Gap-Juntions in the Oocyte

Subjects: Biochemistry & Molecular Biology
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Connexins are proteins that form membrane channels and gap-junctions, and more precisely, these proteins enable the exchange of some ions and molecules, and therefore they do play a fundamental role in the communication between the oocyte and accompanying cells.

Keywords: pannexin ; connexin ; oocyte ; developmental competence ; oogenesis ; maturation ; fertilization

1. Introduction

A breakthrough in human reproductive medicine was the birth of world's first in vitro fertilized It is generally accepted that human oocytes have reduced developmental competence and increased aneuploidy with advancing maternal age. Therefore, the outstanding need for adequate biomarkers of oocyte developmental competence has become a high priority for research and fertility clinics around the globe since women's first attempt at childbearing has increased in the last three decades [1]. As previously mentioned Connexins are membrane channels forming gap-junctions, and more precisely, these intercellular proteins enable the exchange of some ions and molecules and therefore playing a crucial role in the communication between cells [2].

The unique gating and permeability of gap-junction channels is defined by their Connexin alignment [3][4], where channels can be (1) homomeric-homotypic, (2) homomeric-heterotypic, or (3) Noteworthy, heterotypic channels are mainly built by two homomeric hexamers and only some Connexins are compatible for interrelation [5]. However, gap junction channels can also be formed by two heteromeric hexamers but so far there are no reports available which could describe heteromeric-homotypic gap-junctions in oocytes or surrounding cells, although gap junction channels can be formed containing more than one Connexin isoforms [6][7]. Consequently, these differences in the Connexin composition imply the multi-faceted task for the physiology and developmental competence of an oocyte.

Expression of Panx1 was confirmed in the male and female reproductive tract, but its role in reproductive cells, especially in the oocyte, still needs further elucidation. Therefore, acquiring a deeper knowledge of Pannexin's and Connexin's importance for the mammalian oocyte is of high interest for the research field of reproductive medicine and for assisted reproductive technologies. This review provides an overview of current evidence on the link between oocyte developmental competence and the intercellular communication upon the Pannexin and Connexin channel proteins. Herein, their role for the processes of oogenesis, folliculogenesis, oocyte maturation and fertilization will be discussed, and at the end of this review, Pannexin and Connexin related pathologies and their impact on the oocyte's viability and fertility will be provided.

2. Pannexin and Connexin Involvement in Oogenesis and Folliculogenesis

Oogenesis in mammalian species is defined as the formation and maturation of female gametes during embryogenesis and starts on the first days of the embryonic period [8]. Primordial germ cells differentiate into oogonia which proliferate to form primary oocytes. In this state, the oocyte of women can remain even until reaching menopause. Successful oogenesis requires full cooperation of oocytes and those cells surrounding them, namely granulosa and cumulus cells [9] [10].

Furthermore, Pannexin 1 has been recently described to be involved in oocyte development and growth [11]. It has been shown that Pannexin 1 is localized in cumulus cells with ubiquitous expression pattern. The expression of the PANX1 gene in bovine oocytes and cumulus cells is differential with higher expression in smaller antral follicles compared to larger antral follicles, which suggests that the expression of Pannexin 1 decreases in vivo during antral follicle development [11]. The involvement of Pannexins in oocyte development is not precisely described, while the involvement of Connexins has been investigated widely.

Granulosa cells express Cx43, whereas Cx37 is present in both cumulus cells and oocytes at all stages of follicular development [3][12]. Cx43 enables the communication among granulosa cells, and its lack leads to the arrest of oocyte development at primary stages. Furthermore, the deletion of the Gja4 gene, which encodes for Cx37, resulted in a lack of gap-junctions between oocytes and cumulus cells, and additionally, in the granulosa cells, characteristics of a premature luteinization were observed [13]. These hemichannels regulate the release of molecules and ions from cells to interact with receptors on surrounding cells.

Interestingly, when a wild-type murine oocyte was fused with granular cells bearing a mutation of Cx43 Additionally, folliculogenesis was diminished suggesting the pivotal role of Cx43 in forming gap-junctions in murine granulosa cells [14]. Noteworthy, the PDZ-binding domain of Cx43 may play an important role in oogenesis, and it was reported that PDZ-binding domain deletion homozygote mice rarely survive, were infertile, and their follicles were morphologically impaired [13][15].

It is worth noting that the stimulation of GJA1 mRNA abundance upon FSH in granulosa cells appears to be regulated by protein kinase A (PKA) and through the Wnt/β-catenin pathway, which suggests that FSH may upregulate steady-state levels of these mRNAs by increasing their transcription [16]. The gene GJA4 which encodes for Cx37 is also upregulated in oocytes upon FSH, thereby their gap-junctional communication with cumulus cells is increased [17]. A recently published study revealed that the aberrant GJA1 gene appears to provoke an arrest of follicular development in women suffering from polycystic ovary syndrome (PCOS) [10].

