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Review Article

The Insight into Developmental Capacity of Mammalian Cocs and Cumulus-Granulosa Cells-Recent Studies and Perspectives

Wiesława Kranc[1], Adrian Chachuła[2], Katarzyna Wojtanowicz-Markiewicz[3], Sylwia Ciesiółka[2], Edyta Ociepa[3], Dorota Bukowska[3], Sylwia Borys[4], Hanna Piotrowska[4], Artur Bryja[1], Paweł Antosik[3], Klaus. P. Brüssow[3], Michał Nowicki[2], Bartosz Kempisty[1,2]* and Małgorzata Bruska[1]

[1]Department of Anatomy, Poznan University of Medical Sciences, Poland

[2]Department of Histology and Embryology, Poznan University of Medical Sciences, Poland

[3]Institute of Veterinary Sciences, Poznan University of Life Sciences, Poland

[4]Department of Toxicology, Poznan University of Medical Sciences, Poland

***Corresponding author:** Bartosz Kempisty, Department of Histology and Embryology, Anatomy, Poznań University of Medical Sciences, 6 Święcickiego St., 60-781 Poznań, Poland

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Abstract

The mammalian oocyte maintains in the follicular environment that significantly influences its ability to growth and development. The complex process of mammalian oocytes development involves stages of gamete nuclear and cytoplasmic maturation, which finally must lead to formation of a fully mature female gamete ready for successful monospermic fertilization. Although there are a lot of studies indicating the role of the oocyte status on the embryo development in the Preimplantation stage, there are still no data related to gamete-surrounding somatic cells, called Cumulus oophorus (CCs) and follicular Granulosa Cells (GCs). Moreover, the role of surrounding CCs and/or GCs is in many cases discriminated during the routine procedure of *in vitro* Fertilization (IVF). The application of GCs primary culture, soon after their recovery from preantral follicles, is highly related to the increasing proliferation index, which is correlated with further GCs changed into Luteal Cells (LC). Luteinization of GCs may be recognized as the cellular potency of these cells and is associated with expression of specific markers such as Vanin-2 (VNN2), Regulator of G protein Signaling-2 (RGS2), Pentraxin-3 (PTX3), and Prostaglandin-endoperoxide Synthase-2 (PTGS2).

Therefore, this review demonstrated the present knowledge regarding biology of somatic cells that surrounds oocytes (CCs and GCs) during their growth in oogenesis as well as highlighting the possible further use of these both cells populations in reproductive diagnostics and assisted reproductive techniques.

Keywords: Cumulus cells; Granulosa cells; Follicle; Developmental capacity

Introduction

Mammalian oocyte-leaving in the shadow of cumulus-granulosa cells

The function and role of oocytes are determined by processes called folliculogenesis and oogenesis, which finally leads to formation of the full mature female gamete that is able to be fertilized by one single spermatozoon. The process of folliculogenesis includes several morphological and biochemical changes that depend on the formation of antrum in primary follicles [1-5]. Following differentiation to the tertiary follicle, there is the Graafian follicle with fully developed antrum and oocyte inside. However, the folliculogenesis also involves the process of Primordial-Granulosa (PGCs) and Granulosa Cells (GCs) differentiation [5]. The Graafian follicle is composed from highly differentiated granulosa cells layers: (1) Theca Cells (TCs), (2) membrane granulosa, (3) Cumulus Cells (CCs) and (4) the granulosa cells that are formed soon after ovulation, corona radiata [6-8]. Thus, the in fully developed antral follicle oocyte is surrounded by the cumulus oophorus that forms a differentiated group of granulosa cells [9-11].

The developmental capacity and/or potency of mammalian COC are often defined as the ability of female gametes to maturation and successful monospermic fertilization [2,12-15]. It was found in several studies that the formation of zygote as well as proper growth and development of embryos are regulated and determined by proper communication between cumulus and granulosa cells (previously also called the cumulus oophorus) and the female gamete [11,13,16]. This unique bidirectional communication is formed by proteins called Connexins (Cxs) that build the ultra structure of protein "corridor" described as Gap Junction (GJC) connections. This communication, metabolic pathways allowed to transfer small substances with the total mass less than 1kDa in two directions, from the oocyte to cumulus cells, and from cumulus cells into the oocytes [16,17]. It was showed by many authors that the GJC activity highly determined the maturation ability of mammalian oocytes. Moreover, it was also found that disrupted GJC activity may lead to lack of ability of mammalian oocytes to reach the MII stage, which must be always followed by nuclear and cytoplasmic maturation of the gamete [3,18]. However, the growth and development of somatic surrounding cumulus granulosa cells and especially their role in the proper function of oocytes are often discriminated. There is still lack of data indicating the developmental capacity of GCs and/or CCs as well as describing the ability of these cells to proliferation and cultivation *in vitro*. Our recent studies have shown that both of

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these cells populations; GCs and differentiated CCs may be kept and proliferated during in vitro culture in a short-, (48h) and long term-, (168h) cultivation model [9,10,17,19].

