REVIEW ARTICLE

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Granulosa cells and follicular development: a brief review

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INTRODUCTION

The main consequence of delaying motherhood is compromising a woman's reproductive life with a decline in fertility. A critical step in assisted human reproduction is the evaluation of the quality of oocytes and embryos before embryo transfer, and age is a predominant factor in that capacity. In fact, granulosa cells (GCs) have been proposed as fundamental for the quality of oocytes due to their close biodynamic interrelationship[1,2]. Also, GCs with *theca cells* are the main steroidogenic cells of the ovary. The development of follicles in the ovaries begins with the proliferation of GCs, which change their shape from flat to cubic and from a single layer to a multilayer, depending on the follicular phase[3-5]. The aim of the present document was to perform a narrative review, focusing on the role of GCs in follicle maturation and oocyte quality, as well as in the molecular mechanisms involved in this process.

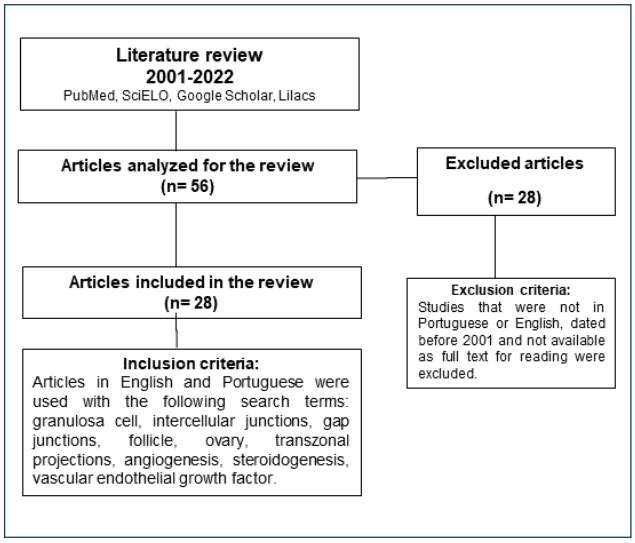

Figure 1. Algorithm of search strategy for the narrative review.

METHODS

This is a narrative review of experimental studies on the physiology of GCs.

Data sources and search strategy

To identify the relevant studies, databases, namely, PubMed, SciELO, Google Scholar, and Lilacs, were accessed from January 1997 to November 2022 with no constraint on the year of publication. Retrieval of the articles was carried out using the search strategies described in Figure 1. To supplement the search, references from retrieved articles were also examined for further data recovery (Figure 1).

Study selection and quality assessment

Study selection and assessment of titles and abstracts were conducted independently by two blinded researchers (G.S.C. and C.S.F.) with strict observation of the inclusion and exclusion criteria. In the next stage, the selected articles were critically assessed for inclusion or non-inclusion in this review. When there was disagreement between the two researchers, a third reviewer (J.M.S. Jr) was consulted.

The inclusion criteria were as follows: (a) availability of complete text; (b) articles written in Portuguese, English, Spanish, and French; and (c) in vitro and histological studies related to GCs.

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RESULTS

Granulosa cells

Granulosa cells are characterized by having a squamous and cuboid shape, which gradually becomes cubic. Although GCs are sources of pro-angiogenic factors for the developing follicle, these cells make up an avascular layer that surrounds the oocytes in the cortical region of the ovary and are separated from the theca cells (which are vascularized) by a basal lamina[6-13].

During puberty, with the production and secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by the anterior pituitary gland, the development and maturation of primordial follicles begin in the peripheral region of the ovarian cortex, a process called folliculogenesis[8,12,13]. The first stage of follicular development is marked by egg enlargement (the diameter increases from two to three times the original size), proliferation, and morphological alteration of the GCs, which change from epithelial to cuboid cells. Studies show that this change in GC morphology is closely linked to the regulation of steroidogenesis and cell proliferation in follicles[8,12-16].

The process of follicular development proceeds with a succession of mitoses and the formation of other cell layers composed of GCs. The primary follicle is then called a secondary follicle. Subsequently, spindle cells cluster around GCs, giving rise to a second cluster of cells called the theca. This cell layer, unlike the one composed of GCs, is divided into two layers (internal and external). In the theca interna, cells are responsible for the production of androgenic steroids, namely, androstenedione and testosterone, in response to LH[8,17].

The GCs produce a follicular fluid that has multiple functions, including oocyte maturation regulation and follicle growth control. This follicular fluid is composed of estrogen, progesterone, egg maturation inhibitor (EMI), melatonin, and inhibin[8,17]. The accumulation of this liquid inside the follicle induces the formation of an antrum. At this stage, the growing follicle becomes an antral follicle (Figure 2), also called a tertiary or pre-ovulatory follicle[8,17].

