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Cultivating a Support Network: Granulosa Cells and the Needed Knowledge on their Role in Oocyte Maturation

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Abstract

Maintaining and restoring full developmental competence of oocytes after a process of cryopreservation to preserve fertility is a growing concern in the field of assisted reproductive technologies (ART). Unfortunately, attempts so far have been characterized by low quality and a poor yield of live births. A promising avenue of research is to replicate the microenvironment of the follicles to achieve careful cryopreservation and successful *in vitro* maturation. A key step for this is to understands the interactions between somatic granulosa cells and the oocyte that are required for maturation and developmental competence. In the present work we present a succinct review of these molecular interactions, including endocrine and paracrine signaling, gene regulation and cell communication. We focus primarily on the role of both types of granulosa cells: mural granulosa cells, which perform endocrine functions, including hormone production and cumulus cells, which provide metabolic support and establish bi-directional communication with the oocyte through various pathways, including direct contact. The coordinate development of both granulosa cell types is necessary to fulfill oocyte requirements. Being able to reproduce those conditions *in vitro* could lead to successful oocyte maturation and positive fertilization outcomes.

Keywords: Granulosa cells; Cumulus; Mural cells; Cumula-oocyte complex; Oocyte maturation; Granulosa cell culture

Introduction

Maintaining and restore the full developmental competence of either mature or immature oocytes after cryopreservation processes to preserve women fertility is a goal in assisted reproductive technologies (ART). This goal arises from the need of some patients to preserve their oocytes before arriving to a reproductively mature age, such as patients who need to undergo chemotherapy. Although *in vitro* fertilization (IVF) can be performed to store embryos for future use with cumulative pregnancy rates >60% [1], this may not be an option for single women or patients without time to complete ovarian stimulation prior to cancer therapy. For those patients, oocyte cryopreservation can be considered instead [1-4]. Data from 21 peer-reviewed journals show that mean survival rate of frozen-thawed mature oocytes is 47%, while mean fertilization rate is 52.5% with a mean pregnancy rate for thawed oocytes of only 1.52% [1]. It has been suggested that immature oocytes may be more resistant to cryodamage due to lower cell volume and lack of metaphase spindle. Even though high rates of nuclear maturation have been reported with cryopreserved immature oocytes instead [1,3], the developmental capacity has been generally low [1].

The processes involved in oocyte maturation includes resumption of meiosis form prophase I, when the oocyte is in Germinal Vesicle stage (GV), to the extrusion of the first polar body, in Metaphase II (MII); the expansion of somatic cells surrounding the oocyte and the maturation of the cytoplasm to support fertilization and early embryonic development [5]. Whether the maturation happens *in vivo* or *in vitro*, the environment to which the oocyte and its surrounding cells are exposed, affects the developmental competence of the oocyte and subsequent embryonic development [5]. Oocytes within the ovarian follicle are surrounded by mural and cumulus granulosa cells, which perform both endocrine and developmental functions, respectively. The coordinate function of both granulosa cell types is necessary to fulfill oocyte requirements. A critical difference between cryopreserving MII or GV stage oocytes centers on the importance of the intercellular contacts between germinal and somatic cells. MII-stage oocytes do not rely on support from surrounding somatic cells, while cryopreservation of GV-stage oocytes requires maintenance of their communication with viable somatic cells to preserve a mutual interaction [4]. In both cases, being able to reproduce the

conditions *in vitro* by recreating the follicle microenvironment using cultured granulosa cells may be necessary for successful oocyte maturation, either before or after freezing.

Mural granulosa cells line the follicle wall and form a stratified epithelium with the basal lamina [6]. This cell type express luteinizing hormone receptors (LHCGR), its expression is induced after human chorionic gonadotropin stimulation. LH signaling induces the expression of progesterone receptor and other ovulation-related genes, including the protease ADAMTS-1 and its substrate Versican, a large chondroitin-sulphate-substituted aggregating proteoglycan of the lectican family. ADAMTS-1 and Versican are secreted and selectively relocated to the Extracellular Matrix (ECM) during cumulus expansion [7]. The principal physiological functions of MGC are the production of estradiol and progesterone [6-8]: CYP11A1 converts cholesterol to pregnenolone, next HSD3B1 converts it into progesterone. On the other hand, CYP19A1 uses androstenedione and testosterone as precursors to produce estrone and estradiol, also HSD17B converts estrone to estradiol [9-11].

Cumulus cells are highly specialized cells that establish direct contact with the oocyte to promote its growth and development though bi-directional interactions [6-8]. The cumulus cells have transzonal cytoplasmic processes, which penetrate through the zona pellucida and abut the oocyte membrane, forming the cumulus-oocyte complex (COC) [8]. The oocyte depends on cumulus cells for metabolic support. Those cells provide the oocyte the nutrients and regulatory signals to facilitate the progression of maturation, while oocyte secreted factors acts as signaling molecules to allow cumulus cell differentiation from mural granulosa cells [5,7,8]. Cumulus cells exhibit a high capacity to metabolize glucose to use it both as an energy source or to produce HA for cumulus expansion [5,12,13]. Cumulus cells glucose uptake is mediated by two separate glucose transporters: SLC2A1 and SLC2A4 [5]. Once inside CCs, glucose is directed to one of four metabolic pathways. A high proportion of this glucose enters the glycolytic pathway and by the action of enzymes such as phosphofructokinase is metabolized to produce ATP, pyruvate and lactate, metabolites that can be easily used as energy sources for both cumulus cells and the oocyte [5,12]. The remaining fraction is metabolized by the hexosamine biosynthesis pathway, where glucose-6-phosphatetofructose-6-phosphate is converted to glucosamine-6-phosphate by glucosamine fructose-6-phosphate transaminase (GFPT), and the end product of the pathway is UDP-N-acetyl glucosamine [5]. UDP-N-acetyl glucosamine is then used for cumulus matrix expansion as it is converted to hyaluronic acid (HA) by hyaluronic acid synthase 2 (HAS2) or to protein posttranslational modification via β-O-linked glycosylation by the action of O-linked glycosylation transferase [5,13]. Cumulus cells also express TGF-beta type I receptor (ALK5) and Bone Morphogenetic Protein Receptor 2 (BMPR2). These receptors allow for the activation of pathways mediated by TGFβ related proteins secreted by the oocyte like the growth differentiation factor 9 (GDF9) and bone morphogenetic protein (BMP15), which are important mediators for the follicle growth, cumulus cells differentiation and cumulus expansion [7,8,14,15].

