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UNDERSTANDING ENDOMETRIOSIS: A THREAT TO FERTILITY

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Abstract
Endometriosis is a gynaecological disease that affects female reproductive organs by the growth of endometrium-like tissues inside and outside the pelvic cavity. This paper explored how endometriosis can lead to increased chances of infertility through a dysregulated immune system and impaired oocyte quality.

Running title: Understanding endometriosis

Keywords: endometriosis, inflammatory cascade, interleukin, oocyte, granulosa cells, cytotrophoblasts

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Introduction

Endometriosis is a common chronic gynecological disease that affects approximately 1 in 10 women [1]. It is characterised by the growth of endometrium-like tissues inside and outside the pelvic cavity, mainly affecting female reproductive organs such as the uterus and ovaries. It can cause symptoms such as pelvic pain and heavy menstrual bleeding. Since these symptoms are non-specific and often associated with a typical menstrual cycle, this condition is often misdiagnosed or not diagnosed at all. In fact, in patients between 18 and 45 years of age, the average delay of diagnosis is 6.7 years [2]. According to the WHO, endometriosis affects around 10% (190 million) of reproductive age women and girls globally and specifically 14 million women affected across the EU [3]. In Asian countries, prevalence is high and affects approximately 6.8% to 15.7% [4]. If endometriosis is left untreated, symptoms will worsen and can lead to infertility. Studies have shown that 30 to 50% of women affected with this disorder are infertile and endometriosis accounts for up to 25% of general infertility cases [5]. This increase chance in infertility can be connected to how endometriosis affects the patients' immune system through generation of reactive oxygen species and recruitment of immunocompetent cells, as well as how it impairs oocyte quality by inhibiting follicular granulosa cells.

Immune system dysregulation

In patients with endometriosis, increased percentages of immunocompetent cells such as macrophages and neutrophils are observed. This points to the involvement of the immune system in endometrial lesions. In the pelvic cavity of those affected with endometriosis, macrophages are accumulated and can lead to the dysfunction of the immune response [6]. When macrophages are activated, they recruit neutrophils and stimulate an inflammatory response through the release of cytokines which promote the angiogenesis of endometrial cells. Such cytokines include IL-1, IL-6, and IL-8.

IL-1

IL-1 is an interleukin typically responsible for regulation of the inflammatory cascade. Through binding to receptors on target cells, they can illicit inflammatory responses by recruiting immune cells such as macrophages and neutrophils [7]. However, when there is over-secretion such as in endometriosis, IL-1 can cause tissue damage. IL-1β is a particular subtype of this cytokine involved in pathogenesis by forming new blood vessels in "tissues surrounding endometriotic lesions by producing vascular endothelial growth factor (VEGF)" [8]. In physiology, IL-1 Ra (receptor antagonist) binds to IL-1 receptors and acts against these cytokines, diminishing their amounts before inflammation occurs [9]. However, in patients with endometriosis, decreased concentration of IL-1 Ra is apparent and has even been shown to cause dysmenorrhea [10].

IL-6

Similar to IL-1, secretion of IL-6 is also intensified in the immune dysfunction found in endometrial pathology. IL-6 helps to develop and sustain endometrial foci and implants by up-regulating secretion of a glycoprotein similar to haptoglobin called endometriosis protein-I (ENDO-1) [11]. Similar to how haptoglobin binds to free haemoglobin in the blood, ENDO-1 adheres itself to macrophages and creates a feed-forward loop that protects the endometrial implants by blocking the phagocytic function of macrophages [8]. This diminished phagocytosis allows for the survival of these implants as well as an increase in secretion of IL-6, which will thereby increase ENDO-1 production, resulting in the feed-forward loop.

IL-8

IL-8 is a "chemotactic factor" which recruits immune cells such as neutrophils and macrophages to the site of inflammation [8]. It is found that when IL-8 concentrations are increased, there is a high proliferation of "ovarian endometrioma-derived stromal cells" which can be linked to the growth of these cells in the peritoneum [12]. It has been speculated that increased concentrations of these immune cells in the peritoneal fluid are "toxic to embryo survival and sperm function" due to the "alterations of decidual microenvironment" [12]. Specifically, through the interaction between macrophages and cytotrophoblasts. Cytotrophoblasts are integral cells making up the outer layer of a blastocyst (the early stage of an embryo), providing nutrients to the growing embryo and later developing into the placenta [13]. Activated macrophages in patients with endometriosis disrupt these important cells by inhibiting their invasiveness, thereby being detrimental to embryo growth [14].

ROS and OS

Another effect of an unregulated immune system is the overproduction of reactive oxygen species (ROS) through stimulation by cytokines and macrophages during inflammation. Although these species are present in regular physiological states and are even associated with supporting female fertility processes such as "folliculogenesis (the development of a follicle needed for release of a mature oocyte), oocyte maturation, and hormone signalling" [12], they must be held in a careful balance with antioxidants or else oxidative stress will occur.

When this balance is lost, oxidative stress (OS) can lead to infertility through damaging oocyte DNA and driving post-ovulatory oocytes to apoptosis [12]. This pathomechanism is driven by the

reduction of glutathione levels by OS. Glutathione is a key antioxidant regulating apoptosis, therefore its decrease leads to morphological changes in the cell and eventually cell death [15]. Oxidative species also cause oocyte ageing and cell cycle arrest in follicular oocytes, thus ultimately hindering pregnancy. It is clear that in patients with endometriosis, the dysregulation of the immune system and the consequent ROS production is not only involved in the pathogenesis of the condition, but can lead to infertility as well.

