New Insights into Mechanisms of Berberine in Alleviating Reproductive Disorders of Polycystic Ovary Syndrome: Anti-inflammatory Properties

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Abstract

Polycystic ovary syndrome (PCOS) is a complex reproductive disorder which seriously harms female reproductive health and decreases their quality of life. Although spontaneous or assisted ovulation, women with PCOS suffer from poor-quality oocytes and embryos, lower fertilization and final pregnancy rate. Therefore, it is urgent to reveal new pathological mechanisms and discover the underlying therapeutic targets for the reproductive disorders in PCOS. Berberine, one of the famous traditional Chinese medicines, has been shown to improve ovulation and live birth rates in women with PCOS. The effects of berberine on insulin resistance and abnormal glucose and lipid metabolism for restoring reproductive health of PCOS are well recognized and widely studied, but much less attention has been paid to its anti-inflammatory properties. Chronic low-grade inflammation as the unifying feature of PCOS may contribute to reproductive disorders in PCOS. Berberine can tune the inflammatory state of ovaries and uterus in PCOS. The anti-inflammatory properties of berberine may provide new insight into mechanisms of berberine in alleviating reproductive disorders of PCOS. Here, we summarize the most recent insights into anti-inflammatory properties of berberine in reproductive disorders of PCOS, inspiring researchers to go in new study directions of berberine.

Full Text

Background

Polycystic ovary syndrome (PCOS) is a complex reproductive disorder affecting 5–10% of women with childbearing age [1, 2]. Although spontaneous or assisted ovulation, there are still some questions regarding the paucity of high-quality oocyte, low fertilization and final pregnancy rate in women with PCOS, which seriously harms women's reproductive health and affects their quality of life [3, 4]. Therefore, revealing the new pathological mechanism of reproduction in women with PCOS and discovering new therapeutic targets are very urgent for the treatment of reproductive disorder in PCOS.

Inflammation is an innate defense responses that can cause activated immune cells to release various pro-inflammatory cytokines which are involved in stimulating chemokines and cytokines, as well as regulating gene expression in cell meiosis and apoptosis to devour damaged tissue and induce remodeling of the tissue [5, 6]. Inflammation is related to multiple aspects of female reproductive physiology, such as oocyte maturation and embryo implantation [7–9]. However, in terms of reproduction related diseases, inflammatory response subject to underlying pathology may be pushed to present a negative impact in female reproduction [10–12]. Chronic low-grade inflammation has been proposed as the unifying feature in PCOS, although the phenotype of PCOS is diverse [13]. Chronic low-grade inflammation may contribute, at least in part, to reproductive disorders in PCOS. Therefore, anti-inflammatory drugs may provide potential strategies for treating impaired oocyte quality and early pregnancy loss in PCOS.

Berberine has been used as an anti-inflammatory drug in the clinical treatment of many diseases, such as gastroenteritis [1, 14]. It is a bioactive alkaloid isolated from various medicinal herbs such as Coptis chinensis Franch. and Hydrastis canadensis L. [15, 16]. The molecular formula of berberine is C_{20}H_{18}NO_4 with a molar mass of 336.36 g/mol and four major metabolites have been identified, including berberrubine (M1), thalifendine (M2), demethylene-berberine (M3) and jatrorrhizine (M4) [17]. Berberine has also been empirically used in PCOS treatment alone or combined with other drugs improving reproductive disorders. However, only a few studies have preliminarily explored the mechanisms of berberine in restoring reproductive health of PCOS. More importantly, the anti-inflammatory properties of berberine are commonly ignored, though are possibly significant [18–20]. This paper summarizes the most recent insights of relationship among berberine, chronic low-grade inflammation and reproductive disorders in PCOS, and discusses the anti-inflammatory properties of berberine on reproductive disorders of PCOS. Furthermore, discussing suppression of chronic low-grade inflammation using berberine may provide new potential therapeutic approaches for reproductive disorders.

