—Review—

Histomorphological aspects of the ovarian cortex regarding ovarian reserve and local pelvic inflammation

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Abstract: The ovaries of women of reproductive age may show specific histological structures that may relate to the maintenance of primordial follicles and the regulation of early follicular development, which are keys to understanding the dynamics of ovarian reserve. The pelvic environment of women is frequently exposed to physiological or pathological inflammatory stimuli. Endometriosis is a disorder that should be viewed as a chronic inflammatory disease manifested by pelvic pain and infertility. Inflammation surrounding the normal ovarian cortex may alter the histological structure which possesses primordial and early growing follicles. Fibrotic changes in histological niches in the nest of primordial follicles may provoke activation of dormant follicles and concomitant atresia. Along with decline in AMH, which is produced by early growing follicles, fibrotic changes may accelerate the demise of primordial follicles which has been described as "burn-out by inflammation". As a result, women with endometriosis may suffer from diminished ovarian reserve, a possible cause of endometriosis-related infertility.
Key words: Inflammation, Ovarian reserve, Endometriosis, Follicle dormancy, Burn-out hypothesis

Introduction

The mammalian ovary consists of germ cells and somatic cells. There are a variety of cellular lineages in the ovary, which support oocyte viability and hormonal production for the maintenance of reproductive functions [1]. The human ovary possesses a unique anatomical

location, and its histological structure may be related to specific reproductive processes and certain pathological conditions. As a moiety exposed to the pelvic cavity, the human ovary may be prone to subtle changes in the local pelvic environment.

The term ovarian reserve is currently defined as the number and quality of follicles left in the ovary at any given time [2]. Acquired and environmental conditions, such as genetic inheritance, pathological diseases, and iatrogenic disturbances including pharmacological, radiological and surgical interventions may affect ovarian reserve [3]. Although the mechanisms that maintain ovarian reserve in the human ovary are not fully elucidated, physiological or pathological inflammation in the local pelvic environment may affect the dormancy of primordial follicles, the status of which directly relates to ovarian reserve.

In this review, we focus on the relationship between the histological structure of the human ovary and ovarian reserve. The possible role of local inflammation in the regulation of early follicular growth is also discussed. We hypothesize that endometriosis adversely affects ovarian reserve through a burn-out phenomenon induced by chronic pelvic inflammation.

Normal Histology of Human Ovary

Tissue and cellular components of human ovary

The cellular components of human ovaries are similar to many other mammalian species that are utilized as laboratory animals [1]. However, the relative size, anatomical location, and histological structure may not completely coincide across species. Histologically, the adult human ovary can be separated into two parts, the cortex and the medulla. The ovarian cortex is the outer shell of the human ovary and it is covered by a monolayer of ovarian surface epithelium (OSE), which is a continuum

of the peritoneum. The OSE may show a flat to cuboidal to columnar appearance in different areas of the same ovary. The epithelium can be invaginated to form epithelial inclusion glands. They become cystic, resulting in epithelial inclusion cysts, which have been postulated as the origin of ovarian endometrioma based on the metaplasia hypothesis [4, 5]. Follicles are functional units which maintain the competence of dormant and growing oocytes via surrounding granulosa cells and theca cells. Folliculogenesis in human ovaries begins in the inner part of the cortex at 14 to 20 weeks of gestation. At birth, approximately 400,000–800,000 primordial follicles are packed into the ovarian cortex. In women of reproductive age, primordial follicles are found scattered irregularly in clusters (nests) throughout a narrow band in the superficial cortex (Fig. 1). Surrounding the follicles, there are fibroblast like stromal cells and blood vessels. Spindle-shaped ovarian stromal cells are typically arranged in a storiform pattern. The appearance and density of stromal cells may differ according to the histological sites of the ovarian cortex and medulla [6, 7]. The characteristics of these cellular components may be affected by menstrual cycles and the growth stage (maturation) of follicles that differ in sensitivity to gonadotropin or juxtacrine stimuli. Stromal cells surrounding early follicles may act as mediators of nutrients and molecular signals as well as a source of somatic cells for growing follicles [8–11]. Extracellular matrix in the ovarian cortex, such as reticulin, and specific collagen fibers, may serve as a rigid frame to supporting follicles and stromal cells [1]. Microfibrils, a subset of the collagen fibers that form extracellular matrix may also serve as signal transduction mediators for dormant and early growing follicles that lack vascular channels [12, 13].

