ISSN 2228-9860 eISSN 1906-9642 CODEN: ITJEA8

International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies

http://TuEngr.com

Investigating the Effects of Endocrine-Disrupting Chemicals/Toxicants on the Human Ovary & Its Inflammatory Signalling Pathways

Feras Sulaiman Aljizani[1*], Fahad Eidah Al Thubaiti[1], Faisal Ali Althobyani[1], Abdullkareem Alamri[1]

[1]King Fahad Armed Forces Hospital. SAUDI ARABIA.
**Corresponding Author (Email: FAljezani@ kfafh.med.sa).*

Paper ID: 13A12C

Volume 13 Issue 12

Received 19 May 2021
Received in revised form 25 July 2022
Accepted 01 August 2022
Available online 08 August 2022

Keywords:
Endocrine-Disrupting Chemicals (EDCs); Ovary; Polycystic Ovary Syndrome (PCOS); Ovarian Cancer (OC); Ovarian dysfunction; Inflammatory signalling pathways

Abstract

The ovary is a vital organ susceptible to various illnesses when disrupted. Concerns have been raised about the effect of endocrine-disrupting chemicals (EDCs) on the reproductive organs, especially the ovaries. EDCs, mainly synthetic, can be present in copious amounts in the environment, contributing to daily human exposure. Studies have established a positive correlation between these chemicals and abnormal reproductive functioning. Ovary functions are undertaken through inflammatory signalling pathways – e.g. cytokines; CRP; ROS; and COX-2 – which in turn alter the functionality of the organ. This study investigates the effect of EDCs on the human ovary through these inflammatory signalling pathways and determines the consequential ovarian dysfunctions. A literature review was to search the EMBASE and MEDLINE databases for in-depth studies of EDCs exposure & subsequent ovarian inflammasome effects. It revealed the precise role of the effective process of EDCs on ovarian inflammatory mediators is complicated. Experimental evidence demonstrated the ability of EDCs (e.g. BPA, HPTE, β-HCH, DDE, PBDEs, PCBs, PFASs, Phthalates) to survive in the female ovary and its component cells through the blood–follicle barrier. Further, studies presented evidence of the association between EDCs exposure and some ovarian dysfunction conditions like ovarian cancer (OC), polycystic ovarian syndrome (PCOS) and cell ageing due to its impact on the endpoint of the ovaries through the inflammation pathways specifically cytokines (TNF-α, ILs and NF-kB), CXCL12, ROS, OS and COX-2. Many studies assumed a higher concentrated level of EDCs exposure with adverse ovarian inflammatory effects. However, these studies did not provide adequate or conclusive evidence that ovarian disruptions through the inflammatory mediators are triggered solely by EDCs exposure. Further experimental studies that test different levels of exposure in the general human population are needed to better understand the possible risks of EDCs on the role of ovarian inflammation.

Discipline: Endocrinology.

Cite This Article:

Aljizani, F.S., Thubaiti, F.E.A., Althobyani, F.A., Alamri A. (2022). Investigating the Effects of Endocrine-Disrupting Chemicals/Toxicants on the Human Ovary & Its Inflammatory Signalling Pathways. *International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies, 13*(12), 13A12C, 1-17. http://TUENGR.COM/V13/13A12C.pdf DOI: 10.14456/ITJEMAST.2022.234

1 Introduction

1.1 Background

1.1.1 Overview of the Human Ovary and Related Inflammatory Pathways

The endocrine system in both sexes consists of organs that are highly vascularized and ductless. These organs are responsible for the production and secretion of chemical signals called hormones into the bloodstream. These hormones, in turn, thereby affect a vast set of cellular functions, such as development and reproduction (La Perle & Dintzis, 2018; La Merrill et al., 2019). The ovaries are the female reproductive glands. They are considered one of the most important glands in the endocrine system and consist of two types of cells: primordial germ cells and somatic cells. Together, they are responsible for producing oocyte-containing follicles, managing ovulation and forming corpus luteum (Richards & Pangas, 2010).

The ovarian cycle is a dynamic endocrine system. It is inclusive of all the events in the ovaries, the interaction between the hypothalamus and pituitary and the changes in the endometrium and myometrium during the menstrual cycle and pregnancy (Richards, 2018). Production and maturation of the female gamete (oocyte) and synthesis of the reproductive and non-reproductive hormones are the primary functions of the ovary (Ding *et al.*, 2020). Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are the two main hormones in the regulation of the menstrual cycle. They are thus an essential aspect of the female reproductive system. Both hormones are referred to as gonadotropin or sex steroids. In addition to these are other hormones which are responsible for the development of female sex characteristics (Campbell & Jialal, 2019).

The physiologic functions of the ovary (folliculogenesis and ovulation) are initially induced by the inflammatory signals, which are mostly focused around this region and play a crucial role in normal ovary function. The inflammatory cycle includes direct and indirect acts that impact the main functions of the ovarian system. For instance, inflammatory signalling mediates the process of ovulation and tissue remodelling if the ovarian tissue is ruptured. Such ruptures can allow mature oocyte expulsion, which can lead to vasodilation, hyperaemia, oedema, collagenolysis and cell proliferation (Boots & Jungheim, 2015).

Figure 1 shows that there are several inflammatory mediators involved in ovarian physiology through a complex succession of molecular signals. The mediators include cytokines, chemokines (Chen *et al.*, 2018), C-reactive protein (CRP) (Wander *et al.*, 2008), natural killer cells (NKs), dendritic cells (DCs) (Savant *et al.*, 2018), cyclooxygenase 2 (COX-2) (Boots & Jungheim, 2015), reactive oxygen species (ROS) (Savant *et al.*, 2018). Cytokines are small cell-signalling proteins that aid in cellular interaction and communication in immune responses and stimulate cellular movement towards sites of inflammation (Zhang & An, 2007). For instance, interleukin 1 (IL-1), interleukin 6 (IL-6), tumour necrosis factor-alpha (TNF-α) and chemokines are all proinflammatory mediators. They play a prominent role in the female reproductive system during folliculogenesis,

which helps to induce ovulation. COX pathways and prostaglandin E2 (PGE2) synthesis are increased by TNF-α and the ILs as well (Vannuccini *et al.*, 2016).

Many studies have shown that concentrations of CRP depend more or less on changes in follicle dynamics (Clancy *et al.*, 2013). Furthermore, ROS has a beneficial effect on the ovary as a major physiological signal molecule (Savant *et al.*, 2018). Pasqualotto *et al.* (2004) found that oxidative stress (OS) levels are connected with pregnancy rates. Finally, the COX family are considered the main enzyme that is promoting PGE2. Several animal model studies have shown the significant role of COX-2 in ovulation regulation (Fang *et al.*, 2015). However, evidence shows that degenerated inflammation can change the normal dynamics of ovarian follicular processes. This can then result in anovulation, impaired oocyte quality and associated infertility along with other ovarian issues (Boots & Jungheim, 2015).