Efficacy And Safety Of Berberine For Reproductive Disorders Of PCOS

Basic scientific researches and clinical trials had clearly established the therapeutic benefit of berberine on reproductive disorders in PCOS but with different treatment regimens. Wang, et al. administered berberine 100 mg/kg•d or 200 mg/kg•d for 8 weeks. The
ovulation and endometrial receptivity in rat model of PCOS were ameliorated after administration by regulating LHCGR, CYP19A1, LPAR3 and αvβ3 [19]. Besides, Li et al. administered berberine 400 mg three times daily for 16 weeks. After treatment, 51 of 98 PCOS women had at least 1 ovulatory cycle [21]. An et al. administered berberine 500 mg three times daily for 12 weeks. Compared with placebo, the biochemical pregnancy rate (54.1%), clinical pregnancy rates (59.5%) and live birth rates (48.6%) were significantly higher in PCOS women who received berberine. However, there were no obvious differences in the mean number of oocytes collected, diploid fertilization rate and embryo utilization rate between placebo and berberine group [22]. Wu et al. administered berberine in a daily dose of 1500 mg for 24 weeks. There were 36.3% ovulation, 28.5% conception, 22.4% clinical pregnancy and 22.0% live birth rates in PCOS women who received berberine. Besides, the ovulation (61.0%), conception (48.8%), clinical pregnancy (37.7%) and live birth (34.4%) rates in PCOS women who received berberine combined with letrozole were higher than berberine group but similar to letrozole group [23] (Table 1). Berberine did not add fecundity in reproductive disorders with PCOS when used in combination with letrozole. Nevertheless, this finding suggests new directions for future research concerning the combination of berberine with other agents to support the potential applicability of berberine in restoring reproductive health of PCOS. Furthermore, traditional Chinese medicines as complementary and alternative medicines have gradually gained researchers' attention due to their powerful therapeutic effects and minimal or no side effects. Berberine as one of the famous traditional Chinese medicines with standard dose has been shown to be well tolerated and no apparent side effects, with the exception of a weak side effect of on the gastrointestinal system [15, 17, 24]. However, despite the side effect of berberine in the female reproductive system have yet to be discovered, further assess the safety of berberine is still necessary. Berberine treatment significantly affected oocyte maturation and subsequent development in a dose-dependent manner. In vitro studies revealed that pretreatment of mouse oocytes preincubated with 5 or 10 µM of berberine for 24 h exerted an increase in apoptosis compared with the control group, whereas oocytes with 2.5 µM berberine showed no significant differences. Preincubation of oocytes with 5 or 10 µM of berberine negatively impacted mouse oocyte development, fertilization, embryonic development and even implantation. In contrast, preincubation with 2.5 µM berberine caused significantly enhanced oocyte development, embryonic development, and no deleterious effects on oocyte fertilization and implantation [25]. Similar dose-dependent effects of berberine were also demonstrated in mouse blastocysts [26]. Moreover, intravenous injection of berberine via the tail vein with 3 or 5 mg/kg exerted a significant decrease in oocyte development and caused harmful effects on fertilization and early embryonic development [25]. Instead, there was a significant positive impact in oocyte development and embryos that developed to the blastocyst stages with intravenous injection of berberine at a dose of 1 mg/kg. Dual effects of berberine appeared to be attributable to promotion or inhibition of reactive oxygen species induced apoptosis, which dependent on the dose of berberine [25]. The safety of berberine on female reproductive system is particularly important (Table 2). Notably, bioavailability of berberine may be affected through co-administration with other drugs because of potential interactions among drugs. Clinical dose adjustment based on drug monitoring is recommended. Further evaluation to confirm the safety and efficacy of berberine for PCOS is warranted.
Table 1
The efficacy of berberine alone or combined with other drugs on reproductive disorders in PCOS

