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OXIDATIVE STRESS-DRIVEN PATHOPHYSIOLOGY OF ENDOMETRIOSIS: INSIGHTS FROM 12Z CELL LINE MODELS AND EMERGING ANTIOXIDANT THERAPEUTIC STRATEGIES

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ABSTRACT

Endometriosis is a chronic estrogen-dependent condition where endometrial-like tissue (the tissue that lines the uterus) grows outside the cavity of the uterus, leading to chronic pelvic pain, infertility, inflammation, and a decrease in quality of life. The mechanisms of disease initiation and progression that have emerged are multifaceted including retrograde menstruation, iron overload, mitochondrial dysfunction, immune dysregulation, chronic inflammation, angiogenesis, epithelial–mesenchymal transition, and progesterone resistance with oxidative stress acting as a central pathogenic mechanism. The role of the 12Z endometriotic epithelial cell line in *in vitro* studies of oxidative stress-mediated mechanisms and evaluation of antioxidant therapeutics are emphasized. The molecular mechanisms of ROS and RNS induced lipid peroxidation, protein oxidation, DNA damage, activation of redox-sensitive signaling mechanisms, such as NF-κB, MAPK, PI3K/Akt, and VEGF-mediated angiogenesis are discussed in detail. Experimental induction of oxidative stress (such as hydrogen peroxide, tert-butyl hydroperoxide, menadione and hypoxia–reoxygenation, iron overload, and inflammatory stimuli) and commonly used analytical endpoints (cell viability, lactate dehydrogenase release, intracellular ROS, mitochondrial superoxide, TBARS/MDA, 4-HNE, antioxidant enzyme activity, cytokine profiling, apoptosis markers, qPCR, Western blotting, and immunofluorescence) are summarized. In addition, complementary non-hormonal strategies (such as the antioxidant compounds,

curcumin, resveratrol, quercetin, epigallocatechin gallate (EGCG), N-acetylcysteine, and melatonin) are discussed for their therapeutic potential in decreasing oxidative damage, inhibiting inflammatory signaling, and for future personalized treatment of endometriosis. In summary, this review highlights oxidative stress as a potential therapeutic target and supports the use in the further investigation of mechanisms and the discovery of new antioxidant-based interventions.

KEYWORDS: Endometriosis; oxidative stress; 12Z cell line; reactive oxygen species; inflammation; antioxidant therapy.

1. INTRODUCTION

Endometriosis is a complex condition of the female reproductive system characterized by the presence of glands and stroma of the endometrium in various locations in the body, such as the ovary, ligaments and the pelvic peritoneum, the most frequent location. The prevalence of the disorder is about 10% of women between the reproductive ages and it is related to chronic pelvic pain and dysmenorrhea, dyspareunia, and health-care burden in terms of infertility.[1–3] Endometriosis is typically said to be an estrogen-dependent disorder, but the accompanying review is focused on that disease's meaning that goes beyond hormone biology. It is a systemic inflammatory and metabolic disease in which the immune, endocrine, oxidative and environmental mechanisms are in a constant interaction.[4–6] Such interactions have a clear clinical significance as they relate to two issues: diagnostic delay and need for specific, less toxic treatment options after therapy and their ongoing requirement.[7,8]

The classical theory of retrograde menstruation still is the most accepted cause of the initiation of diseases. This theory suggests that the endometrial cell reflux into the fallopian tube during menstruation and may implant, invade and get fixed in the peritoneal cavity.[4,5] But, retrograde menstruation is a normal occurrence and some women develop endometriosis.[5,6] This difference indicates that there are more factors that decide if refluxed cells will be eliminated or remain alive. Genetic predisposition, epigenetic change, immune dysfunction, hormonal imbalance, environmental exposure and mitochondrial damage are all important modifiers of disease risk and progression that are listed in the review.[6–12]

Of these mechanisms, oxidative stress is shown as a common pathophysiological mechanism.[12,13] Oxidative stress is the imbalance between production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and antioxidant defence

mechanisms.[14,15] Endometriosis patients have increased levels of ROS in the peritoneal fluid, ectopic lesions, follicular fluid and circulation.[11,13,16] This is an oxidative imbalance which results in lipid peroxidation, protein oxidation, DNA damage and mitochondrial dysfunction. It also induces activation of redox-sensitive pathways like NF-kB, MAPK and PI3K/Akt.[17,18], which results in a greater release of cytokines, angiogenesis, cell survival and invasiveness of the lesions.[19]

The 12Z endometriotic epithelial cells strain is used, since this cell line is characterized by many of the properties of ectopic endometriotic epithelium such as inflammatory responsiveness, estrogen receptor-beta dominance, decreased progesterone sensitivity, oxidative-stress sensitivity and high invasive potential.[19–22]

2. Pathophysiological Network of Endometriosis

The process of developing an endometriosis involves several steps in which cells of the endometrium need to survive in the peritoneum, adhere to the mesothelial surfaces, invade the surrounding tissue and gain a blood supply.[7] It describes the convergence of the following factors that are known to be related to hormonal imbalance and to take part in the development of ovarian cancer: immune dysfunction, chronic inflammation, oxidative stress, angiogenesis, neurogenesis, epithelial-mesenchymal transition and ECM remodeling, all of which are known to be mutually reinforcing. All of these mechanisms must be viewed not in isolation. Rather, they create a disease network and enhance each other.[2,4,11,19]

In endometriosis, immune dysfunction and oxidative stress reinforce each other. Reduced natural killer (NK)-cell cytotoxicity and altered macrophage activity allow ectopic endometrial cells to escape immune clearance.[23] Activated macrophages and lesion cells release tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6), interleukin-1 beta (IL-1 beta), and monocyte chemoattractant protein-1 (MCP-1), which activate NF-kB and STAT3 signaling.[24] This creates a persistent inflammatory microenvironment that promotes pain, cell proliferation, angiogenesis, lesion survival and resistance to apoptosis.[7,23,24]

In implanted lesions, angiogenesis is responsible for maintaining lesions. Newly formed vessels bring oxygen to and nourish ectopic tissue, through vascular endothelial growth factor (VEGF).[5] Low oxygen levels in lesions further stabilize HIF-1 alpha that further increases the expression of VEGF and ROS production.[30] Symptoms also are caused by neurogenesis. The greater the nerve fibre density and nerve growth factor activity in endometriotic lesions,

the more likely that the women are to experience chronic pain and increased nociceptive signaling.[9]

The hormonal imbalance adds to the disease process. Normally, estrogen inhibits growth and inflammation of endometriotic cells and progesterone inhibits growth and inflammation, but estrogen encourages these cells to grow, become inflamed, form blood vessels and live. Progesterone resistance in endometriosis can be attributed to the alteration of the progesterone receptor signal transduction and imbalance in the isoforms of the receptor.[18] This resistance will affect anti-inflammatory and anti-proliferative pathways, which will leave estrogen driven pathways as the winners. This imbalance can be exacerbated by environmental chemicals that disrupt the actions of estrogen, like dioxins, PCBs and bisphenol A.[25]

Table 1: Major Pathophysiological Processes in Endometriosis.