Specific histological findings of reproductive-age women

As mentioned above, in the ovaries of women of reproductive age, primordial follicles are present as a nest in the cortex. The distance from the surface of the ovary to the nest of primordial follicles is within the range of one to two mm, which suggest subtle environmental alterations surrounding the ovarian cortex may affect the maintenance of the nest of primordial follicles (Fig. 1).

The human ovary has a layered histological structure. The ovarian cortex is composed of ovarian surface epithelial cells, a zone of hypocellular connective tissue (tunica albuginea), and an area of cortex-specific stroma with early follicles in a layered structure [6, 14]. Outermost is the OSE, which is derived from peritoneal mesothelium. Beneath the OSE, tunica albuginea, which may not be as a distinct histological structure in the fe-

male gonad as in the homonymous structure in the male gonad, consists of hypo-cellular connective tissue. The cortex-specific stroma is made up of tightly bound fascicles, identified as a strip of strongly hematoxylin and eosin–stained cells due to increased cellular density at the border of the corticomedullary junction [6]. Areas containing advanced follicular structures, such as antral follicles, corpus luteum and corpus albicans, and edematous stroma with spiral vessels or large arterioles may be considered as medullar regions [6, 14]. Incessant ovulation after menarche may structurally alter the ovarian cortex. In women of reproductive age, dormant primordial follicles are present as nests in the cortical specific stroma. To maintain dormancy, primordial follicles may need rigid surroundings, which may be formed by extracellular matrix, such as specific collagen and microfibrils. Mechanical or biochemical breakdown of the rigid surroundings may provoke activation of dormant primordial follicles [15]. Once activated, the majority of primordial follicles are destined to proceed to atresia. Atresia of early follicles begins with degradation of the oocytes, followed by degradation of the surrounding granulosa cells. These atretic early follicles may show specific histological features, such as chromatin condensation, piknosis, fragmentation, cytoplasmic vacuolation, and detachment of the basement membrane [16]. Histological atretic features may vary in follicles at advanced stages.

Inflammation of Tissues Surrounding the Human Ovary

Physiological conditions

During menstruation, menstrual blood may enter the pelvic cavity by retrograde flow through the Fallopian tubes and fimbriae. Menstrual blood may contain blood cells, such as erythrocytes, cell debris, humoral mediators, and contaminated pathogens. The contaminants in retrograde menstruation may induce local inflammation which may be regarded as a physiological process that can be observed in most women [17]. The ovarian surface may be exposed to retrograde menstruation every month in eumenorrheic women. An immunologically competent pelvic environment may be able to eradicate these contaminants, and activated macrophages may accumulate on the surfaces of ovaries (Fig. 2).