<table>
<thead>
<tr>
<th>Model/Design</th>
<th>Sample size</th>
<th>Administration protocol</th>
<th>Ovulation</th>
<th>Biochemical pregnancy/ Conception</th>
<th>Clinical Pregnancy</th>
<th>Live birth</th>
<th>Miscarriage</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berberine</td>
<td>98</td>
<td>400 mg, tid, 16 weeks</td>
<td>51/98</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(52.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Berberine</td>
<td>37</td>
<td>500 mg, tid, 12 weeks</td>
<td>NA</td>
<td>20/37</td>
<td>19/37</td>
<td>18/37</td>
<td>NA</td>
<td>[22]</td>
</tr>
<tr>
<td>Metformin</td>
<td>38</td>
<td>500 mg, tid, 12 weeks</td>
<td></td>
<td>(54.1)</td>
<td>(59.5)</td>
<td>(48.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>34</td>
<td>1 pill, tid, 12 weeks</td>
<td></td>
<td>20/38</td>
<td>17/38</td>
<td>14/38</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(52.6)</td>
<td>(47.4)</td>
<td>(36.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14/34</td>
<td>10/34</td>
<td>7/34</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(41.2)</td>
<td>(29.4)</td>
<td>(20.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Berberine</td>
<td>215</td>
<td>Berberine or Berberine</td>
<td>302/831</td>
<td>61/214</td>
<td>48/214</td>
<td>47/214</td>
<td>14/61</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>placebo:</td>
<td>(36.3)</td>
<td>(28.5)</td>
<td>(22.4)</td>
<td>(22.0)</td>
<td>(23.0)</td>
<td></td>
</tr>
<tr>
<td>Letrozole +</td>
<td>215</td>
<td>1500 mg, qd, 24 weeks</td>
<td>473/796</td>
<td>98/215</td>
<td>84/215</td>
<td>78/215</td>
<td>17/98</td>
<td></td>
</tr>
<tr>
<td>Berberine</td>
<td></td>
<td>Berberine or Letrozole</td>
<td>(59.4)</td>
<td>(45.6)</td>
<td>(39.1)</td>
<td>(36.3)</td>
<td>(17.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>placebo:</td>
<td></td>
<td>(59.4)</td>
<td>(45.6)</td>
<td>(39.1)</td>
<td>(36.3)</td>
<td></td>
</tr>
<tr>
<td>Berberine</td>
<td>215</td>
<td>2.5 mg, qd, days 3–7 of</td>
<td>486/797</td>
<td>105/215</td>
<td>81/215</td>
<td>74/215</td>
<td>27/105</td>
<td>(25.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>the first three treatment cycles; 5 mg, qd, days 3–7 of the last three cycles if not pregnant</td>
<td>(61.0)</td>
<td>(48.8)</td>
<td>(37.7)</td>
<td>(34.4)</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
The safety of berberine on female reproductive system

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Apoptosis</th>
<th>Proliferation</th>
<th>Oocyte development</th>
<th>Fertilization</th>
<th>Embryonic development</th>
<th>Implantation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mouse cumulus-oocyte complexes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5µM, 24h</td>
<td>NO</td>
<td>NA</td>
<td>Positive</td>
<td>No deleterious effects</td>
<td>Positive</td>
<td>No deleterious effects</td>
<td>[25]</td>
</tr>
<tr>
<td>5µM, 24h</td>
<td>Positive</td>
<td>NA</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>10 µM, 24h</td>
<td>Positive</td>
<td>NA</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td><strong>Mouse blastocysts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5µM, 24h</td>
<td>NO</td>
<td>NO</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>[26]</td>
</tr>
<tr>
<td>5µM, 24h</td>
<td>Positive</td>
<td>Negative</td>
<td>NA</td>
<td>NA</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>10 µM, 24h</td>
<td>Positive</td>
<td>Negative</td>
<td>NA</td>
<td>NA</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td><strong>Mouse</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mg/kg, tail vein injection, 4 days</td>
<td>NA</td>
<td>NA</td>
<td>Positive</td>
<td>No deleterious effects</td>
<td>Positive</td>
<td>NA</td>
<td>[25]</td>
</tr>
<tr>
<td>3 mg/kg, tail vein injection, 4 days</td>
<td>NA</td>
<td>NA</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>NA</td>
</tr>
<tr>
<td>5 mg/kg, tail vein injection, 4 days</td>
<td>NA</td>
<td>NA</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>NA</td>
</tr>
</tbody>
</table>