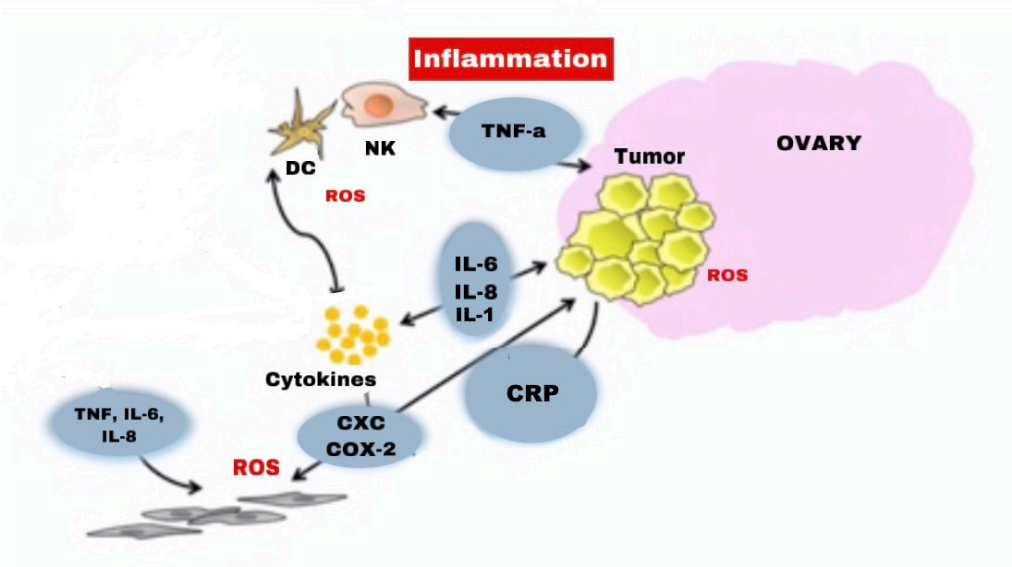

Figure 1: The inflammatory mediators of the ovary [edited] (Savant *et al*., 2018)

1.1.2 Endocrine-Disrupting Chemicals

Endocrine-disrupting chemicals (EDCs) include both natural and artificial compounds. These are defined as any type of molecule from either a man-made external source or exogenous chemicals, which disrupt or cause interference and alteration of hormone functions and/or homeostatic regulation (Gore *et al.*, 2015; Ding *et al.*, 2020). EDCs can contaminate the human body and its systems via environmental exposure. The most common sources of environmental exposure include eating contaminated food or inhalation of or direct dermal contact with EDCs (Figure 2) (Connolly, 2009). Some of these chemicals bioaccumulate in the human body over the years (Balaguer *et al.*, 2017). EDCs are known for inducing adverse health outcomes in infants. They can pass from the mother either directly into the blood of a foetus via the placenta or embryonic exposure or indirectly to the infant through breastfeeding (Figure 2) (Duursen *et al.*, 2020; Yang *et al.*, 2015).

Industrial and waste materials, flame retardants, plant chemicals, pharmaceuticals and food additives have all been identified as sources of EDCs (Piazza & Urbanetz, 2019), as have pesticides, fungicides, herbicides and cosmetic products (Rachoń, 2015). The most common types of endocrine-disrupting toxicants include bisphenol A (BPA), dioxins, per-/polyfluoroalkyl substances

(PFAS), phthalates, phytoestrogens, polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) (National Institute of Environmental Health Sciences, 2020).

1.1.3 Clinical Evidence of Ovarian Toxicity Disorders Caused by EDCs

1.1.3.1 Carcinogenicity of the Ovary

Many EDCs are considered potential carcinogens because of their ability to cause epigenetic changes that possibly play a fundamental role in ovarian cancer (OC) development by targeting inflammatory pathways (Samtani *et al.*, 2018). BPA is an EDC that was first introduced as a synthetic oestrogen (Ness *et al.*, 2000). More than eight billion pounds of estrogenic monomer BPA are used annually in the manufacture of plastic products (Romani *et al.*, 2013). However, after significant research and data collection, it was found that this compound has the potential to cause OC in females. OC is one of the leading causes of death of all gynaecological malignancies. It is closely linked with inflammation pathways such as TNF-α (Ness *et al.*, 2000; Kulbe *et al.*, 2007).

1.1.3.2 Infertility

Disruption of the female hormone system due to EDCs can also lead to infertility or subfertility. Some epidemiological studies have shown that exposure to heavy metals as constant exposure to mercury is one of the leading causes of infertility (Rattan *et al.*, 2017). Additionally, PFAS is known to cause ovarian toxicity at all stages of the ovarian cycle and, consequently, infertility (Ding *et al.*, 2020).

1.1.3.3 Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is another disorder of the ovarian system that consists of several clinical conditions, including hyperandrogenism, obesity, insulin resistance and chronic anovulation. According to two recent studies, many women who were exposed to chemical compounds found in plastics had a higher susceptibility to developing PCOS by the methylation process of the DNA (Piazza & Urbanetz, 2019; Neel & Sargis, 2011).

1.1.3.4 Premature Ovarian Failure

Premature ovarian failure (POF) is another concern for female reproductive health as it is the cause of premature infertility and can increase the risk of other disorders and even death due to the environmental toxicants that constitute a significant source of EDCs (Patel *et al.*, 2015).

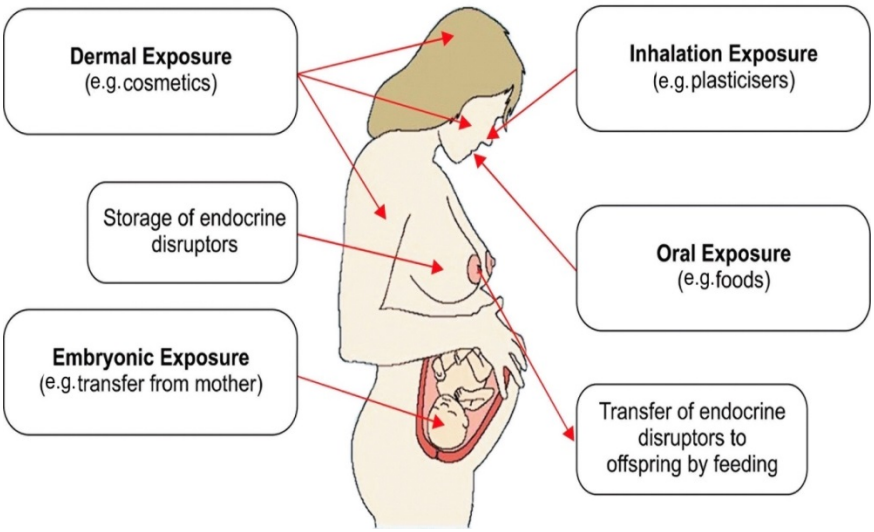

Figure 2: Main routes of human exposure to endocrine disruptors in humans (Yang et al., 2015).