Process	Core features	Contribution to disease
Retrograde menstruation	Reflux of viable endometrial cells into the peritoneal cavity.[4,5]	Initiates exposure of peritoneal surfaces to endometrial cells but requires other factors for lesion formation.
Immune dysfunction	Reduced NK cytotoxicity and altered macrophage function.[7,23]	Allows ectopic cells to evade clearance and supports chronic inflammation.
Inflammation	TNF-alpha, IL-6, IL-1 beta and MCP-1 activate NF-kB and STAT3.[24]	Promotes pain, proliferation and survival signaling.
Angiogenesis	VEGF and HIF-1 alpha drive new vessel formation.[26]	Supplies oxygen and nutrients to developing lesions.
Hormonal imbalance	Estrogen dominance and progesterone resistance.[18,27]	Enhances proliferation and weakens anti-inflammatory regulation.
Oxidative stress	ROS/RNS cause damage and activate redox-sensitive pathways.[5,13]	Links injury, inflammation, immune evasion and lesion persistence.

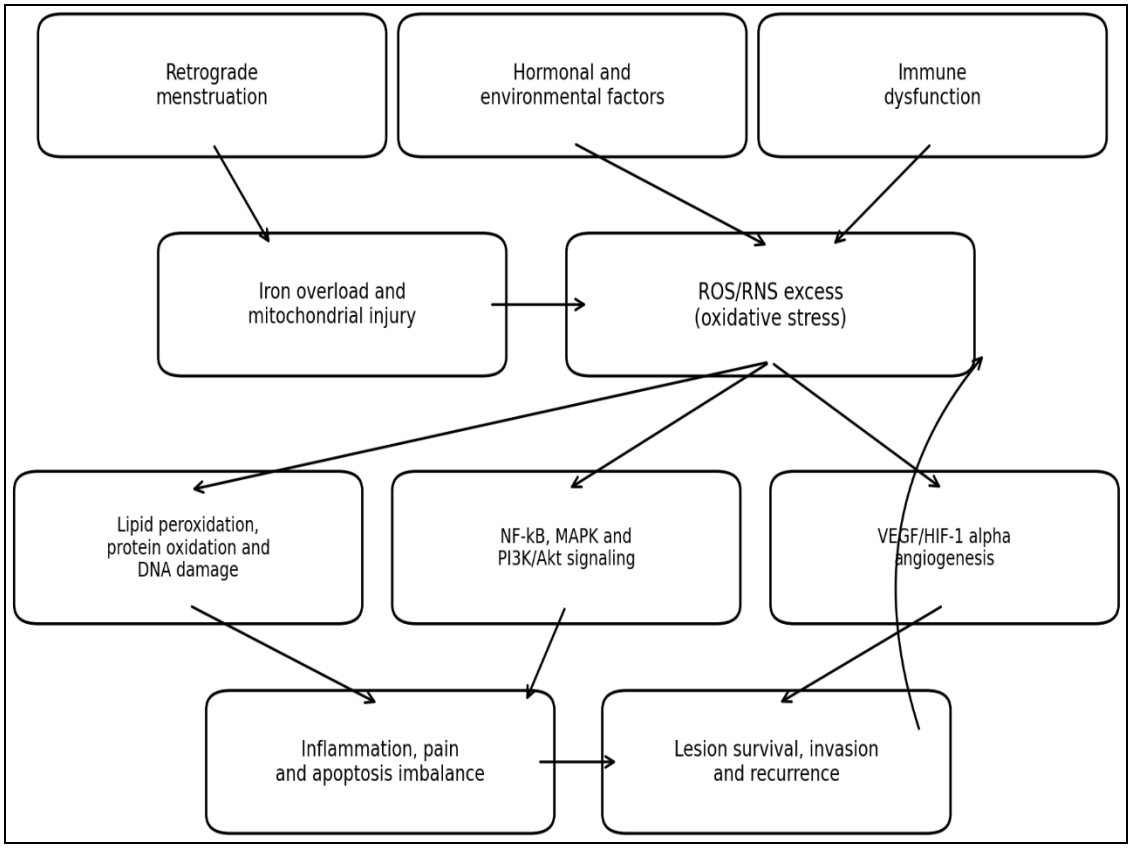

Fig. 1: Oxidative-stress-centered network of endometriosis pathophysiology.

3. Oxidative Stress as a Central Mechanism

Later repercussions of chronic inflammation are fibrosis and extracellular-matrix remodeling. The expression of matrix metalloproteinases is increased which allows invasion, formation of adhesion and tissue distortion.[25,27] The possibility of recurrence from the progenitor/stem cells, which have high proliferative and migratory capacity, is another reason that stem cells are critical.[28] In this network oxidative stress does not occur as a result. What is it that links up accumulation of iron, mitochondrial dysfunction, release of cytokines, angiogenesis, EMT and survival of lesions?

Oxidative stress functions as the central connecting mechanism between iron overload, mitochondrial dysfunction, immune activation, angiogenesis, epithelial-mesenchymal transition and lesion survival.[29] Instead of repeating ROS production, NF-kB activation and inflammatory signaling in separate sections, these events should be read as one continuous sequence: repeated bleeding and iron release increase ROS generation[26]; ROS damage lipids, proteins and DNA.[21,30] damaged cells and activated macrophages release cytokines; and these cytokines activate NF-kB, MAPK and PI3K/Akt pathways. This redox-inflammatory loop sustains lesion growth, survival, pain and recurrence.