The anti-inflammatory properties of berberine on reproductive disorders of PCOS

Inflammation is related to multiple aspects of female reproductive physiology. However, in terms of reproduction related diseases, inflammatory response subject to underlying pathology may be pushed to present a negative impact in female reproduction, such as the paucity of high-quality oocyte, low fertilization and final pregnancy rate. Chronic low-grade inflammation as the unifying feature of PCOS may contribute, at least in part, to reproductive disorders in PCOS. Anti-inflammatory strategies may become a priority for the prevention and treatment of reproductive disorders in PCOS. However, most of studies exploring the mechanisms of berberine in restoring reproductive health of PCOS focus on its effects on insulin resistance and abnormal glucose and lipid metabolism, and less attention has been paid to its anti-inflammatory properties [19, 27–29].

Inflammatory Response Involved In Female Reproductive Physiology

In female reproductive system, reproductive function such as oocyte quality and implantation are thought to be attributable, at least in part, to an inflammatory response, which includes direct and indirect actions to cause vasodilation, hyperemia, edema, collagenolysis and cell proliferation [7–11]. Follicle as the fundamental unit of the ovary has key physiologic roles in female reproductive function. The development of follicles depends heavily on the microenvironment constituted by follicular fluid, which is an exudate from blood and exhibits positive correlations with a series of different serum inflammatory factors. Proinflammatory cytokines are also produced throughout folliculogenesis [30–32]. The quality of oocytes is important for embryonic development. In mammals, the developing oocyte undergoes germinal vesicle breakdown (GVBD), spindle assembly, and polar body extrusion. Each month, only one dominant oocyte undergo directly to meiosis resumption. During meiosis, microtubules are organized into barrel-shaped bipolar spindles, with chromatin condensed and all chromosomes arranged neatly. Meiosis I (MI) is completed until ovulated, with the first polar body (PB1) extruded. Increased reactive oxygen species (ROS) and
Considerable evidence indicates in the ovulation process, adequate amounts of ROS regulate the normal physiologic process, but of granulosa cells and reduce nutrient supply to oocytes, and eventually influencing oocyte quality and maturation. Telomere shortening and chromosome segregation disorder, eventually leading to oocyte fragmentation and fertilization failure. Chromosome arrangement which increase the risk for oocyte aneuploidy. The recovery of meiotic division in diploid terminated oocyte is severely affected. IL-1 can inhibit plasminogen activators, which in turn interfere with normal ovulation. IL-1 induced production of prostaglandins in the dominant follicle can mimic the effect of the LH surge and trigger ovulation. However, IL-1 can inhibit plasminogen activators, which in turn interfere with normal ovulation. Despite not required for successful ovulation, IL-1 has negative effects on oocyte maturation and the number of fertilized oocytes. TNF-α can control ovarian repair and remodeling by affecting apoptosis and autophagy of unruptured follicles. This pro-inflammatory cytokine can directly affect the chromatin structure and spindle morphology in oocytes. This series of inflammatory factors coupled with the changing follicular microenvironment can directly affect oocyte and embryo development, ovulation, and fertilization. IL-6 as a pro-inflammatory cytokine can directly affect the chromatin structure and spindle morphology in oocytes. TNF-α can control ovarian repair and remodeling by affecting apoptosis and autophagy of unruptured follicles. This pro-inflammatory cytokine can also stimulate hyperplasia of follicles and affect steroidogenesis and luteolysis of thecal and granulosa cells. CRP is a marker of systemic inflammation in response to TNF-α and IL-6 produced by hepatocytes. Systemic CRP levels fluctuate in follicular dynamics. IL-1 induced production of prostaglandins in the dominant follicle can mimic the effect of the LH surge and trigger ovulation. However, IL-1 can inhibit plasminogen activators, which in turn interfere with normal ovulation. Despite not required for successful ovulation, IL-1 has negative effects on oocyte maturation and the number of fertilized oocytes. Of note, there is a close connection between inflammation and oxidative stress (OS). Elevated OS has been shown to induce an inflammatory state, and inflammation has been associated with OS, which is presumed as a perpetuating cycle. OS is viewed as an imbalance between pro-oxidants and antioxidants. Increased ROS and/or reactive nitrogen species (RNS), or decreased antioxidant defense mechanisms can break the balance and create an environment which is unsuitable for normal female physiological reactions. Extensive research has shown that women with PCOS, even if matched for age, BMI, and insulin resistance, showed significantly elevated oxidative stress as well as decreased antioxidant capacity, with the subsequent activation of inflammatory pathways. The concentrations of several promoters and byproducts of oxidative stress were significantly increased in patients with PCOS compared with control women, and by increasing NADPH oxidase and decreasing thioredoxin, homocysteine induces oxidative stress by promoting ROS production. When the level of ROS exceeds the normal physiological level, the recovery of meiotic division in diploid terminated oocyte is severely affected. Increased levels of ROS can attack microtubules and interfere with spindle formation, eventually leading to abnormal chromosome arrangement which increase the risk for oocyte aneuploidy. Increased levels of ROS can also contribute to telomere shortening and chromosome segregation disorder, eventually leading to oocyte fragmentation and fertilization failure. Moreover, changes of the anti-apoptosis factor BCL2 expression levels can emerge under OS, which mediate apoptosis of granulosa cells and reduce nutrient supply to oocytes, and eventually influencing oocyte quality and maturation. Considerable evidence indicates in the ovulation process, adequate amounts of ROS regulate the normal physiologic process, but

