

## Radiation-induced premature ovarian failure: focus on the role of ovarian inflammatory signaling pathways

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### ABSTRACT

Radiation therapy, an effective treatment modality for many types of cancer, often carries a heavy cost for young women. In particular, those undergoing pelvic radiotherapy mostly experience sudden menopausal symptoms and premature ovarian failure (POF) impacting their reproductive health and overall quality of life. Upon radiation, excessive reactive oxygen species (ROS) are produced causing a state of oxidative stress to tissues. Following this, DNA is damaged augmenting subsequent inflammatory responses and further tissue damage. Mild inflammation is essential for the development and release of oocytes. However, prolonged inflammation is associated with poor quality oocytes and sometimes complete dysfunction of the ovaries, as excessively produced pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukins (IL) lead to compromised ovary functionality. This work aims at considering the radiation-induced POF as an inflammatory process. It also covers key molecular pathways involved in this response like nuclear factor kappa-B (NF- $\kappa$ B), transforming growth factor- $\beta$  (TGF- $\beta$ ), mitogen-activated protein kinase (MAPK), sirtuin-1 (SIRT-1), poly [ADP-ribose] polymerase-1 (PARP-1) and insulin-like growth factor-1 (IGF-1) in regulating inflammation. Eventually, understanding these interrelated pathways can provide researchers with valuable molecular targets that can be used to develop new protective agents for radiation-induced POF thus improving the quality of life for these patients.

**Keywords:** *Premature ovarian failure; Ionizing radiation; Inflammation; NF- $\kappa$ B; TGF- $\beta$ ; SIRT-1; PARP-1; IGF-1.*

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Citation | Mohamed DH, Mantawy EM, Said RS, Kassem DH, Gad AM, El-Demerdash, 2023. Radiation-induced premature ovarian failure: focus on the role of ovarian inflammatory signaling pathways. Arch Pharm Sci ASU 7(2): 522-538

DOI: [10.21608/aps.2023.251808.1147](https://doi.org/10.21608/aps.2023.251808.1147)

Print ISSN: 2356-8380. Online ISSN: 2356-8399.

Received 03 December 2023. Accepted 12 December 2023.

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Published by: Ain Shams University, Faculty of Pharmacy

### 1. Introduction

Premature ovarian failure (POF) is a distressing medical condition concerning women of reproductive age. It can be identified by either a marked decline in follicular count or follicles

being less sensitive to hormones before turning 40 [1]. These events lead to a feedback response making the pituitary gland stimulated to secrete more follicle-stimulating hormone (FSH) (hyper gonadotropic) to force the non-functioning follicles to secrete estrogen (hypogonadism) [2].

Unfortunately, this damage is irreversible due to the limited supply of ovarian follicles leading to a range of negative impacts on both fertility and long-term quality of life [3, 4].

Unfortunately, a lack of awareness and moderate clinical signs might lead to a delayed diagnosis of this condition. It is mostly characterized by missed periods before the age of 40, hot flashes, vaginal dryness, night sweats, and difficulty conceiving [1]. Hypergonadotropism is confirmed by FSH levels > 20 IU/L along with low estradiol (E2) levels, about 20 pg/mL, on two distinct occasions separated by at least four weeks. Furthermore, low levels of anti-Müllerian hormone (AMH) less than 1 ng/mL signal a depleted ovarian reserve [5].

In parallel, ovarian health relies on a complex network of processes and interrelated signaling pathways [6]. Among these processes, inflammation plays a dual role in both the maintenance of physiological ovarian function and the onset of POF. A controlled inflammatory environment contributes to the balance of ovarian dynamics from follicular rupture to ovulation. All of which guarantees proper menstrual cycles and support fertility. However, the exact pathways that ensure the normal functioning of the ovaries can become agents of dysfunction being subjected to excessive activation [7].

Radiation exposure has been reported to disrupt this delicate environment inside the ovaries. Given the extensive use of radiation therapy for childhood cancers, it became one of the most prevalent reasons for decreased ovarian reserve and hormonal dysregulation. 90% of young females who received total body irradiation (TBI) and 97% of those who received abdominal radiation experienced POF [8, 9].

Several studies have demonstrated the damaging effects of radiation on ovarian tissues. However, the exact mechanisms beyond this

damage are still under investigation. Interestingly, increasing attention was applied to the role of inflammation in disrupting the follicular environment and the development of POF. Ionizing radiation (IR) can initiate broader inflammatory pathways ending with the accumulation of proinflammatory cytokines rather than only oxidative damage [10]. Thus, in this review, we aimed to elucidate the molecular interactions involving radiation exposure and inflammation and their relevance to POF focusing on nuclear factor kappa-B (NF- $\kappa$ B), transforming growth factor- $\beta$  (TGF- $\beta$ ), mitogen-activated protein kinase (MAPK), sirtuin-1 (SIRT-1), poly ADP-ribose polymerase 1 (PARP-1) and insulin-like growth factor-1 (IGF-1). Eventually, understanding the molecular pathogenesis causing ovarian damage after radiation provides a map toward possible biomarkers and novel targeted therapeutic approaches. This is particularly significant for female cancer survivors offering hope to preserve their fertility and improve their overall quality of life.

