Review

Pannexins and Connexins: Their relevance for oocyte developmental competence

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Abstract: The oocyte is the major determinant of embryo developmental competence in all mammalian species. Although fundamental advances have been generated in the field of reproductive medicine and assisted reproductive technologies in the past three decades, researchers and clinicians are still trying to elucidate molecular factors and pathways which could be pivotal for the oocyte's developmental competence. The cell-to-cell and cell-to-matrix communications are crucial not only for oocytes but also for multicellular organisms in general. This latter mentioned communication is among others possible due to the Connexin and Pannexin families of large-pore forming channels. Pannexins belong to a protein group of ATP-release channels, therefore of high importance for the oocyte due to its requirements of high energy supply. An increasing body of studies on Pannexins provided evidence that these channels not only play a role during physiological processes of an oocyte but also during pathological circumstances which could lead to the development of diseases or infertility. 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 playing a fundamental role in the communication between the oocyte and accompanying cells. Herein, the role of Pannexins and Connexins 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 developmental competence of oocytes will be provided.

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 (IVF) baby, Louise Joy Brown who was born in 1978. Today, there is a widespread use of various assisted reproductive technologies. It is generally accepted that the developmental competence of a human oocyte and the ability to be fertilized begins to decrease drastically at around ten years before menopause. Therefore, the outstanding need for adequate biomarkers of oocyte developmental competence has become

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high priority for research and fertility clinics around the globe since women's first attempt at childbearing has increased in the last three decades (te Velde & Pearson, 2002). One possible pathway which is worth having a closer look at when intending to find such biomarkers could be the cellular communication between the oocyte and surrounding cells inside of a follicle. The cell-to-cell and cell-to-matrix communications are crucial not only for oocytes but also for multicellular organisms in general. This latter mentioned communication is among others possible due to the Connexin and Pannexin families of large-pore forming channels. Connexin and Pannexin have a comparable 3D structure, although there is no sequence homology. Furthermore, six Connexins form a connexon and six Pannexins form a pannexon which both are hemichannels (Figure 1A). The Connexin family constitutes a group of homologous proteins (21 in humans), encoded by different genes (Söhl & Willecke, 2003). The protein's size of the members of the Connexin family is different, ranging from the smallest size of 23 kD (Connexin 23) to the largest protein size of this family at 62kD (Connexin62) (Winterhager & Kidder, 2015). 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 (Harris & Contreras, 2014). The permeability of gap-junction channels is defined by their Connexin alignment (Harris, 2007; Winterhager & Kidder, 2015), where channels can be 1) homomeric-homotypic, 2) homomeric-heterotypic, or 3) heteromeric-heterotypic (Figure 1B). Consequently, these differences in the Connexin composition imply the multi-faceted task for the physiology and developmental competence of an oocyte. Two decades ago, Panchin and co-workers (Panchin *et al*, 2000) discovered the Pannexin family which constitutes a group of three glycoproteins, Pannexin 1, Pannexin 2, and Pannexin 3 (Baranova *et al*, 2004; Penuela *et al*, 2007). 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. Numerous studies have been dedicated to Pannexin 1, which is encoded by the gene PANX1 (Penuela *et al*, 2013) and has a wide range of involvement in several physiological and pathophysiological functions (Qu *et al*, 2011; Sang *et al*, 2019). Therefore, acquiring 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.

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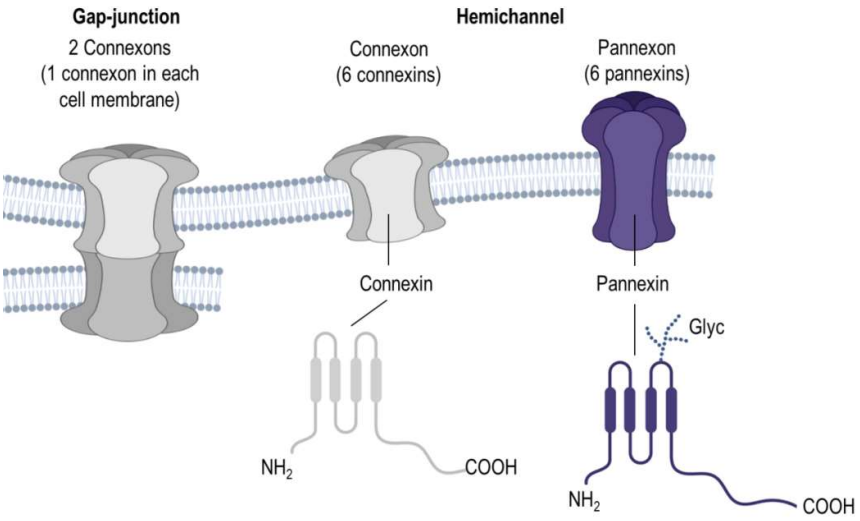

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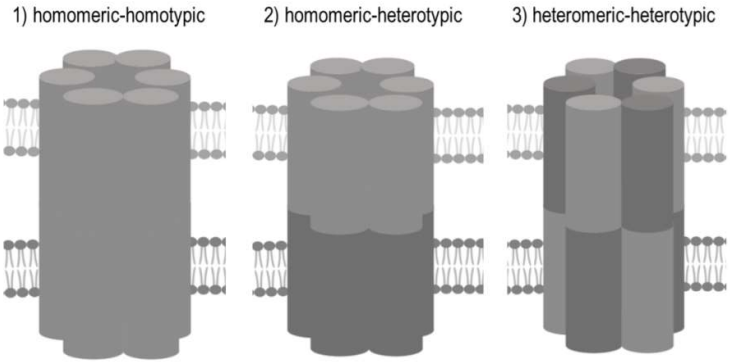

Figure 1. (A) Connexin and pannexin share a similar structure, despite the absence of sequence homology. Connexin and pannexin form functional connexon and pannexon hemichannels, respectively. Connexins and pannexins are transmembrane proteins with four transmembrane domains, two extracellular loops, one cytoplasmic loop, and cytoplasmic N- and C-terminal domains. Connexin channels can assemble into a gap junction that mediates intercellular communication, while pannexin's extracellular loop can have different degrees of glycosylation in mammalian cells, which prevent the formation of gap junctions, three variants of glycosylation are known: GLY0, GLY1, and GLY2. **(B)** The permeability properties of gap junction channels depend on their connexin composition: channels can be (1) homomeric-homotypic, (2) homomeric-heterotypic, or (3) heteromeric-heterotypic. *Abbreviation:* Glyc= glycosylation