Ovulation may cause inflammatory reactions surrounding the ovarian cortex. Follicular fluid may contain large amounts of pro-inflammatory prostaglandins, growth factors, cytokines and chemokines [17, 18]. These factors may provoke local inflammation in tissues surrounding the ovaries at the time of ovulation, and leakage of fol-
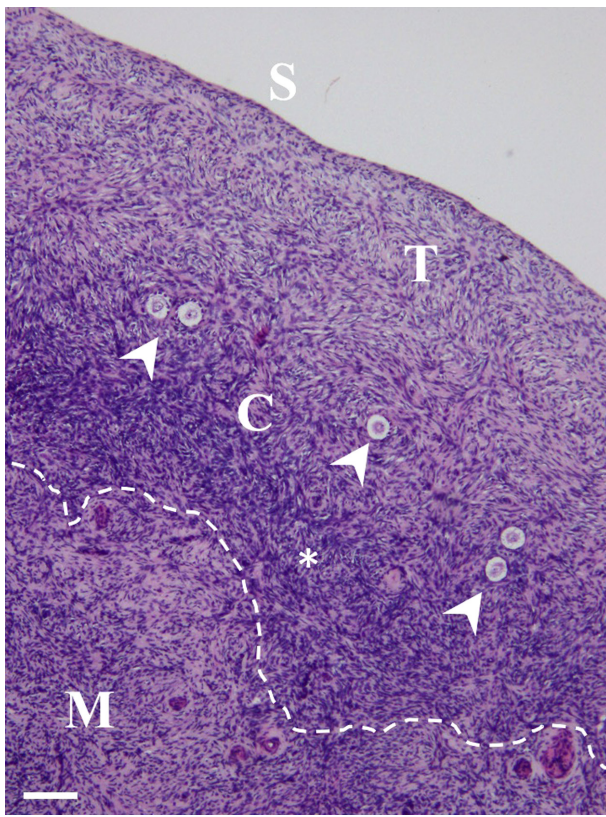
Fig. 1. Representative photomicrograph of ovarian tissue derived from women of reproductive age (hematoxylin-eosin [H&E] staining). Histology of the ovary shows a layered structure. The ovarian cortex harbors primordial follicles with dense specific stroma. This ovarian tissue was harvested in the follicular phase. S: surface of the ovary, the ovarian surface epithelium is denuded and absent from this specimen. T: tunica albuginea, C: cortex, M: medulla, *: cortex specific stroma. Arrowheads indicate primordial follicles. The dotted line indicates the inferred corticomedullary border. Bar: 0.1 mm
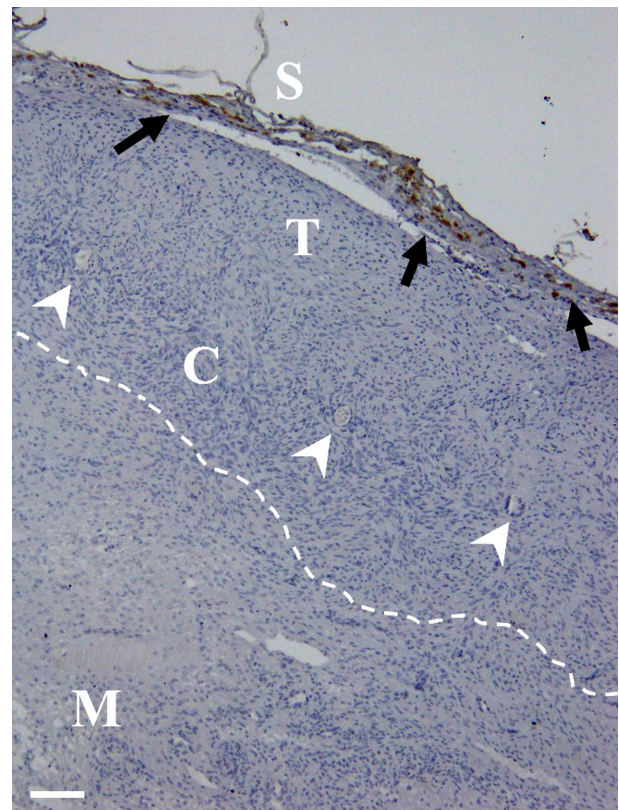
Fig. 2. Representative photomicrograph of immunohistochemical staining of CD68, which may detect activated macrophages. CD68-positive macrophages (black arrows) are present on the ovarian surface with fibrous deposits which may indicate local inflammation surrounding the ovary. This ovarian tissue was harvested in the follicular phase. S: ovarian surface, T: tunica albuginea, C: cortex, M: medulla. White arrowheads indicate primordial follicles. The dotted line indicates the inferred corticomedullary border. Bar: 0.1 mm

licular fluids. The rupture of ovulatory follicles may also cause bleeding. The inflammation may be self-limiting [17], however, if an intra-pelvic pathology, such as endometriosis, is present, and pro-inflammatory mediators may influence the development and progress of the disease. These processes may also cause structural alterations in tissues surrounding ovulatory follicles where primordial follicles are situated, and ovulation may affect the dormancy of these resting follicles by disrupting the rigid stromal environment.

Pathological conditions

Endometriosis may be viewed as a chronic inflammatory disease of the pelvis. The ovary is a common site of endometriosis, and an ovarian lesion may form a cystic lesion, which is called an endometrioma or ovarian chocolate cyst. The pathogenesis of endometrioma is still a matter of debate, however, metaplasia of OSE is one of the hypotheses proposed to explain the formation of endometrioma [4, 5]. In retrograde menstruation, desquamated endometrial cells are transported into the pelvic cavity. A superficial ovarian endometriotic lesion, a very early form of ovarian endometriosis, may be formed by attachment of endometrial cells to the OSE which may cause local destruction of the normal histological structure. Ovarian superficial endometriosis may be associated with accumulation and activation of pelvic macrophages. Progression of the formation of an endometriotic lesion in the superficial ovarian cortex may provoke invagination of the OSE, and may facilitate cyst forma-

tion. Accumulated macrophages may secrete repertoires of growth factors and pro-inflammatory cytokines and chemokines, including hepatocyte growth factor (HGF), which would promote cell migration, metaplasia, and angiogenesis [19]. HGF and cooperation with other pro-inflammatory macromolecules may induce metaplastic changes in the linings of inclusion cysts resulting in the formation of an epithelial inclusion cyst, which can evolve into an ovarian epithelial neoplasm. Recently, it has been reported that ovarian endometrioma is a precursor lesion of ovarian epithelial carcinomas, such as clear cell, endometrioid, and low-grade serous carcinomas [20]. Pelvic inflammation may also be involved in this carcinogenic process [20, 21].