It is a state of imbalance between the production of ROS and their antioxidant defence.[15,30] There are several ROS (superoxide anion, hydrogen peroxide and hydroxyl radicals) and RNS (nitric oxide and peroxynitrite).[31] Low concentrations of ROS are involved in physiological pathways involved in normal signaling, while high levels or prolonged generation of ROS leads to harmful effects on cells and alter gene expression.[15,31]

One of the key factors in the development of oxidative stress in endometriosis is iron overload, as a result of repeated retrograde menstruation.[11] Hemoglobin and heme in the peritoneum cause free iron to be produced. Free iron catalyzes the formation of the highly reactive hydroxyl radicals, by the Fenton reaction[31,32] This mechanism accounts for the possibility of oxidative stress to be ongoing without any acute inflammatory insult. This vicious cycle continues - menstrual debris causes an increase in iron, iron causes an increase in ROS, ROS causes inflammation, inflammation causes an influx of macrophages and activated macrophages cause more ROS.[32,33]

Molecular effects are the peroxidation of lipids, damage to DNA and oxidation of proteins Lipid peroxidation induces damage to the polyunsaturated fatty acids (PUFAs) of cell membrane and produces toxic lipid peroxidation products like malondialdehyde and 4 hydroxynonenal.[21,30,31] These compounds disrupt the integrity of the membrane, and can serve as secondary messengers and extend the period of injury. The damage to DNA consists of strand breaks, modification of bases and genomic instability. Protein oxidation decreases the enzymatic activity, changes the structural proteins and impacts the mitochondrial enzymes. These changes act together to disrupt the events of apoptosis, proliferation and survival of the ectopic endometrial cells.[16,34]

Another aspect of the role of ROS is their ability to act as signaling molecules. The activation of the NF-kB leads to the upregulation of the transcription of inflammatory factors, such as TNF-alpha, IL-6 and IL-1 beta, which enhance inflammation.[22,35–37] The stresses and proliferation are associated with MAPK signaling, while PI3K/Akt signaling is associated with survival. VEGF is triggered by oxidative and hypoxic stress and leads to angiogenesis and provision of nutrients towards the ectopic tissue.[26] ROS can cause mitochondrial dysfunction and this in turn can lead to ROS buildup. The damage of the electron transport chain causes an increase in the production of ROS in mitochondria, decrease in ATP production and can trigger apoptotic pathways.[38] This can be exacerbated by mitochondrial

DNA damage as damaged mitochondrial DNA increases the pro-oxidant activity and decreases the efficiency of the mitochondria.[15,38]

4. In Vitro Models and the 12Z Endometriotic Cell Line

Oxidative stress also alters the functions of the immune system. ROS inhibit NK activities and modulate macrophage functions resulting in an immunosuppressive microenvironment which is not conducive to clearance of ectopic cells.[15,23] Additionally, oxidative stress has been suggested to lead to epigenetic changes in the DNA methylation and/or histone change, which can affect genes involved in inflammatory pathways, apoptosis and hormonal signaling processes.[31] This is significant because it can be a sign of resistance and/or recurrence, which may not be apparent simply by the number of lesions.[39,40]

In vitro models are essential as they enable the study of mechanisms which are hard to isolate in patients, under controlled conditions. The review does not provide a comprehensive overview of the models used but focuses on epithelial, stromal and three-dimensional models, including the 12Z endometriotic epithelial cell line. Cell models offer unique advantages over animal or patient-only models in that the dose, duration, hormone concentrations, inflammatory agents and antioxidant treatment of the model can be carefully controlled. They are also beneficial for high throughput screening and to the elucidation of the mechanism of action.[21,41,42]

The 12Z cell line is a subline of the 12FS cell line that was obtained from lesions found in the peritoneum and still has certain characteristics of the ectopic endometriotic epithelium.[15,21] These include increased inflammatory responsiveness, cytokine secretion following stimulation (including tumor necrosis factor (TNF) and interleukin (IL) 6), increased predominance of the estrogen receptor beta pathway, decreased sensitivity to progesterone, increased migratory and invasive capacities and sensitivity to oxidative stress.[5,22] The properties of 12Z cells make them ideal for lesion establishment studies, ROS mediated damage studies, NF-kB and MAPK activation studies, mitochondrial dysfunction studies, drug response and antioxidant protection studies.

Endometrial stromal cell models are also useful to complement the 12Z epithelial models, as stromal cells are involved in the decidualization, extracellular-matrix remodeling, fibrosis and paracrine inflammatory signaling processes.[42] Patient-derived primary stromal cells are useful for studying patient variability, but are in short supply and are short-lived. Thus, an

adequate experimental design should include patient-derived cells or advanced systems for the translational relevance and standardized cell lines for good reproducibility.[5,41]

Table 2: In Vitro Models Described for Endometriosis Research.

Model	Strengths	Limitations or considerations	Best use
12Z epithelial cell line	Stable, reproducible, inflammatory, invasive and oxidative-stress sensitive.[21,22]	Cell-line behaviour must be validated against patient-derived systems.	Mechanistic signalling, ROS injury and antioxidant screening.
Endometrial stromal cells	Relevant to decidualization, fibrosis, ECM remodelling and cytokine signaling.[42]	Primary cells show donor variability and limited lifespan.	Hormone response, fibrosis, adhesion and paracrine interactions.
3D spheroids	Better mimic cell-cell contact, oxygen gradients, hypoxia and drug penetration.[41]	More complex culture conditions and analysis.	Lesion-like architecture, invasion, angiogenesis and therapeutic response.
Organ-on-chip/future models	Integrate flow, multi-cellular interactions and omics analysis.[43]	Requires technical standardization.	Translational modelling and personalized testing.

5. Oxidative Stress Induction Strategies

In addition to these 2D culture limitations, there are also limitations in cell-cell contacts, cell-matrix interaction, oxygen and nutrient gradients, hypoxia and drug penetration barriers that are not addressed by 2D culture, all of which can be addressed by 3D spheroids. The changes are very applicable to the architecture of the endometriotic lesions, which also experience local hypoxia and changes to the matrix that affect the survival of the lesions and response to treatment. To close this gap, the future models should combine the multicellular spheroids, organoids, microfluidic organ-on-chip platforms and omics technologies, which are not combined in them today.[5,44]

The experimental system has to mimic the oxidative environment of endometriosis in a predictable and quantifiable fashion to study oxidative stress. The following are listed as beneficial induction methods: hydrogen peroxide, tert-butyl hydroperoxide, menadione, hypoxia-reoxygenation, iron overload and inflammatory stimuli.[30,44–47] The different models represent different aspects of disease biology - the choice of the disease inducing agent should be related to the disease question that is being asked.

Hydrogen peroxide is the most frequently used inducer as it is relatively stable, permeable to the membrane and can induce the production of ROS inside the cell.[48] Hydrogen peroxide is able to generate hydroxyl radicals in the presence of transition metals, via the Fenton reaction. Exposure leads to mitochondrial dysfunction, decreased membrane potential, lipid, protein, and DNA oxidative damage, and cell death, by apoptosis and/or necrosis, depending on dose and duration of exposure.[4,31,47] Other pro-oxidant agents, such as tert-butyl hydroperoxide and menadione, are also useful to use and induce ROS.