## 2. Physiological role of inflammation during ovulation

For ovulation to occur, mature follicles must rupture allowing the release of the formed oocyte. Previous research hypothesized that this rupturing process takes place due to increasing intrafollicular pressure [11]. In parallel, physiological inflammation plays a crucial role in weakening the follicle walls resulting in their rupture. Significant tissue remodeling occurs during ovulation as the follicle grows in size and the thecal layers enclosing the oocyte merge and become thinner to facilitate follicular rupture [12].

It is hypothesized that inflammation can trigger both ovulation and tissue remodeling. Inflammatory processes result in hyperemia, edema, vasodilation, cell proliferation, and

collagen lysis, all of which are reflected in the ovulation process [13]. Prostaglandin levels peak during ovulation and rise in response to luteinizing hormone (LH). Prostaglandins also release proteolytic enzymes like collagenases to break down the connective tissues of the follicle and trigger ovulation [14]. The overuse of nonsteroidal anti-inflammatory drugs inhibits prostaglandin-endoperoxide synthase and is linked to reversible infertility in women. This is most likely due to ovulation failure [15]. Tumor necrosis factor alpha (TNF- $\alpha$ ) and IL-6 generated by macrophages increase C-reactive protein (CRP), an inflammatory biomarker, whose levels are known to vary during a woman's menstrual cycle, particularly around ovulation [16].

Proinflammatory cytokines are produced during the folliculogenesis process and are thought to help inducing ovulation [13]. IL-18, for example, is vital to follicular expansion and oocyte growth. A strong association between IL-18 follicular levels and the number of oocytes recovered and successful implantation supported the involvement of IL-18, while women suffering unexplained infertility exhibited decreased levels of IL-18 [17]. These findings signify that a controlled inflammatory response is vital for the proper ovulation process.

### **3. Uncontrolled inflammation contributes to ovarian dysfunction**

On the other hand, emerging evidence shows that misguided inflammation can interfere with normal ovarian dynamics, resulting in infertility. External activators, such as radiation, can disrupt this delicate equilibrium in the ovaries, resulting in a cascade of inflammatory events that are responsible for the excessive release of proinflammatory mediators [18]. Excessive inflammation not only impairs the quality and number of oocytes but also increases the risk of POF [19].

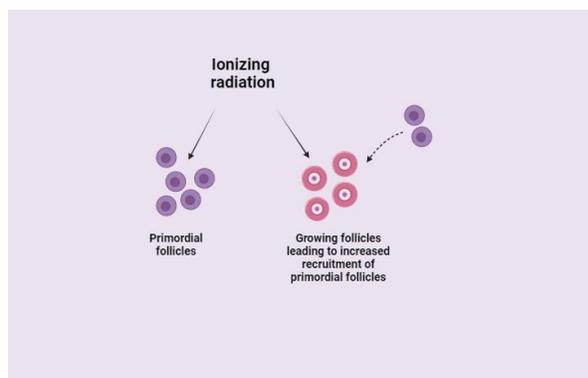
Lower chances of conception can be attributed to increased content of IL-6. Besides, increased follicular levels of TNF- $\alpha$  were reported to result in poorer quality oocytes and thereby, decreased fertility [20]. Furthermore, IL-15 is one of the important ILs that negatively impact oocyte development. Its levels were reported to increase in women who failed assisted reproductive techniques multiple times than in women who achieved full pregnancy [21].

### **4. Radiotherapy-induced ovarian follicle loss**

Radiation therapy is commonly used as it can cause DNA damage and inhibit further replication and cell growth. However, the radiation field may involve healthy tissues near the tumor that are unintentionally exposed [3]. In general, cells that experience frequent cell division and active DNA replication are more liable to damage by radiation. Whereas those with a lower rate of division appear to be more resistant to it. In terms of the ovaries, are affected by radiation because they consist of rapidly dividing cells, especially during the early stages of follicular development. These rapidly multiplying cells can suffer DNA damage resulting in dysfunction of these cells or complete death [22].

Within 6 months of gestation, oocytes are held inside primordial follicles, relying on neighboring somatic cells to survive. At any moment, a small portion of follicles are recruited out from this resting pool and begin to mature. Only one of these activated follicles will survive to form a Graafian follicle. At this point, LH triggers this Graafian follicle to rupture and liberate fully developed oocytes into fallopian tubes boosting the likelihood of effective fertilization. The others will die from atresia [23]. The presence of growing follicles affects the rate at which follicles exit the resting primordial pool [24].

As seen in **Fig. 1**; radiation may have a direct impact on the primordial follicles resting pool or the population of growing follicles. Because growing follicles restrict the recruitment of other primordial follicles to the growing pool, the loss of this population will result in higher activation of primordial follicles and therefore the loss of ovarian reserve [25].



**Fig. 1.** Ionizing radiation targets inside the ovary

Ionizing radiation can directly affect the primordial follicles' resting pool or affect the population of growing follicles, leading to higher activation of primordial follicles and subsequent depletion of ovarian reserve.