3. Pannexin and Connexin Involvement in Oocyte Maturation

The first step of oocyte maturation contains the resumption triggered naturally by the luteinizing hormone (LH) [8]. The second step of oocyte maturation is the cytoplasmic molecular maturation where recruitment of specific transcripts for translation takes place. The aim of the third step is cytoplasmic organelles' maturation, meaning the adequate distribution of among others cortical granules, and mitochondria, during the transition to metaphase II (MII) [9]. All in all, oocyte maturation is a precisely orchestrated process in which an undisturbed communication between the oocyte and surrounding cumulus cells as well as among granulosa cells is necessary.

Recent studies have shown that PANX1 expression in human oocytes and eight-cell embryos is higher in comparison with cells of somatic tissues [18]. The localization of Pannexin 1, taking into consideration only localizations related to oocytes, was detected mainly on the cell membrane of human oocytes, zygotes, and at cell-cell interfaces in early embryos (Table 1) [18]. This study also revealed that the cause of oocyte death phenotype was due to alternations in Pannexin 1 channel activity and led to aberrant ATP release followed by oocyte death [18]. This research suggests that changes in the degree of Panx1 channel activity may lead to oocyte death even at a stage before fertilization.

Table 1. Pannexins involved in oocyte development and female fertility. See text for references.

Channel Protein	Encoding Gene	Reported Function for Female Fertility	Localization
Pannexin 1	***PANX1***	**Expression of PANX1 in bovine oocyte cumulus cells is differential with higher expression in smaller antral follicles compared to larger antral follicles. The expression of PANX1 is downregulated in vivo during folliculogenesis and oocyte maturation. PANX1 channel inhibition during in vitro maturation resulted in temporarily delayed meiotic maturation and improved in vitro developmental outcomes while decreasing intercellular reactive oxygen species. PANX1 inhibition during in vitro maturation led to maintaining elevated cAMP levels and modulation of ATP release, which delayed maturation and improved developmental competence. The mutation in PANX1 appeared to affect maturation potential in the oocytes—very few oocytes were mature, with the majority being immature and all degenerated or died very shortly after fertilization. The mutation in PANX1 led to an altered PANX1 glycosylation pattern and influenced the subcellular localization of PANX1in cultured cells. The result was the aberrant PANX1 channel activity and abnormal ATP release in oocytes. Oocytes having the mutation of PANX1, degenerated soon after retrieval due to the release of more adenosine 5′-triphosphate (ATP) to the extracellular space.**	**Oocytes, zygotes, early embryonic cleavage stages**
Pannexin 2	***PANX2***	**unknown**	**unknown**
Pannexin 3	***PANX3***	**unknown**	**unknown**

In the same study, it was reported that after six hours of 10Panx treatment significantly more oocytes remained at the germinal vesicle (GV) stage with significantly higher cAMP concentrations when compared to untreated counterparts, whereas after 22 h of treatment no changes in the number of oocytes reaching MII was observed [11]. Finally, they demonstrated that during the maturation of oocytes with inhibited Pnx1 channels Cumulus cells tightly surround oocytes forming a cumulus-oocyte-complex, as mentioned before. Signals from the microenvironment of the ovary have to be transferred to oocytes through communication channels.

Molecules and signals essential for molecular maturation of most mammalian oocytes are presumably transferred between cells during the first four hours of in vitro maturation (IVM) [19]. However, endothelin-1 (ET-1) may downregulate cAMP transfer from cumulus cells to the oocyte through Cx26, which is fundamental for the initiation of oocyte maturation [20]. The same study revealed that ET-1 affects cAMP levels in oocytes. Treatment with ET-1 significantly increased cAMP concentration in cumulus cells, whereas a decreased cAMP level in oocytes [20].

4. Pannexin and Connexin Involvement in Oocyte Fertilization

In humans, Cx43 gene expression was decreased in cumulus cells surrounding mature oocytes, comparing to their counterparts surrounding immature ones [21]. In case of embryonic development, there was no correlation between Cx43 expression in cumulus cells and fertilization or cleavage rate [22]. in cumulus cells was related to better embryo morphology on day 3 of the in vitro culture and improved blastocyst development [22][23]. Its protein expression was decreased in cumulus cells enclosing the oocytes more competent for embryo development, when compared to the less competent oocytes [11].

In the study of Zhou and co-workers [24], removal of cumulus cells before insemination of the in vitro matured murine oocytes led to a decrease of the fertilization rate, whereas this effect could be reversed upon the supplementation of dispersed cumulus cells to the insemination medium. These results show that gap junctions are not essential during fertilization, confirming at the same time that cumulus cells can be an important source of chemotactic factors guiding the spermatozoa to the oocyte [25][26]. There are findings in Caenorhabditis elegans, indicating the role of innexins (gap junction proteins in invertebrates) [27], particularly innexin-14, in the sperm recruitment to the site of sperm storage [28][29]. CD44 may act on tyrosine phosphorylation of Cx43—found predominantly in cumulus cells, which results in the closure of gap junctions and the activation of meiosis resumption [30].

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