Physiologically relevant because there is the possibility of varying oxygen tension in endometriotic lesions, during hypoxia-reoxygenation. When reoxygenating, there is an "ischemia-reperfusion" like surge of ROS that occurs as a result of the sudden increase in oxygen availability.[26,49] A great model for working on HIF-1 alpha, angiogenesis, mitochondrial stress and inflammatory activation. Retrograde menstruation has a direct connection with an iron overload. Free iron is capable of catalyzing into Fenton and Haber-Weiss reactions which result in hydroxyl radicals, lipid peroxidation and fibrosis-injury.[33,47]

Table 3: Oxidative-Stress Induction Methods for 12Z-Based Studies.

Induction method	Mechanism represented	Experimental value
Hydrogen peroxide (H2O2)	Membrane-permeable ROS inducer; hydroxyl radical generation through Fenton chemistry.[47]	Standardized oxidative injury and dose-response testing.
t-BHP and menadione	Chemical induction of intracellular ROS.[38]	Comparison of oxidant-specific pathways and antioxidant protection.
Hypoxia-reoxygenation	Fluctuating oxygen conditions and reoxygenation ROS burst.[49]	Study of HIF-1 alpha, angiogenesis, mitochondrial stress and inflammation.
Iron overload	Free iron from hemoglobin/heme catalyzes ROS formation.[48]	Models peritoneal iron accumulation caused by repeated retrograde menstruation.
LPS, TNF-alpha and IL-1 beta	Inflammatory activation indirectly increases ROS.[35]	Links cytokine signaling with oxidative stress and NF-kB activation.

Fig. 2. Workflow for oxidative-stress studies in 12Z endometriotic epithelial cells.

6. Experimental Parameters and Analytical Endpoints

The inflammatory stimuli (LPS, TNF-alpha and IL-1 beta) activate immune and inflammatory signaling pathways, which in turn cause the indirect activation of oxidative stress.[35] These models can aid to understand the relationship between inflammation and ROS production. It is appropriate that this review makes a distinction between acute high dose exposures and chronic low dose exposures.[14,36] Acute high dose oxidants can lead to acute necrosis and may not be a measure of the progression of the disease. The advantage of chronic low dose exposure is that it is more useful for modelling the endometriosis as it

involves chronic and sublethal oxidative stress, adaptive antioxidant response and long term pathway remodeling.

A good oxidative-stress study should have several readings: no one single test will be able to measure the entire process. The following review is an arrangement of experimental parameters in terms of cell viability, ROS measurement, lipid peroxidation, cytokine analysis, antioxidant defense, apoptosis, gene expression and protein expression. There is a first requirement that is experimental quality control. Cells should be authenticated, mycoplasma tested, limited to a number of passages and be seeded at the same cell density . To minimize bias due to overgrowth, the ratios of confluency should be around 70-80% prior to treatment. They should include controls for the vehicle, time matched controls, biological replicates and randomized well allocation.[30,43,46,47,49,50]

MTT is traditionally used to measure mitochondrial metabolic activity by formazan production, and is a good method for the evaluation of cell viability. Another other suggestion from the review is to enhance the interpretation of the results using the following three methods: WST-1, XTT, ATP-based luminescence and Trypan Blue exclusion. LDH release is another indicator of membrane damage and necrotic cytotoxicity which complements the other indicators. Mitochondrial metabolic assays are not sufficient on their own and using at least two viability methods is important to ensure that changes in metabolism, due to oxidants, are not interpreted as changes in cell number.[30,47]

The measurement of ROS is a key factor. DCFH-DA is a general intracellular ROS detector, which can detect ROS through fluorescence.[48] A more accurate quantification at the single cell level can be achieved by flow cytometry, and direct measurement of free radicals can be performed by electron spin resonance, and detection of mitochondrial superoxide by MitoSOX Red.[36,37] The measurement of TBARS/MDA, 4-HNE and lipid hydroperoxides are all methods used to measure lipid peroxidation.[30,36] These markers indicate biochemistry damage, rather than just transient signaling, from ROS production.

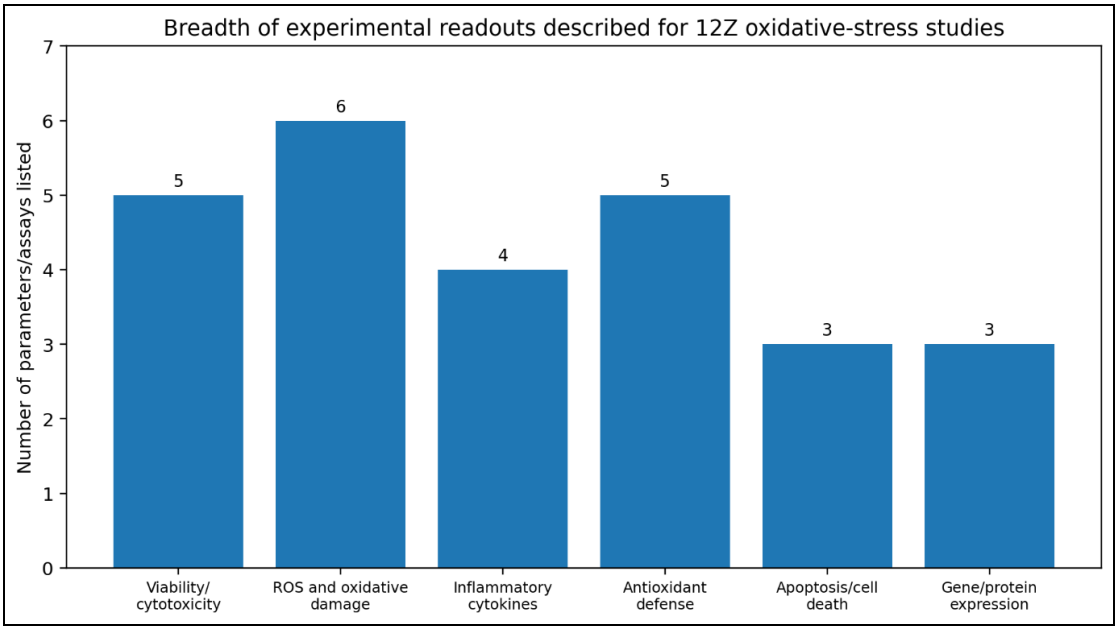

Fig. 3: Summary of major experimental domains assessed in oxidative stress studies using the 12Z cell line.

Table 4: Experimental Parameters and Assays.