## 5. Factors affecting ovarian radiosensitivity

Radiosensitivity in the ovaries is affected by several factors, with radiation dose being a key factor determining the severity of damage. Fractionation of this dose of radiation, the irradiated field, age of the patient at treatment, targeted cells, and individual variations all contribute to the susceptibility of the ovarian cells to radiation damage. Lower doses of radiation are associated with a lower number of damaged follicles. That is besides the potential of DNA repair of follicles affected by radiation. Higher doses of  $\gamma$ -irradiation accelerate DNA damage [26]. The dose of radiotherapy sufficient to damage 50% of total ovarian follicles was estimated and calculated to be within 2 Gy [8, 27].

Besides, the toxicity of single large doses of radiotherapy is more damaging in comparison to

low fractionated ones [28]. Subjecting the total body to irradiation with fractionated doses exhibited a lower incidence of ovarian failure even after using higher total doses of radiation. High doses tend to kill cells which leads to a quicker response from the whole body. cells:s low doses are more likely to cause mutations inside these cells; thus, the outcomes might not be noticed for many years [29].

Furthermore, the location of the ovaries concerning the field of radiotherapy has a role in risking POF stating that around 68% of patients with both ovaries inside the abdominal radiation fields suffered from POF, in comparison to only 14% of patients that had their ovaries located at the edges of radiation field exhibited ovarian damage [30]. Moreover, females having both of their ovaries exposed to IR experienced an increased risk of POF than those who had only one ovary radiated [31].

In addition, the severity of radiotoxicity in the ovaries was reported to change with age. Younger patients are stated to be more likely to withstand radiation damage as they usually have a larger pool of primordial follicles [29, 30]. Besides, past research used a model to estimate the age at which POF is expected to occur after radiation exposure using the age of the patient and the dose of radiation. They assessed the effective sterilizing dose (ESD) to be at birth 20.3 Gy, while at 10 years the dose is likely 18.4 Gy. At 20 years, the dose is decreased to 16.5 Gy, and 14.3 Gy at 30 years [29].

The type of targeted cells also shapes the impact on ovarian function. Cells that divide rapidly are more vulnerable to radiation than those cells that divide at a slower rate. The germ cells supporting the growth and maturation of the oocytes are more radiosensitive than the stromal cells that give structural support to the ovary [32].

Genetic factors also play a role in individual radiosensitivity, signifying the importance of personalized risk assessment. For example, mutations in the genes responsible for DNA repair or cell signaling pathways may make some patients more vulnerable to ovarian damage than others. For example; females lacking the ataxia telangiectasia mutated gene (ATM) responsible for DNA damage response (DDR) may lead to numerous defects in checkpoints and ultimately increased apoptosis [33].

Recognizing these factors is vital for tailoring strategies to mitigate radiation toxicity to the ovaries providing insights to preserve fertility in females undergoing radiation treatment.

## **6. Molecular targets implicated in radiation-induced ovarian failure**

### **6.1. Oxidative stress and inflammation**

The most generally accepted explanation for ovarian damage after radiation exposure lies beyond granulosa cells' capacity to combat reactive oxygen species (ROS) [34]. Water is the most abundant molecule within cells and is thus a target for radiolysis by gamma radiation to form ROS [35]. ROS like hydrogen peroxide ( $H_2O_2$ ), superoxide ( $O_2^-$ ) and hydroxyl radical ( $OH^\cdot$ ) are chemically unstable. When they exceed the levels of cellular antioxidants, they cause a state of oxidative stress damaging cellular structures like proteins and DNA [36]. The increase in ROS levels in the ovaries affects granulosa cells [37]. This decreases the interaction between granulosa cells and oocytes decreasing the availability of components and nutrients necessary for maturation thus leading to poor-quality oocytes [38]. The physiological processes involved in oocyte maturation, fertilization, and growth of embryo development up to pregnancy are all impacted by ROS [39]. Recently, emerging evidence has been focusing on oxidative stress as the main player in radiation-induced POF [37].

However, misguided inflammatory response is closely linked to the formation of ROS [43]. This can be confirmed by the increased production of inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 [40]. Further research revealed elevated levels of IL-1 $\beta$ , IL-4, and IL-6 levels in the follicular fluid and granulosa cells (GCs) in POF patients. In particular, IL-4 was further reported to inhibit GC proliferation in *in vitro* studies. Besides, ROS may induce excessive inflammation by initiating inflammatory signaling pathways such as NF- $\kappa$ B, which contributes to ovarian dysfunction. Ultimately, excessive oxidative stress and inflammation reactions are the main causes of the detrimental effects of IR [41].

Investigating the role of ROS in inflammation gives a deeper view of the complex interplay of cellular processes offering insights about developing viable radioprotective strategies for these patients. In parallel, the ovarian microenvironment represents an atmosphere of cell survival in which numerous intercellular components influence ovarian function. It includes an array of signals that aid in intercellular communication [42]. Oxidative stress-induced damage to biomolecules can result in the generation and release of cytokines by activating endogenous damage-related biochemical pathways in the body. Cytokines identify receptors and activate downstream signaling pathways, resulting in increased cytokines and inflammatory factors production, recruitment, and activation of additional inflammatory cells, as well as a persistent inflammatory response in the body [43].