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 already at the first days of the embryonic period (Bukovsky *et al*, 2005). Primordial germ cells differentiate into oogonia which proliferate to form primary oocytes. At the end of the fetal period, they begin the meiosis which is arrested at the prophase stage in many mammalian species and humans. In this state, the oocyte of women can remain even until reaching menopause. Just before each ovulation the first meiotic division is resuming (Sanchez & Smitz, 2012). Successful oogenesis requires full cooperation of oocytes and those cells surrounding them, namely granulosa and cumulus cells (Sanchez & Smitz, 2012; Liu *et al*, 2020). Differentiated cumulus cells are essential for oocyte nuclear and cytoplasmic maturation. They supply

oocytes with nutrients and regulatory signals needed to further development (Bukovsky *et al*, 2005; Kordowitzki *et al*, 2020; Liu *et al*, 2020). There are conserved homologies of up to 94% between human and murine Pannexins, and Panx1 knockout mice have shown to be viable and fertile (Lee *et al*, 2018; Penuela *et al*, 2013). Furthermore, Pannexin 1 has been recently described to be involved in oocyte development and growth (Dye *et al*, 2020). 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 what suggests that the expression of Pannexin 1 decreases *in vivo* during antral follicle development (Dye *et al*, 2020). The role of Pannexin 2 and Pannexin 3 in oogenesis needs to be elucidated. The involvement of Pannexins in oocyte development is not precisely described, while the involvement of Connexins has been investigated widely. The most well-studied Connexins in oocyte-cumulus-complexes (COCs) are Connexin 43 and 37 (Cx43 and 37, respectively) (Penuela *et al*, 2007; Winterhager & Kidder, 2015). Both of them have a pivotal role in all stages of folliculogenesis and oocyte maturation (Ackert *et al*, 2001; Kidder & Mhawi, 2002). Granulosa cells express Cx43, whereas Cx37 is present in both cumulus cells and oocytes at all stages of follicular development (Winterhager & Kidder, 2015). Due to their differential localization (Figure 2), they are responsible for slightly different functions. Cx43 enables the communication among granulosa cells, and its lack leads to arrest of oocyte development at primary stages. Cx37 is essential for the gap-junctional communication between oocyte and cumulus cells (Figure 2) (Gittens & Kidder, 2005). Previous research provided evidence that the lack of Cx37 in female mice led to diminished follicular development at early antral stages, as well as to a reduced meiotic competence (Simon *et al*, 1997). 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 (Simon *et al*, 1997). Consequently, it can be assumed that Cx37 might be also involved in the signal transduction responsible for the prevention of granulosa cell luteinization prior to ovulation (Winterhager & Kidder, 2015). The knockout of Cx43 in mice was lethal, therefore it was necessary to transplant the ovaries lacking Cx43 into a kidney of another adult wild-type mouse to follow up its further postnatal development (Gittens & Kidder, 2005; Tong *et al*, 2006). Loss of Cx43 led to an arrest of follicle development before antrum formation. Moreover, the absence of Cx43 impaired the follicle growth and decreased the sensitivity of granulosa cells upon growth differentiation factor 9 (GDF9) (Wang *et al*, 2013) Cx43 also facilitates oogenesis by providing connexons for the plasma membranes of granulosa cells (Leybaert *et al*, 2003). These hemichannels regulate the release of molecules and ions from cells to interact with receptors on surrounding cells. This pathway may also be supportive and crucial for folliculogenesis (Leybaert *et al*, 2003). Interestingly, when a wild-type murine oocyte was fused with granular cells bearing a mutation of Cx43 no intercellular gap-junction could be formed, although hemichannels were present in the plasma membrane. Additionally, folliculogenesis was diminished suggesting the pivotal role of Cx43 in forming gap-junctions in murine granulosa cells (Tong *et al*, 2007). 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 (Simon *et al*, 1997; Gershon *et al*, 2008). The Follicle-stimulating hormone (FSH) secreted by the posterior lobe of the pituitary gland has its receptors among other localizations on granulosa cells (Erickson *et al*, 1979). FSH is required for the proper growth of more advanced follicular stages (antral follicles) which during their development become responsive to FSH (Kumar *et al*, 1997). It was demonstrated that FSH regulates the Cx43 protein expression (Figure 2), and its encoding mRNA (*Gja1*) expression (Figure 2) (Wiesen & Midgley, 1994; Grummer *et al*, 1999). Noteworthy, the stimulation of *Gja1* mRNA abundance upon FSH in granulosa cells appears to be regulated by protein kinase A (PKA), and through Wnt/β-catenin pathway what suggests that FSH may up-

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regulate steady-state levels of these mRNAs by increasing their transcription (Yun *et al*, 20212; Wang *et al*, 2013). Granulosa cell communication in an cumulus-oocyte-complex (COC) is increased by FSH through phosphorylation and translocation of Cx43 (Figure 2) (Sommersberg *et al*, 2000; Johnson et al, 2002). The gene *Gja4* which encodes for Cx37 is also upregulated in oocytes upon FSH, thereby their gap-junctional communication with cumulus cells is increased (Chakraborty *et al*, 2010; El-Hayek & Clarke, 2015). Studies on the FSH dependent regulation of Connexins suggest that free Connexins form new gap-junctions once the hormone is present, and presumably the direct effect of FSH on *Gja1* expression in granulosa cells is responsible for the increase of Cx43. A recently published study revealed that the *Gja1* gene appears to provoke an arrest of follicular development in women suffering from polycystic ovary syndrome (PCOS) (Liu *et al*, 2020). This female endocrine disorder may lead to infertility due to ovulatory dysfunction and polycystic ovary morphology. These data demonstrated that *Gja1* is downregulated in oocytes in women with PCOS. Another research showed impaired endothelin-1 (ET-1) expression in granulosa cells from patients with PCOS. ET-1 can promote the oocyte maturation but more research about the importance of this protein in PCOS patients is needed (Cui *et al*, 2018).