Molecular and cellular potency of mammalian ovarian granulosa cells

The developmental capacity of mammalian oocytes is described as the potency to reach the MII stage and formation of the gamete ready for successful monospermic fertilization [3,20]. It was found in several studies that reaching the MII stage by oocytes is achieved during long stages of maturation *In Vivo* or *In Vitro* (IVM). The process of oocytes maturation involved molecular and cellular maturation, which consists of induction of several morphological and biochemical changes as well as activation of metabolic pathways crucial for sustained gamete function and cell survival [21,22]. The change that leads to the formation of the fully mature gamete is also called cytoplasmic and nuclear oocyte maturation. There are many available data indicating the internal external factors involved in proper mammalian oocyte maturation [23]. The main internal factor, which is necessary for sustaining proper oocyte maturation is accumulation of the proper amount of mRNA and proteins that are further used as a template in transcription and translation for new nucleic acids and the proteins synthesis soon after fertilization [2,14]. These new molecules are necessary for the zygote formation and transition of maternal into the zygotic genome (MZT, Maternal-Zygote Transition) [24]. The most important external factors crucial for oocytes maturation involve a bidirectional "dialog" between oocyte and surrounding somatic ovarian granulosa cells. This specific cross-talk is necessary for achieving the MII stage by the oocyte and sustaining the proper growth and development of the female gamete during oogenesis, folliculogenesis, and formation of antrum and cumulus, as well as corona radiate cells proliferation and differentiation [12,6,19]. This communication pathway is possible by the Gap Junctions Connections (GJC) formed by proteins called Connexins (Cx's) [25]. The Granulosa Cells (GCs) belongs to the population of cells that build the follicle and are differentiated into mural granulosa cells and the cumulus oophorus that form the first layer of granulosa cells surrounding the oocytes in the antral follicle. The molecular potency of ovarian-follicular granulosa cells may be defined as the ability of GJC to transport small substances between oocytes and surrounded cells and as the expression and activity of Cxs that form the GJC connections [19,26]. The proper expression of Cxs genes and/or related proteins highly influenced GJC activity in cumulus cells. However, our recent study indicated the expression of Cxs mRNA and proteins in porcine follicular granulosa cells, which is a proof of GJC activity also in non-differentiated GCs [17,27]. On the other hand, it was also found that follicular granulosa cells after 48-72 hours of *In Vitro* Culture (IVC) differentiated into luteal cells, that are regulated by secretion of hormones (LH following fertilization and hCG during pregnancy) and depend on the stage of the oestrus cycle. The process of granulosa cells luteinization may be recognized as the cellular potency of these cells and is associated with expression of specific markers such as Vanin-2 (VNN2), Regulator of G protein Signaling-2 (RGS2), Pentraxin-3 (PTX3), and Prostaglandin-endoperoxide Synthase-2 (PTGS2).

Characteristics of GCs potency markers

All these markers are actually involved in vascularization, oxidative stress, inflammatory processes, and in membrane signaling pathways around ovulation. The role of these genes and encoded proteins in regulation of the proper course of folliculogenesis and oogenesis still remains to be elusive. However, there are some suggestions indicating that expression of these proteins is highly associated with the expansion process of cumulus-granulosa cells during COCs maturation *in vivo* and *in vitro*. Below the biological function of these proteins with special regards to their role as markers in reproductive processes in mammals is described. The first marker is Vanin 2 (VNN2), which encodes VNN2 protein containing the hydrophobic regions that are required to form a complex with a glycosylphosphatidylinositol-anchored cleavage site, present within a hydrophilic spacer region and subsequent attachment to the cell membrane, but lacks a leader peptide [28]. The VNN2 protein exists in a soluble and membrane-associated form. In the mammalian organism the vanins are only one known source of the pantheteinase activity, which involves the hydrolysis of pantetheine to pantothenic acid (vitamin B5) and cysteamine [29]. Moreover, VNN2 protein is involved in leukocyte adhesion and migration to inflammatory sites. The second marker, Regulator of G protein Signaling 2 (RGS2) is a protein encoded by RGS2 gene. The RGS2 protein acts as GTPase Activating Protein (GAP) for Gα subunits of heterotrimeric G proteins. The RGS2 protein drives G proteins into their inactive GDP-bound forms [30]. Moreover, RGS2 functions as a mediator of myeloid differentiation and plays a potential role in leukemogenesis. Another marker, Pentraxin 3 (PTX3) is a protein encoded by PTX3 gene and is a member of pentraxin superfamily. It is characterized by the cyclic multimeric structure [31]. The PTX3 protein is quickly synthesized and released in response to primary inflammatory signals [32]. PTX3 is also secreted by cumulus cells and stabilizes TNFAIP6 protein to maintain the expanded matrix during COCs maturation *in vivo* and *in vitro* [33]. The Prostaglandin-endoperoxide Synthase 2 (PTGS2), also known as Cyclooxygenase-2 (COX-2), is an enzyme encoded by PTGS2 gene. It is an essential enzyme in the prostaglandin biosynthesis – it converts Arachidonic Acid (AA) to prostaglandin endoperoxide H2. PTGS2 acts as a peroxidase and as a dioxygenase [34]. Yenuganti et al. reported in 2015 that VNN2 and RGS2 genes are upregulated in the luteinization process after the LH surge. Moreover, Yenuganti et al. (2015) found that high plating density of GC drives to up-regulation of VNN2 and RGS2 transcripts, thus high plating density can minimize the LH effects. Diaz et al. described that PTGS2 and PTX3 genes are crucial for the expansion of the cumulus oophorus, which is induced by the preovulatory surge of LH, which induces MAPK3/1-dependent up-regulation of PTX3 and PTGS2 genes [35].

Future perspectives

In this article we described the role of expression of several genes and proteins. We concentrated on the regulation of Cxs expression with special respect to proliferation and differentiation (luteinization) of mammalian follicular granulosa cells. Moreover, we characterized GCs potency markers and they role during folliculogenesis and oogenesis [36,37].

While exact role of proteins encoded by GC potency marker genes is elusive, it is very important discover and describe precisely their functions during folliculogenesis and oogenesis. The discriminated role of surrounding somatic cells (both CCs and GCs)

is restricted to the perspectives based on their ability to sustain the oocyte development. However, it opens new gates in research on the biological function in the aspect of endocrine activity of these cells and the application of this knowledge in assisted reproductive techniques.

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