### **6.2. Nuclear factor kappa-B (NF- $\kappa$ B)**

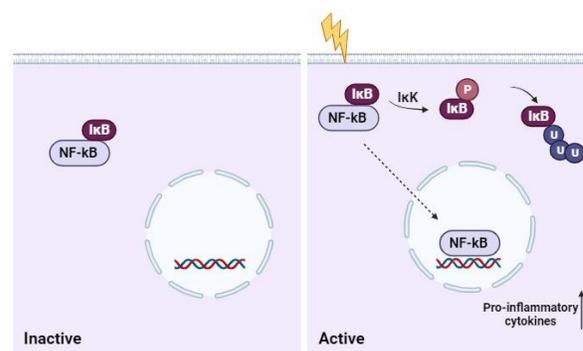
Reviewing literature about radiation-induced damage to the ovaries, studies reported several signaling pathways being exaggerated upon radiation exposure. Among these pathways is NF- $\kappa$ B signaling [44]. NF- $\kappa$ B; a protein specific

to B-cells; is capable of binding to a specific site in the immunoglobulin kappa light chain gene enhancers. It consists of five related transcription factors: p50, p52, RelA (p65), c-Rel, and RelB1. It plays significant roles in cell survival, immune response, and DNA repair [45]. Its regulation involves translocation into the nucleus and acetylation. However, excessive activation of NF- $\kappa$ B is a potent contributor to the production of inflammatory genes, including TNF- $\alpha$ , IL-1, IL-6, and IL-8, which are involved in inflammation, and further tissue injury. In normal conditions, the inhibitor of the kappa-B (I $\kappa$ B) protein is responsible for sequestering NF- $\kappa$ B in the cytoplasm [46]. However, upon stimulation, it is released from I $\kappa$ B and then binds to DNA to regulate gene transcription. Upon radiation exposure, a kinase complex (IKK) comprising; I $\kappa$ B kinase (IKK $\alpha$  and/or IKK $\beta$ ) and regulatory non-enzymatic scaffold protein NEMO (NF- $\kappa$ B essential modulator also known as IKK $\gamma$ ) is activated [47].

As shown in **Fig. 2**; IKK then phosphorylates I $\kappa$ B, which induces its degradation by the proteasome and activates the NF- $\kappa$ B dimers. The active NF- $\kappa$ B transcription factor subunits thus can enter the nucleus to induce the expression of target genes as pro-inflammatory cytokines, chemokines, and adhesion molecules [48].

ROS production after irradiation is directly related to the progression of inflammatory processes [39]. Excessive ROS binds to integral membrane receptors that trigger IKK $\beta$  kinase which phosphorylates I $\kappa$ B $\alpha$  resulting in it being detached from NF- $\kappa$ B. Once activated, NF- $\kappa$ B is relocated to the nucleus and produces inflammation markers like ILs and TNF- $\alpha$  [49]. In parallel, activation of NF- $\kappa$ B itself stimulates a set of enzymes that lead to more ROS generation, for example, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, cyclooxygenase-2 (COX-2), and

inducible NO synthase (iNOS) [50]. Ovarian injury and follicular atresia were noticed after the initiation of this inflammatory response in a model of radiation-induced POF in rats [51]. This was demonstrated by the elevated protein expressions of the inflammatory markers: NF- $\kappa$ B, COX-2, iNOS, and TNF- $\alpha$ , in the ovaries of the irradiated animal group. Besides, ILs can act as signals binding to specific cell surface receptors initiating the activation of NF- $\kappa$ B and subsequent signaling cascades till the generation of further inflammatory cytokines [52]. Thereby, targeting NF- $\kappa$ B could regulate inflammation and slow down the progression of POF.



**Fig. 2.** NF- $\kappa$ B signaling between the inactive state and active one in response to radiation exposure

NF- $\kappa$ B is, normally bound to I $\kappa$ B in the cytoplasm, when activated, it is released from I $\kappa$ B and binds to DNA to regulate gene transcription. I $\kappa$ B, inhibitor of nuclear factor- $\kappa$ B; IKK, inhibitor of nuclear factor- $\kappa$ B kinase; NF- $\kappa$ B, Nuclear factor kappa-B.

### 6.3. Mitogen-Activated Protein Kinase (MAPK)

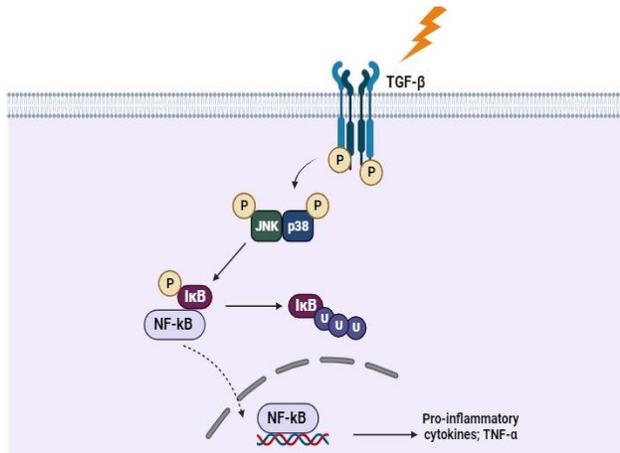
MAPK is an intracellular enzyme that plays a crucial role in converting extracellular signals into various physiological reactions, including cell proliferation and inflammation. However, for these processes to start, ligand molecules such as growth factors and cytokines must attach to cell membrane receptors, such as G protein-coupled receptors or receptor-tyrosine kinases [53]. Conventional MAPKs typically have three family members, which include c-Jun N-terminal kinases (JNK), and extracellular signal-regulated