1.2 Risk Assessment of EDCs to the Ovary and Its Inflammatory Pathways

A growing number of women worldwide are experiencing fertility problems. Clinical findings suggest that many women of reproductive age are affected by ovarian disorders (FREIA Project, 2020). EDCs exposure is known to cause transgenerational effects resulting in dysregulation of normal reproductive health, such as early menopause, irregular menstrual cycles, infertility, POF (Patel *et al.*, 2015), early or delayed puberty (Dietert, 2012) (Figure 3), ovarian dysgenesis syndrome (Khan *et al.*, 2020), OC (Hall & Korach, 2013) and even PCOS resulting from endocrinopathy (Palioura & Diamanti-Kandarakis, 2015). Therefore, the detection of early life exposure to EDCs is a significant public health challenge.

Some animal studies have proven that harmful chemicals such as PCB reduce ovarian weight, adversely affecting the growth of follicles (Bhattacharya & Keating, 2012; Petroff *et al.*, 2001). Furthermore, these chemicals have been shown to decrease the rate of *in-vitro* fertilisation (IVF) and the development of oocytes into embryos (Rattan *et al.*, 2017).

Therefore, the investigation and detection of EDCs are necessary because their presence can manifest in dangerous health outcomes in the future, not only for the exposed individuals but also for subsequent generations. Nevertheless, studies of how EDCs affect the human ovary system, particularly through the inflammatory pathways, have been scarce. Epigenetic endpoints are used to identify alterations or modifications to the epigenetic system, specifically those related to adverse health effects and an unhealthy lifestyle (Jacobs *et al.*, 2017). In terms of clinical inflammatory testing for the ovary, these endpoints can help to provide sensitive markers of toxicity. Moreover, they can also help in the identification of new inflammatory biomarkers of toxicity in the future and can even detect exposure to low doses of a toxin (Jacobs *et al.*, 2017).

The European-funded FREIA Project (2020) aims to identify human biomarkers that can help to identify ovarian toxicity beginning with the development of the foetus and continuing through adulthood (Duursen *et al.*, 2020). In the FREIA system, human tissues are cultured and then exposed to diethylstilboestrol (DSE) and ketoconazole. After that, *in-vitro* analyses are conducted to test the microscopic morphological, endocrine, and transcriptional effects. These methods can help in the identification of biomarkers that are specific to humans.

1.3 Aim and Objective of the Study

Growing evidence suggests that the ovaries are a possible target for EDCs toxicity. This presents profound implications for the reproductive health of humans. This thesis aims to highlight and evaluate human toxicological studies to determine the likely associations between EDCs exposure and detrimental impacts on the ovaries and their inflammatory signalling pathways. A better understanding of the complex mechanisms involved in the impact of EDCs on the human ovarian system will help to establish a sustainable direction for future research as well as strategies to promote a healthier society and improve women's health before, during and after gestation.

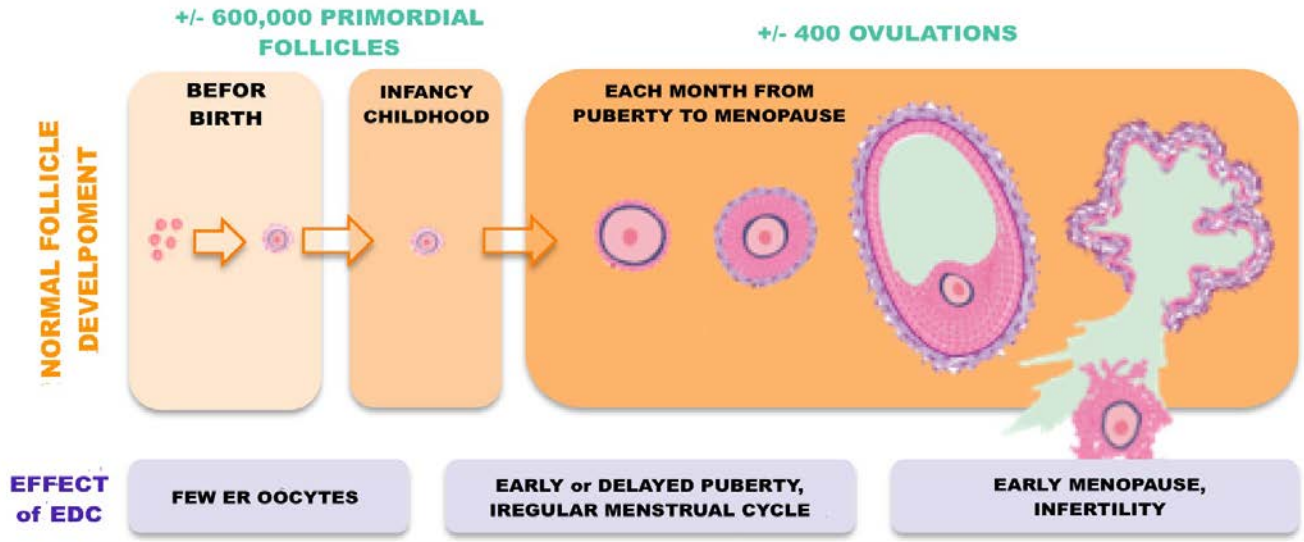

Figure 3: EDC disturbance at different oocyte stages contributes to various reproductive effects on women; the number of total follicles and ovulations over life span are noted as well (Duursen et al., 2020)

2 Materials and Methods

2.1 Methodology

This section illustrates the method used to conduct a review of the relevant studies. Academic and scientific databases were searched to provide a detailed analysis of studies investigating exposure to EDCs and their impacts on the ovary and its inflammatory pathways. The search was limited to relevant articles from 2010 until now found in the Ovid EMBASE and Ovid MEDLINE databases. Medical subject headings (MeSH) were employed where available; otherwise, keywords were used. The search terms and keywords included: endocrine-disrupting chemicals (EDCs), persistent organic pollutants (POPs), endocrine disruptors, ovary, inflammation signalling pathways, inflammation mediators, inflammation, primary ovarian insufficiency (POI), infertility, polycystic ovary syndrome (PCOS), ovarian dysfunction, ovarian health issues, ovarian cancer (OC) and ovarian cysts. The inclusion criteria were studies providing descriptive, experimental, and analytical data generated for any of the variables of EDCs exposure and the effects on the ovary and inflammatory pathways, particularly ovarian disorders among women. The studies were limited to research published in the English language from 2010 until now (2020) with human female participants. A manual search of review articles and cross-references was also conducted, and this research can be found in the PubMed search engine (http://www.ncbi.nlm.nih.gov./entrez/query.fcgi) and Scholar database as well. This was done to enhance the search and yield clear guidelines on the most appropriate outcomes from both scientific and medical-biological studies done by different scholars.

This research is limited to a small number of the published articles in the database that covered the main study elements. This limited research scope may have inhibited the ability to observe associations between the key research terms. May the COVID-19 pandemic have a role in this limitation.

