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Mini-Review

Developments in reproductive biology and medicine

Immune system regulation of physiological and pathological aspects of the ovarian follicle pool throughout the female reproductive lifespan

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ABSTRACT

The immune system plays a major role in ovarian physiology by regulating the ovarian follicle pool through complex signaling of different growth factors, cytokines, and chemokines. These may promote follicle activation and further growth but could also trigger follicle atresia and clearance of aging or damaged cells within the ovarian cortex. Moreover, extraglandular steroidogenesis potentially occurring in different immune cells like macrophages and natural killer cells might be another way of modulating follicle growth. Ovarian macrophages have recently been found to contain two different populations, namely resident macrophages and monocyte-derived cells, with potentially different roles. The immune system also plays a role in the development of pathological conditions, including premature ovarian insufficiency (POI). Indeed, autoimmune activation against various ovarian antigen targets results in lymphocytic oophoritis mainly targeting early growing follicles, but later leading to complete follicle pool depletion. Immune-mediated ovarian damage may also be caused by viral infection or be the consequence of iatrogenic damage. Certain novel cancer immunotherapies like checkpoint inhibitors have recently been shown to induce ovarian reserve damage in a murine model. Studies are needed to corroborate these findings and further investigate the potential of newly developed immunotherapies to treat POI. Technological advances such as single-cell analyses of less represented cell populations like immune cells inside the ovary are now contributing to valuable new information, which will hopefully lead to the development of new therapeutic strategies for women with fertility issues.

Keywords: immune system / ovarian reserve / follicle activation / steroidogenesis / macrophages / premature ovarian insufficiency / immunotherapy / female fertility

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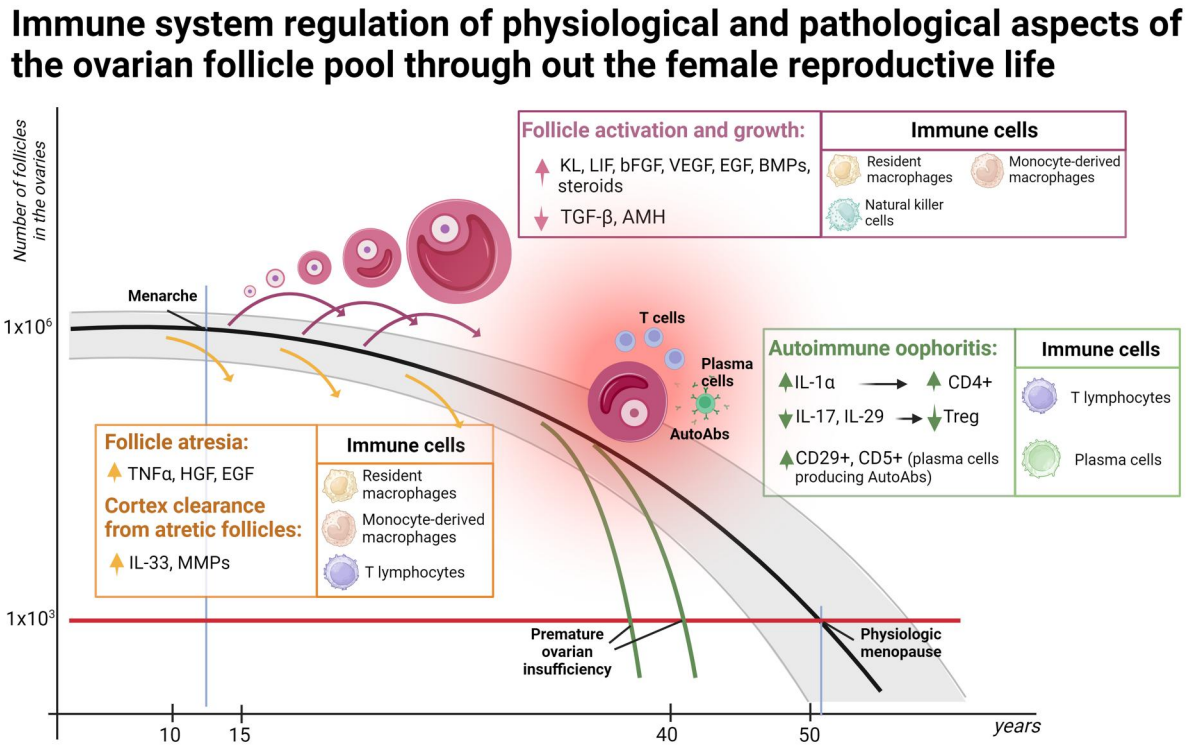
GRAPHICAL ABSTRACT



The impact of the immune system of the ovarian reserve involves different aspects, including (i) regulatory activity on initial follicle activation and growth by macrophages and natural killer cells, (ii) regulation of follicle atresia and clearance of cortex form atretic follicles by integrated action of macrophages and T lymphocytes, and (iii) pathologic immune system activation with adaptive response by T lymphocytes and plasma cells, causing immune oophoritis.