Domain	Representative assays or markers	Interpretation
Cell viability and cytotoxicity	MTT, WST-1, XTT, ATP luminescence, Trypan Blue, LDH release.[30,47]	Measures metabolic activity, cell survival and membrane damage.
ROS and oxidative damage	DCFH-DA, flow cytometry, MitoSOX, ESR, TBARS/MDA, 4-HNE.[30,36,48]	Confirms intracellular and mitochondrial ROS plus biochemical injury.
Inflammatory cytokines	TNF-alpha, IL-6, IL-1 beta and MCP-1 by ELISA/multiplex.[24,36]	Shows activation of inflammatory networks and NF-kB/STAT3 signaling.
Antioxidant defense	SOD, catalase, GSH, GPx and Nrf2 pathway.[9,49]	Assesses redox compensation and cellular antioxidant capacity.
Apoptosis and death	Annexin V/PI, caspases, TUNEL.[5,11]	Distinguishes early apoptosis, late apoptosis, necrosis and DNA fragmentation.
Molecular expression	qPCR, Western blotting and immunofluorescence.[23,39]	Identifies genes, proteins and localization changes in signalling pathways.

7. Protective Role of Selected Antioxidant Compounds

To avoid duplication, ROS measurement and inflammatory pathway analysis should be treated as linked but distinct endpoints. ROS assays such as DCFH-DA, MitoSOX, ESR, TBARS/MDA and 4-HNE confirm oxidative burden and biochemical injury. Cytokine assays such as TNF-alpha, IL-6, IL-1 beta and MCP-1 demonstrate inflammatory

amplification.[26,30,36] Molecular assays such as qPCR, Western blotting and immunofluorescence should then confirm whether NF-kB, MAPK, PI3K/Akt, Nrf2, HIF-1 alpha and VEGF pathways are activated or suppressed after oxidant or antioxidant treatment.[5,8,26,37]

Due to the fact that oxidative stress is related with the survival of the lesions and inflammatory amplification, antioxidant therapy is a logical approach. The review covers various natural and synthetic antioxidants as alternative and/or additional treatment options. These agents could act as antioxidants, boost the body's antioxidant reserves, prevent inflammation signals, control mitochondria, block the growth of new blood vessels and normalize the cell death in abnormal cells.[5,9,11,43,50,51]

An antioxidant, anti-inflammatory and anti-proliferative effect of curcumin, a component of turmeric, is emphasized.[43] It possesses the ability to scavenge free radicals, boost antioxidant enzyme activity, suppress NF-kB signaling, diminish the level of such inflammatory cytokines as TNF-alpha and IL-6, downregulate COX-2 and prostaglandin synthesis, inhibit VEGF-mediated angiogenesis and can induce the apoptosis of endometriotic cells.[52] Grapes and berries contain a polyphenol named resveratrol, which is also able to decrease the level of ROS and the proliferation of endometriotic cells. It stimulations the SIRT1 and the AMPK, enhances mitochondrial function, inhibits estrogen-dependent proliferation and decreases cytokine and angiogenic factors.[53,54]

Quercetin is a flavonol that is able to scavenge free radicals.[48] The review categorizes it to all of these functions, which include diminished lipid peroxidation, inhibition of PI3K/Akt and MAPK signaling, decreased release of inflammatory mediators and protection against mitochondrial dysfunction.[55] The major catechin of green tea is epigallocatechin gallate, which is said to be anti-angiogenic and anti-inflammatory due to its anti-VEGF mediated angiogenesis activity. N-acetylcysteine is able to restore glutathione levels and boost cellular antioxidant defence.[5] Melatonin could directly and indirectly act as a free-radical scavenger; protect against DNA damage; and may have a positive effect on the hormones, by regulating the circadian rhythm.[56]

Table 5: Antioxidant Candidates and Principal Mechanisms.

Compound	Mechanisms	Potential therapeutic value
Curcumin	Scavenges radicals, enhances antioxidant enzymes, inhibits NF-kB, COX-2 and VEGF, induces apoptosis.[43]	Anti-inflammatory, antioxidant, anti-angiogenic and anti-proliferative support.
Resveratrol	Activates SIRT1/AMPK, improves mitochondria, suppresses estrogen-induced proliferation and cytokines.[54]	Mitochondrial protection and reduced lesion growth.
Quercetin	Reduces lipid peroxidation, inhibits PI3K/Akt and MAPK, protects mitochondria.[55]	Limits oxidative damage and survival signalling.
EGCG	Inhibits VEGF-mediated angiogenesis and inflammatory processes.[16]	Restricts lesion blood supply.
N-acetylcysteine	Replenishes glutathione and increases antioxidant capacity.[9]	Strengthens intracellular redox defence.
Melatonin	Direct and indirect free-radical scavenging; DNA protection; circadian regulation.[11]	Protects against oxidative DNA injury and may support hormonal balance.

8. Therapeutic Implications and Future Perspectives

Antioxidant compounds should not be discussed repeatedly under each pathway. Their mechanisms can be grouped into four major therapeutic actions: reduction of ROS and lipid peroxidation, restoration of antioxidant defense through Nrf2-linked enzymes[8], suppression of NF-kB-mediated cytokine release[37,57], and inhibition of angiogenesis through VEGF/HIF-1 alpha regulation. Curcumin, resveratrol, quercetin, EGCG, N-acetylcysteine and melatonin may therefore be presented as complementary redox-modulating agents rather than as separate repeated examples of the same ROS-inflammation mechanism.[44,55]

The current treatment is limited due to chronic and recurrent nature of the endometriosis. Hormonal treatment with GnRH agonists/antagonists, oral contraceptives, progestins and aromatase inhibitors can be used to reduce the effects of estrogenic stimulation but may have unwanted side effects and may not be recommended for some patients who want to preserve their fertility. Although it may be possible to remove visible disease and adhesions during surgery, there is a high risk of recurrence due to microscopic disease, stem-like cells, inflammation and oxidative reprogramming can all continue.[3,8,28]

Targeted antioxidant therapy will thus provide a complementary approach in addition to conventional care. The delivery using nanoparticles is especially attractive as it may offer greater bioavailability, the ability to target antioxidants or anti-inflammatory agents to lesions, as well as reduce systemic toxicity and offer sustained release.[3,8] Anti-angiogenic

and anti-inflammatory strategies such as VEGF inhibition, EGCG, NSAIDs and cytokine inhibitors may help to decrease the vascularization and inflammation of the lesions.[11,58] Other treatments (lifestyle/dietary) also help with redox balance. It includes a list of antioxidant-rich foods, vitamin C, vitamin E, polyphenols, omega-3 fatty acids, avoiding processed food or high-fat food, physical activity, stress management, yoga and meditation as aids to the process.[53] The strategies discussed should be considered as complementary and should help alleviate the oxidative burden including the quality of life.