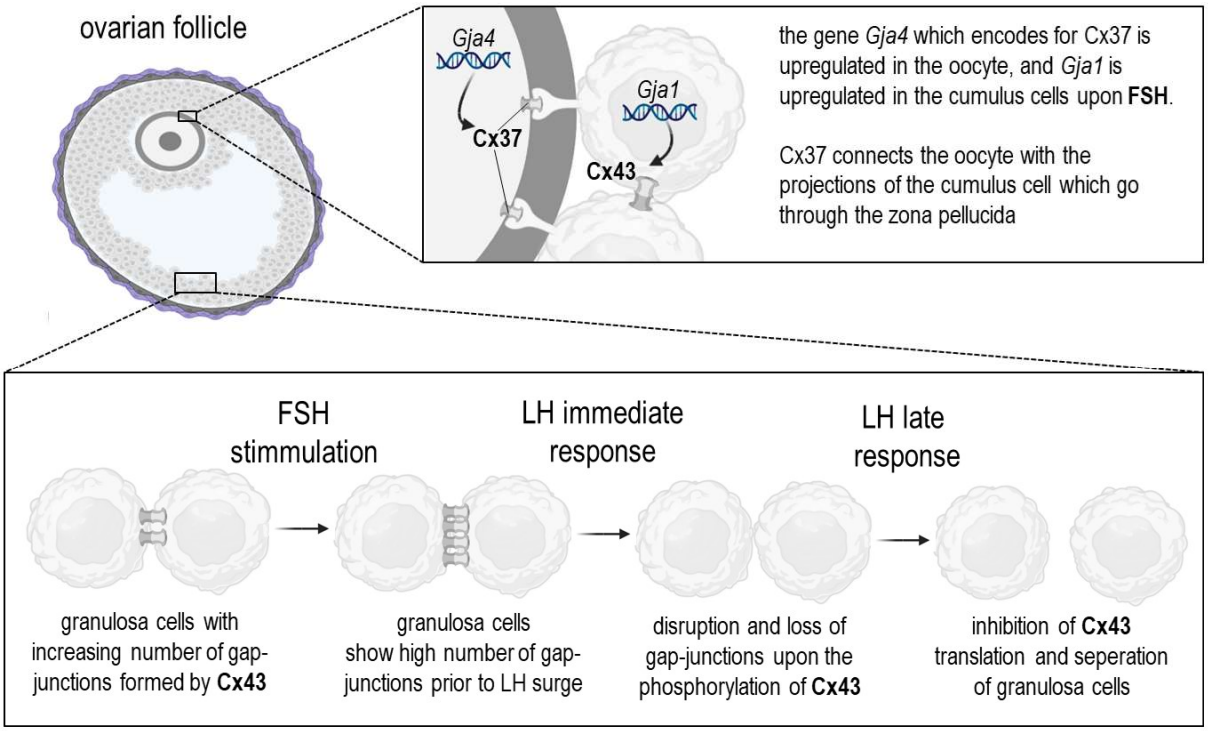

Figure 2. Scheme showing the regulation of gap-junctional communication (Cx43, Cx37) during the development of antral follicles prior to ovulation. FSH hormone stimulates mRNA expression that codifies the Cx43/Cx37 synthesis of the gap-junctions, the amplification of functional channels, and consequently the integration to the metabolic activity. Mediated by the family mitogen-activated protein kinases, pre-ovulatory LH levels interrupt cell-cell communications by means of phosphorylation and modification of Cx43 protein conformation. This leads to interruption of the intercellular channels. The primary effect of the immediate response to LH is accompanied by elimination of the Cx43 protein, disappearance of gap-junction separation of the GCs from the oocyte. *Abbreviations:* Cx=Connexin, FSH=follicle stimulating Hormone, GJA= gene encoding for connexins, LH= luteinizing hormone.

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

Channel Protein	Encoding Gene	Reported Function in Females	Localization
Connexin 26 (Cx26)	*Gjb2*	Knockout female mice died 11 days *post coitum*. Cx26 may be involved in local cellular mechanisms in oocytes among the peri-ovulation time. The expression of Cx26 is upregulated during the LH surge in the bovine follicle but the exact mechanism is not fully understood. Endothelin-1 (ET-1) may downregulate cAMP transfer from cumulus cells to oocyte via Cx26 to induce oocyte maturation. Mutations may lead to implantation failure.	Oocytes, granulosa cells, theca cells of several species.
Connexin 32 (Cx32)	*Gjb1*	Knockout females remained viable and fertile.	Porcine ovary, especially theca cells. In cattle in granulosa cells of arthritic but not healthy follicles.
Connexin 37 (Cx37)	*Gja4*	Cx37 is essential for the gap-junctional communication between granulosa cells and oocyte. Cx37 localizes to gap junctions at the oocyte surface and is thereby responsible for oocyte-granulosa cell metabolic coupling. It has been shown that in mice ovaries lacking Cx37 folliculogenesis is impaired at early antral stages, as well as meiotic competence. Mutations may lead to complication with conceiving due to impaired folliculogenesis.	In murine oocytes, cumulus cells, in cumulus cells of *corona radiata*. Cx37 is present on oocytes at all stages of follicle forming.
Connexin 43 (Cx43)	*Gja1*	Cx43 provides communication among granulosa cells and its lack leads to arrest oocyte development at the primary stages. Marker potentially associated with oocyte maturation. Mutations may lead to complications with conceiving due to deficiency of oogonia and/or impaired oocyte/follicle development.	Within the follicles of numerous species. Strongly expressed in cumulus cells.
Connexin 45 (Cx45)	*Gja7*	Knockout females mice died in utero.	In pig, mouse and rat oocytes, granulosa cells and cumulus cells.

3. Pannexin and Connexin involvement in oocyte maturation

Oocyte maturation reflects the process which is mandatory for the mammalian female gamete to be fertilized by the male gamete (Sanchez & Smitz, 2012). Oocyte maturation can be divided in four main steps, namely into (1) the nuclear maturation, (2) the cytoplasmic molecular maturation, (3) the cytoplasmic organelle maturation, and (4) the

epigenetic maturation (Sanchez & Smitz, 2012). In perinatal stages, oocytes of the most mammalian species are arrested at the prophase of meiosis I (prophase I). The first step of oocyte maturation contains the resumption triggered naturally by the luteinizing hormone (LH) (Bukovsky *et al*, 2005). The second step of oocyte maturation is cytoplasmic molecular maturation where a recruitment of specific transcripts for translation takes place [1, 2]. 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) (Sanchez & Smitz, 2012). The epigenetic maturation step is crucial for the regulation of gene expression, nuclear architecture and chromosome stability (Bukovsky *et al*, 2005). 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 (Sang *et al*, 2019). 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 2) (Sang *et al*, 2019). Pannexin 1 may form three variants, the no glycosylated protein (GLY0), a high-mannose glycoprotein (GLY1), and a fully mature glycoprotein (GLY2) (Figure 1A) (Penuela *et al*, 2007). Four independent families suffering from female infertility, in which mutations of Panx1 were detected, were recently analyzed (Sang *et al*, 2019). A different Panx1 gene mutation has been identified in each family. All four mutations (Figure 3) altered glycosylation of pannexin 1 resulting in a lack of GLY2, whereas GLY1 was maintained. Furthermore, the localization of the mutation of Pannexin 1 was in all investigated cases on the cytoplasmic side of the protein (Figure 3). This study also revealed that the cause of oocyte death phenotype was due to alternations in Panx1 channel activity, and led to aberrant ATP release followed by oocyte death (Sang *et al*, 2019). This research suggests that changes in the degree of Panx1 channel activity may lead to oocyte death even at a stage before fertilization. These findings clearly demonstrated the pivotal role of Pannexin 1 in human oocyte development. The Pannexin 1 inhibition with the help of an inhibitor named 10Panx was conducted during *in vitro* maturation of bovine COCs (Dye *et al*, 2020). This study revealed that Pannexin 1 inhibition decreased cumulus cells expansion when compared to the untreated (without 10Panx) control. 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 22h of treatment no changes in the number of oocytes reaching MII was observed (Dye *et al*, 2020). This study demonstrated clearly that oocyte maturation can be delayed upon the inhibition of the Pannexin 1 hemichannel. These effects seemed to be temporary, but a higher proportion of treated oocytes reached the blastocyst stage suggesting that this delay, related to maintaining elevated cAMP levels, improve *in vitro* oocyte developmental competence. Finally, they demonstrated that during the maturation of oocytes with inhibited Pnx1 channels significantly less reactive oxygen species (ROS) were produced in comparison with the untreated control oocytes (Dye *et al*, 2020). Consequently, the *in vitro* development of the embryo was protected against the negative effects of ROS. The role of Pannexins in oocyte maturation is crucial but not clearly defined, whereas Connexins have been well studied. An adequate cellular exchange between oocyte and follicular cells is important for oocyte maturation and development. The loss or reduction of these membrane proteins affect negatively fertility in various mammalian species (Gabriel *et al*, 1998; Kruger *et al*, 2000; Ackert *et al*, 2001; Tong *et al*, 2007; Hasegawa *et al*, 2007; Gershon *et al*, 2008; Berisha *et al*, 2009; Dobrowolski & Willecke, 2009; Nitta *et al*, 2010; Santiquet *et al*, 2013). Several Connexins were identified as playing a role in the functioning of the female reproductive tract, including Cx26, Cx32, Cx37, Cx43 and Cx45 (Grazul-Bilska *et al*, 1997; Wang *et al*, 2009; Winterhager & Kidder, 2015). Cumulus cells tightly surround oocytes forming a cumulus-oocyte-complex, as mentioned before. Sig-

nals from the microenvironment of the ovary have to be transferred to oocytes through communication channels. Besides providing nutrients, Connexins enable the exchange of molecules, and thereby gap-junctions are responsible for maintaining a stable pH inside the oocyte and promote chromatin structure remodeling during oocyte maturation (Wigglesworth *et al*, 2013). This is mandatory for proper proliferation of the oocyte and its survival. Moreover, Connexins are required to regulate cGMP concentration in oocytes which is relevant for the meiotic arrest and later for the resumption of meiosis (Norris *et al*, 2009).