Introduction

The female reproductive lifespan is tied to its resting pool of ovarian primordial follicles, referred to as the ovarian reserve, which is determined at birth, having already undergone drastic degeneration. Indeed, about 85% of its potential oocytes are lost *in utero*, leaving ~300 000 at birth (Wallace and Kelsey, 2010). During a woman's reproductive lifetime, the primordial reservoir contains the majority of follicles within the ovary, which are maintained in a quiescent state in the cortex awaiting recruitment for cyclic growth. It serves as a continuous supply of developing follicles for potential mature oocytes, while experiencing a steady physiological decline until menopause (Grosbois *et al.*, 2020).

Follicle recruitment, activation, and further development are gradual processes independent of the gonadal axis, requiring orchestrated and sequential secretion of autocrine and paracrine factors from different cell populations within the ovary (Skinner, 2005). Among specific molecular pathways known to play a crucial role in follicle activation, the phosphoinositol-3-kinase (PI3K)/protein kinase B (Akt) and Hippo pathways have been widely investigated as key players in initial follicle growth (Kawamura *et al.*, 2013; Zhang and Liu, 2015; Grosbois *et al.*, 2020). As such, their dysregulation leads to abnormal follicle activation followed by follicle burn-out in pathological conditions, like after chemotherapy exposure (Grosbois *et al.*, 2020).

The entire process of folliculogenesis has been estimated to take around 200 days in humans, of which only the last 15 are gonadotropin-dependent, before culminating in ovulation (Gougeon, 1996). Follicle recruitment is controlled by different pathways regulating initial hormone-independent follicle growth (like PI3K/Akt and Hippo) and various paracrine signals from a number of growth factors (Grosbois *et al.*, 2020). Other general determinants, including nutrition, chronic stress, and exposure to endocrine disruptors, also have an influence on follicle dynamics, having been found to correlate with accelerated follicle depletion in mouse models (Wang *et al.*, 2014; Hu *et al.*, 2018). In any case, only a small proportion of follicles actually reach ovulation, while the vast majority undergo atresia, a mechanism of programmed cell death, through either activation of the caspases cascade culminating in DNA fragmentation and cell death, or accumulation of autophagosomes ending in autophagic cell death (Cacciottola *et al.*, 2023). Although a number of autocrine and paracrine signals able to regulate follicle growth have been discovered over the years, the precise factors responsible for determining the fate of each follicle (activation for growth or atresia) are still unknown.

Evidence of the possible impact of immune cells and their secretome composed of cytokines and growth factors on ovarian reserve regulation first emerged a few decades ago (Skinner, 2005; Grosbois *et al.*, 2020). Among various immune system functions, including protection from pathogens and immune surveillance for cancer prevention, tissue maintenance, and renewal are vital to regulating cell growth and differentiation, and removing aged and dying cells from the ovary. Given the dynamic nature of ovarian function, characterized by cyclical development and regression of tissue structures to facilitate ovulation, the immune system has to play a crucial role in regulating these periodic changes to maintain homeostasis (Duffy *et al.*, 2019; Qi *et al.*, 2023). It is also involved in modulating the somatic aging process, which is characterized by a chronic inflammatory environment

that targets cells expressing aging markers like epigenetic alterations and genomic instability (Zeng *et al.*, 2024).

In the ovaries, there is a specialized immune microenvironment characterized by the presence of diverse immune cells, including those of the innate (monocytes/macrophages, natural killer [NK] cells, and dendritic cells) and adaptive (T and B lymphocytes/plasma cells) response (Fan *et al.*, 2019). Investigating their role in regulating the ovarian reserve, has nevertheless been quite challenging, as these immune cells represent just a small fraction of the ovary compared to other cell types and organs. Technological advances using single-cell analyses to explore less-represented cell populations in tissues are now contributing to valuable new information to this field. The aim of this review is to integrate the latest findings, looking to shed light on biological mechanisms that may be involved in ovarian reserve regulation in both physiological and pathological conditions.

Paracrine signaling to regulate folliculogenesis

The ovarian microenvironment contains a number of cytokines and growth factors secreted by resident immune cells to exert their function of immune surveillance and organ function maintenance. These interact with the ovarian follicle pool to keep the right balance between stimulatory and inhibitory signals. Stimulatory factors are involved in primordial follicle activation and transition to the primary stage, while other factors exert inhibitory effects to avoid massive follicle activation, as may occur in non-physiological conditions like after transplantation of ovarian cortical strips for fertility restoration (Masciangelo *et al.*, 2019; Cacciottola *et al.*, 2021). Their degree of involvement varies greatly, making some of them indispensable, and others just amplifiers of signaling pathways.

Stem cell factor, also known as kit ligand (KL), is one of the key factors regulating initial follicle recruitment and growth. Its absence in mutant mice homozygous for the hypomorphic KL $Steel^{panda}$ allele decreases ovarian expression of KL, blocks follicles at the primary stage (Huang *et al.*, 1993), and impairs ovulation (John *et al.*, 2009). KL binds to its receptor c-kit to activate different signaling pathways, including PI3K for the regulation of cell survival and proliferation (Parrott and Skinner, 2000; Hutt *et al.*, 2006). In human ovarian tissue, higher c-kit expression in primordial follicles indicates loss of quiescence, as observed in xenotransplantation models used for long-term follow-up of human folliculogenesis occurring in ovarian cortical strips transplanted to immunodeficient mice (Gallardo *et al.*, 2018; Cacciottola *et al.*, 2020).