The need for personalized medicine is at the core of the future needs. This is likely because there are genetic, epigenetic, hormonal, metabolic and immune differences between patients that may cause the two to be affected differently by the same treatment. Multi-omics can be used to identify the oxidative stress subtypes, biomarkers and therapeutic targets. The 3D spheroids and organ-on-chip models or organoids could facilitate an evaluation of therapy prior to patient testing. There is a need for strong preclinical proof of efficacy and safety, standardized oxidative-stress assays and large trials to show the efficacy, recurrence, pain, and quality-of-life data for both safety and efficacy.[3,5,8]

9. Proposed Methodological Framework

Well characterized cell stocks should be used as a good starting point for a refined methodology in future studies of oxidative stress in 12Z.[21,42] 12Z cells must be recovered from a master-bank and re-authenticated, confirmed mycoplasma free and be cultured in a standard serum and culture environment. To minimise genetic drift, numbers of passages should be kept to a minimum. Seed cells in controlled cell density, reach 70-80% confluency and then are randomly divided into control, vehicle, oxidative stress and treatment groups.[30,47] Concentration and duration of exposure are important considerations for oxidative stress and it is important that there are dose-response and time-course arms in the design.

The IC50 as well as the no-observed-adverse-effect level (NOAEL), effective concentration (EC), therapeutic index and Hill coefficient for both oxidants and protective compounds should be determined at the level of pilot experiments.[36] As well as the models of acute injuries, chronic low dose exposure models should be incorporated to obtain a more physiological relevance.[31] Depending on the mechanism, hydrogen peroxide can be used as a standard oxidant, t-BHP, hypoxia-reoxygenation, iron overload or cytokines can be used.[47–

[49] Antioxidant controls e.g. N-acetylcysteine or vitamin C should be used. Reagents must be freshly made; and pH of the media checked during treatment.[5,59]

A panel of several assays at multiple endpoints should be used.[36]-[50] Methods for testing viability include MTT and ATP. For cytotoxicity, LDH release can be used[39], and ROS can be determined using DCFH-DA and flow cytometry. Mitochondrial superoxide can be tested by the use of MitoSOX[36], while lipid peroxidation may be tested by using the MDA/TBARS and 4-HNE.[30] Antioxidant defense can be determined by the use of the cell's SOD, catalase, GSH and GPx.[49] ELISA or multiplex cytokine assays can be used to test inflammation, and Annexin V/PI, caspase activity and TUNEL can all be used to test apoptosis.[9,24] To confirm the molecular mechanisms, use qPCR and western blotting, immunofluorescence for NF-kB, MAPK, PI3K/Akt, Nrf2, HIF-1 alpha and VEGF.[5,26,57] Biological replicates from various passages, technical triplicates, pre-established exclusion criteria and reporting of these criteria should be used in statistical analysis.[30,47]

10. CONCLUSION

Oxidative stress has an important role in the pathogenesis of endometriosis, connecting the different steps of retrograde menstruation, iron overload, mitochondrial dysfunction, chronic inflammation, immune dysfunction, hormonal imbalance, angiogenesis and ectopic lesion formation. Overproduction of reactive oxygen and nitrogen species (ROS/RNS) leads to lipid peroxidation, DNA and protein damage, activation of several redox-sensitive signaling pathways like NF-κB, MAPK, and PI3K/Akt pathways which promote cellular proliferation, invasion, angiogenesis, immune escape, and apoptosis resistance. Overall, there is evidence that supports oxidative stress as a mechanism that is associated with disease progression and may be a potential therapeutic target for the treatment of endometriosis.

The 12Z endometriotic epithelial cell line still represents one of the most useful in vitro models to study oxidative stress-associated mechanisms as it has been cultured to mimic many pathological features of ectopic endometrial epithelial cells, such as inflammatory responsiveness, estrogen receptor-β predominance, progesterone resistance, increased invasiveness and enhanced sensitivity to oxidative injury. As an epithelial monoculture it cannot, however, fully recapitulate the complex interactions between the stromal, immune, endothelial, neural and extracellular matrix components characteristic of endometriotic lesions. Thus, conclusions drawn from 12Z cells should be considered to be of a mechanistic

nature and should be supported by more physiologically representative systems like multicellular spheroids, organoids and organ-on-chip systems.

Experimental evidence is emerging that antioxidant compounds, such as curcumin, resveratrol, quercetin, epigallocatechin gallate (EGCG), N-acetylcysteine and melatonin can ameliorate oxidative damage by inhibiting the generation of ROS, maintaining mitochondrial function, decreasing the production of inflammatory cytokines, blocking angiogenesis, and restoring apoptosis. However, there are many hurdles to be overcome before these agents can be put into routine clinical use. Standardized experimental protocols, well-designed clinical trials, and rigorous dose optimization and long-term safety evaluation should be undertaken in future investigations to determine the efficacy of antioxidant-based interventions and specific patient populations that are likely to benefit.

Future studies are also needed to integrate advanced 3D disease models, multi-omics technologies into the study of disease heterogeneity, and study oxidative stress biomarkers and nanoparticle-based drug delivery systems to enable personalized therapeutic strategies. There is a need for the standardization of oxidative stress models such as oxidants used, conditions of exposure, number of passages, serum status and outcome measures to enhance the reproducibility and comparison between studies. Finally, the application of mechanistic insights, clinically relevant models and precision medicine strategies might shift the paradigm of oxidative stress research from descriptive biology to more targeted and personalized treatments to help alleviate pain, improve fertility and long-term disease management in women with endometriosis.

REFERENCES

1. Giudice LC. Clinical practice. Endometriosis. N Engl J Med, 2010; 362: 2389–98. https://doi.org/10.1056/NEJMcp1000274.
2. Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. Fertil Steril, 2012; 98: 511–9. https://doi.org/10.1016/j.fertnstert.2012.06.029.
3. Zondervan KT, Becker CM, Missmer SA. Endometriosis. New England Journal of Medicine, 2020; 382: 1244–56. https://doi.org/10.1056/NEJMra1810764.
4. Nisolle M, Donnez J. Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities. Fertil Steril, 1997; 68: 585–96. https://doi.org/10.1016/S0015-0282(97)00191-X.