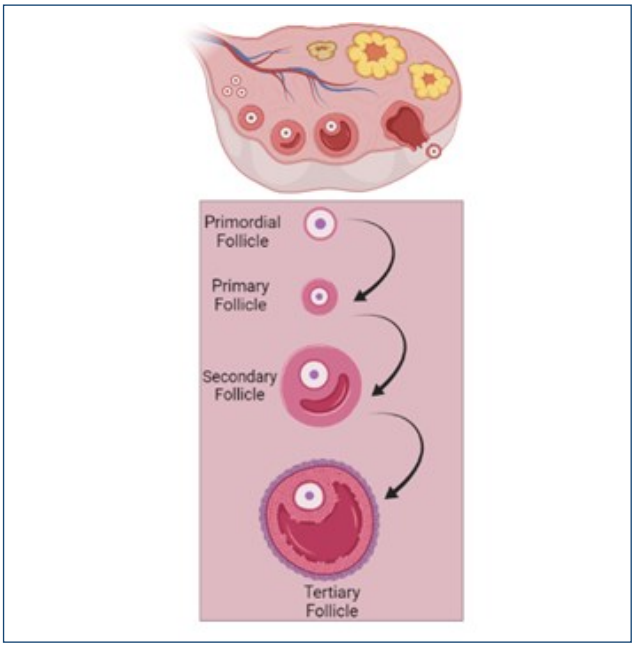

Figure 2. Schematic representation of follicular development. Source: Biorender (https://app.biorender.com/) developed by the authors.

The role of granulosa cells in folliculogenesis

The role of GCs in regulating the development of oocytes depends on their stage of differentiation and the communication between them. This intercellular communication can be mediated by paracrine, autocrine, or endocrine signaling and is responsible for metabolic cooperation that involves the transport of glucose, nucleotides, amino acids, and metabolites to the egg[14,15]. The communication between the developing oocyte and GCs is facilitated by membrane processes rich in microtubule structural proteins that support the active movement of organelles called transzonal projections (TZP). These extensions originate from GCs and reach the egg membrane through the zona pellucida, allowing cell signaling and the transport of substances between the two cells, such as growth factors, for example, through gap cell junctions of the GAP type (Figure 3A). Mora et al.[18] reported that during follicular growth, with the emergence of the zona pellucida, TZPs become abundant, accompanying the increase in the number of microvilli in the ovule. Also, the electrodense regions at the contact points between TZPs and microvilli may represent cell GAP junctions between GCs and oocytes, which are essential for follicle development and oocyte quality[12,13,18-20].

In antral follicles, GCs play a key role in modulating signaling for energy production during the processes of glycolysis and the tricarboxylic acid cycle. This follicular development determines the response of the egg in the emission of signals that culminate with the synthesis of adenosine triphosphate (ATP)[21,22].

The GCs participate in the formation of new blood vessels from those existing around each follicle. Endothelial and mural cells are destabilized, later migrate toward angiogenic stimuli, and proliferate, forming a new vessel, in a process called angiogenesis. This process requires the participation of the VEGF (vascular endothelial growth factor), which is responsible for stimulating endothelial cell

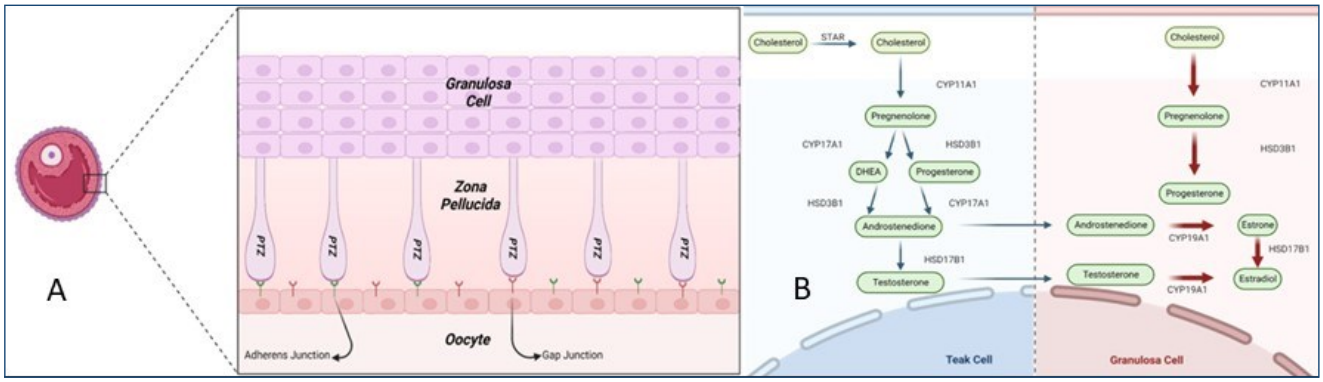

Figure 3. (A) Schematic representation of the transzonal projections between the ovule and the granulosa cells. (B) Steroidogenic pathway (theca cells and granulosa cells). Source: Biorender (https://app.biorender.com/) developed by the authors.

proliferation, endothelial cell migration, and blood vessel formation. The production of VEGF is stimulated by FSH and LH. Also, theca cells contribute to VEGF production during follicle growth[23-25,29].