Leukemia inhibitory factor is expressed in granulosa cells of early-stage ovarian follicles and its addition to *in vitro* culture of rat ovaries was shown to promote primordial-to-primary follicle transition (Nilsson *et al.*, 2002). It acts via Janus kinases (JAKs), which in turn phosphorylate docking sites for signal transducers and activators of transcription (STATs), phosphorylated by JAKs. Phosphorylated STAT 3 dimerizes and then migrates to the nucleus, where it binds to specific regulatory sequences to activate transcription of cytokine-responsive genes like SOCS proteins (Croker *et al.*, 2008; Sutherland *et al.*, 2012).

Basic fibroblast growth factor (bFGF) was used to boost primary follicle growth rates in cultured rat ovaries, and its neutralizing antibody to inhibit such activation (Nilsson *et al.*, 2001). Its suppression in mutant mice, homozygous for loss of function of the FGF-2 allele, was not found to impair folliculogenesis (Dono *et al.*, 1998), pointing to a role in follicle growth rather than initial activation. In humans, bFGF supplementation had a protective effect on the follicle pool after ovarian tissue xenotransplantation, suggesting a favorable impact on both primordial follicle survival and promotion of further growth. Follicle protection could either be the direct result of bFGF acting on the follicle pool via paracrine signaling, or indirect due to increased vascularization sustained by bFGF within the ovarian cortex (Tanaka *et al.*, 2018).

Several members of the transforming growth factor beta (TGFβ) superfamily have been extensively investigated in different animal models as well as humans and shown to exert crucial functions in folliculogenesis. Among them, anti-Müllerian hormone (AMH) was found to negatively regulate primordial follicle development in mice (Roness *et al.*, 2019; Sonigo *et al.*, 2019) and humans (Man *et al.*, 2017). This growth factor is differentially expressed in granulosa cells according to follicle stage, being initiated at the primary stage, reaching its peak during the late secondary/early antral stage, and then dropping with accelerating antral follicle growth (di Clemente *et al.*, 2021). AMH plays a role in follicle recruitment over time by inhibiting signals for follicle activation and growth in the primordial follicle pool (Grosbois *et al.*, 2020; di Clemente *et al.*, 2021). Other members of the TGFβ family are less specific and may be produced by different cell types, including immune cells. Bone morphogenetic protein (BMP)-7 and -15 were shown to regulate primordial follicle growth (Dube *et al.*, 1998; Lee *et al.*, 2004), and BMP-4 to promote primordial-to-primary transition (Nilsson and Skinner, 2003).

While growth factors have often been linked to positive effects in terms of follicle growth and development, some cytokine dysregulation causing a significant upturn, as in case of acute or chronic inflammation, can have a detrimental impact on the ovarian reserve. One of the most widely investigated in the context of ovarian follicle depletion is tumor necrosis factor α (TNFα), signaling cytokine for ovulation, and corpus luteum formation (Chen *et al.*, 2022a), which may also cause increased follicle atresia in murine models (Yamamoto *et al.*, 2015; Winship *et al.*, 2022).

Growth factors and cytokines acting as signaling molecules may be synthesized by a wide range of cell types, including non-immune cells like stromal cells and granulosa cells in growing follicles (Adamczak *et al.*, 2021). This makes it difficult to determine to what extent resident immune cells contribute to this network of follicle activation. There is, however, some evidence of a link between the immune cell pool and ovarian reserve status. Accumulation of immune cells, especially CD3+ lymphocytes in borderline ovarian tumors, appeared to be correlated with a decrease in ovarian follicle activation from the follicle pool surrounding a tumoral mass, according to a recently published paper (Cacciottola *et al.*, 2024). The immune microenvironment also looks to be altered around ovarian endometriotic lesions, characterized by an increase in M2 macrophage polarization and a decrease in NK cell activity compared to native endometrial tissue (Quan *et al.*, 2024). M2 macrophages inhibit cell clearance and promote tissue remodeling, leading to chronic inflammation and fibrosis deposition around lesions. This has direct repercussions on the ovarian reserve, as primordial follicles undergo accelerated depletion with increased apoptosis rates in response to fibrosis (Kitajima *et al.*, 2011, 2014).

Irrespective of which cell population is responsible for initial cytokine secretion, immune cells are responsive to this signaling, possibly amplifying and coordinating different tissue functions (Wu *et al.*, 2004; Lliberos *et al.*, 2021; Ben Yaakov *et al.*, 2023).

Interactions within the ovarian follicle pool may therefore occur on many levels, either through crosstalk with resident immune cells or after signal amplification and recruitment of circulating immune cells.

