [108]
TNFR	Aberrant expression in endometrium of obese and normal weight PCOS women [108]
TNFAIP3	Increased expression in different cell populations from proliferative-phase endometrium of obese PCOS women, versus obese controls [94]. Increased uterine gene expression in a PCOS model [106]
TNFAIP6	Increased expression in different cell populations from proliferative-phase endometrium of obese PCOS women, versus obese controls [94]
Transferrin	Identified as possible proteomic biomarkers for PTB in PCOS [104]
VEGF	Lower gene and protein expression in endometrium of PCOS women, comparing to control patients [100]
Vimentin	Identified as possible proteomic biomarkers for PTB in PCOS [104]. Increased expression in proliferative-phase endometrium of PCOS women [105]

CCL-2/MCP1, chemokine (C-C motif) ligand 2/monocyte chemoattractant protein 1; CCL5, chemokine (C-C motif) ligand 5; COX2, cyclooxygenase 2; cPLA2a, cytosolic phospholipase A2; GM-CSF, granulocyte-colony stimulating factor; Hif1A, hypoxia-inducible factor 1-alpha; HOXA10, Homeobox A10; HOXA11, Homeobox A11; HSPB1, heat shock protein b 1; ICAM-1, intercellular adhesion molecule 1; PGE2, prostaglandin E2; PGF2, prostaglandin F2; PIBF, progesterone-induced blocking factor 1; PTB, preterm birth; S100P, S100 calcium-binding protein P; sPLA2a, secretory phospholipase A2; TNFR, tumor necrosis factor receptor; TNAIFP3, TNFA-induced protein 3; TNFAIP6, TNFA-induced protein 6; VEGF, vascular endothelial growth factor.

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inflammatory markers such as IL-6 and 8, monocyte chemoattractant protein-1, and granulocyte-macrophage colony-stimulating factor. Further evidence comes from a murine PCOS model in which endometrial tissue presented an altered immune, oxidative, and apoptotic state [98]. There was a lack of expression of the known immunological mediator of progesterone, PIBF, together with an increase in COX2 expression, both on implantation sites [98]. Moreover, low serum and uterine PIBF concentrations of pregnant women suggest a risk for spontaneous pregnancy termination [127], and blocking PIBF during implantation in mice impaired the inflammatory profile along with increased resorption rates in later pregnancy [128]. Defective expression of COX enzymes and PG production may contribute to poor endometrial receptivity for embryo implantation. This is, in part, attributed to endometrial PG synthesis being altered in women with several pathologies who have experienced repeated IVF failure, including PCOS women [84]. In that sense, Li et al. [129] found in follicular fluids of PCOS women, altered levels of several arachidonic acid metabolites, and derived prostanoids, including PGE2 and PGF2A. Also, increased endometrial levels of PGF2A and decreased levels of PGE2 were found in a PCOS murine model [109]. In addition, the endometrium of PCOS women presents an altered expression of homeobox genes, particularly HOXA-10 [81, 101], a gene essential for endometrial receptivity [101]. HOXA-10 has been identified as a mediator of progesterone-controlled expression of the PG receptors E-series prostanoid (EP)3 and EP4 [130]. In that sense, mice lacking HOXA-10 gene presented abnormal regulation of progesterone-regulated genes, i.e., progesterone resistance [130]. In women with PCOS, elevated endometrial concentrations of androgens may prevent or delay the timing of HOXA-10 gene activation [101]. Interestingly, the ovarian drilling employing laparoscopy increases the expression of HOXA-10 and HOXA-11 and improves the endometrial receptivity in PCOS women [103]. It has been shown that cytokines such as IL-1B and coagulation factors such as thrombin regulate the endometrial HOXA-10 expression [131].

Another important cytokine involved in the implantation process is LIF [121]. In the endometrium of PCOS women, Savaris et al. [91] found an association between progesterone resistance and downregulation of LIF expression. Deregulation of endometrial LIF expression has been proposed as a possible marker of unexplained infertility and repetitive failures of implantation [132]. Moreover, in PCOS and control women, increased levels of LIF were associated with increased rates of successful implantation after IVF [72]. Altogether, aberrant expression of cytokines, LIF, HOX genes, particularly HOXA-10, and progesterone dysfunction (i.e., progesterone resistance and PIBF expression), might be implicated in the pathological endometrial alterations and implantation failure in PCOS women. Table 3 provides a summary of inflammatory mediators in PCOS (see also FIGURE 1).