5. Gupta S, Agarwal A, Krajcir N, Alvarez JG. Role of oxidative stress in endometriosis. Reprod Biomed Online, 2006; 13: 126–34. https://doi.org/10.1016/S1472-6483(10)62026-3.
6. Szczepańska M, Koźlik J, Skrzypczak J, Mikołajczyk M. Oxidative stress may be a piece in the endometriosis puzzle. Fertil Steril, 2003; 79: 1288–93. https://doi.org/10.1016/S0015-0282(03)00266-8.
7. Shaaban M, Ryan JT, Adediran E, Farland L V., Pollack AZ, Peterson CM, et al. Relationship between endometriosis diagnosis, staging, and typology and inflammatory cytokines. F S Rep, 2026; 7: 134–42. https://doi.org/10.1016/j.xfre.2026.02.007.
8. Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. Nat Rev Endocrinol, 2014; 10: 261–75. https://doi.org/10.1038/nrendo.2013.255.
9. Augoulea A, Mastorakos G, Lambrinoudaki I, Christodoulakos G, Creatsas G. The role of the oxidative-stress in the endometriosis-related infertility. Gynecol Endocrinol, 2009; 25: 75–81. https://doi.org/10.1080/09513590802485012.
10. Olcha P, Hawryluk I, Depciuch J. Decoding the Endometriosis-Associated Infertility Microenvironment: A Review of FTIR and Raman Spectroscopic Insights into Follicular Fluid. Curr Issues Mol Biol, 2026; 48: 303. https://doi.org/10.3390/cimb48030303.
11. Van Langendonckt A, Casanas-Roux F, Donnez J. Oxidative stress and peritoneal endometriosis. Fertil Steril, 2002; 77: 861–70. https://doi.org/10.1016/S0015-0282(02)02959-X.
12. Saleh R, Sallam H, Elsuity MA, Dutta S, Sengupta P, Nasr A. Antioxidant therapy for infertile couples: a comprehensive review of the current status and consideration of future prospects. Front Endocrinol (Lausanne), 2025; 15. https://doi.org/10.3389/fendo.2024.1503905.
13. Giudice LC, Kao LC. Endometriosis. The Lancet, 2004; 364: 1789–99. https://doi.org/10.1016/S0140-6736(04)17403-5.
14. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. Oxford University Press;, 2015. https://doi.org/10.1093/acprof: oso/9780198717478.001.0001.
15. Sies H. Oxidative stress: a concept in redox biology and medicine. Redox Biol, 2015; 4: 180–3. https://doi.org/10.1016/j.redox.2015.01.002.
16. Scutiero G, Iannone P, Bernardi G, Bonaccorsi G, Spadaro S, Volta CA, et al. Oxidative Stress and Endometriosis: A Systematic Review of the Literature. Oxid Med Cell Longev, 2017; 2017. https://doi.org/10.1155/2017/7265238.

17. Vannuccini S, Clifton VL, Fraser IS, Taylor HS, Critchley H, Giudice LC, et al. Infertility and reproductive disorders: impact of hormonal and inflammatory mechanisms on pregnancy outcome. Hum Reprod Update, 2016; 22: 104–15. https://doi.org/10.1093/humupd/dmv044.
18. Bulun SE. Endometriosis. New England Journal of Medicine, 2009; 360: 268–79. https://doi.org/10.1056/NEJMra0804690.
19. Artini PG, Scarfò G, Marzi I, Fusi J, Obino ME, Franzoni F, et al. Oxidative Stress-Related Signaling Pathways Predict Oocytes' Fertilization In Vitro and Embryo Quality. Int J Mol Sci, 2022; 23: 13442. https://doi.org/10.3390/ijms232113442.
20. Olivia J. Carpinello; Lauren W. Sundheimer; Connie E. Alford; Robert N. Taylor; Alan H. DeCherney. Endometriosis. Endotext [Internet]. MDText.com, Inc.;, 2017.
21. Zeitvogel A, Baumann R, Starzinski-Powitz A. Identification of an Invasive, N-Cadherin-Expressing Epithelial Cell Type in Endometriosis Using a New Cell Culture Model. Am J Pathol, 2001; 159: 1839–52. https://doi.org/10.1016/S0002-9440(10)63030-1.
22. Banu SK, Lee J, Speights VO, Starzinski-Powitz A, Arosh JA. Cyclooxygenase-2 regulates survival, migration, and invasion of human endometriotic cells through multiple mechanisms. Endocrinology, 2008; 149: 1180–9. https://doi.org/10.1210/en.2007-1168.
23. Lebovic DI, Mueller MD, Taylor RN. Immunobiology of endometriosis. Fertil Steril, 2001; 75: 1–10. https://doi.org/10.1016/s0015-0282(00)01630-7.
24. Harada T, Iwabe T, Terakawa N. Role of cytokines in endometriosis. Fertil Steril, 2001; 76: 1–10. https://doi.org/10.1016/s0015-0282(01)01816-7.
25. Makabe T, Koga K, Nagabukuro H, Asada M, Satake E, Taguchi A, et al. Use of selective PGE2 receptor antagonists on human endometriotic stromal cells and peritoneal macrophages. Mol Hum Reprod, 2021; 27. https://doi.org/10.1093/molehr/gaaa077.
26. Young VJ, Ahmad SF, Duncan WC, Horne AW. The role of TGF-β in the pathophysiology of peritoneal endometriosis. Hum Reprod Update, 2017; 23: 548–59. https://doi.org/10.1093/humupd/dmx016.
27. Maybin JA, Critchley HOD. Progesterone: a pivotal hormone at menstruation. Ann N Y Acad Sci, 2011; 1221: 88–97. https://doi.org/10.1111/j.1749-6632.2011.05953.x.
28. Brosens I, Benagiano G. Endometriosis, a modern syndrome. Indian J Med Res, 2011; 133: 581–93.
29. Asghari S, Valizadeh A, Aghebati-Maleki L, Nouri M, Yousefi M. Endometriosis: Perspective, lights, and shadows of etiology. Biomedicine & Pharmacotherapy, 2018; 106: 163–74. https://doi.org/10.1016/j.biopha.2018.06.109.