3 Results and Main Findings

3.1 Researching the Epidemiologic Effects of EDCs on the Ovary Via Its Inflammatory Pathways

In the past two decades, there has been increased attention regarding environmental toxins and their adverse effects on female reproductive systems (Soave *et al.*, 2020). Daily exposure to EDCs has raised concerns for women, as it has been observed that EDCs tend to primarily target the ovary. As the primary organ responsible for reproduction and endocrine function in females, this translates into a myriad of health problems, such as infertility, POF, OC and PCOS (Silbergeld & Flaws, 2002). Table 2 presents the research results of numerous studies on the effects of these chemicals on the ovary through the inflammatory signalling pathways, for instance, cytokines (TNF-α, IL-1, IL-10, IL-6 and nuclear factor kappa B [NF-kB]), chemokine C-X-C motif ligand 12 (CXCL12), OS, ROS, COX-2 and CRP.

3.2 The Effective Role of EDCs on Ovarian Inflammatory Pathways

The results obtained by Hall and Korach (2013) (Table 2) showed a three- to a four-fold increase of CXCL12 in a cultured human ovarian carcinoma cell line (BG-1) for all treatments – oestradiol (E2), genistein, BPA and 2,2-bis(4-hydroxyphenyl)-1,1,1-trichloroethane (HPTE) – in 24 hours. A 10-fold increase was observed for E2, genistein and HPTE and a seven-fold increase for BPA in a 48-hour period. This means that the robustness of the EDCs – including genistein, BPA and HPTE – increases CXCL12 protein signals (via the cell surface receptor, which is C-X-C chemokine receptor type 4 (CXCR4). Where, how much the CXCL12 signal is boosted depends on the dose of each EDC, along with the effect elapsed time (Hall & Korach, 2013). Thus, the EDCs investigated in this study were shown to boost CXCL12 expression and the development of OC cells based on the exposure time and POP concentration.

Shah *et al*. (2020) reported statistically significant associations between increased exposure to organochlorine pesticides (OCPs) – namely beta hexachlorocyclohexane (β-HCH), dichlorodiphenyldichloroethylene (DDE) and dieldrin – and the increased risk prediction of OC through the up-regulation of messenger ribonucleic acid expressed in inflammatory cytokines (TNF-α, IL-1b, IL-6 and NF-kB), COX-2 and ROS production in human ovarian surface epithelial cells (HOSE When HOSE cells were exposed to 20 µM of β-HCH, DDE and dieldrin for seven days, the expression levels of inflammatory mediators increased by different folds (Table 1-A). Also, ROS which can increase the risk of OC increased after three days of exposure to the same concentration of 20 µM of β-HCH, DDE and dieldrin, respectively (Table 1-B) (Shah et al., 2020). This study presented evidence indicating that the existence of EDCs in the human body could induce carcinogenicity in the ovary through inflammatory pathways like cytokines (TNF-α, IL-1b, IL-6, NF-kB), OS, ROS and COX-2.

Soave *et al.* (2020) assessed the effects of exposure to EDCs and advanced glycation end products (AGEs). AGEs originally come from foods that are overheated and cooked (Ravichandran *et*

al., 2019). The latter of which results from the biomagnification of chemicals in the food chain, on the pathogenesis of PCOS. AGEs are capable of binding to cell surface receptors, cross-linking with body proteins and communicating with and activating proinflammatory signalling pathways, namely ROS, OS and the cytokines IL-6 and TNF-α (Soave *et al.*, 2020). A significant outcome was found in this study that the interaction of AGEs with inflammatory signalling pathways and OS substantially leads to ovarian dysfunction, PCOS and OC (Table 2).

Table 1: Effect of OCPs (β-HCH, DDE and dieldrin; 20-µM concentration) (A) for seven days on inflammatory signalling of HOSE cells IL-6, IL-1β, TNF-α, NF-kB and COX-2. (B) for three days on ROS levels of HOSE cells.

OCP effects on the levels of expression of pro-inflammatory cytokines							
OCPs	Concentration	Exposure period	IL-6 ↑	IL-1b ↑	TNF-α ↑	NF-kB ↑	COX-2 ↑
β-HCH	20 µM	7 days	10.35-fold ($p < .01$)	2.75-fold ($p < .01$)	4.78-fold ($p < .05$)	8.43-fold ($p < .05$)	7.89-fold ($p < .005$)
DDE	20 µM	7 days	10.82-fold ($p < .01$)	3.60-fold ($p < .01$)	4.78-fold ($p < .05$)	11.66-fold ($p < .01$)	11.52-fold ($p < .05$)
Dieldrin	20 µM	7 days	7.57-fold ($p < .05$)	2.38-fold ($p < .05$)	4.78-fold ($p < .05$)	6.36-fold ($p < .05$)	15.83-fold ($p < .01$)

ROS level in HOSE cells treated by OCPs			
OCPs	Concentration	Exposure period	ROS ↑
β-HCH	20 µM	3 days	71.06% ($p < 0.01$)
DDE	20 µM	3 days	80.82% ($p < 0.01$)
Dieldrin	20 µM	3 days	65.59% ($p < 0.05$)

ROS elevation is known to distort the redox balance, which then leads to OS (Agarwal *et al.*, 2005). OS can affect the entire duration of a woman's ovulation and even menopause (Savant *et al.*, 2018). Huang *et al.* (2020) stated that the single most striking observation to emerge from their study was a significant correlation between the exposure to different concentrations of BPA and its analogues, bisphenol S (BPS), bisphenol F (BPF) and bisphenol AF (BPAF) on the levels of calcium ions (Ca) in ovarian granulosa cell tumour line (KGN). These unwanted changes in the Ca level lead to damage or macromolecular deterioration, which is reflected in the physiological characteristics of ovarian granulosa cells (GCs). They observed that the highest concentration (1-100 mM) of BPA and its analogues, especially BPAF, in KGN cells elevated the ROS significantly, which affected the viability of the KGN cells. The toxicity impact for KGN cells varied depending on the EDC substance, with BPS and BPF showing slight toxicity compared to other chemical substances (Huang *et al.*, 2020). Therefore, this study indicated that certain EDCs could result in ovarian follicular development disorders depending on the concentration (Table 2).

Zota *et al.* (2018) conducted their study among 103 overweight or obese pregnant women from the San Francisco Bay area who were in their first or second trimester of pregnancy. They observed an association of prenatal exposure to certain types of EDCs (e.g., PBDEs, PCBs and PFASs) with reproductive cellular ageing and inflammatory signalling biomarkers in pregnant and postpartum women. The most striking observation to emerge from the data comparison was the positive correlation between exposure to these substances and inflammatory cytokine (IL-6 and

TNF-α) levels with time, and continuing their height linked to doubling concentrations (Zota *et al.*, 2018). Thus, they determined that ongoing exposure to EDCs can increase inflammatory mediator biomarkers during and after pregnancy, and these changes in inflammatory signalling increase cellular ovarian age, which could affect ovary function (Table 2).

A similar study (Table 2) by Kelley *et al.* (2019) investigated a sample of 56 women in their first trimester who had been exposed to mixtures of EDCs. They found a positive correlation between this exposure and cytokine levels, specifically interferon-gamma (IFN-γ), macrophage inflammatory protein (MIP) 1α and 1β, TNF-α, vascular endothelial growth factor (VEGF), IL-1β, IL-6, IL-8 and IL-17α. These increased cytokine levels can increase a woman's reproductive age, thus having clinical and health implications for pregnant and postpartum women (Kelley *et al.*, 2019).