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) (Santiquet *et al*, 2013). It is well documented that the meiosis arrest depends on the level of cAMP in mammalian oocyte. However, endothelin-1 (ET-1) may downregulate cAMP transfer from cumulus cells to the oocyte through Cx26 what is fundamental for the initiation of oocyte maturation (Cui *et al*, 2018). Recent studies suggest that ET-1 determines oocyte maturation via endothelin receptor type B (ETRB) by downregulation Cx26 expression in cumulus cells (Cui *et al*, 2018). Whole genome transcriptome microarray analyses of cumulus cells were performed. The study reported a significant lower Cx26 protein expression upon ET-1 treatment. This effect could be reversed due to a co-treatment with an ETRB antagonist (Cui *et al*, 2018). 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 (Cui *et al*, 2018). Interestingly, ET-1 did not affect Cx43 and Cx37. Moreover, Cx26 in oocytes may be involved in local cellular mechanisms during the peri-ovulation time. The expression of Cx26 is upregulated during the luteinizing hormone (LH) surge in bovine follicles but the exact mechanism is not fully understood yet (Berisha *et al*, 2009). After the LH surge, progesterone production is upregulated and the gap-junctions start to reduce the passage of cyclic nucleotides, what contributes to initiation of the meiotic resumption (Shimada *et al*, 2003; Santiquet *et al*, 2013). In porcine oocytes, it was demonstrated that Cx43 is highly controlled by gonadotropins (Granot & Dekel, 1994; Sasseville *et al*, 2009). Thereby, the decrease in the concentration of cGMP in oocytes induces hydrolysis of cAMP, which promotes the meiotic process and maturation of oocytes (Shinohara *et al*, 2018).

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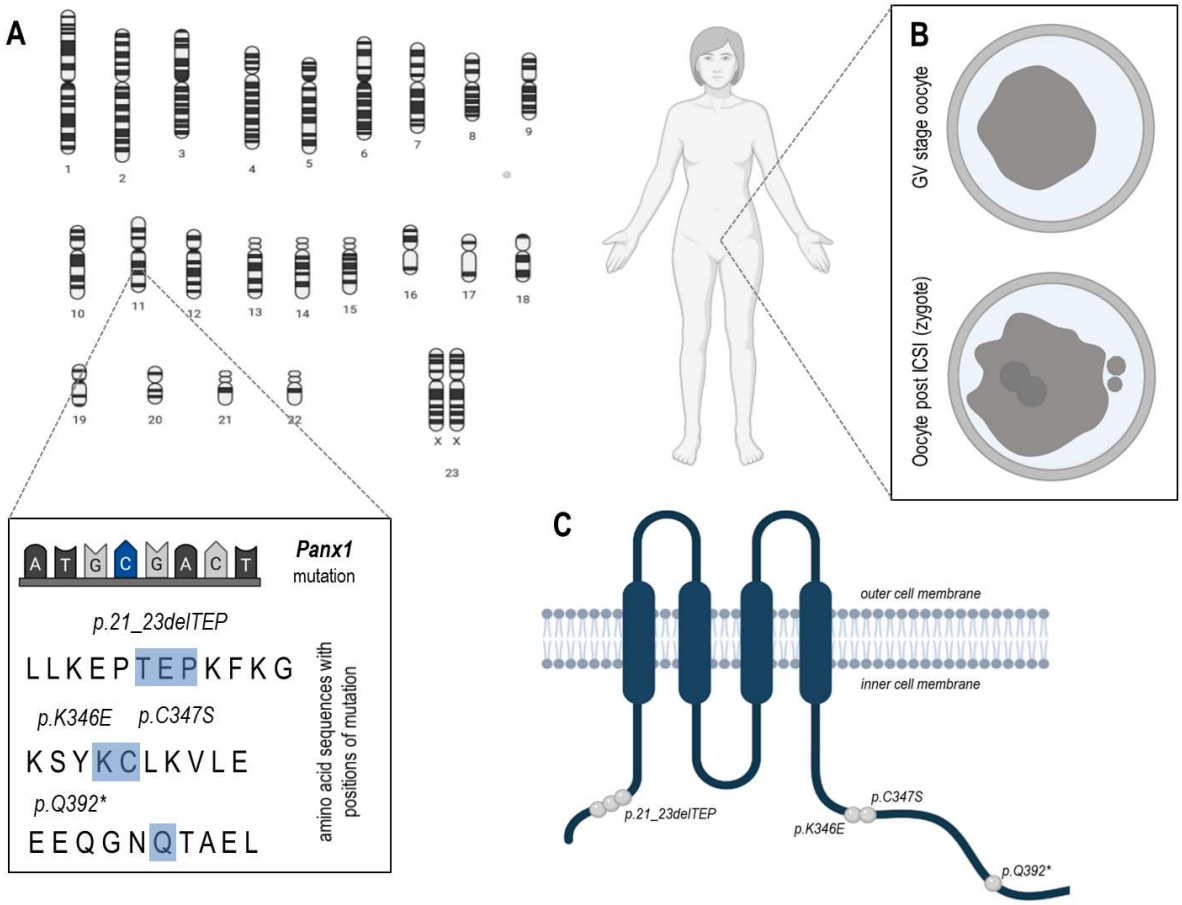

Figure 3. Mutation distribution of PANX1 and morphological appearance of oocytes retrieved from PANX1 channelopathy patients. **(A)** The gene Panx1 which encodes for PANX1 is located on chromosome 11 in humans. In four investigated families, four different mutations loci have been identified which led to a change in the amino acid sequence (highlighted in blue). **(B)** The upper part shows an exemplary degenerated oocyte in the germinal vesicle (GV) stage with shrunken ooplasma (dark grey), directly after oocyte retrieval; the lower part shows an exemplary degenerated zygote (fertilized oocyte) 30h after Intra-Cytoplasmic-Sperm-Injection=ICSI, with the two pronuclei and extruded polar-bodies visible, but all specimens died at this stage. **(C)** Distribution of four disease-causing mutations in PANX1. All mutations were located in the cytoplasmic region and are highlighted as grey dots (according to Sang *et al*, 2019).