Macrophages inside the ovary: origin, function, and interaction with the follicle pool

Macrophages are the most widely represented immune cell population in the ovary (Wu et al., 2004). Ovarian macrophages were recently found to express different phenotypes depending on their origin, either monocyte-derived macrophages or tissue-resident cells. Recent studies in mice were able to distinguish these two distinct populations using specific markers (Jokela et al., 2020; Zhang et al., 2020; Li et al., 2022) (Fig. 1). The former originate from circulating bone-marrow-derived monocytes and migrate from the bloodstream to the ovary in response to paracrine signaling in both physiological and pathological settings. The latter, more recently identified as originating from the yolk sac, migrate to the differentiating organ during embryogenesis. There they reside long term in a steady state, thanks to their self-renewal capacity and independence from circulating cells (Hoeffel et al., 2012; Shaw et al., 2018; Dick et al., 2019).

These different macrophage populations are both present in the ovaries and have overlapping functions. However, recent findings from single-cell analysis of immune cells from murine ovaries at different ages showed that these two distinct populations are not stable over time. Indeed, the number of tissue-resident cells decreases with increasing age, leaving more space for monocyte-derived macrophages (Zhang et al., 2020). Higher expression levels of chemokines (like CCL2, CCL3, CCL5, CCL7, and CCL12) were observed in the ovaries of older mice and they were able to recruit more monocyte-derived macrophages and myeloid cells (Zhang et al., 2020).

Macrophages may participate in different activities, including amplification of immune signaling through secretion of growth factors, cytokines, and chemokines for different purposes, but also phagocytosis and extracellular matrix remodeling. Ovarian macrophage phagocytosis is mainly oriented toward recognizing and removing aging, damaged or apoptotic cells (Itoh et al., 1999). As part of the innate immune system, they also work alongside NK cells for surveillance of viral infections and other pathogens (Tomac et al., 2021).

In addition, macrophages clear the ovarian cortex of follicles at advanced stages of atresia (Zhang et al., 2020). Their recruitment appears to be mediated by different signaling, including the secretion of IL-33 and metalloproteinases (MMPs). IL-33 is a cytokine belonging to the IL-1 superfamily and part of the inflammatory signaling cascade acting upon various immune cell activity depending on the specific tissues in which it resides. It plays a key role in tissue healing, as demonstrated by significantly slower wound healing in IL-33 knockout mice (Oshio et al., 2017). In the ovary, IL-33 is expressed by pericytes around the microvasculature and its absence (in IL-33−/− mice) has been associated with abnormal accumulation of tissue waste in murine ovarian cortex (Carlock et al., 2014; Wu et al., 2015). Macrophage-secreted MMP3 was found to be upregulated in ovarian cortex around atretic follicles (Hagglund et al., 1999). However, it is yet to be determined whether macrophage-driven signaling is also involved