30. Siddique YH, Ara G, Afzal M. Estimation of Lipid Peroxidation Induced by Hydrogen Peroxide in Cultured Human Lymphocytes. Dose-Response, 2012; 10. https://doi.org/10.2203/dose-response.10-002.Siddique.
31. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol, 2007; 39: 44–84. https://doi.org/10.1016/j.biocel.2006.07.001.
32. Bulun SE. Endometriosis. Yen and Jaffe's Reproductive Endocrinology, Elsevier;, 2019, p. 609-642.e7. https://doi.org/10.1016/B978-0-323-47912-7.00025-1.
33. Fortin M, Lépine M, Merlen Y, Thibeault I, Rancourt C, Gosselin D, et al. Quantitative Assessment of Human Endometriotic Tissue Maintenance and Regression in a Noninvasive Mouse Model of Endometriosis. Molecular Therapy, 2004; 9: 540–7. https://doi.org/10.1016/j.ymthe.2003.12.012.
34. Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative stress on female reproduction: a review. Reproductive Biology and Endocrinology, 2012; 10: 49. https://doi.org/10.1186/1477-7827-10-49.
35. Richter P, Macovei LA, Mihai IR, Cardoneanu A, Burlui MA, Rezus E. Cytokines in Systemic Lupus Erythematosus—Focus on TNF-α and IL-17. Int J Mol Sci, 2023; 24: 14413. https://doi.org/10.3390/ijms241914413.
36. Naka T, Nishimoto N, Kishimoto T. The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. The Lancet, 2012; 379: 1214–24. https://doi.org/10.1016/S0140-6736(12)60110-X.
37. Pakjoo M, Ahmadi SE, Zahedi M, Jaafari N, Khademi R, Amini A, et al. Interplay between proteasome inhibitors and NF-κB pathway in leukemia and lymphoma: a comprehensive review on challenges ahead of proteasome inhibitors. Cell Communication and Signaling, 2024; 22: 105. https://doi.org/10.1186/s12964-023-01433-5.
38. Aitken RJ, Roman SD. Antioxidant Systems and Oxidative Stress in the Testes. Oxid Med Cell Longev, 2008; 1: 15–24. https://doi.org/10.4161/oxim.1.1.6843.
39. Brosens I, Benagiano G. Endometriosis, a modern syndrome. Indian J Med Res, 2011; 133: 581–93.
40. Zondervan KT, Becker CM, Missmer SA. Endometriosis. N Engl J Med, 2020; 382: 1244–56. https://doi.org/10.1056/NEJMra1810764.
41. Pascoal E, Wessels JM, Aas-Eng MK, Abrao MS, Condous G, Jurkovic D, et al. Strengths and limitations of diagnostic tools for endometriosis and relevance in diagnostic

test accuracy research. Ultrasound in Obstetrics & Gynecology, 2022; 60: 309–27. https://doi.org/10.1002/uog.24892.

42. Zeitoun KM, Bulun SE. Aromatase: a key molecule in the pathophysiology of endometriosis and a therapeutic target. Fertil Steril 1999; 72: 961–9. https://doi.org/10.1016/S0015-0282(99)00393-3.
43. Gupta SC, Patchva S, Aggarwal BB. Therapeutic Roles of Curcumin: Lessons Learned from Clinical Trials. AAPS J, 2013; 15: 195–218. https://doi.org/10.1208/s12248-012-9432-8.
44. D'Hooghe TM, Kyama C, Debrock S, Meuleman C, Mwenda JM. Future directions in endometriosis research. Ann N Y Acad Sci, 2004; 1034: 316–25. https://doi.org/10.1196/annals.1335.034.
45. Roohie RK, Umesha S. Identification of genes associated with black rot resistance in cabbage through suppression subtractive hybridization. 3 Biotech, 2015; 5: 1089–100. https://doi.org/10.1007/s13205-015-0311-8.
46. Kruger NJ. The Bradford Method for Protein Quantitation. Protein Protocols Handbook, The, New Jersey: Humana Press; n.d., p. 15–22. https://doi.org/10.1385/1-59259-169-8: 15.
47. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65: 55–63. https://doi.org/10.1016/0022-1759(83)90303-4.
48. LeBel CP, Ischiropoulos H, Bondy SC. Evaluation of the probe 2',7'-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. Chem Res Toxicol 1992; 5: 227–31. https://doi.org/10.1021/tx00026a012.
49. ELLMAN GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82: 70–7. https://doi.org/10.1016/0003-9861(59)90090-6.
50. Myung-Ji Seo. Antioxidant Activity of Fermented Foods and Food Microorganisms. MDPI;, 2025. https://doi.org/10.3390/books978-3-7258-2925-5.
51. Yang H, Kuhn C, Kolben T, Ma Z, Lin P, Mahner S, et al. Early Life Oxidative Stress and Long-Lasting Cardiovascular Effects on Offspring Conceived by Assisted Reproductive Technologies: A Review. Int J Mol Sci, 2020; 21: 5175. https://doi.org/10.3390/ijms21155175.
52. Swarnakar SPSumit. Curcumin arrests endometriosis by downregulation of matrix metalloproteinase-9 activity. Indian Journal of Biochemistry & Biophysics, 2009; 46.

53. Jiang T, Chen Y, Gu X, Miao M, Hu D, Zhou H, et al. Review of the Potential Therapeutic Effects and Molecular Mechanisms of Resveratrol on Endometriosis. Int J Womens Health, 2023; Volume 15: 741–63. https://doi.org/10.2147/IJWH.S404660.
54. Baur JA, Sinclair DA. Therapeutic potential of resveratrol: the in vivo evidence. Nat Rev Drug Discov, 2006; 5: 493–506. https://doi.org/10.1038/nrd2060.
55. Boots AW, Haenen GRMM, Bast A. Health effects of quercetin: From antioxidant to nutraceutical. Eur J Pharmacol, 2008; 585: 325–37. https://doi.org/10.1016/j.ejphar.2008.03.008.
56. Yildirim G, Attar R, Ozkan F, Kumbak B, Ficicioglu C, Yesildaglar N. The effects of letrozole and melatonin on surgically induced endometriosis in a rat model: a preliminary study. Fertil Steril, 2010; 93: 1787–92. https://doi.org/10.1016/j.fertnstert.2009.09.021.
57. Hayden MS, Ghosh S. Shared Principles in NF-κB Signaling. Cell, 2008; 132: 344–62. https://doi.org/10.1016/j.cell.2008.01.020.
58. Xu H, Lui WT, Chu CY, Ng PS, Wang CC, Rogers MS. Anti-angiogenic effects of green tea catechin on an experimental endometriosis mouse model. Human Reproduction, 2008; 24: 608–18. https://doi.org/10.1093/humrep/den417.
59. Porpora MG, Brunelli R, Costa G, Imperiale L, Krasnowska EK, Lundeberg T, et al. A Promise in the Treatment of Endometriosis: An Observational Cohort Study on Ovarian Endometrioma Reduction by N-Acetylcysteine. Evidence-Based Complementary and Alternative Medicine, 2013;, 2013: 1–7. https://doi.org/10.1155/2013/240702.