kinases (ERK), in addition to MAPK p38. The initiation of this pathway occurs through a series of MAPK kinases [54]. Different stimuli activate different MAPK members, ERK is triggered with growth factors, stress is reported to activate JNK, while p38 is stimulated *via* stress as well as inflammatory cytokines [53]. After activation, MAPK moves into the nucleus phosphorylating transcription factors and subsequently influences the expression of inflammatory genes such as COX-2, TNF- $\alpha$ , and IL-1 $\beta$  [55]. Moreover, it was also noted that both JNK and p38 enhance NF- $\kappa$ B signaling by promoting the degradation of I $\kappa$ B resulting in the release and expression of more proinflammatory mediators [56]. Moreover, TNF- $\alpha$  as well as other released pro-inflammatory cytokines will bind to their receptors and then activate both MAPK and NF- $\kappa$ B. As a result, a circle of inflammatory reactions is repeated leading to more tissue damage [57]. When it comes to radiation exposure, radiation acts as a stressor triggering the MAPK pathway and setting off cellular reactions that eventually cause cellular malfunction due to inflammation and apoptosis [58]. In another study, irradiated rats showed upregulated levels of active forms of p38 and JNK in comparison to normal rats in a model of radiation-induced POF confirming the involvement of MAPK signaling in radiation-induced ovarian damage [51]. Consequently, both MAPK and NF- $\kappa$ B are linked to one another. Therefore, in the context of POF, targeting NF- $\kappa$ B and MAPK signaling can be considered.

#### 6.4. Transforming growth factor- $\beta$ (TGF- $\beta$ )

The TGF- $\beta$  receptor is involved in various cell processes like cell growth, immune responses, and tissue repair [59]. Despite its anti-inflammatory effects on immune responses, over-activation of TGF- $\beta$  led to exaggerated levels of pro-inflammatory mediators. It can interfere with

other signaling pathways, such as MAPK, resulting in various consequences on the activity of cells [60]. Besides, in another study, the authors observed increased contents of TGF- $\beta$  in the ovarian tissues of irradiated rats [61]. Subsequently, TGF- $\beta$  activation prompted the activation of downstream MAPKs as mirrored in elevated amounts of phosphorylated forms of p38 and JNK in a model of radiation-induced POF in rats. TNF- $\alpha$ , NF- $\kappa$ B, COX-2, and iNOS levels were also measured in the ovaries of the irradiated rats, exhibiting higher levels in comparison to normal rats. All of which suggested the association of TGF- $\beta$  in inflammation induction following radiation exposure. In another work, the authors used human corneal epithelial cells being pre-treated with TGF- $\beta$  to demonstrate the relevance of TGF- $\beta$  to NF- $\kappa$ B activation [62]. At the end of the experiment, TGF- $\beta$  activation was associated with a significant decrease in (I $\kappa$ B), indicating higher NF- $\kappa$ B translocation into the nucleus and thus increased inflammatory response. Moreover, they observed that NF- $\kappa$ B p65 subunit levels were higher in TGF- $\beta$ -treated cells, stating that TGF- $\beta$  signaling stimulation activated the NF- $\kappa$ B pathway. Building on the previous findings, TGF- $\beta$  activation might be involved in the inflammatory response associated with radiation-induced ovarian injury. The induction of inflammation *via* TGF- $\beta$  following radiation exposure can be illustrated in **Fig. 3**. Accordingly, targeting the TGF- $\beta$ /MAPK cascade can be considered as a potential therapeutic approach regarding radiation-induced POF.



**Fig. 3.** TGF- $\beta$  activation in response to radiation exposure

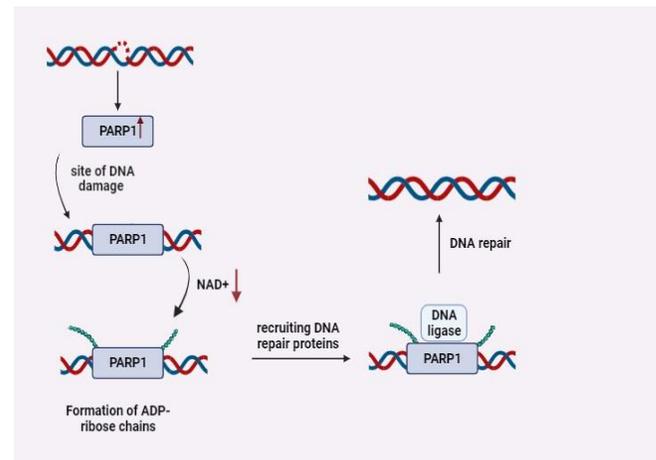
Radiation stimulates TGF- $\beta$  signaling that in turn activates downstream p38 and JNK, stimulating the degradation of NF- $\kappa$ B inhibitory proteins. I $\kappa$ B, inhibitor of nuclear factor- $\kappa$ B; JNK, c-Jun N-terminal kinases; NF- $\kappa$ B, Nuclear factor kappa-B; p38, mitogen-activated protein kinase MAPK p38; TGF- $\beta$ , transforming growth factor- beta.