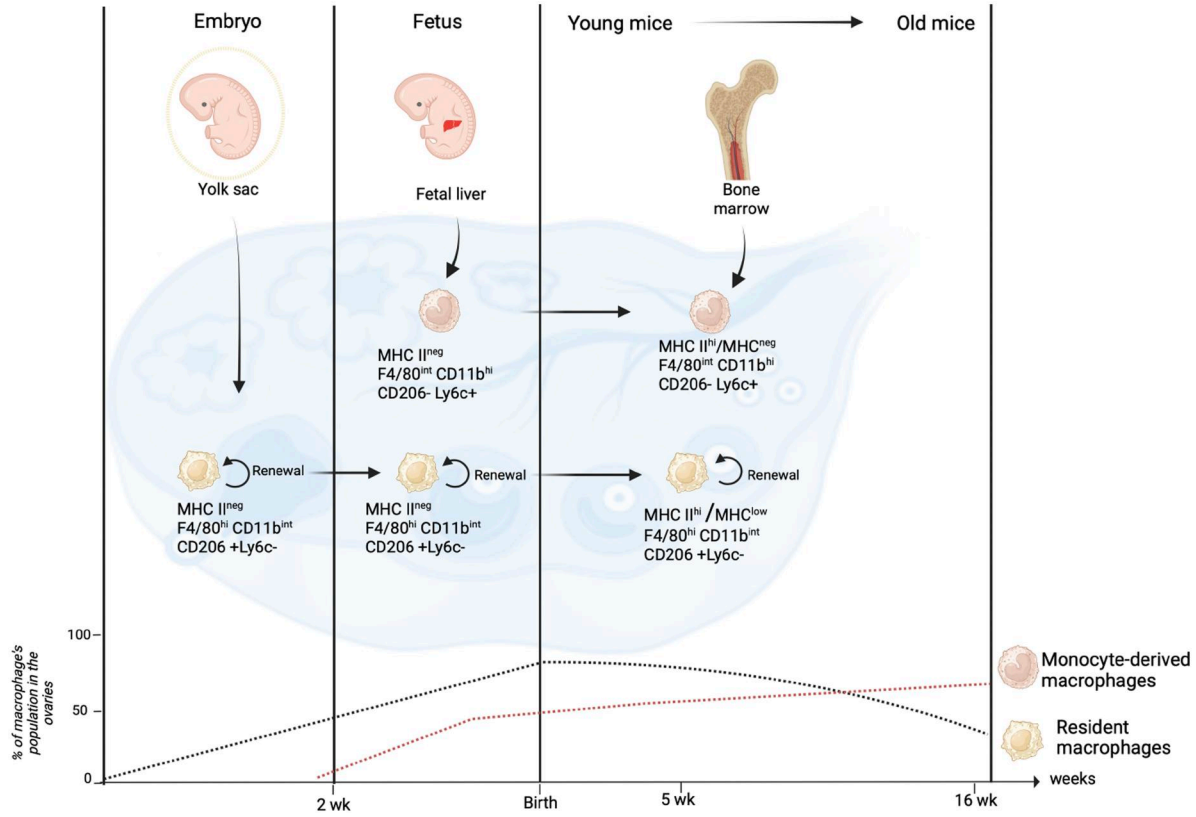


Figure 1. Resident and monocyte-derived macrophages in ovarian tissue. Representation of embryologic origin and distribution inside the ovaries over time of two macrophage populations (monocyte-derived macrophage and resident macrophages) in mice. MHC, major histocompatibility complex.

in initiating the cascade leading to follicle atresia, whose regulation in humans is still largely unknown.

Since numerous cytokines and growth factors have been shown to regulate granulosa cell survival and proliferation *in vitro* and *in vivo*, it is possible that follicle atresia may also be at least partially governed by immune cells in the ovary. Signaling by bFGF, vascular endothelial growth factor and epithelial growth factor have a pro-survival effect on granulosa cells (Yamamoto *et al.*, 1997; Mao *et al.*, 2004; Kang *et al.*, 2017), while others like TNFα and hepatocyte growth factor appear to promote apoptotic signaling in murine granulosa cells (Uzumcu *et al.*, 2006; Yamamoto *et al.*, 2015; Winship *et al.*, 2022).

Macrophage migration inhibitory factors (MIFs), produced by different immune cells including macrophages themselves as well as T cells to regulate macrophage migration, also have an impact on folliculogenesis. Indeed, inhibition by polyclonal anti-MIF antibodies was associated with a decrease in follicle growth and an increase in follicle morphological aberrations in mice (Matsuura *et al.*, 2002). A different tendency was also observed in macrophage polarization according to increasing age (Zhang *et al.*, 2020). Although able to express a broad phenotypic spectrum based on the local tissue microenvironment, activation signaling, and expected function, macrophages commonly polarize toward two phenotypes, M1 initiating a pro-inflammatory response and M2 linked to wound healing and immunomodulation (Ginhoux *et al.*, 2016). M2 polarization in the ovary, activated by a number of cytokines including IL-4 and IL-13, did indeed appear to be more related to monocyte-derived macrophages at an older age. This aging phenotype therefore looks more oriented to tissue remodeling, as IL-4 and IL-13 are critical factors for collagen deposition, resulting in increased ovarian fibrosis (Zhang *et al.*, 2020).

On the other hand, M1 polarization in ovarian macrophages was shown to be related to their proangiogenic function. Rapid cyclical angiogenesis occurs inside the ovary for microvessel enrichment in the theca layer to support follicle growth, ovulation, corpus luteum formation, and regression. In this context, M1-like macrophages and dendritic cells support new vessels by remodeling the surrounding extracellular matrix, further promoting vessel stability. This provides structural support for perivascular pericytes through platelet-derived growth factor B signaling (Hellström *et al.*, 1999; Lindblom *et al.*, 2003). M1 and M2 polarization therefore appear to change with increasing age, the former in favor of proangiogenic activity for good follicle growth and ovulation at a young age, and the latter responsible for tissue remodeling occurring later in the reproductive lifespan.

Elimination of CD11c+ cells, which include M1-like macrophages and dendritic cells, was found to reduce the numbers of pericytes and endothelial cells surrounding follicles in mice treated with Cb11-diphtheria toxin receptor (DTR) injection for macrophage depletion (Ono *et al.*, 2018). However, the same effect was not observed when CD206+ M2-like macrophages were depleted using CD206-DTR injections, suggesting the involvement of M1-like macrophages in this specific function (Ono *et al.*, 2018; Zhang *et al.*, 2021). Indeed, macrophages play a key role throughout follicle growth until corpus luteum formation by sustaining follicle vasculature remodeling for optimal growth. This was established by experiments investigating the effect of macrophage ablation with Cb11-DTR, which resulted in extensive hemorrhaging affecting both theca and luteal tissue, as well as ovarian damage and necrosis (Turner *et al.*, 2011).

Extraglandular steroidogenesis and steroid metabolism to regulate ovarian function

Immune cells in the body are able to metabolize sex steroids, like tissue-resident macrophages converting androstenedione to potent androgens through 5α-reductase activity in the lungs (Milewich *et al.*, 1983) and monocyte-derived macrophages converting androgens to estrogens through aromatase activity (Watanabe *et al.*, 2017). Peripheral immune cells involved in type 2 responses are also capable of *de novo* steroidogenesis (Wang *et al.*, 2020), which has recently been evidenced in humans, but its exact function and context are still subject to investigation (Chakraborty *et al.*, 2021). Steroids can indeed act as immunomodulatory signals, including androgens and estrogens playing pro- or anti-inflammatory roles depending on cell type, microenvironment, and endocrine regulation interactions (Dragin *et al.*, 2017).