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Table 2. 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)	*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 what 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)	*Panx2*	unknown	unknown
Pannexin 3 (PANX3)	*Panx3*	unknown	unknown

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4. Pannexin and Connexin involvement in oocyte fertilization

It was found that the interruption of gap-junctional communication within COCs is gonadotropin-dependent and decreases after germinal vesicle break-down (GVBD). The resumption of meiosis was associated with the reduction of Cx43 protein level in porcine and rat cumulus cells (Shimada *et al*, 2001, Kalma *et al*, 2004, Sassevile *et al*, 2009). Analogical findings were made in bovine. The expression of Cx43 mRNA in cumulus cells was down-regulated 6 hours after LH release (Assidi *et al*, 2010). Disappearance of small Cx43-positive gap junctions, interconnecting the corona radiata cells with the oocyte, was related to germinal vesicle break down (GVBD) as revealed by immunofluorescence and electron microscopy (Sutovsky *et al*, 1993). In humans, Cx43 gene expression was decreased in cumulus cells surrounding mature oocytes, comparing to their counterparts surrounding immature ones (Li *et al*, 2015). In case of embryonic development, there was no correlation between Cx43 expression in cumulus cells and fertilization or cleavage rate (Hasegawa *et al*, 2007). However, it was revealed that lower expression of Cx43 in cumulus cells was related to better embryo morphology on day 3 of the *in vitro* culture and improved blastocyst development (Hasegawa *et al*, 2007, Feuerstein *et al*, 2007). Similar results were obtained with regard to Panx1. Its protein expression in bovine granulosa cells diminished along with follicular growth. Moreover, it was decreased in cumulus cells enclosing the oocytes more competent for embryo development, when compared to the less competent oocytes (Dye *et al*, 2020).

In the study of Zhou and co-workers (Zhou *et al*, 2016), 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 neatly, 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 (Sun *et al*, 2005; Guidobaldi *et al*, 2008). There are findings in *Caenorhabditis elegans*, indicating the role of innexins (gap junction proteins in invertebrates) (Phelan, 2005), particularly innexin-14, in the sperm recruitment to the site of sperm storage (Whitten & Miller, 2007; Edmonds *et al*, 2011). Cumulus cells support the fertilizing ability of the spermatozoa by creating the specific microenvironment around the oocyte. Their presence during *in vitro* fertilization increases spermatozoa penetration rate in domestic animals (Van Soom *et al*, 2002). Preovulatory LH surge induces the extensive production of hyaluronic acid (HA) in cumulus cells through the increase in hyaluronan synthase 2 (HAS2) expression, what leads to the deposition of a large amount of hyaluronan in their extracellular matrix and expansion (Fouladi-Nashta *et al*, 2017). This expanded matrix plays an important role in the fertilization process, participating in the complex mechanism controlling the access of the spermatozoa to the oocyte (Kimura *et al*, 2006). It was revealed that the interaction between HA and its main surface receptor CD44, may act on tyrosine phosphorylation of Cx43 – found predominantly in cumulus cells, what results in closure of gap junctions and activation of meiosis resumption (Sato & Yokoo, 2005). In their plasma membrane, spermatozoa possess the hyaluronic-binding proteins that can either bind directly with HA or facilitate their infiltration through cumulus cells via hyaluronidase activity. In clinical practice, their ability to bind HA is used to select physiologically mature spermatozoa for ICSI. It was revealed that HA bound spermatozoa are viable, characterize with proper morphology, low rates of DNA fragmentation and aneuploidy (Jakab *et al*, 2005, Huszar *et al*, 2007). In bovine, HA supplementation of the culture media influenced spermatozoa capacitation status, resulting in the increased number of motile, rapid and progressively moving (da Fonseca Junior *et al*, 2020) and improved embryo development to the blastocyst stage (Furnus *et al*, 1998). Production of highly competent spermatozoa is prerequisite for successful fertilization. Similarly as in case of oogenesis, gap-junctions and their constituent proteins are crucial for normal tes-

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ticular development and spermatogenesis (Pointis *et al*, 2005; Cyr, 2011, Giese *et al*, 2012). The main gap junctional protein in testes is Cx43. In experiments on mice, it was revealed that lack of Cx43 resulted in neonatal death and testes of the pups were lacking developing germ cells (Juneja *et al*, 1999). Moreover, changes in Cx43 expression were noted in different spermatogenesis disorders in men (Brehm *et al*, 2006; Kotula-Balak *et al*, 2007), for example low level of Cx43 mRNA and protein was related to azoospermia (Defamie *et al*, 2003). The secretory ability of the Leydig cells was not changed upon the absence of Cx43 as shown in mice (Kahiri *et al*, 2006). Contrary to this, in case of intercellular connections between Sertoli cells (SCs), and SCs and germ cells, Cx43 is absolutely required. By coordination of SCs junctional permeability Cx43 enables functional communication which provides nutrients and other molecules supporting maturation and proliferation of germ cells and its deletion in SCs resulted in the block of spermatogenesis (Risley *et al*, 1992; Brehm *et al*, 2007; Hilbold *et al*, 2020). Connexin-43-based gap junctions linking SCs are found in basal compartment of the seminiferous epithelium and together with proteins such as cadherin and occludin are known to participate in constituting blood-testis barrier (Risley *et al*, 1992; Batias *et al*, 1999; Li *et al*, 2009; Li *et al*, 2010). Two other connexins were also detected in testes – Cx26 and 32, both localized in the apical region of the seminiferous epithelium (Risley *et al*, 1992). As for the Cx43 expression in the testes, it is regulated mainly by thyroid hormones, what is common observation among different species (St-Pierre *et al*, 2003, Gilleron *et al*, 2006, Sridharn *et al*, 2007). The epididymis characterizes with the presence of several connexins: Cx26, Cx30.3, Cx31, Cx32, Cx37, Cx40, Cx43 and Cx45. Their expression is segment-specific (Dufresne *et al*, 2003, Han & Lee, 2013; Lee, 2013), what may be the consequence of the epididymis morphological and functional differentiation into three main regions: caput, corpus and cauda. The main role of the epididymis is to provide the optimal environment for the proper post-testicular sperm maturation, protection and storage (Lee, 2014). Epididymal epithelial cells secret wide range of proteins which interact with maturing spermatozoa, enabling them acquisition of two basic features, motility and fertilization capability (Skerget *et al*, 2015). Numerous knock-out experiments revealed that various dysregulations of epidydimal microenvironment lead to subfertility or even infertility (Joseph et al., 2010, Weissgerber *et al*, 2012; Wang *et al*, 2017). The role of androgens and estrogens in regulation of gap junctional communication in epididymis has been shown previously. The studies of Lee (2014, 2015) revealed, that the treatment of neonatal male rats with androgen antagonist (flutamide) and estradiol agonist (estradiol benzoate) modulated the expression of most connexin genes, typical for the initial segment and cauda of adult rat epididymis. Exposure to flutamide induced a decrease of Cx43 expression in the stromal cells of the corpus and cauda regions of boar epididymis (Lydka *et al*, 2011). Epidermal growth factor (EGF) was suggested to impact epididymal function (Tomsig *et al*, 2006). It was assumed that in men suffering from azoospermy a decreased expression of EGFR could provoke diminished levels of Cx43 (Dube *et al*, 2012).