**Impact of inflammation in reproductive disorders of PCOS**

The inflammatory response engaged in repetitive cyclic changes in the ovaries and uterus are notably disrupted in PCOS (Table 3). Increased evidence demonstrates that women with PCOS, even if matched for body mass index (BMI), showed significantly higher levels of C-reactive protein (CRP), interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-17 (IL-17), interleukin-18 (IL-18), monocyte chemotactic protein-1 as well as soluble endothelial leukocyte adhesion molecule and soluble intercellular adhesion molecule than normal women. This series of inflammatory factors coupled with the changing follicular microenvironment can directly affect oocyte and embryo development, ovulation, and fertilization. IL-6 as a pro-inflammatory cytokine can directly affect the chromatin structure and spindle morphology in oocytes. TNF-α can control ovarian repair and remodeling by affecting apoptosis and autophagy of unruptured follicles. This pro-inflammatory cytokine can also stimulate hyperplasia of follicles and affect steroidogenesis and luteolysis of thecal and granulosa cells. CRP is a marker of systemic inflammation in response to TNF-α and IL-6 produced by hepatocytes. Systemic CRP levels fluctuate in follicular dynamics. IL-1 induced production of prostaglandins in the dominant follicle can mimic the effect of the LH surge and trigger ovulation. However, IL-1 can inhibit plasminogen activators, which in turn interfere with normal ovulation. Despite not required for successful ovulation, IL-1 has negative effects on oocyte maturation and the number of fertilized oocytes. Of note, there is a close connection between inflammation and oxidative stress (OS). Elevated OS has been shown to induce an inflammatory state, and inflammation has been associated with OS, which is presumed as a perpetuating cycle. OS is viewed as an imbalance between pro-oxidants and antioxidants. Increased ROS and/or reactive nitrogen species (RNS), or decreased antioxidant defense mechanisms can break the balance and create an environment which is unsuitable for normal female physiological reactions. Extensive research has shown that women with PCOS, even if matched for age, BMI, and insulin resistance, showed significantly elevated oxidative stress as well as decreased antioxidant capacity, with the subsequent activation of inflammatory pathways. The concentrations of several promoters and byproducts of oxidative stress were significantly increased in patients with PCOS compared with control women, and by increasing NADPH oxidase and decreasing thioredoxin, homocysteine induces oxidative stress by promoting ROS production. When the level of ROS exceeds the normal physiological level, the recovery of meiotic division in diploid terminated oocyte is severely affected. Increased levels of ROS can attack microtubules and interfere with spindle formation, eventually leading to abnormal chromosome arrangement which increase the risk for oocyte aneuploidy. Increased levels of ROS can also contribute to telomere shortening and chromosome segregation disorder, eventually leading to oocyte fragmentation and fertilization failure. Moreover, changes of the anti-apoptosis factor BCL2 expression levels can emerge under OS, which mediate apoptosis of granulosa cells and reduce nutrient supply to oocytes, and eventually influencing oocyte quality and maturation. Considerable evidence indicates in the ovulation process, adequate amounts of ROS regulate the normal physiologic process, but
an increase in ROS production alters the physiologic ovarian dynamics, and adversely affects reproductive functions [10]. The mitochondrial caspase pathway induced by OS can also lead to the deterioration of oocyte quality after ovulation [67]. Therefore, we argue that aberrant concentration of inflammatory factors in PCOS may result in predisposition to poor oocyte and embryo qualities, ovulatory dysfunction and fertilization failure.