Reactive oxygen species (ROS) have been implicated in the pathogenesis of many human diseases and aging [22, 23]. ROS may act physiologically in normal female reproductive functions, such as oocyte maturation, ovarian steroidogenesis [24], ovulation [25], luteal function and luteolysis [24]. Chronic inflammation may cause oxidative stress in the local pelvic environment. Peritoneal fluids from women with endometriosis may show increased oxidative stress [26]. Activated macrophages in the peritoneal cavity potentially promote the production of ROS and reactive nitrogen species, cytokines, growth factors and prostaglandins [22, 26]. These factors are involved in cellular proliferation and apoptosis pathways. In women with endometriosis, the ovarian superficial cortex may be exposed to increased oxidative stress, which may be partly involved in disturbances in the maintenance of early follicular development and the stability of tissues surrounding follicles [27].

Structural Changes in Human Ovarian Histology Associated with Inflammation

In the ovarian cortex, certain histological alterations associated with inflammation in the surrounding pelvic environment may occur. On the ovarian surface, inflammation evoked by mechanical or biochemical stimulus may cause macrophage accumulation (Fig. 2). Limited inflammation due to physiological processes such as ovulation and menstruation may converge with the stable histological structure by tissue remodeling. If inflammation leads to a pathological reaction mainly due to an altered local pelvic environment, such as one induced by endometriosis, it may cause overproduction of fibrous deposits rather than physiological tissue remodeling. Chronic and repeated inflammatory reactions on the ovarian surface may cause accumulation of fibrous deposit results in fibrosis in the ovarian cortex and altera-

tions to the histological structure, such as loss of cortical specific stroma and vascular channels [7]. These structural alterations may affect the dormancy of primordial follicles situated in the ovarian cortex.

Inflammation Is a Possible Cause of Follicle Activation

In ovaries under the influence of chronic pelvic inflammation, depletion of primordial follicles may occur. Indeed, some women with small endometriomas (before surgery) may show diminished ovarian reserve [28]. Even women with early endometriosis without endometriomas may show lower serum levels of anti-Müllerian hormone (AMH) than women without endometriosis [29].

Early follicular development may be activated and follicular atresia increased in the presence of chronic local pelvic inflammation. In a normal cortex surrounding small endometriomas, the number of primordial follicles is less and the number of primary follicles is higher than in ovaries without endometriomas [30]. At the same time, the number of morphologically atretic follicles is higher in ovaries with endometriomas [30]. These results indicate that upregulated recruitment and the subsequent demise of early follicles through atresia may result in focal exhaustion of primordial follicles. Activation of follicular recruitment and subsequent atresia has also been reported in studies of ovarian cortical graft implantation [31, 32]. The association between fibrosis and reduced ovarian reserve was also demonstrated in these studies and in a study of women with gonadotoxic anti-cancer chemo-therapy [33]. Hypoxic stress and reduced vascularization after the implantation of ovarian tissue in the host has been postulated as a cause, and inflammation and fibrosis may be associated with reduced vascularization and increased oxidative stress, and may also be responsible for follicle activation with subsequent follicle loss [34, 35]. The involvement of oxidative stress in the pathogenesis of infertility related to endometriosis has also been reported [26, 36–38].

The loss of follicular dormancy may be described as burn-out of early follicles by local inflammation [30]. As discussed above, chronic inflammation may cause activation of primordial follicles and atresia thereafter. The decline in early developing follicles may result in local loss of AMH. AMH serves as an inhibitory paracrine factor maintaining follicle dormancy in the ovarian micro-environment. Reduction of AMH in the tissues surrounding a follicular nest may lead to further activation of primordial follicles that subsequwntly undergo atresia, and this dysregulation of early folliculogenesis may cause a vi-

cious cycle leading to over-activation and enhanced atresia, which may eventually result in diminished ovarian reserve in women with endometriosis [30].

Conclusions

The histological features of human ovaries should be taken into consideration to understand species-specific reproductive processes and pathologies related to infertility. Chronic local pelvic inflammation may lead to the destruction of the normal histological structure of the ovarian cortex which nourishes dormant primordial follicles. Inflammation may cause focal fibrosis in the ovarian cortex, and alterations in the rigid histological structure surrounding follicles may activate primordial follicles and concomitant atresia. Decreased vascularization and oxidative stress may be responsible for these events, and repeated occurrences eventually result in diminished ovarian reserve in women with endometriosis, a chronic inflammatory disease.

Conflicts of Interest

None.

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