With extraglandular steroidogenesis emerging as a topic of interest in attempting to understand the mechanisms regulating the immune system, synergy between the latter and all steroid-responsive tissues has reached new levels of complexity. Resident immune cells like macrophages and NK cells are potentially able to modulate estrogen levels locally in growing follicles, hence controlling their growth. This interaction was demonstrated in investigations into polycystic ovarian syndrome pathogenesis using a rat model, where macrophage activation altered ovarian steroid production by increasing androstenedione and decreasing estradiol (Figueroa *et al.*, 2012). Moreover, depending on their polarization, depletion of M1-like macrophages was also associated with reduced serum estradiol levels, as they negatively regulate folliculogenesis, while no such effect was observed after M2-like macrophage depletion (Ono *et al.*, 2018).

Immune response and premature ovarian insufficiency

The blood–follicle barrier, where granulosa cells grow in an avascular environment and provide oocytes with nutrients, confers a certain degree of protection upon immune reactions against the germ line. However, it is sometimes eluded, as in the case of viral infections with ovarian tropism or the occurrence of an autoimmune response, resulting in potential immune-mediated ovarian damage. This inflammatory reaction is called oophoritis and is characterized by different levels of infiltration of the innate (macrophages and dendritic cells) and adaptive (T lymphocytes and plasma cells) immune system, mainly in the theca layer of growing follicles (Silva *et al.*, 2014; Domniz and Meirow, 2019; Sharif *et al.*, 2019).

Some viral infections can cause ovarian oophoritis with long-term detrimental effects on the ovaries, like mumps that increase the risk of premature ovarian insufficiency (POI) (Hviid *et al.*, 2008) and coxsackie virus B that intensifies follicle atresia in a murine model (Shim *et al.*, 2015). Other viruses like hepatitis B (Mak *et al.*, 2020), Epstein-Barr (Virant-Klun and Vogler, 2018), and Zika (Caine *et al.*, 2019), while detected inside the ovaries during acute infections, have no such consequence.

The other main cause of oophoritis is autoimmune activation against various ovarian antigens, potentially leading to POI) due to both follicle dysfunction, namely the inability of growing follicles to develop further and mature, and depletion at a later stage. By then, the ovarian reserve may have been exhausted by immune cell-mediated damage and abnormal follicle activation occurring in the absence of inhibitory stimuli from growing follicles (Chen *et al.*, 2022a). POI is clinically defined by the European

Society of Human Reproduction and Embryology (ESHRE) as menstrual disturbance (amenorrhea or oligomenorrhea) for at least 4 months in the presence of increased FSH levels of >25 IU/l on two occasions more than 4 weeks apart (European Society for Human Reproduction and Embryology (ESHRE) Guideline Group on POI et al., 2016). It can be considered as a continuum state, sometimes overlapping with diminished ovarian reserve (DOR) status and showing an impaired ovarian response to hormonal stimulation. POI fluctuates in a substantial number of cases (up to 20%), with spontaneous irregular ovulations arising after diagnosis (Sharif et al., 2019).

Evidence of a role for the immune system in controlling the ovarian reserve is supported by the high prevalence of concurrent autoimmune diseases in women diagnosed with POI, present in up to 30% of cases (Tucker et al., 2016). Autoimmune conditions most commonly associated with POI are autoimmune polyglandular syndrome type one (APS-1) and type 2 (APS-2), in which around 30% of subjects are affected (Domniz and Meirow, 2019), and Addison's disease, with around 10% (Vogt et al., 2021). POI may also be linked to other non-adrenal autoimmune disorders, such as type 1 diabetes mellitus, thyroiditis, rheumatoid arthritis, systemic lupus erythematosus, and Sjögren's syndrome (Silva et al., 2014).

Autoimmune oophoritis is characterized by lymphocytic infiltration of the ovary, targeting growing follicles but sparing the primordial follicle pool, which may explain the fluctuant nature of ovarian insufficiency. The immune population comprises T cells as major players as well as plasma cells responsible for antibody secretion, with increasing infiltration density depending on follicle maturation stage (Sedmak et al., 1987; Sharif et al., 2019). With respect to systemic T cell activity and local infiltration, a significant drop in Treg cells and upturn in proinflammatory T cells and their associated cytokines was demonstrated in murine models, where an antibody-mediated response against zona pellucida glycoprotein 3 (ZP3) was induced in oocytes to mimic autoimmune POI (Yin et al., 2018; Lu et al., 2019). Moreover, injection of CD4+ T lymphocytes, but not CD8+ T lymphocytes, appeared to trigger oophoritis in immunodeficient (SCID) mice (Ohno et al., 1999). Similar evidence has been mainly reported in human studies reporting small case series (Levit et al., 2024). Greater proportions of CD4+ lymphocytes and proinflammatory T cells along with fewer Treg cells, known to suppress CD4+ activity, have been detected in peripheral blood of POI patients (Kobayashi et al., 2019; Chen et al., 2022a).