### 6.5. Poly (ADP-ribose) polymerase 1 (PARP-1)

Multiple studies have repeatedly demonstrated that radiation exposure activates PARP-1, particularly in the context of repairing DNA damage. PARP-1 is a nuclear enzyme participating in DNA damage repair mechanisms. As shown in **Fig. 4**; PARP-1 senses single-strand breaks (SSBs) in DNA and then becomes activated. Through its DNA binding domain, PARP-1 binds to these gaps. Following that, it goes through a conformational change that allows it to attach to nicotinamide adenine dinucleotide (NAD<sup>+</sup>) and catalyze the transfer of ADP-ribose from NAD<sup>+</sup> to nucleoprotein targets. As a result, poly (ADP-ribose) chains are formed, serving as signals for recruiting DNA repair proteins, and subsequently, accelerating the restoration of genomic integrity. Yet, overstimulation of PARP-1 can deplete cellular energy stores of NAD<sup>+</sup> leading to cellular death [63].

Compelling evidence has linked PARP-1 inhibition to lower inflammatory responses, implying a relationship between DNA damage repair and the activation of pro-inflammatory

signaling cascades. In particular, there is a direct correlation between the activation of PARP-1 and NF- $\kappa$ B following radiation exposure [64]. PARP-1 can activate NF- $\kappa$ B by preventing its deacetylation ending with more expression of inflammatory genes [65]. Besides, PARP-1 enhances the activity of NF- $\kappa$ B which can further result in upregulated tumor suppressor protein (p53) expression which subsequently increases PARP-1 expression [62].



**Fig. 4.** PARP-1 activation in response to DNA damage after radiation exposure

PARP-1 is activated when it detects DNA single-strand breaks (SSBs) and binds to damaged DNA through its binding domain using NAD<sup>+</sup> as a substrate. After that, it forms poly (ADP-ribose) chains which recruit DNA repair enzymes and restore genomic integrity. NAD, Nicotinamide adenine dinucleotide; PARP-1, poly ADP-ribose polymerase-1.

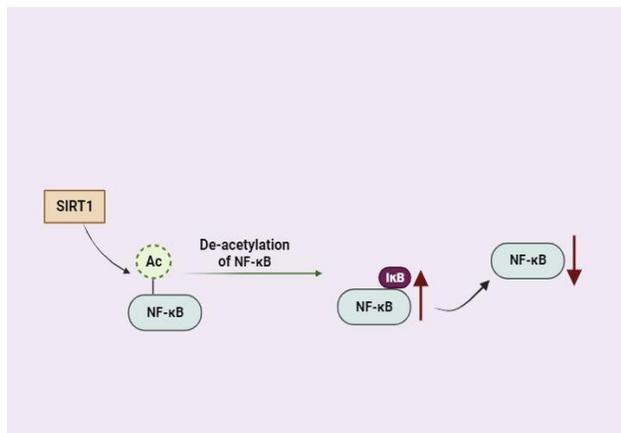
In addition, it was reported that IR causes DNA damage, which increases the activation of PARP-1 [66]. Besides, PARP-1 was also stated to promote NF- $\kappa$ B binding to DNA enhancing its transcription of inflammatory genes [67]. In another study done on a model of radiation-induced POF in rats, irradiated ovaries showed higher PARP-1 and NF- $\kappa$ B expression, as well as enhanced production of IL-6 and TNF- $\alpha$  following radiation exposure confirming the involvement of PARP-1 and NF- $\kappa$ B in radiation-induced POF [68]. It was also reported that

PARP-1 activation has a role in the pathophysiology of immunological ovarian failure [69].

As a result, the observed effects of PARP-1 inhibition on decreasing radiation-induced inflammation suggest the possibility of including PARP-1 inhibitors as adjuvants in radiation toxicities.

### 6.6. Sirtuin-1

SIRT-1 is a histone deacetylase from class III that depends on  $\text{NAD}^+$  to control metabolism, cellular survival, cell development, and stress responses. NF- $\kappa$ B undergoes post-translational acetylation increasing its activity [70]. As shown in Fig. 5.



**Fig. 5.** SIRT-1 inhibiting NF- $\kappa$ B activation in physiological conditions

NF- $\kappa$ B undergoes acetylation, a post-translational change that increases its activity. SIRT-1 deacetylates NF- $\kappa$ B, making NF- $\kappa$ B binds more to I $\kappa$ B, and inhibiting its release and transcriptional function. Ac, acetyl group; I $\kappa$ B, an inhibitor of nuclear factor- $\kappa$ B; NF- $\kappa$ B, Nuclear factor kappa-B; SIRT-1, sirtuin-1.