Ferguson *et al.* (2016) conducted a nested case-control study of preterm births (130 cases, 352 controls) in Boston, Massachusetts, from 2006 through 2008. They found that pregnant women exposed to BPA had increased levels of CRP, inflammatory mediators (TNF-α, IL-6, IL-1 and IL-10) and OS. In particular, IL-6 concentrations in blood plasma were extremely high. These increased levels can have adverse impacts on pregnancy and can alter the ovarian dynamic post-partum (Table 2) (Ferguson *et al*., 2016).

Table 2: Summary of selected studies on the correlation between EDCs exposure and ovarian inflammatory pathways.

EDCs→ Ovarian Inflammatory Signalling Pathways → Ovarian Health Impacts					
Authors	Aim	Design	Inflammatory Signals Targeted	Exposure	Health Outcome
Hall & Korach 2013	To determine and emphasise the possible role of EDCs on the oestrogen receptors and CXCL12 signalling as an aetiology of OC	An experimental study (*in vitro*)	CXCL12	EDCs: genistein, BPA and HPTE	Induction of OC
Shah *et al.* 2020	To evaluate the pro-inflammatory response of OCPs in HOSE and their effect on OC risk prediction	An experimental study (*in vitro*)	Cytokines (TNF-α, IL-1b, IL-6, NF-kB), ROS and COX-2	OCPs: β-HCH, DDE and dieldrin	Development of OC
Soave *et al.* 2020	To investigate the effect of EDCs from environmental toxin exposure on the pathogenesis of PCOS	Systematic review and meta-analysis	Cytokines (IL-6, TNF-α), OS and ROS	AGEs resulting from the excitation of EDCs	PCOS pathogenesis
Huang *et al.* 2020	To evaluate the OS in ovarian KGN cells caused by BPA and its substitutes	An experimental study (*in vitro*)	ROS	BPA, BPS, BPF and BPAF	Ovarian follicular development disorders
Zota *et al.* 2018	To evaluate the impacts of prenatal EDC exposure on cellular ageing and inflammatory mediators during and after pregnancy	Cross-sectional study	IL-6 and TNF-α	PBDEs, PCBs and PFASs	Inflammation and cellular ageing
Kelley *et al.* 2019	To test different cytokines in samples of women exposed to EDCs during early pregnancy	Cohort study	IFN-γ, MIP-1α, MIP-1β, TNF-α, VEGF, IL-1β, IL-6, IL-8 and IL-17α	Phthalates, phenols and other metals	Clinical implications for women in the gestational spectrum
Ferguson *et al.* 2016	To research the correlations of exposure to BPA with OS and inflammatory pathways	Case-control study	CRP, TNF-α, IL-6, IL-1, IL-10 and OS	BPA	Adverse results for pregnancy and foetal development

The results of these studies provide insight into the effects of EDCs on ovarian cells through inflammatory signalling pathways, specifically cytokines (TNF-α, ILs and NF-kB), CXCL12, ROS, OS and COX-2. These cellular effects can lead to various adverse disorders, for example, OC, PCOS, follicular development disorders and cellular ageing. Additionally, these studies showed that the type of EDC, its concentration and the duration of exposure played a critical role in determining the potency of these materials on the stimulation of inflammatory pathways.

4 Discussion

4.1 Correlation between EDCs and OC

OC is highly lethal due to its aggressive metastasised in the peritoneal cavity and its often-late diagnosis. The overwhelming majority (80–90%) of OC tumours originate from the ovarian epithelial cells (Ptak *et al.*, 2014). There are a variety of OC aetiologies; this study focused on the disruption of the inflammatory pathways (such as cytokines) due to environmental and occupational exposure to EDCs, including pesticides (Shah *et al.*, 2020) and BPA (Ptak *et al.*, 2014).

Unfortunately, the literature on the effects of EDCs on ovarian inflammation mediators is scant. Hall and Korach's (2013) study showed a significant positive relationship between exposure to EDCs (E2, genistein, HPTE and BPA) for 24 and 48 hours and ovarian function disruption through an increase in the CXCL12 protein in BG-1 cells. Elevation in ovarian CXCL12 can result in the development of OC cells (Hall & Korach, 2013). This finding further supports the idea of the involvement of chemokines as central regulators in advanced ovarian malignancy (Muralidhar & Barbolina, 2014). The CXCL12–CXCR4 axis is increasingly considered to be a vital marker for ovarian tumour cells (Popple *et al.*, 2012).

Shah *et al.*, (2020) *in-vitro* study was designed to determine the pro-inflammatory response to OCPs in HOSE cells. The OCPs (β-HCH, DDE and dieldrin) increased TNF-α, IL-1b, IL-6, NF-kB and COX-2 expression in HOSE after 7 days of exposure. Additionally, they found elevated ROS levels after 3 days of exposure to the OCPs. These elevated ovarian inflammatory mediators provide evidence of epithelial OC risk (Shah *et al.*, 2020).

Possible explanations for the findings in both of these studies could be that EDCs are capable of aggressively disrupting ovarian cells via various inflammatory pathways. However, how much adverse effect EDCs can have on ovarian cells is dependent on the direct proportion with concentration. However, concentrations of the exposures to these OCPs in the study of Shah *et al.* (2020) look relatively high and contradicted compared to real-life exposure concentrations. This concentration of 20 µM cannot correspond to the daily exposure value, because the United Nations Environment Program recognised that a very low concentration of OCPs could present high toxicity (Wang et al., 2011). Researchers also have not sufficiently extrapolated the effects of exposure time to EDCs. Therefore, further studies in this field that focus on the effects of different EDCs concentrations and exposure times are needed.

4.2 Correlation between EDCs and PCOS

PCOS affects about 5–10% of the world's population of reproductive-age females (Diamanti-Kandarakis & Dunaif, 2012). PCOS is a diverse disorder with myriad contributors and is categorised by numerous endocrine instabilities. Recent studies have focused on whether EDCs within the environment are associated with PCOS (Barrett & Sobolewski, 2014). The list of potentially critical environmental factors that may influence the pathogenesis and/or presentation of PCOS has grown to include environmental chemicals like BPA (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Working Group, 2004; Akgül *et al.*, 2019).

Other EDC homologues, such as AGEs, may also have the potential to disturb the homeostasis of the ovarian system, although the evidence remains inconclusive. Soave *et al.* (2020) found that AGEs are a significant PCOS aetiology. It is possible that their hallmark results were due to the direct effect of AGEs on the stimulation of inflammatory pathways in ovarian cells, specifically ROS, OS and the cytokines IL-6 and TNF-α (Soave *et al.*, 2020). Their finding seems to be consistent with other research that found that increasing people's exposure to AGEs could be responsible for boosting OS and disrupting hormones, resulting in PCOS (Ravichandran *et al.*, 2019). Soave *et al.* (2020) findings would have been more convincing and useful if the study had provided evidence on the exposure time and dosage and linked these to the main study aim. Despite this limitation, this study provides compelling evidence suggesting that PCOS could be the result of EDCs damaging ovarian cells by targeting inflammatory signalling pathways.