### Table 3

<table>
<thead>
<tr>
<th>Inflammation factors</th>
<th>Level</th>
<th>Impact</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>Elevated</td>
<td>I Affect the chromatin structure and spindle morphology in oocytes I Alter paracrine signaling throughout the endometrium, which in turn interrupt the signal of lumen epithelium and affect the crosstalk of maternal fetal interface</td>
<td>[49] [51] [71] [73] [74]</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Elevated</td>
<td>I Stimulate ovarian repair and remodeling by affecting apoptosis and autophagy of unruptured follicles I Affect steroidogenesis and luteolysis of thecal and granulosa cells I Prevent the endometrial epithelial cells from adhering to mesothelial cells, which in turn impair the expression of adhesion molecule complex cadherin/ beta-catenin, and ultimately cause the dyscohesion of the endometrial epithelial cells</td>
<td>[52] [53] [54] [78] [79]</td>
</tr>
<tr>
<td>IL-1</td>
<td>Elevated</td>
<td>I Affect oocyte maturation and the number of fertilized oocytes I Increase postovulatory progesterone production which is pivotal for maintenance of the pregnancy</td>
<td>[55]</td>
</tr>
<tr>
<td>ROS</td>
<td>Elevated</td>
<td>I Affect the recovery of meiotic division in diploid terminated oocyte I Attack microtubules and interfere with spindle formation, eventually leading to abnormal chromosome arrangement which increase the risk for oocyte aneuploidy I Contribute to telomere shortening and chromosome segregation disorder, eventually leading to oocyte fragmentation and fertilization failure I Mediate apoptosis of granulosa cells and reduce nutrient supply to oocytes, and eventually influencing oocyte quality and maturation I Lead to the deterioration of oocyte quality after ovulation I Lead to impaired implantation and loss of embryos</td>
<td>[34] [37] [65] [66] [67] [68] [81] [82]</td>
</tr>
</tbody>
</table>