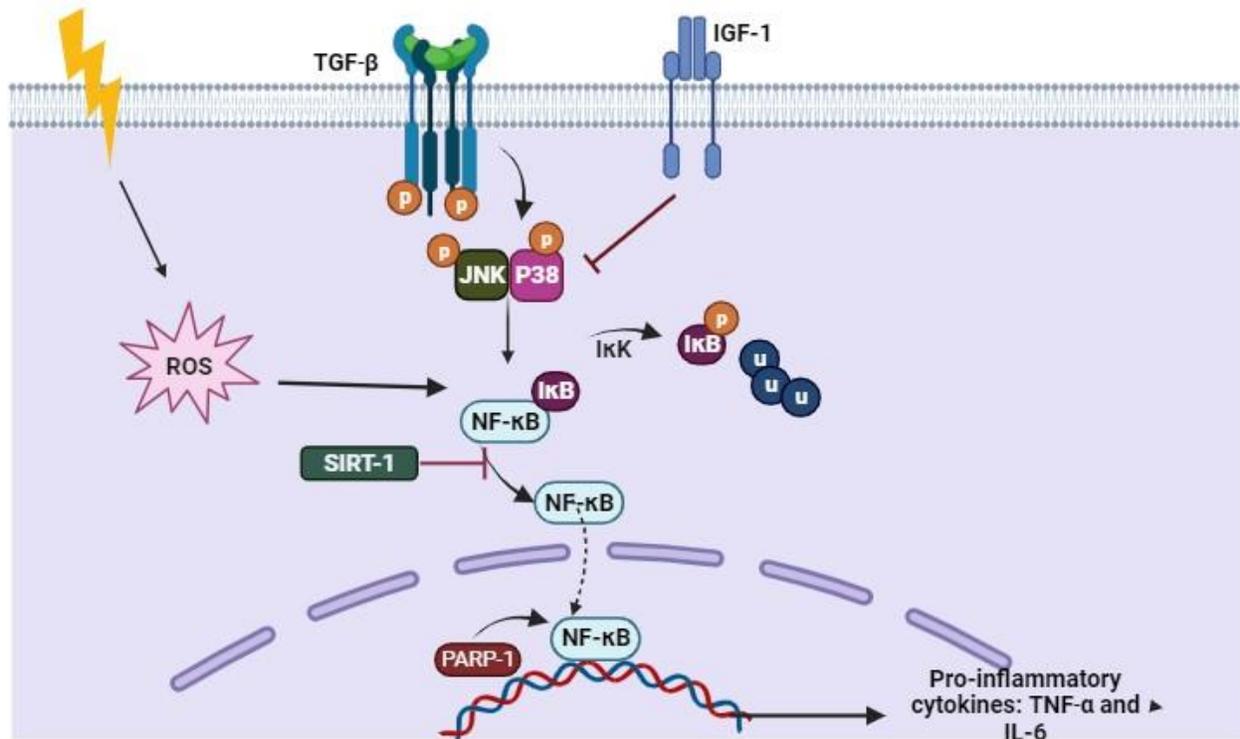
In normal conditions, SIRT-1 deacetylates NF- $\kappa$ B, which makes NF- $\kappa$ B bind more to its inhibitor protein, I $\kappa$ B, and thereby its transcriptional function decreases. PARP-1 and SIRT-1 were documented to inhibit each other [71]. They compete together for cellular  $\text{NAD}^+$  needed for NF- $\kappa$ B activation [64]. Activation of SIRT-1 by resveratrol led to cellular growth and

increased progesterone levels [67]. It was also shown that activating SIRT-1 resulted in a substantial reduction in radiation-induced inflammation in the irradiated ovaries of female rats. Previous observations regarding the activation of SIRT-1 and the decrease of radiation-induced inflammation imply the potential of using SIRT-1 activators as adjuvants in radiation-induced ovarian injuries.

### 6.7. Insulin growth factor-1 (IGF-1)

IGF-1 is another important peptide essential for cellular growth and maintenance [72]. Several studies have related IGF-1 to the regulation of ovary function increasing the proliferation of granulosa cells and ovarian follicles, as well as enhancing the synthesis of sex hormones [73]. Studies involving gene knockout techniques have confirmed the fundamental role of IGF-1 in reproductive physiology. Mice lacking the IGF-1 gene exhibited difficulty in conceiving, as they were unable to ovulate even after gonadotropin injection [74]. IGF-1 signaling has been shown to protect against radiation-induced damage [75]. IGF-1 was also documented to inhibit P38/JNK in MAPK signaling decreasing inflammatory responses [76]. In addition, in another study; there was an inverse relationship between IGF-1 and TNF- $\alpha$  in the ovaries of irradiated rats [10]. The irradiated rats exhibited a decrease in IGF-1 expression with higher TNF content in comparison to normal rats. In parallel, the treatment with carvacrol led to a significant decrease in TNF- $\alpha$  levels and counteracted the downregulation of IGF-1 following radiation exposure confirming the IGF-1 anti-inflammatory effects. Accordingly, enhancing IGF-1 activity offers a potential radioprotective intervention regarding radiation-induced ovarian damage.

In the end, Fig. 6 summarizes all the inflammatory pathways involved in radiation-induced POF.