4.3 Correlation between EDCs and Ovarian Complications

EDCs have been shown to interfere with ovarian follicular development, resulting in adverse ovarian outcomes (Craig *et al.*, 2011). Huang *et al.* (2020) provided significant findings regarding increased ROS in KGN cells after exposure to different concentrations of BPA, BPS, BPF and BPAF, demonstrating that these EDCs can disrupt ovarian follicular development. These results imply the possibility that follicular developmental disorders like PCOS are the result of this ovarian follicular disruption (Rutkowska & Rachoń, 2014). This finding is essential for developing further research to study different EDC concentrations and monitor their effects on inflammatory pathways of ovarian cells.

In addition to ovarian follicular disorders, another concern regarding ovarian exposure to EDCs like PBDEs, PCBs and PFASs is increased inflammation biomarkers, such as cytokines IL-6 and TNF-α, as well as cellular ageing in women during and after pregnancy (Zota *et al.*, 2018). Findings suggest that exposure to specific EDCs is associated with increased inflammation, resulting in ovarian ageing (Ding *et al.*, 2020).

A study of 482 pregnant women demonstrated higher levels of TNF-α, IL-6, IL-1, IL-10, CRP and OS after exposure to BPA (Ferguson *et al.*, 2016). Similarly, in a study of 56 women, Kelley *et al.* (2019) found that exposure to EDC mixtures correlated significantly with the increased expression of inflammasomes like IFN-γ, MIP-1α, MIP-1β, TNF-α, VEGF, IL-1β, IL-6, IL-8 and IL-17α. This can result in inflammatory pregnancy (Kelley *et al.*, 2019). Taken together, the results of these two

studies suggest that certain EDCs play a crucial role in triggering inflammatory mediators in pregnant women. However, these studies only observed a significant association, and the number of cases in each study was small. Thus, the evidence is insufficient to assess the effects of EDCs on the ovary.

5 Conclusion

There are significant associations between EDCs exposure and ovarian function like interference and alteration of hormone functions, targeting some immune pathways and cell carcinogenesis due to the epigenetic changes such as methylation of DNA, all of which can impact public health. The ovary is a primary regulator of the reproductive, endocrine and general health functions of the female population. Because millions of women around the world are exposed to EDCs daily, a causal association can have severe implications for their reproductive health.

The main goal of the current study was to examine the possible ways in which EDCs affect human ovarian inflammatory signalling pathways. The evidence from the reviewed studies indicates that exposure to different concentrations of EDCs, e.g. (genistein, BPA, HPTE, β-HCH, DDE, dieldrin, AGEs, BPA, BPS, BPF, BPAF, PBDEs, PCBs, PFASs, phthalates and phenols) for prolonged periods could influence the expected performance of female reproductive organs, more specifically the ovaries, which can later lead to dysfunction of the reproductive system. In particular, these effects are transmitted by ovarian inflammatory mediators, for instance, CXCL12, cytokines (TNF-α, IL-1b, IL-6, IL-8, NF-kB, IFN-γ), OS, ROS, CRP and COX-2. Most EDCs have the potential to cause different diseases in the ovary, including distinct types of cancer – OC, PCOS, ovarian dysfunction and the onset of ovarian cell senescence. However, the association between EDCs and ovarian inflammation pathways remains uncertain.

The ovary is the most influential organ in the reproduction process. Humans are very vulnerable to environmental and human-made toxicants. Only limited knowledge has been obtained regarding how EDCs cause ovarian toxicity through inflammatory pathways. More information is required to thoroughly understand what molecular mechanism and how EDCs affect the ovary. Further experimental research should be done on cell lines to analyse the impact of EDCs themselves or even their different concentrations on the ovaries and their abilities in the pathogenesis of ovarian disorders. Examining whether EDCs affect the diversity of inflammatory pathways, which in turn affect the ovary indirectly, can help us to take appropriate action to address this contemporary health event.

Such in-depth studies are one of the ways to establish the causes of impairment to normal ovarian processes. The FREIA Project (2020) which works toward safeguarding female reproductive health against EDCs is an example of such a study. Future work should follow the methodological example of FREIA to provide fresh insights into the aetiology of ovarian disorders, mainly through inflammatory mediators. This can pave the way for innovative modern protocols or approaches to prevent, detect and reduce the toxicity of EDCs that induce human ovarian damage.

6 Availability of Data and Material

Data can be made available by contacting the corresponding author.

7 References

Agarwal, A., Gupta, S. & Sharma, R. K. (2005) 'Role of oxidative stress in female reproduction', *Reproductive Biology and Endocrinology*, 3, p. 28.

Akgül, S., Sur, Ü., Düzçeker, Y., Balci, A., Kizilkan, M. P., Kanbur, N., Bozdağ, G., Erkekoğlu, P., Gümüş, E. & Kocer-Gumusel, B. (2019) 'Bisphenol A and phthalate levels in adolescents with polycystic ovary syndrome', *Gynaecological Endocrinology*, 35, pp. 1084-1087.

Balaguer, P., Delfosse, V., Grimaldi, M. & Bourguet, W. (2017) 'Structural and functional evidence for the interactions between nuclear hormone receptors and endocrine disruptors at low doses', *Comptes Rendus Biologies*, 340, pp. 414-420.

Barrett, E. S. & Sobolewski, M. (2014) 'Polycystic ovary syndrome: do endocrine disrupting chemicals play a role?', *Seminars in Reproductive Medicine*, 32, 3, p. 166.

Bhattacharya, P. & Keating, A. F. (2012) 'Impact of environmental exposures on ovarian function and the role of xenobiotic metabolism during over toxicity', *Toxicology and Applied Pharmacology*, 261**,** pp. 227-235.

BioNinja, 2020. [image] Available at: https://ib.bioninja.com.au/higher-level/topic-11-animal-physiology/114-sexual-reproduction/reproductive-tissue.html (Accessed 30 August 2020).

Boots, C. E. & Jungheim, E. S. (2015) 'Inflammation and human ovarian follicular dynamics', *Seminars in Reproductive Medicine*, 33, 4, pp. 270-275.

Campbell, M. & Jialal, I. (2019) 'Physiology, endocrine hormones', in *StatPearls*. Treasure Island (FL): StatPearls Publishing. Available at: https://www.ncbi.nlm.nih.gov/books/NBK538498/ (Accessed 17 July 2020).

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X. & Zhao, L. (2018) 'Inflammatory responses and inflammation-associated diseases in organs', *Oncotarget*, 9, p. 7204.

Clancy, K. B., Baerwald, A. R. & Pierson, R. A. (2013) 'Systemic inflammation is associated with ovarian follicular dynamics during the human menstrual cycle', *PloS One*, 8(5), e64807.

Colquitt P. J. (1995) 'The effect of occupational exposure to mercury vapour on the fertility of female dental assistants', *Occupational and Environmental Medicine*, 52, 3, p. 214.

Connolly, L. (2009) 'Endocrine-disrupting chemicals: origins, fates and transmission into the food chain', in *Endocrine-disrupting chemicals in food*. Elsevier.