Although the role of pannexins in many physiological and pathological processes was broadly studied (reviewed in Penuela *et al*, 2013; Wicki-Stordeur *et al*, 2014), there are limited data concerning their involvement in spermatogenesis. Pannexin1 was found to be present in SCs in the basal compartment of the seminiferous epithelium, whereas Panx3 in Leydig cells. As for the epididymis, Panx1 was also localized in basal area of the epithelium, while Panx3 in the apical region (Turmel *et al*, 2011). In the studies on orchiedectomized animals it was revealed, that androgens are involved in both the transcriptional regulation and the posttranslational modification of Panx1 and 3 (Turmel *et al*, 2011, Dufrense & Cyr, 2014). The major role of Panx1 is to form ATP releasing channels what was stated for several cell types. Pannexin channels are highly permeable for ATP and their expression correlates with ATP release sites (Dahl *et al*, 2013). Taking into consideration the fact, that epidydimal cells release ATP and that Panx3 is located in the apical region of the epithelium, it can be assumed that they participate in ATP-mediated

sperm maturation. It was found that extracellular ATP plays significant role in epididymis, by modulating spermatozoa transport and motility (Rouan *et al*, 2012). However, the function of pannexins in the testes and epididymis has yet to be clearly established.

5. Pannexin and Connexin related pathologies which impact oocyte developmental competence

Connexins are known to play an important role in a variety of biological processes and disturbances in their functioning may cause different pathologies. Several disorders in humans were classified as related to Connexin mutations (reviewed in Pfenniger *et al*, 2011). The mostly studied are mutations of Cx26 associated with hearing loss (Xu & Nicholson, 2013; Ambrosi *et al*, 2013). In this paragraph, we will concentrate on the Connexin and Pannexin linked pathologies impacting developmental competence of the female gamete. As it was described earlier in this review, gap-junctions between granulosa cells and granulosa cells and the oocyte, regulate cellular communication required for the proper oocyte maturation and ovulation. Connexin-43 and -37 seem to have predominant role in follicular development as confirmed in knockout experiments. Lack of Cx43 in mice ovaries resulted in abnormal folliculogenesis and as a consequence in developmentally incompetent oocytes with morphological anomalies such as poorly developed zona or vacuolated cytoplasm, which failed to be fertilized (Ackert *et al*, 2001). Similar observations were made in case of Cx37 deficiency. Targeted deletion of Cx37 gene was associated with arrested follicle and oocyte growth and ovulation failure (Simon *et al*, 1997, Carabatsos *et al*, 2000). It was revealed that down-regulation of Cx43 was implicated in follicular growth arrest in women with polycystic ovary syndrome (PCOS), which are known to suffer from subfertility (Liu *et al*, 2020). Connexin-43 gene expression was decreased in GV-stage oocytes obtained from PCOS ovaries, in relation to the oocytes recovered from the ovaries of healthy women (Liu *et al*, 2016, Liu *et al*, 2020). Parallel findings were made regarding Cx43 protein – it was very low in oocytes obtained from PCOS patients (Liu *et al*, 2020). Androstenedione dependent up-regulation of Cx43 and gap junctional communication in granulosa cells was suggested to contribute to the pathogenesis of PCOS in rat (Talhouk *et al*, 2012). Both Cx43 protein and Cx37 mRNA expression was found to be affected in COCs of diabetic mice (Chang *et al*, 2005, Ratchford *et al*, 2008). This could generate negative consequences such as delay in follicular growth, oocyte maturation and higher apoptosis rate in ovarian follicles, which were observed previously as linked to diabetes (Colton *et al*, 2002, Chang *et al*, 2005). Connexin-43 knockdown in parthenogenetically activated porcine embryos was responsible for the reduction of the membrane permeability, mitochondrial membrane potential and ATP production, and for the enhanced level of reactive oxygen species. Additionally, blastocyst developmental rate and the total cell number were decreased (Shin *et al*, 2020). The importance of Cx43 for the oocyte quality was also confirmed in bovine. High expression of Cx43 in COCs was correlated with superior developmental competence of the resulting embryos (Read *et al*, 2018). Connexins not only form gap junctions enabling intercellular communication but may also participate in transmembrane communication through formation of unopposed hemichannels. Connexons are involved in release of small metabolites and ions into the extracellular environment thus taking part in paracrine signaling (Pfenniger *et al*, 2010). However, this process has to be precisely regulated because abnormal opening of hemichannels usually leads to uncontrolled efflux of ions and metabolites what may result in cell death. As it was shown in the studies on oocyte vitrification in cat, Cx43 and Cx37 hemichannels may open during vitrification and warming, what ends with the serious injury of the oocytes (Snoeck *et al*, 2018). In case of ovine, Cx43 mRNA was found to be also affected by vitrification and *in vitro* culture (Sampaio da Silva *et al*, 2016).

As mentioned previously, the main role of pannexin channels is ATP transfer from the intra- into the extracellular environment in response to activation by calcium or purinergic receptors (Locovei *et al*, 2006). Pannexins have been known to participate in many physiological processes such as cell differentiation (Penuela *et al*, 2013), apoptotic cell clearance (Chekeni *et al*, 2011), initiation of inflammation (Lohman et al., 2014), HIV infection (Orellana et al., 2013) or neurological functions (Gulbransen *et al*, 2012). There are limited information concerning pannexin channels dysfunctions in relation to the oocyte developmental competence. In bovine, COCs Panx1 expression decreased as follicular growth progressed, i.e. the protein was higher in COCs isolated from small antral follicles in comparison to its levels in large follicles. The oocytes of high developmental potential characterized with lower expression of Panx1 than their less developmentally competent counterparts. In humans, Panx1 channelopathy associated with changes in Panx1 glycosylation pattern and subcellular localization, resulted in abnormal channel activity and ATP release, and oocyte death as previously mentioned (Sang et al., 2019). The majority of oocytes collected from these Panx1 mutated patients dedicated to intra-cytoplasmatic sperm injection (ICSI) were immature and all degenerated or did not show further development very shortly after ICSI (Figure 3B). Interestingly, the mutations showed pattern of paternal inheritance with no negative effect on male fertility what assuming that mutant Panx1 has a specific pathophysiological role during oogenesis (Sang *et al*, 2019).

6. Conclusions and future directions

Pannexins and Connexins act as essential channel proteins enabling the communication in oocytes to support the processes of oogenesis, folliculogenesis, maturation, fertilization, and embryonic development. As oocytes age, their number, quality, and fertilization outcomes decrease. An increasing number of studies showed that the communication as well as the exchange of molecules and ions via the Pannexin and Connexin pore-forming hemichannels and gap-junctions, are a critical determinant of oocyte developmental competence and fitness. This sparked interest within the reproductive molecular medicine field to better understand how both earlier mentioned channel proteins contribute to the determination of the competence related to oocytes' growth, development inside of a follicle, and oocytes' ability to be fertilized. Once the impact of Pannexins and Connexin will be fully elucidated, there will be the possibility with new technologies or treatments to modulated or restore oocyte viability. Technical advances in the three past decades have led to increasingly sophisticated assisted reproductive technologies. Nevertheless, the safe and ethical modulation of channel proteins and oocyte quality requires more comprehensive understanding of their interplay. Taken together, elucidating the molecular mechanisms that contribute to the maturation and fertilization of oocytes, the unique role of Pannexins and Connexins herein, and careful identification of therapeutic targets to improve their function and thereby oocyte health, can contribute to new strategies to enhance and prolong reproductive fitness. Importantly though, our review aimed to stimulate new research ideas on this interesting topic of cell to cell communication what could aid in the design of novel studies and animal models.