Physiological inflammation in parturition and preterm birth in PCOS

Physiological inflammation in parturition and preterm birth

During physiological parturition, there is a massive neutrophil and macrophage influx into the myometrium and cervix, which along with fetal membranes, all release pro-inflammatory cytokines [133]. Although the signals that drive the inflammatory mechanism before parturition are largely unknown, a "functional" progesterone withdrawal before parturition has been proposed, which is initiated by an inflammation-induced trans-repression of PR by nuclear factor NF-kB [134]. Another possible mechanism explaining the apparent loss of progesterone sensitivity at term is the catabolism of progesterone in the uterus into inactive compounds along with alterations of PR levels [134]. The release of progesterone dominance allows for increased E2 sensitivity within the uterus, which in turn increases the expression of contractility-associated genes, such as COX2, connexin-43, and release of pro-inflammatory cytokines that may play a major role via stimulation of myometrium contractions [134]. The inflammatory mechanism leading to parturition might involve the initiation of stimuli signaling via Toll-like receptors, which results in PG and MMP production in addition to leukocyte invasion into reproductive tissues, and culminating in myometrial contractility, rupture of membranes, and cervical ripening, followed by parturition [55]. Cytokines like IL-1B might have an important role in these mechanisms, since IL-1B administration induces preterm labor in mouse models [135] via stimulation of MMPs and is likely involved in the process of collagen breakdown during cervical ripening and in the early phases of parturition [136]. Progesterone withdrawal may also affect the levels of PIBF, which have been found to modulate cytokine production from women with recurrent miscarriage or preterm birth (PTB), inducing a Th1–Th2 cytokine profile shift [137].

Inflammation in parturition and preterm birth in PCOS

Women with PCOS have increased risk of PTB and other pregnancy and neonatal complications [138, 139], and the presence of hyperandrogenism, inflammation, obesity, and IR may contribute to the higher risk of obstetric and neonatal complications in these women. In that sense, Palomba et al. [138] showed that PCOS women present a systemic chronic low-grade inflammatory state during pregnancy, comparing to healthy controls, and this finding was significantly associated with a higher risk of adverse obstetric/neonatal outcomes. In a systematic review study, Galazis et al. [104] identified some protein biomarkers of PTB in PCOS women, including Transferrin, Vimentin, and heat shock protein B1, and these biomarkers were highly associated with oxidative and pro-inflammatory mechanisms in these women. To gain insight into the understanding of PTB, Schatz et al. [82] have examined endometrial tissues from women in preterm labor. In that sense, cellular and molecular derangements in the decidualized endometrium have been associated with PTB [82]. Adverse pregnancy outcomes may be related to a pathological decidual hemorrhage, consequence of an excess of local thrombin production. The excess of thrombin generation may enhance decidual cell MMPs, neutrophil infiltration, and downregulation of PR expression, which are mirrored by TNFA and to a lesser extent by IL-1, linking inflammatory conditions to preeclampsia, promoting the so-called Preterm Premature Rupture of Membranes and/or PTB [82]. Regardless, the etiology of PTB and pregnancy complications in PCOS women is largely unknown, and a need for studies exploring etiologies and strategies to improve pregnancy outcomes in these women is evident. Table 3 summarizes the inflammatory mediators and risk factors identified in uterine tissue in PCOS women and/or animal models (see also FIGURE 1).

Anti-inflammatory markers and mediators in PCOS

We mentioned previously that, given the cyclic nature of reproduction, anti-inflammatory mechanisms regulate and resolute