Cumulus expansion during oocyte maturation involves the synthesis of ECM by cumulus cells in response to the LH surge *in vivo* [5,7,8,13] and epidermal growth factor (EGF) [5,13] or follicle stimulation hormone (FSH) stimulation *in vitro* [5]. After LH stimulation, cumulus cells express the HA surface receptors CD44 and the hyaluronan-mediated motility receptor (RHAMM), which bind to HA and allow the formation of the ECM matrix between cumulus cells. Versican interacts with HA providing strength and elasticity while being specifically cleaved by ADAMTS-1 which is selectively accumulated in the ECM, to allow for normal matrix function. Two other proteins expressed by CC, Pentraxin 3 (PTX3) and TNF-alpha induced protein 6 (TNFAIP6) interact to assemble and stabilize the matrix [7,8,15,16]. Active components of the ECM are synthesized directly by cumulus cells under the instruction of oocyte-derived factors and endocrine factors secreted by mural granulosa cells or enter the follicle through blood plasma [14]. The coordinate development of both granulosa cell types is necessary to fulfill the oocyte's requirements.

Given that mural granulosa cells exhibit hormone responsiveness and gene expression profiles distinct from that of cumulus cells, it is important to establish specific molecular markers of each cell type to be able to characterize them when in culture. For mural granulosa cells hormone receptors, ovulatory-related proteins and steroidogenic enzymes are potential markers. In cumulus cells receptors for oocyte-derived factors, glycolytic and hexosamine biosynthesis pathway (HBP) related enzymes, and proteins necessary for cumulus expansion are ideal markers. Additionally, several genes have been identified to be expressed specifically in each granulosa cell type and to play crucial roles, potentially could be used as well. In Table 1 we summarized the proposed markers that can be used to characterize both mural and cumulus granulosa cells. All the molecules have been reported to be detected in human granulosa cells at least once.

Conclusion

Establishing *in vitro* models for both cumulus and granulosa cells is an essential step in order to provide working material for oocyte maturation. Furthermore, well characterized models to study these cells will provide research tools to understand the complex interplay between these cells and the oocyte. This may open even more leads into understanding how external factors, such as endocrine disruptors and environmental pollutants can affect oocyte quality. Without a doubt, expanding our research on granulosa cells will become fundamental in the field of reproductive biology in the coming years.

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Table 1: Molecular Markers for human granulosa cells.

Cumulus Cells Markers			
Gene	**Name**	**Function**	**Reference**
PTGS2	Prostagandin Sintase 2	Rate-limitant enzyme in prostaglandin production. Required for maximum cumulus expansion and ovulation.	[7,8,14-29]
PTX3	Pentraxin 3	Required for ECM stability and retention.	[7,8,15,16]
TNFAIP6	TNF-alpha induced protein 6	Required for ECM organization and stabilization.	[7,16,18,30]
HAS2	Hyaluronic acid synthase 2	Converts UDP-N-acetyl glucosamine to HA.	[7,8,14-16,18,20,22,26]
CD44	CD44 cell-surface glycoprotein	HA receptor.	[7,25,26]
BMPR2	Bone Morphogenetic Protein Receptor 2	Binds to GDF9 to activate ALK5.	[7,15,23]
ALK5	TGF-beta type I receptor	GDF9 and BMP15 receptor. Activates SMAD2/3 signaling pathway.	[7,28]
RHAMM	Hyaluronan-mediated mobility receptor	HA receptor. Regulates the activation of ERK1/2	[7,27]
AMH	Anti-Mullerian Hormone	Regulator of mammalian follicular development.	[7, 8,21]
GREM1	Gremlin 1	TGFβ agonist. Induced by GDF9.	[14,19,24]
Mural Granulosa Cell Markers			
CYP11A1	Cholesterol side-chain cleavage enzyme	Converts cholesterol to pregnenolone.	[11,18]
HSD17B1	Estradiol 17-beta-dehydrogenase 1	Converts estrone to estradiol	[10,11,31]
CYP19A1	Aromatase	Converts androstenedione and testosterone to estrone and estradiol	[10,11,32]
HSD3B1	Hidroxi-Δ-5-esteroid dehydrogenase	Converts pregnenolone to progesterone.	[10,11,31]
PR	Progesterone Receptor	Regulates gene profile in mural granulosa cells.	[7,33,34]
ADAMTS1	Disintegrin and metalloproteinase with thrombospondin motifs 1	Selectively cleaves Versican in COC matrix.	[7,34]

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