**Fig. 6.** A scheme summarizing all the inflammatory pathways being activated following exposure of the ovaries to ionizing radiation

Radiation exposure induces reactive oxygen species (ROS) generation within ovarian cells. These ROS are reported to activate IκB kinase (IκK) leading to the phosphorylation of inhibitor of kappa-B (IκB) targeting it for subsequent degradation and thus, releasing NF-κB into the nucleus and activating pro-inflammatory genes. TGF-β also is stimulated by radiation exposure, which in turn activates downstream signaling and phosphorylation of JNK and p38 MAPK members, which ultimately enhances NF-κB activity for more inflammatory responses. Simultaneously, the MAPK pathway can be triggered following radiation and activates NF-κB creating amplified inflammatory effects. Likewise, PARP-1 is activated after the DNA damage caused by radiation exposure, which is reported to enhance the binding of NF-κB to DNA and activate the transcription of pro-inflammatory genes. Conversely, SIRT-1 activity is reduced after radiation exposure, leading to a reduction of its anti-inflammatory effects and enhancing NF-κB activation. Likewise, IR exposure alters the expression of various growth factors, including IGF-1 and its receptors which are reported to play a significant role in modulating MAPK pathways. Thereby, MAPK will be activated leading to stimulation of NF-κB and the release of further inflammatory cytokines. All of these processes influence the development of radiation-induced POF. IκB, inhibitor of nuclear factor-κB; IκK, inhibitor of nuclear factor-κB kinase; JNK, c-Jun N-terminal kinases; MAPK, mitogen-activated protein kinase; NF-κB, Nuclear factor kappa-B; PARP-1, poly ADP-ribose polymerase-1; p38, MAPK p38; SIRT-1, sirtuin-1; TGF-β, transforming growth factor- beta

## Conclusion

Individuals with normal ovarian function exhibit a well-coordinated ovarian microenvironment. The activation of NF-κB is finely controlled ensuring an appropriate inflammatory response without excessive damage. TGF-β maintains its regulatory role contributing to immune response and cellular homeostasis. MAPK signaling is quietly

regulated promoting cellular responses without inducing an exacerbated inflammatory state. The interrelation between PARP-1 and SIRT-1 remains balanced, allowing effective inhibition of NF-κB and facilitating DNA repair mechanisms. IGF-1 interactions function optimally promoting cell survival and protecting the tissues from external damage.

In contrast, in females exposed to radiation,

the internal network of inflammatory signaling pathways undergoes dysregulation contributing to a compromised ovarian microenvironment. Exposure to radiation triggers excessive activation of NF- $\kappa$ B resulting in an exacerbated inflammatory response and impacting the function of the ovaries. The regulation of TGF- $\beta$  becomes imbalanced disrupting immune responses and cellular processes. Misguided activation of the MAPK pathway will intensify the expression of pro-inflammatory genes causing more tissue damage. SIRT-1 activity is downregulated, potentially decreasing its ability to modulate inflammation. PARP-1 becomes overstimulated and its opposition with the reduced SIRT-1 decreases, depleting energy stores and causing cell death. IGF-1 interactions may be altered, impacting cell survival mechanisms. Collectively, these dysregulated events create an unfavorable environment inside the ovaries promoting early cessation of ovarian function.

### Abbreviations

Ac, Acetyl group; AMH, Anti-Müllerian hormone; ATM, Ataxia telangiectasia-mutated gene; COX II, Cyclo-oxygenase 2; CRP, C-reactive protein; DDR, DNA damage response; E2, Estradiol; ERK, Extracellular signal-regulated kinases; ESD, Effective sterilizing dose; GCs, Granulosa cells; H<sub>2</sub>O<sub>2</sub>, Hydrogen peroxide; IGF-1, Insulin-like growth factor-1; I $\kappa$ B, Inhibitors of kappa-B; I $\kappa$ K, I $\kappa$ B kinase; IL, Interleukin; iNOS, Inducible NO synthase; IR, Ionizing radiation; JNK, Include c-Jun N-terminal Kinases; LH, Luteinizing hormone; MAP2K, MAP kinase kinase; MAP3K, MAP kinase kinase kinase; MAPK, Mitogen-activated protein kinase; NAD<sup>+</sup>, Nicotinamide adenine dinucleotide; NADPH, Nicotinamide adenine dinucleotide phosphate; NEMO, Regulatory non-enzymatic scaffold protein; NF- $\kappa$ B, Nuclear factor kappa B; O<sup>2-</sup>, Superoxide; OH, Hydroxyl radical; PARP1,

Poly [ADP-ribose] polymerase 1; POF, Premature ovarian failure; ROS, Reactive oxygen species; SIRT1, Sirtuin 1; SSBs, Single strand breaks; TBI, Total body irradiation; TGF- $\beta$ , Transforming growth factor- $\beta$ ; TNF- $\alpha$ , Tumor necrosis factor-alpha.

### Declarations

#### Ethics approval and consent to participate

Not applicable

#### Consent to Publish

Not applicable

#### Availability of Data and Materials

All data generated or analyzed are included in the main manuscript. All figures included in this manuscript were designed by the authors. These figures are uploaded again separately for high resolution.

#### Competing Interests

No competing interests.

#### Funding Statement

No funding source was received.

#### Authors' Contributions

Idea Conceptualization: Eman M. Mantawy, Riham S. Said, Doaa H. Mohamed. Writing the Original Draft: Doaa H. Mohamed. Revision and approval of the final version of the manuscript: Eman M. Mantawy, Riham S. Said, Dina H. Kassem, Amany M. Gad, Ebtehal El-demerdash.

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