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References

1. Ackert, C.L.; Gittens, J.E.I.; O'Brien, M.J.; Eppig, J.J.; Kidder, G.M. Intercellular communication via connexin43 gap junctions is required for ovarian folliculogenesis in the mouse. Dev. Biol. 2001, 233, 258-270. doi: 10.1006/dbio.2001.0216
2. Ambrosi, C.; Walker, A.E.; Depriest, A.D.; Cone, A.C.; Lu, C.; Badger, J.; Skerrett, I.M.; Sosinsky, G.E. Analysis of trafficking, stability and function of human connexin 26 gap junction channels with deafness-causing mutations in the fourth transmembrane helix. PLoS One 2013, 15, e70916. doi: 10.1371/journal.pone.0070916
3. Assidi, M.; Dieleman, S.J.; Sirard, M.-A. Cumulus cell gene expression following the LH surge in bovine preovulatory follicles: potential early markers of oocyte competence. Reproduction 2010, 140, 835-852. doi: 10.1530/REP-10-0248
4. Baranova A, Ivanov D, Petrash N, Pestova A, Skoblov M, Kelmanson I, Shagin D, Nazarenko S, Geraymovych E, Litvin O, Tiunova A, Born TL, Usman N, Staroverov D, Lukyanov S, Panchin Y. The mammalian pannexin family is homologous to the invertebrate innexin gap junction proteins. Genomics. 2004 Apr;83(4):706-16. doi: 10.1016/j.ygeno.2003.09.025. PMID: 15028292
5. Batias, C.; Defamie, N.; Lablack, A.; Thepot, D.; Fenichel, P.; Segretain, D., Pointis, G. Modified expression of testicular gap-junction connexin 43 during normal spermatogenic cycle and in altered spermatogenesis. Cell Tissue Res. 1999, 298, 113-121. doi: 10.1007/s004419900076
6. Berisha, B., et al., Expression and localization of gap junctional connexins 26 and 43 in bovine periovulatory follicles and in corpus luteum during different functional stages of oestrous cycle and pregnancy. Reprod Domest Anim, 2009. 44(2): p. 295-302.
7. Brehm, R.; Rüttinger, C.; Fischer, P.; Gashaw, I.; Winterhager, E.; Kliesch, S.; Bohle, R.M.; Steger, K.; Bergmann, M. Transition from preinvasive carcinoma in situ to seminoma is accompanied by a reduction of connexin 43 expression in Sertoli cells and germ cells. Neoplasia 2006, 8, 499-509. doi: 10.1593/neo.05847
8. Brehm, R.; Zeiler, M.; Ruttinger, C.; Herde, K.; Kibschull, M.; Winterhager, E.; Willecke, K.; Guillou, F.; Lécureuil, C.; Steger, K.; Konrad, L.; Biermann, K.; Failing,K.; Bergmann, M. A Sertoli cell-specific knockout of connexin 43 prevents initiation of spermatogenesis. Am. J. Pathol. 2007, 171, 19-31. doi: 10.2353/ajpath.2007.061171
9. Bukovsky, A., et al., Oogenesis in adult mammals, including humans: a review. Endocrine, 2005. 26(3): p. 301-16.
10. Carabatsos, M.J.; Sellitto, C.; Goodenough, D.A.; Albertini, D.F. Oocyte-granulosa cell heterologous gap junctions are required for the coordination of nuclear and cytoplasmic meiotic competence. Dev. Biol. 2000, 226, 167-179. doi: 10.1006/dbio.2000.9863
11. Chakraborty, S., et al., E-cadherin differentially regulates the assembly of Connexin43 and Connexin32 into gap junctions in human squamous carcinoma cells. J Biol Chem, 2010. 285(14): p. 10761-76.
12. Chang, A.S.; Dale, A.N.; Moley, K.H. Maternal diabetes adversely affects preovulatory oocyte maturation, development, and granulosa cell apoptosis. Endocrinology 2005, 146, 2445-2453. doi: 10.1210/en.2004-1472
13. Chekeni, F.B., Elliott, M.R., Sandilos, J.K., Walk, S.F., Kinchen, J.M., Lazarowski, E.R., Armstrong, A.J.; Penuela, S.; Laird, D.W.; Salvesen, G.S.; Isakson, B.E.; Bayliss, D.A.; Ravichandran, K.S. Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis. Nature 2010, 467, 863-867. doi: 10.1038/nature09413
14. Colton, S.A.; Pieper, G.M.; Downs, S. Altered meiotic regulation in oocytes from diabetic mice. Biol. Reprod. 2002, 67, 220-231. doi: 10.1095/biolreprod67.1.220
15. Cui, L., et al., Endothelin-1 promotes human germinal vesicle-stage oocyte maturation by downregulating connexin-26 expression in cumulus cells. Mol Hum Reprod, 2018. 24(1): p. 27-36.
16. Cyr, D.G. Connexins and pannexins: Coordinating cellular communication in testis and epididymis. Spermatogenesis 2011, 1, 325-338. doi: 10.4161/spmg.1.4.18948
17. da Fonseca Junior, A.M; Gaita, V.; Argumedo, D.R; Signori de Castro, L.; de Agostini Losano, J.D.; Leite, R.F.; Nichi, M; Ortiz d'Ávila Assumpção, M.E.; Ribeiro de Araújo, D.; Ranha Neves, A.A.; Pecora Milazzotto, M. Changes in fertilization medium viscosity using hyaluronic acid impact bull sperm motility and acrosome status. Reprod. Domest. Anim. 2020, 55, 974-983. doi: 10.1111/rda.13739
18. Dahl, G.; Qiu, F.; Wang, W. The bizarre pharmacology of the ATP release channel pannexin1. Neuropharmacology 2013, 75, 583-593. doi: 10.1016/j.neuropharm.2013.02.019