Different signaling molecules with regulatory effects on T cells, like IL-29 and IL-17, were found to be lower in serum from women with POI (Liu et al., 2020). Conversely, those promoting lymphocyte differentiation, like IL-6 and IL-21 (Sun et al., 2018) or activating T cells, like IL-1α, were found to be elevated (Yang et al., 2018).

Autoantibody secretion also participates in autoimmune ovarian damage. Specificity may explain why growing follicles containing metabolically active and steroid-producing cells are the target of an autoimmune response, rather than primordial follicles. Various organ- and non-organ-specific autoantibodies are produced by circulating CD19+ and CD5+ B cells in higher numbers in POI patients (Chen et al., 2022b). Antigens to blame for autoimmune activity against the ovaries and their specific role in ovarian damage have only been partially elucidated. Among organ-specific targets, autoantibodies against oocyte structures like the ZP and gonadotropin receptors have nevertheless been described (Anasti et al., 1995; Takamizawa et al., 2007; Sharif et al., 2019). Regarding non-organ-specific autoantibodies, their main targets are enzymes active in steroid-producing cells, including P450-17α-hydroxylase, 3β-hydroxysteroid dehydrogenase, and 21-hydroxylase, which act on granulosa and theca cells as well as adrenal glands in Addison's disease (Domniz and Meirow, 2019; Sharif et al., 2019). Other non-organ-specific autoantibodies found in POI patients are anti-selenium-binding protein 1, anti-α-enolase, and anti-heat shock protein 90 (Sundblad et al., 2006; Pires and Khole, 2009) (Fig. 2).

Dysregulation of the immune system may also have a deleterious effect on the ovarian reserve through cytokine and chemokine generation, resulting in a proinflammatory environment that may amplify follicle atresia at all follicle stages (Zhao et al., 2023; Domniz and Meirow, 2019). This proinflammatory milieu coincides with an increase in oxidative stress. Indeed, reactive oxygen species are active in immune cell recruitment and intensification of inflammation by triggering cytokine and chemokine expression. There is indeed evidence of proinflammatory features in follicular fluid from women with DOR or POI (van Kasteren et al., 2000), including increased oxidative stress-mediated production of chemokines like CCL5, which result in CD8+ T lymphocyte accumulation (Zhao et al., 2023) and boost CD8+ T cell levels producing IFN-γ (Zhao et al., 2022). Further *in vitro* investigations demonstrated a detrimental effect of CD8+ T lymphocytes on granulosa cell proliferation, showing it to be a possible mechanism of action in impaired fertility (Zhao et al., 2023).

A number of aspects remain still unknown in the pathogenesis of autoimmune POI, as does its exact prevalence. We lack specific diagnostic criteria and treatment guidelines for autoimmune POI. Larger studies and randomized controlled trials are needed to define these fundamentals and develop new therapeutic strategies by testing innovative immunotherapies.

Potential ovarian reserve damage due to novel immunotherapy treatments

Over the past few years, immunotherapy has taken center stage in cancer treatment, including three main categories characterized by their mechanisms of action: monoclonal antibodies, immunomodulators (checkpoint inhibitors), and adoptive cell therapy (chimeric antigen receptor [CAR] T cells). Checkpoint inhibitors strengthen a patient's antitumor immune response by blocking cell signaling pathways that tumor cells use to downregulate immune cell activation and upregulate proinflammatory cytokines (Waldman et al., 2020). CAR T cells are engineered fusion proteins that target T cells against a specific antigen present on tumor cells to generate an antitumor immune response. Acute toxicities related to CAR T cell expansion are widely documented, often manifesting as a systemic inflammatory response referred to as cytokine release syndrome (Waldman et al., 2020).

These novel therapies are able to trigger immune cell activation and systemic inflammatory responses with potential repercussions on various organs, including the ovaries (Roberts and Dougan, 2022). While there have been reports of successful pregnancies following immunotherapy with monoclonal antibodies, much less is known about the potential impact of the other two categories, namely checkpoint inhibitors and adoptive cell therapy. These treatments have recently been approved for cancer treatment, but their repercussions on female fertility remain unexplored. No studies on reproductive toxicity were even conducted before FDA approval was granted (Duma and Lambertini, 2020; Alesi et al., 2021; Ligon et al., 2022).