Craig, Z. R., Wang, W. & Flaws, J. A. (2011) 'Endocrine-disrupting chemicals in ovarian function: effects on steroidogenesis, metabolism and nuclear receptor signalling', *Reproduction*, 142, p. 633.

Diamanti-Kandarakis, E. & Dunaif, A. (2012) 'Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications', *Endocrine Reviews*, 33, pp. 981-1030.

Dietert, R. R. (2012) 'Misregulated inflammation as an outcome of early-life exposure to endocrine-disrupting

chemicals', *Reviews on Environmental Health,* 27, pp. 117-131.

Ding, N., Harlow, S. D., Randolph, J. F., Loch-Caruso, R. & Park, S. K. (2020) 'Perfluoroalkyl and polyfluoroalkyl substances (PFAS) and their effects on the ovary', *Human Reproduction Update*. Available at: https://doi.org/10.1093/humupd/dmaa018 (Accessed 3 July 2020).

Duursen, M., Boberg, J., Christiansen, S., Connolly, L., Damdimopoulou, P., Filis, P., Fowler, P. A., Gadella, B. M., Holte, J. & Jääger, K. (2020) 'Safeguarding female reproductive health against endocrine disrupting chemicals—the FREIA project', *International Journal of Molecular Sciences,* 21, p. 3215.

Fang, L., Chang, H.-M., Cheng, J.-C., Leung, P. C. & Sun, Y.-P. (2015) 'Nitric oxide and cGMP induce COX-2 expression and PGE2 production in human granulosa cells through the CREB signalling pathway', *The Journal of Clinical Endocrinology & Metabolism,* 100, pp. E262-E269.

Ferguson, K. K., Cantonwine, D. E., Mcelrath, T. F., Mukherjee, B. & Meeker, J. D. (2016) 'Repeated measures analysis of associations between urinary bisphenol-A concentrations and biomarkers of inflammation and oxidative stress in pregnancy', *Reproductive Toxicology,* 66, pp. 93-98.

FREIA Project (2020) *Discovering new insights*. Available at: freiaproject.eu/wp/discovering-new-insights/ (Accessed 7 July 2020).

Gore, A. C., Chappell, V., Fenton, S., Flaws, J. A., Nadal, A., Prins, G. S., Toppari, J. & Zoeller, R. (2015) 'EDC-2: the Endocrine Society's second scientific statement on endocrine-disrupting chemicals', *Endocrine Reviews,* 36, pp. E1-E150.

Grindler, N. M., Allsworth, J. E., Macones, G. A., Kannan, K., Roehl, K. A. & Cooper, A. R. (2015) 'Persistent organic pollutants and early menopause in US women', *PloS One,* 10, p. e0116057.

Hall, J. M. & Korach, K. S. (2013) 'Endocrine-disrupting chemicals promote the growth of ovarian cancer cells via the ER-CXCL12-CXCR4 signalling axis', *Molecular Carcinogenesis,* 52, pp. 715-725.

Hoyer, P. B. (2013) *Ovarian toxicology*. Boca Raton-London-New York, CRC Press.

Huang, M., Liu, S., Fu, L., Jiang, X. & Yang, M. (2020) 'Bisphenol A and its analogues bisphenol S, bisphenol F and bisphenol AF, induce oxidative stress and biomacromolecular damage in human granulosa KGN cells', *Chemosphere***.** Available at: https://doi.org/10.1016/j.chemosphere.2020.126707 (Accessed 22 August 2020).

Jacobs, M. N., Marczylo, E. L., Guerrero-Bosagna, C. & Rüegg, J. (2017) 'Marked for life: epigenetic effects of endocrine-disrupting chemicals', *Annual Review of Environment and Resources,* 42, pp. 105-160.

Kelley, A. S., Banker, M., Goodrich, J. M., Dolinoy, D. C., Burant, C., Domino, S. E., Smith, Y. R., Song, P. X. & Padmanabhan, V. (2019) 'Early pregnancy exposure to endocrine-disrupting chemical mixtures are associated with inflammatory changes in maternal and neonatal circulation', *Scientific Reports,* 9, pp. 1-14.

Kahn, L. G., Philippat, C., Nakayama, S. F., Slama, R., & Trasande, L. (2020) 'Endocrine-disrupting chemicals: implications for human health', *The Lancet Diabetes & Endocrinology*, 8(8), pp. 703-718.

Kulbe, H., Thompson, R., Wilson, J. L., Robinson, S., Hagemann, T., Fatah, R., Gould, D., Ayhan, A. & Balkwill, F. (2007) 'The inflammatory cytokine tumour necrosis factor-α generates an autocrine tumour-promoting network in epithelial ovarian cancer cells', *Cancer Research,* 67, pp. 585-592.

La Merrill, M. A., Vandenberg, L. N., Smith, M. T., Goodson, W., Browne, P., Patisaul L, H. B., Guyton, K. Z., Kortenkamp, A., Cogliano, V. J. & Woodruff, T. J. (2019) 'Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification'. *Nature Reviews Endocrinology*, pp. 1-13.

La Perle, K. & Dintzis, S. (2018) 'Endocrine system', in *Comparative Anatomy and Histology*. Academic Press, pp. 251-273.

Lumen Learning (2020) *The ovarian cycle, the menstrual cycle, and menopause*. Available at: https://courses.lumenlearning.com/wm-biology2/chapter/the-ovarian-cycle-the-menstrual-cycle-and-menopause (Accessed 13 August 2020).

Muralidhar, G. G. & Barbolina, M. V. (2014) 'Chemokine receptors in epithelial ovarian cancer', *International Journal of Molecular Sciences*, 15, pp. 361-376.

National Institute of Environmental Health Sciences (2020) *Endocrine disruptors*. Available at: https://www.niehs.nih.gov/health/topics/agents/endocrine/index.cfm (Accessed 1 July 2020).

Neel, B. A. & Sargis, R. M. (2011) 'The paradox of progress: environmental disruption of metabolism and the diabetes epidemic', *Diabetes*, 60, pp. 1838-1848.

Ness, R. B., Grisso, J. A., Cottreau, C., Klapper, J., Vergona, R., Wheeler, J. E., Morgan, M. & Schlesselman, J. J. (2000) 'Factors related to inflammation of the ovarian epithelium and risk of ovarian cancer', *Epidemiology*, 11, pp. 111-117.

Palioura, E. & Diamanti-Kandarakis, E. (2015) 'Polycystic ovary syndrome (PCOS) and endocrine-disrupting chemicals (EDCs)', *Reviews in Endocrine and Metabolic Disorders*, 16, pp. 365-371.

Pasqualotto, E. B., Agarwal, A., Sharma, R. K., Izzo, V. M., Pinotti, J. A., Joshi, N. J. & Rose, B. I. (2004) 'Effect of oxidative stress in follicular fluid on the outcome of assisted reproductive procedures', *Fertility and Sterility*, 81, pp. 973-976.

Patel, S., Zhou, C., Rattan, S. & Flaws, J. A. (2015) 'Effects of endocrine-disrupting chemicals on the ovary', *Biology of Reproduction*, 93(20), pp. 1-9.