Oocyte impairments

Oocyte quality is one of the most important factors in fertility and can be characterised by an oocyte's ability to mature and be fertilised [16]. Oocytes are immature eggs or ovum found in the ovaries that mature within a follicle and have not been fertilised. If there is damage to an oocyte's quality, embryo development may be impaired. In order to describe how endometriosis affects the abilities of an oocyte, we must consider a main factor affecting oocyte growth: granulosa cells. When primordial germ cells become oogonia and later proliferate into oocytes, the oocytes are encapsulated by a layer of granulosa cells.

Granulosa cells

Granulosa cells are somatic cells in the ovaries that surround an oocyte in a follicle [17]. These granulosa cells communicate directly with oocytes in primordial follicles but when the oocytes start to grow, they are separated from the granulosa cells by the zona pellucida. The zona pellucida is a coat of glycoproteinaceous matrix that surrounds the oocyte and is responsible for the "binding of sperm to unfertilised eggs" [18]. Since it completely encapsulates the oocyte, this disrupts the communication between the granulosa cells and the oocyte. Therefore, heterologous gap junctions begin to form on cytoplasmic projections called transzonal projections to restore communication [19,20]. Transzonal projections are follicular cell processes branching from granulosa cells that penetrate the zona pellucida, reaching the oocyte. The connection between the oocyte membrane and these projections is called a gap junction [21]. Through these junctions, granulosa cells provide growing oocytes with nutrients like amino acids and glucose substrates, as well as paracrine signals such as cAMP and cGMP to regulate oocyte proliferation by maintaining oocyte meiotic arrest. Given this, it is clear that functional granulosa cells are crucial in healthy oocyte quality. Endometriosis impairs this quality not by attacking the oocytes directly, but instead by modifying and destroying the granulosa cells.

As mentioned earlier, there are high levels of ROS in the cellular environment of patients with endometriosis. This stress affects the endoplasmic reticulum (ER), an organelle within the cell that is responsible for synthesising and folding proteins. The oxidative stress stimulates ER stress due to the increase demand for protein folding. When this occurs and there is an accumulation of unfolded or misfolded proteins in the ER, signal transduction cascades called the unfolded protein response (UPR) are activated to cope with this increase [22]. In physiological conditions, the UPR is a mechanism used to restore homeostasis by "eliminating slow--folding proteins", allowing the ER to maintain its folding capacity [23]. However, if ER stress persists and is severe, the UPR's adaptive measures will no longer be sufficient and thus terminal UPR is activated [24]. Terminal UPR is designed to remove stressed cells through signalling cell suicide and apoptosis. Specifically, by activating protein kinase R-like endoplasmic reticulum kinase, also known as PERK. PERK is a vital protein in terminal UPR because it can induce the "expression of proapoptotic transcription factors" such as activating transcription factor 4 (ATF4) and C/EBP homologous protein (CHOP) [25].

ATF4 and CHOP

These factors will drive the apoptosis of cells, including the granulosa cells needed for oocyte development. With granulosa cells being destroyed, oocytes are no longer getting the adequate amounts of nutrients needed for healthy growth. It has also been found that oxidative stress can cause damage to mitochondria in granulosa cells which can lead to the dyssynchronisation of nuclear and cytoplasmic maturation [26]. This impacts fertility because it may result in embryo development failure. Given the importance of oocyte quality in fertility and granulosa cells vital role in maintaining it, it is evident that the ER stress caused by endometriosis negatively impacts fertility.

Discussion

It is clear that there is a multitude of factors caused by endometriosis that can lead to infertility. The primary focus of treatment research should be on eliminating these consequences before it can permanently negatively impact fertility. Drugs that have anti-inflammatory effects or specific inhibitors of driving interleukins should be studied as these methods can eliminate the root pathomechanisms of endometriosis. However, these solutions may only be helpful if patients are diagnosed before the disease causes irreparable damage to the reproductive system. To this day, far too many women are living with endometriosis and do not realise. Proper awareness and education of reproductive health will allow women and girls to recognise irregularities in their own menstrual cycles and detect this

disease. Healthcare professionals must also not dismiss symptoms as typical menstrual manifestations as endometriosis can worsen with time. Regarding areas of research, future studies should investigate if there are any external factors that can lead to the pathogenesis of endometriosis so that the public can be aware and avoid harmful agents. For a disease that impacts so many lives, little is known about the exact causes for its pathogenesis. Areas for research regarding endometriosis is wide and it is with hope that the further we study this disease, the sooner we can develop more effective treatments and possibly a cure.

Conclusions

In conclusion, endometriosis can induce a pro-inflammatory state in which the immune system is unregulated and leads to the proliferation of immune cells and reactive oxygen species, creating a toxic environment for embryo development. Endometriosis also affects the quality of oocytes by stimulating terminal UPR and destroying the vital granulosa cells needed for oocyte maturation. Through these mechanisms, it can be concluded that untreated endometriosis creates conditions unsuitable for embryo growth and can therefore be linked to infertility.

Ethical approval
The study was a descriptive one. No humans or animals were a subject of examinations.

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Conflict of interest statement
The authors declare no conflict of interest.

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