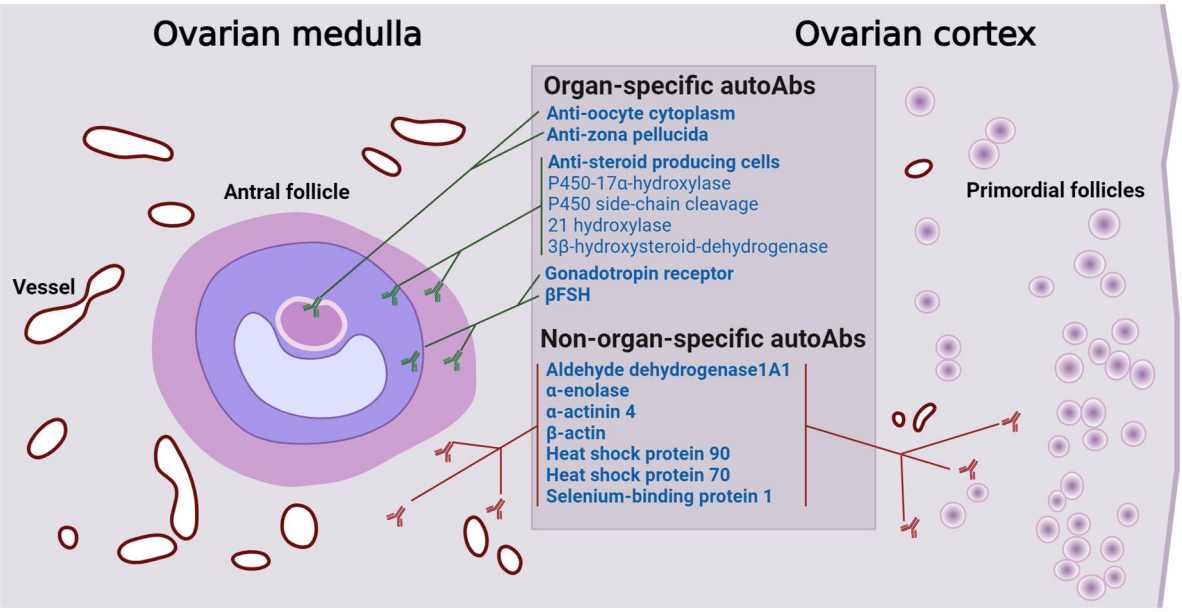


Figure 2. Autoantibodies and their ovarian antigen targets. Representative graph of organ-specific and non-organ-specific autoantibodies targeting different ovarian structures, including primordial and growing follicles, stroma, and vessels. AutoAbs, autoantibodies.

To date, only a few preclinical studies have been undertaken in immunocompetent mice, revealing a reduction in the primordial follicle pool and diminished numbers and quality of mature oocytes after checkpoint inhibitor administration (Xu *et al.*, 2021; Winship *et al.*, 2022). In this animal model, the ovarian reserve appears to be affected through a mechanism potentially involving inflammation-mediated follicle depletion via activation of the extrinsic apoptosis pathway (Xu *et al.*, 2021; Winship *et al.*, 2022). Indeed, CD3+ T cell infiltration and elevated levels of proinflammatory cytokine TNF-α have been encountered in mouse ovaries following immune checkpoint inhibition. Higher intraovarian levels of TNF-α have actually been shown to induce follicle depletion in mice both *in vitro* and *in vivo*. The impact of TNF-α is mitigated in knock-out mice by the BH3-only protein (BID), a proapoptotic protein that mediates amplification of the extrinsic apoptosis pathway, pointing to the key role of BID-mediated apoptosis in follicle depletion after checkpoint inhibitor treatment (Winship *et al.*, 2022). Although the mouse model is a valuable tool to help elucidate the pathophysiology of immunotherapy toxicities, animal models have limitations, as they cannot perfectly replicate complex human immune systems. Further studies should investigate the possibility of translating these results to a clinical setting, as well as testing all novel immunotherapies already in use.

Conclusions

A more in-depth characterization of tissue-specific immunity inside the ovary has recently been made available, generating new hypotheses on its role in regulating different ovarian functions. These include folliculogenesis and hormone generation, but also long-term regulation of the ovarian follicle pool and the impact of cell aging on different ovarian compartments. New and interesting research topics have emerged from this evidence, including (i) differing roles of macrophage subpopulations based on their origin (tissue residents or monocyte-derived cells) in various aspects of ovarian function, (ii) growth factor and cytokine production in regulating the ovarian follicle pool in terms of its physiological growth and follicle selection, (iii) extraglandular steroidogenesis and steroid metabolism implemented by resident immune cells to modulate ovarian function, and (iv) involvement of T lymphocyte infiltration in ovarian follicle pool regulation and potential impairment.

These are all crucial aspects, not only to shed light on the physiological mechanisms of ovarian function that still need to be explored, but to help develop therapeutic strategies to protect the ovarian reserve from abnormal and early depletion. Moreover, the introduction of novel immunotherapy agents like checkpoint inhibitors and CAR T cells will certainly require an assessment of potential iatrogenic damage and the development of appropriate gonadoprotective strategies. Pathological conditions like DOR and POI, be they of autoimmune origin, caused by iatrogenic damage or still unexplained, have been found to affect a significant number of patients referred for infertility treatments. There is therefore a need to raise awareness among healthcare professionals and patients and find solutions to address this clinical issue, which affects both the health and quality of life of increasing numbers of women.

Data availability

No new data were generated or analyzed in support of this research.

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Authors' roles

L.C.: conceptualization, manuscript design, writing and editing; A.C.: conceptualization and manuscript writing; M.-M.D.: manuscript critical revision.

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Conflict of interest

None of the authors has any conflict of interest to disclose.

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