Growing evidence suggests that an inflammatory milieu in PCOS endometrium with concomitant changes in immune cell chemoattraction, pro-inflammatory cytokine and matrix metalloproteinase (MMP) release may contribute to embryo implantation failure and pregnancy loss [69–72]. As a multifunctional cytokine, IL-6 expressed in endometrial stromal cells and decidua play an important role in coordinating trophoblast invasion and communication between endometrial stromal fibroblasts (eSF) and endometrial epithelium or leukocytes. However, when aberrant production of IL-6 occurs, paracrine signaling throughout the endometrium is altered, which in turn interrupt the signal of lumen epithelium and affect the crosstalk of maternal fetal interface [71, 73, 74]. TNF-α synthesized by decidual and trophoblast cells also plays an important role in embryo implantation and pregnancy maintenance [75, 76]. TNF-α can significantly inhibit trophoblast cell motility, but not alter trophoblast cell adhesion [77]. Moreover, aberrant production of TNF-α can prevent the endometrial epithelial cells from adhering to mesothelial cells, which in turn impair the expression of adhesion molecule complex cadherin/ beta-catenin, and ultimately cause the dyscohesion of the endometrial epithelial cells [78, 79]. IL-15 and IL-18 can induce uNK cells activation during implantation [80]. IL-1 is pivotal for maintenance of the pregnancy as it is thought to increase the production of postovulatory progesterone [55]. Furthermore, OS can contribute to changes in local immune function in the uterus, leading to impaired implantation and loss of embryos [81, 82].
Therefore, we argue that implantation failure and early pregnancy loss in PCOS are partly attributable to an aberrant inflammatory milieu in PCOS endometrium.

**The anti-inflammatory properties of berberine for the inflammatory state of PCOS**

The evidence obtained thus far indicates that anti-inflammatory strategies can be a valuable choice for the prevention and therapy of reproductive disorders in PCOS. Berberine with anti-inflammatory properties can reduce the expression of IL-6, TNF-α and other inflammatory factors, so as to tune the inflammatory state of ovaries and uterus in PCOS [29, 83, 84]. Anti-inflammatory mechanisms of berberine through several cell kinases and signal transduction pathways such as PI3K/Akt and AMPK pathway have also been described [20, 27]. Berberine can inhibit activation of NF-κB signaling pathways and its subsequent upregulation of the inflammatory response in PCOS by regulating the expression of TLR4 and LYN [29] (Table 4). Moreover, berberine can regulate the generation of ROS and the exhaustion of antioxidant system associated with inflammation [27]. Recent studies have found that elevated AMPK mRNA level and protein amounts as well as phosphorylated FOXO3a protein levels were observed paralleling increased expression of antioxidant enzymes (SOD, CAT and GSR) in berberine treated KGN cells, suggesting that berberine may upregulate antioxidant enzymes in response to oxidative stress through the AMPK/FOXO3a pathway [85]. FOXO3a, the forkhead-box O family, plays an important role in the control of cell death, cell cycle progression, metabolism and longevity [86]. Moreover, FOXO3a is a direct downstream target of AMPK, whose activation causes FOXO3a phosphorylation, increasing its DNA-binding activity and inducing the expression of its various target genes [87, 88]. Elevated mRNA and protein expression amounts of SOD, CAT and GSR may be due to the phosphorylation of FOXO3a by AMPK, even autophagy [89]. Meanwhile, decreased SIRT3 protein amounts and enhanced SIRT3 protein ubiquitination-degradation were observed after berberine treatment [85]. SIRT3 is a soluble protein deacetylase located in the mitochondrial matrix [90]. As a key molecule regulating autophagy, FOXO3a is not only regulated by phosphorylation but also by deacetylation [91]. SIRT3 deacetylated FOXO3a and phosphorylated AMPK, thus up-regulating antioxidant enzymes in response to oxidative stress and regulating autophagy [85, 92] (Table 4).
Table 4
The anti-inflammatory effects of berberine in PCOS

<table>
<thead>
<tr>
<th>Pathway and mediator</th>
<th>Anti-inflammatory effects</th>
<th>Reference</th>
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| NF-κB                | I Inhibit activation of NF-κB signaling pathways by regulating the expression of TLR4 and LYN in berberine treated PCOS rats  
I Inhibit the inflammatory response  
I Reduce the expression of IL-6, TNF-α and IL-1  
I Inhibit cell apoptosis of ovary granulosa cells | [29] |
| SIRT3/AMPK/FOXO3a    | I Increase AMPK mRNA level and protein amounts as well as phosphorylated FOXO3a protein levels which were observed paralleling increased expression of antioxidant enzymes (SOD, CAT and GSR) in berberine treated KGN cells  
I Uregulate antioxidant enzymes in response to oxidative stress  
I Decrease SIRT3 protein amounts and enhance SIRT3 protein ubiquitination-degradation  
I Regulate the generation of ROS and the exhaustion of antioxidant system associated with inflammation | [85] [86] [92] |
| ROS-/caspase-3       | I Diminish ROS-/caspase-3-dependent apoptosis and NF-κB mediated proinflammatory factors in LPS-treated blastocysts  
I Alleviate LPS-induced embryo damage by mitigating apoptosis and oxidation and suppressing proinflammatory cytokines during mouse preimplantation embryonic development  
I Rescue the skewed cell lineage specification under LPS challenge, safeguarding the formation of competent embryos for future development. | [93] |