Along with the continuous production of VEGF, endothelial cell proliferation occurs, and during this period of remodeling, high concentrations of VEGF support endothelial cell survival. During the development of the corpus luteum, endothelial cells reconnect and align to form tubules under the influence of VEGF. The newly formed vessels are stabilized by the recruitment of pericytes through the production of platelet-derived growth factor B (PDGFB) and the activity of the production of angiopoietin. Consequently, luteal cells are now fully vascularized, and therefore VEGF concentrations must remain elevated throughout luteal development to maintain immature vessel survival[26,29].

Granulosa cells and steroid hormones

In follicles, FSH stimulates GC proliferation and the production of steroid hormones. This process begins with the uptake of cholesterol (Figure 3B) into cells (GC and theca interna) and its conversion into pregnenolone by cytochrome P450 (CYP450) in the mitochondria. The formed pregnenolone diffuses from the mitochondria to the smooth endoplasmic reticulum, where it is processed and converted into progesterone when it undergoes the action of the 3β-hydroxysteroid dehydrogenase (HSD3B) enzyme and can also be converted into dehydroepiandrosterone sulfate (DHEA-S) by the action of the 17α-hydroxylase (CYP17A). HSD3B and CYP17A catalyze the conversion of DHEA and progesterone to androstenedione, respectively. Androstenedione can be converted to testosterone within the theca interna, by the action of 17β-hydroxysteroid dehydrogenase (HSD17B), or into estrone, testosterone, or 17-beta-estradiol (E2), within the GC, by the action of the aromatase enzyme (CYP19A1). Furthermore, in GCs, estrone can be converted into E2 through the action of HSD17B[17-19]. The synthesized estrogens in turn are also pro-proliferative factors for GCs, potentiating their action in the synthesis of steroids[23-25,27].

Angiogenesis

Ovulation and luteal development require the coordinated activity of several angiogenic factors and various cell types. VEGF regulates angiogenesis by stimulating endothelial proliferation, migration, and survival and is very necessary at all stages from a secondary follicle to a mature corpus luteum[27,28]. The accumulation of VEGF in the follicle and the consequent diffusion to the capillaries generate an angiogenic gradient that may regulate the development of a blood vessel network with the participation of the theca cell layer, located between the basal membrane and the granular layer, resulting in the potentiation of the supply of nutrients, oxygen, and hormones to the GC and oocyte[26,28].

In the preovulatory follicle, the granulosa layer remains avascular. During follicular development, VEGF and FGF (fibroblast growth factor) accumulate. Proteolytic activity increases after the LH surge, as does heparanase, which degrades the basement membrane, releasing sequestered angiogenic factors such as FGF and allowing vascular cells to migrate under the influence of VEGF. Also, the large increase in FGF levels may stimulate the disassembly of the vasculature and the dispersion of endothelial cells. The initial angiogenic step is the creation of capillaries in the thecal vasculature toward GCs that produce VEGF. Blood flow then resumes as these capillary networks start to connect with one another, create tubules, and attract pericytes. After ovulation, the process of maturation of

the vasculature continues with the corpus luteum. Also, there is extensive endothelial cell proliferation and migration in order to re-establish connections with other endothelial and luteal cells. Fibronectin participates in this process. After that, FGF concentrations decrease, and the capillary beds are rebuilt. Consequently, blood flow and progesterone production increased with this process[23,27,28].

DISCUSSION

The angiogenic process and intercellular communications of cells in the ovarian follicle are essential for follicular maturation quality as well as adequate sex steroid production. The analyzed studies report that GCs provide nutrition to the oocytes, and this action is directly related to good oocyte quality[14,15].

The communication between the GCs and follicular cells is mediated by PTZ and gap junctions, which are involved in the processes of follicular development and oocyte maturation. These projections are essential to maintain polarity and communication between cells, and the absence of those structures interrupts oocyte maturation as well as folliculogenesis. Therefore, these junctions are extremely important for the study of folliculogenesis[13].

Vascular endothelial growth factor is required for the development of the secondary follicle into the mature corpus luteum. In this process, the FGF plays a role in angiogenesis and the transformation of follicular cells into luteal ones. The increase in FGF levels stimulates the extensive tissue remodeling that accompanies the rapid angiogenesis after ovulation, which is essential for progesterone production[21-23,26-28].

Although studies evidence the importance of GCs, their knowledge regarding research is still limited since most information about this cell population has been obtained from animal models and from the primary culture of human follicular aspirate cells during *in vitro* fertilization (IVF) procedures. During this scenario, a few cells are obtained, which are usually under the influence of supraphysiological amounts of hormones, generally used for IVF.

CONCLUSION

Vascular endothelial growth factor and FGF are involved in angiogenesis, follicular growth, and steroid production. In addition, this information can be important for developing new therapies in the assisted reproduction techniques.

AUTHORS' CONTRIBUTIONS

GSC: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **KCC:** Validation, Writing – original draft, Writing – review & editing. **CSF:** Formal Analysis, Writing – original draft, Writing – review & editing. **PC:** Writing – original draft, Writing – review & editing. **PAAM:** Writing – original draft, Writing – review & editing. **ECB:** Writing – original draft, Writing – review & editing. **JMS:** Project administration, Supervision, Validation, Writing – review & editing.

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