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physiological reproductive inflammation and are also targeted in the treatment of chronic inflammatory disease. Besides producing inflammatory PGs, COX enzymes also produce several other prostanoids, including PGD2 and 15-Deoxy-delta-12_14-prostaglandin-J2 (15d-PGJ2), which might play an active role in the resolution of physiological inflammation [140]. PGD2 suppresses pro-inflammatory cytokine production and, in the placenta, inhibits IL-6 and IL-8 production [141]. In addition, 15d-PGJ2 inhibits NF-kB signaling and downregulates COX2 and TNFA expression in cultured trophoblasts [142] and in LPS-stimulated amnion, choriodecidual, and placental cells in vitro [143]. Conversely, recent evidence from Li et al. showed increased levels of 15d-PGJ2 and PGD2 in follicular fluids of PCOS women, so there is a need for further studies accounting for the precise role of these PGs during reproductive events in PCOS women [129]. 15d-PGJ2 also serves as a natural ligand for peroxisome proliferator-activated receptors (PPARs) [144], especially the gamma isoform (PPARG), which is extensively expressed in gestational tissues [145]. PPARG might play key roles in both the regulation of metabolic pathways (carbohydrate, lipid, protein) and the pathophysiology of inflammatory responses [146]. Activation of PPARs inhibits inflammatory response genes (including COX2, IL-2, IL-6, IL-8, TNFA, and MMPs) by repressing NF-kB signaling pathways [147]. In the ovary, PPAR gamma is downregulated in response to LH stimulation in physiological conditions, further implicating this transcription factor as an anti-inflammatory receptor mediator [148]. In this regard, in PCOS women and animal models, it was shown that synthetic PPARs activators reduce circulating levels of pro-inflammatory mediators such as CRP, along with a reduction in ovarian/adrenal androgen levels [149, 150]. The use of synthetic PPARG agonists in adult women with PCOS indeed improves metabolic and reproductive profiles, although there are serious concerns that the overall risks of these drugs exceed their benefits [151].

Natural activators of PPARs are found in nutrients and bioactive compounds that are ingested with diet, such as polyunsaturated fatty acids (PUFAs), which exert anti-inflammatory actions by binding PPARs and also by acting as competitive inhibitors of inflammatory PGs [152]. PUFAs, particularly those of the n-3 and n-6 families, are perhaps the most potent fatty acid regulators of metabolic function and are implicated in a diverse range of processes in vivo [153]. The current evidence establishes that omega-3 PUFAs promote the generation of the 3-series PGs (such as PGE3 and PGF3A), which are categorized as anti-inflammatory, while omega-6 PUFAs are precursors to pro-inflammatory PGs [154]. Recently, Chiu et al. [155] showed that higher serum long-chain omega-3-PUFA levels may improve reproductive outcomes in women undergoing infertility treatment. Furthermore, in PCOS women, dietary consumption of PUFAs, particularly those of the n-3 series, has beneficial effects lowering androgens, lipid levels, and inflammatory markers [156]. Moreover, Wathes et al. found that PUFA-induced anti-inflammatory effects in the ovary may be mediated in part by an increase in the production of PGE1, a competitive inhibitor of PGE2 synthesis [157]. Also, it was shown that PUFAs may exert some of their anti-inflammatory effects via PPARG activation [158].

Finally, there are endogenous protein hormones with known anti-inflammatory effects. In line with this, the adipocytokine adiponectin regulates insulin sensitivity and may be an important predictor of the metabolic syndrome in PCOS women [159], and it has been shown to exert anti-inflammatory effects [160]. Receptors for adiponectin are expressed in the epithelial and stromal cells of the endometrium with expression levels of the receptors peaking during the window of implantation [161]. In this regard, adiponectin has been shown to inhibit endometrial IL-1B-induced expression of IL-6 and IL-8, suggesting that adiponectin signaling plays a role in regulating pro-inflammatory pathways during implantation [161]. Accordingly, PCOS women present low levels of circulating adiponectin, even when adjusted by BMI [159].

Conclusions

The present work is intended to provide current evidence available regarding inflammatory mediators and risk factors in PCOS women, with special emphasis on reproductive function. This is a complex and multifactorial endocrine disorder, and women with PCOS frequently present reproductive abnormalities along with IR, cardiovascular complications, dyslipidemia, and obesity. Notably, chronic inflammation overlaps these comorbidities in these women (see FIGURE 1). Although we were not intended to discuss in detail the inflammatory setting as a cause or consequence of PCOS, data presented here showed that PCOS women present a permanent and altered profile of inflammatory markers in circulation and reproductive tissues, impairing follicular maturation, and subsequent ovulation. Even if ovulation succeeds, there is a high rate of implantation failures, early pregnancy loss, and pregnancy complications in PCOS women, and the establishment of a chronic pro-inflammatory profile might have a pivotal role in these reproductive derangements. On the other hand, there is a need for a better understanding of the inflammatory alterations in women with PCOS, to dissect physiological versus pathological inflammatory mechanisms in reproductive functions, which could have implications in the pharmacological and therapeutic approach in women with this syndrome. Ongoing and future studies in human and animal models are expected to shed light on the reproductive dysfunctions of women with PCOS, thus helping to develop tools to improve reproductive health in these patients.

Conflict of interest

The authors declare that there is no conflict of interest.

Data availability

No new data were generated or analyzed in support of this research.

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