Berberine used as an anti-inflammatory drug may provide potential strategies for reproductive disorders in PCOS (Fig. 1). Importantly, several studies have already directly or indirectly demonstrated this conjecture. Miao and Cui found that berberine can effectively alleviate LPS-induced embryo damage by diminishing ROS-/caspase-3-dependent apoptosis and NF-κB mediated proinflammatory factors during preimplantation embryonic development [84]. Shen, et al. found that berberine can inhibit cell apoptosis of ovary granulosa cells and regulate the expression of TLR4, LYN, IL-6, TNF-α and IL-1 in ovarian tissues of PCOS rats [29] (Table 4).

Conclusions

Chronic low-grade inflammation may contribute to reproductive disorders in PCOS. As an anti-inflammatory strategy, berberine can be valuable for restoring reproductive health of PCOS. Moreover, PCOS is a chaotic disease with multiple factors and various clinical manifestations, multi-target drug or multi-drug combination is also needed. Berberine as an alternative and complementary strategy with anti-inflammatory properties may provide strong future insights regarding controlling reproductive disorders in PCOS. We hope to present the impact of inflammation on reproductive disorders in PCOS and the beneficial effects of berberine in order to inspire researchers to go in new study directions of berberine with anti-inflammatory properties for reproductive disorders in PCOS. Currently available studies have provided evidence to support the use of the anti-inflammatory properties of berberine for restoring reproductive health of PCOS. However, no human clinical trial has been done to evaluate the therapeutic potential of the anti-inflammatory properties of berberine on reproductive disorders of PCOS, despite showing promising anti-inflammatory activities in both in vitro and animal studies. Thus, further experiments and large-scale randomized controlled studies are required to elucidate the mechanism of berberine actions at inflammation that directly impacts the reproductive health of PCOS.

Abbreviations
PCOS
Polycystic ovarian syndrome
GVBD
Germinal vesicle breakdown
ROS
Reactive oxygen species
CRP
C-reactive protein
MII
Metaphase II
WOI
Window of implantation
uNK
Uerine-specifc natural killer
MMP
Matrix metalloproteinase
eSF
Endometrial stromal fibroblasts
RNS
Reactive nitrogen species

Declarations

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Author's contributions
All authors contributed to the article. Dayong Wang and Xu Gao had the idea for the article. The first draft of the manuscript was written by Qing Xia and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References


Figures
Figure 1

Possible impact of berberine on inflammation-mediated reproductive disorders in PCOS. The perpetuating cycle between inflammation and OS can affect oocyte and embryo development, ovulation, fertilization and implantation in PCOS. Berberine can inhibit activation of NF-κB signaling pathways and its subsequent upregulation of the inflammatory response. Berberine may upregulate antioxidant enzymes in response to OS through the AMPK/FOXO3a pathway. Sirt3 deacetylated Foxo3a and phosphorylated AMPK, thus up-regulating antioxidant enzymes in response to OS and regulating autophagy. Berberine can decrease SIRT3 protein amounts and enhance SIRT3 protein ubiquitination-degradation. NF-κB, nuclear factor-κB; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin 6; AMPK, adenosine monophosphate activated protein kinase; mTOR, mammalian target of rapamycin; SIRT3, sirtuin 3; FOXO3a, forkhead box O3a; OS, oxidative stress.