Petroff, B. K., Roby, K. F., Gao, X., Son, D.-S., Williams, S., Johnson, D., Rozman, K. K. & Terranova, P. F. (2001) 'A review of mechanisms controlling ovulation with implications for the anovulatory effects of polychlorinated dibenzo-p-dioxins in rodents', *Toxicology*, 158, pp. 91-107.

Piazza, M. J. & Urbanetz, A. A. (2019) 'Environmental toxins and the impact of other endocrine-disrupting chemicals in women's reproductive health', *JABRA Assisted Reproduction*, 23, p. 154.

Popple, A., Durrant, L., Spendlove, I., Rolland, P., Scott, I., Deen, S. & Ramage, J. (2012) 'The chemokine, CXCL12, is an independent predictor of poor survival in ovarian cancer', *British Journal of Cancer*, 106, pp. 1306-1313.

Ptak, A., Hoffmann, M., Gruca, I. & Barć, J. (2014) 'Bisphenol A induce ovarian cancer cell migration via the MAPK and PI3K/Akt signalling pathways', *Toxicology Letters*, 229, pp. 357-365.

Rachoń, D. (2015) 'Endocrine-disrupting chemicals (EDCs) and female cancer: informing the patients', *Reviews in Endocrine and Metabolic Disorders*, 16, pp. 359-364.

Rattan, S., Zhou, C., Chiang, C., Mahalingam, S., Brehm, E. & Flaws, J. A. (2017) 'Exposure to endocrine disruptors during adulthood: consequences for female fertility', *Journal of Endocrinology,* 233, pp. R109-R129.

Ravichandran, G., Lakshmanan, D. K., Raju, K., Elangovan, A., Nambirajan, G., Devanesan, A. A. & Thilagar, S. (2019) 'Food advanced glycation end products as potential endocrine disruptors: an emerging threat to contemporary and future generation', *Environment International,* 123, pp. 486-500.

Richards, J. S. (2018) 'The ovarian cycle', in *Vitamins and hormones.* Academic Press, 107, pp. 1-25.

Richards, J. S. & Pangas, S. A. (2010) 'The ovary: basic biology and clinical implications', *The Journal of Clinical Investigation,* 120, pp. 963-972.

Romani, F., Tropea, A., Scarinci, E., Russo, C. D., Lisi, L., Catino, S., Lanzone, A. & Apa, R. (2013) 'Endocrine disruptors and human corpus luteum: in vitro effects of phenols on luteal cells function', *Journal of Environmental Science and Health, Part C,* 31, pp. 170-180.

Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Working Group (2004) 'Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome', *Fertility and Sterility,* 81, pp. 19-25.

Rutkowska, A. & Rachoń, D. (2014) 'Bisphenol A (BPA) and its potential role in the pathogenesis of the polycystic ovary syndrome (PCOS)', *Gynaecological Endocrinology,* 30, pp. 260-265.

Samtani, R., Sharma, N. & Garg, D. (2018) 'Effects of endocrine-disrupting chemicals and epigenetic modifications in ovarian cancer: a review', *Reproductive Sciences,* 25, pp. 7-18.

Savant, S. S., Sriramkumar, S. & O'Hagan, H. M. (2018) 'The role of inflammation and inflammatory mediators in the development, progression, metastasis, and chemoresistance of epithelial ovarian cancer', *Cancers,* 10, p. 251.

Shah, H. K., Sharma, T. & Banerjee, B. D. (2020) 'Organochlorine pesticides induce inflammation, ROS production, and DNA damage in human epithelial ovary cells: an in vitro study', *Chemosphere,* 246, 125691.

Silbergeld, E. K. & Flaws, J. A. (2002) 'Environmental exposures and women's health', *Clinical Obstetrics and Gynaecology,* 45, pp. 1119-1128.

Soave, I., Occhiali, T., Assorgi, C., Marci, R. & Caserta, D. (2020) 'Environmental toxin exposure in polycystic ovary syndrome women and possible ovarian neoplastic repercussion', *Current Medical Research and Opinion,* 36, pp. 693-703.

Vannuccini, S., Clifton, V. L., Fraser, I. S., Taylor, H. S., Critchley, H., Giudice, L. C. & Petraglia, F. (2016) 'Infertility and reproductive disorders: impact of hormonal and inflammatory mechanisms on pregnancy outcome', *Human Reproduction Update,* 22, pp. 104-115.

Wander, K., Brindle, E. & O'Connor, K. A. (2008) 'C-reactive protein across the menstrual cycle', *American Journal of Physical Anthropology,* 136, pp. 138-146.

Wang, H. S., Sthiannopkao, S., Du, J., Chen, Z. J., Kim, K. W., Yasin, M. S. M., Jamal, H. H., Wong, K-C. C. & Wong, M. H. (2011) 'Daily intake and human risk assessment of organochlorine pesticides (OCPs) based on Cambodian market basket data', *Journal of hazardous materials,* 192(3), pp. 1441-1449.

Yang, O., Kim, H. L., Weon, J.-I. & Seo, Y. R. (2015) 'Endocrine-disrupting chemicals: a review of toxicological mechanisms using molecular pathway analysis', *Journal of Cancer Prevention*, 20, p. 12.

Zhang, J.-M. & An, J. (2007) 'Cytokines, inflammation and pain', *International Anesthesiology Clinics*, 45, p. 27.

Zota, A. R., Geller, R. J., Romano, L. E., Coleman-Phox, K., Adler, N. E., Parry, E., Wang, M., Park, J.-S., Elmi, A. F. & Laraia, B. A. (2018) 'Association between persistent endocrine-disrupting chemicals (PBDEs, OH-PBDEs, PCBs, and PFASs) and biomarkers of inflammation and cellular ageing during pregnancy and postpartum', *Environment International*, 115, pp. 9-20.

Feras Aljizani is a Molecular Geneticist in the Medical Molecule biology Laboratory at King Fahad Armed Forces Hospital with over a decade of experience in the field of diagnostic of infectious, genetic diseases and cancer bio marks. He holds a Bachelor of Laboratory Medicine degree from Umm Al Qura University, KSA and a master's degree in molecular biology from Queens University Belfast, UK .He is a member of the Saudi Society for Molecular Biology and Biotechnology.

Fahad Al Thubaiti is a Microbiologist in the Medical Microbiology Laboratory at King Fahad Armed Forces Hospital with over fifteen years of experience in the field of diagnostic of Bacterial and fungal diseases. He holds a Bachelor's and Master of Microbiology degrees from King Abdullaziz University, KSA. He is Head of Phlebotomy department at King Fahad Armed Forces Hospital.

Faisal Ali Althobyani is Chief of Blood Bank Department. He was Chief of Flowcytometry in the Medical Laboratory, King Fahad Armed Forces Hospital .Member of Trauma team and the Society of Transfusion Medicine.

Abdulkareem Majed Alamri is a medical laboratory technician. He graduated from the Faculty of Science, King Abdulaziz University. He worked in King Fahad Armed Forces Hospital. He is Chief of the DNA Fingerprinting and Profiling Committee.