19. Defamie, N.; Berthaut, I.; Mograbi, B.; Chevallier, D.; Dadoune, J.P.; Fenichel, P.; Segretain, D.; Pointis, G. Impaired gap junction connexin 43 in Sertoli cells of patients with secretory azoospermia: a marker of undifferentiated Sertoli cells. Lab. Invest. 2003, 83, 449-456. doi: 10.1097/01.lab.0000059928.82702.6d
20. Dobrowolski, R. and K. Willecke, Connexin-caused genetic diseases and corresponding mouse models. Antioxid Redox Signal, 2009. 11(2): p. 283-95.
21. Dubé, E.; Dufresne, J.; Chan, P.T.K.; Cyr, D.G. Epidermal growth factor regulates connexin 43 in the human epididymis: role of gap junctions in azoospermia. Hum. Reprod. 2012, 27, 2285-2296. doi: 10.1093/humrep/des164
22. Dufresne, J.; Cyr, D.G. Regulation of the pannexin-1 promoter in the rat epididymis. Biol. Reprod. 2014, 91, 1-13. doi: 10.1095/biolreprod.114.122168
23. Dufresne, J.; Finnson, K.W.; Gregory, M.; Cyr, D.G. Expression of multiple connexins in the rat epididymis indicates a complex regulation of gap junctional communication. Am. J. Physiol. Cell Physiol. 2003, 284, C33-43. doi: 10.1152/ajpcell.00111.2002
24. Dye, Z.T.; Rutledge, L.W.; Penuela, S.; Dyce, P.W. Pannexin 1 inhibition delays maturation and improves development of Bos taurus oocytes. J. Ovarian Res. 2020, 13, 98. doi: 10.1186/s13048-020-00704-w
25. Edmonds, J.W.; McKinney, S.L.; Prasain, J.K.; Miller, M.A. The gap junctional protein INX-14 functions in oocyte precursors to promote C. elegans sperm guidance. Dev. Biol. 2011, 359, 47-58. doi: 10.1016/j.ydbio.2011.08.014
26. El-Hayek, S. and H.J. Clarke, Follicle-Stimulating Hormone Increases Gap Junctional Communication Between Somatic and Germ-Line Follicular Compartments During Murine Oogenesis. Biol Reprod, 2015. 93(2): p. 47.
27. Erickson, G.F., C. Wang, and A.J. Hsueh, FSH induction of functional LH receptors in granulosa cells cultured in a chemically defined medium. Nature, 1979. 279(5711): p. 336-8.
28. Esseltine, J.L. and D.W. Laird, Next-Generation Connexin and Pannexin Cell Biology. Trends Cell Biol, 2016. 26(12): p. 944-955.
29. Feuerstein, P.; Cadoret, V.; Dalbies-Tran, R.; Guerif, F.; Bidault, R.; Royere, D. Gene expression in human cumulus cells: one approach to oocyte competence. Hum. Reprod. 2007, 22, 3069-3077. doi: 10.1093/humrep/dem336
30. Fouladi-Nashta, A.A.; Raheem, K.A.; Marei, W.F.; Ghafari, F.; Hartshorne, G.M. Regulation and roles of the hyaluronan system in mammalian reproduction. Reproduction 2017, 153, R43-R58. doi: 10.1530/REP-16-0240
31. Furnus, C.C.; de Matos, D.G.; Martinez, A.G. Effect of hyaluronic acid on development of in vitro produced bovine embryos. Theriogenology 1998, 49, 1489-1499. doi: 10.1016/s0093-691x(98)00095-8
32. Gabriel, H.D., et al., Transplacental uptake of glucose is decreased in embryonic lethal connexin26-deficient mice. J Cell Biol, 1998. 140(6): p. 1453-61.
33. Gershon, E., V. Plaks, and N. Dekel, Gap junctions in the ovary: expression, localization and function. Mol Cell Endocrinol, 2008. 282(1-2): p. 18-25.
34. Giese, S.; Hossain, H.; Markmann, M.; Chakraborty, T.; Tchatalbachev, S.; Guillou, F.; Bergmann, M.; Failing, K.; Weider, K.; Brehm, R. Sertoli-cell-specific knockout of connexin 43 leads to multiple alterations in testicular gene expression in prepubertal mice. Dis. Model. Mech. 2012, 5, 895-913. doi: 10.1242/dmm.008649
35. Gilleron, J.; Nebout, M.; Scarabelli, L.; Senegas-Balas, F.; Palmero, S.; Segretain, D.; Pointis, G. A potential novel mechanism involving connexin 43 gap junction for control of Sertoli cell proliferation by thyroid hormones. J. Cell. Physiol. 2006, 209, 153-161. doi: 10.1002/jcp.20716
36. Gittens, J.E. and G.M. Kidder, Differential contributions of connexin37 and connexin43 to oogenesis revealed in chimeric reaggregated mouse ovaries. J Cell Sci, 2005. 118(Pt 21): p. 5071-8.
37. Granot, I. and N. Dekel, Phosphorylation and expression of connexin-43 ovarian gap junction protein are regulated by luteinizing hormone. J Biol Chem, 1994. 269(48): p. 30502-9.
38. Grazul-Bilska, A.T., L.P. Reynolds, and D.A. Redmer, Gap junctions in the ovaries. Biol Reprod, 1997. 57(5): p. 947-57.
39. Grummer, R., O. Traub, and E. Winterhager, Gap junction connexin genes cx26 and cx43 are differentially regulated by ovarian steroid hormones in rat endometrium. Endocrinology, 1999. 140(6): p. 2509-16.
40. Guidobaldi, H.A.; Teves, M.E.; Unates, D.R.; Anastasia, A.; Giojalas, L.C. Progesterone from the cumulus cells is the sperm chemoattractant secreted by the rabbit oocyte cumulus complex. PLoS ONE 2008, 3, e3040. doi: 10.1371/journal.pone.0003040
41. Gulbransen, B.D.; Bashashati, M.; Hirota, S.A.; Gui, X.; Roberts, J.A.; MacDonald J.A.; Muruve, D.A.; McKay, D.M.; Beck, P.L.; Mawe, G.M.; Thompson, R.J. Activation of neuronal P2X7 receptor-pannexin-1 mediates death of enteric neurons during colitis. Nat Med. 2012, 18, 600-604. doi: 10.1038/nm.2679
42. Han, S.Y.; Lee, K.-H. The expression patterns of connexin isoforms in the rat caput epididymis during postnatal development. J. Anim. Sci. Tech. 2013, 55, 245-255. doi.org/10.5187/JAST.2013.55.4.249
43. Harris AL, Contreras JE. Motifs in the permeation pathway of connexin channels mediate voltage and Ca (2+) sensing. Front Physiol. 2014 Mar 31;5:113. doi: 10.3389/fphys.2014.00113. PMID: 24744733
44. Harris, A.L., Connexin channel permeability to cytoplasmic molecules. Prog Biophys Mol Biol, 2007. 94(1-2): p. 120-43.
45. Hasegawa, J.; Yanaihara, A.; Iwasaki, S.; Mitsukawa, K.; Negishi, M.; Okai, T. Reduction of connexin 43 in human cumulus cells yields good embryo competence during ICSI. J. Assist. Reprod. Genet. 2007, 24, 463-466. doi: 10.1007/s10815-007-9155-4

46. Hilbold, E., Distl, O.; Hoedemaker, M.; Wilkening, S.; Behr, R.; Rajkovic, A; Langeheine, M.; Rode, K.; Jung, K.; Metzger, J.; Brehm, R.H.J. Loss of Cx43 in murine Sertoli cells leads to altered prepubertal Sertoli cell maturation and impairment of the mitosis-meiosis switch. Cells 2020, 9, 676. doi: 10.3390/cells9030676
47. Huszar, G.; Jakab, A.; Sakkas, D.; Celik-Ozenci, C.; Cayli, S.; Delpiano, E.; Ozkavukcu S. Fertility testing and ICSI sperm selection by hyaluronic acid binding: clinical and genetic aspects. Reprod. Biomed. Online 2007, 14, 650-663. doi: 10.1016/s1472-6483(10)61060-7
48. Itahana, K., Y. Morikazu, and T. Takeya, Differential expression of four connexin genes, Cx-26, Cx-30.3, Cx-32, and Cx-43, in the porcine ovarian follicle. Endocrinology, 1996. 137(11): p. 5036-44.
49. Jakab, A.; Sakkas, D.; Delpiano, E.; Cayli, S.; Kovanci, E.; Ward, D.; Ravelli, A.; Huszar, G. Intracytoplasmic sperm injection: a novel selection method for sperm with normal frequency of chromosomal aneuploidies. Fertil. Steril. 2005, 84, 1665-1673. doi: 10.1016/j.fertnstert.2005.05.068
50. Johnson, M.L., et al., Expression of gap junctional proteins connexin 43, 32, and 26 throughout follicular development and atresia in cows. Endocrine, 1999. 10(1): p. 43-51.
51. Johnson, M.L., et al., Gap junctional intercellular communication of bovine granulosa and thecal cells from antral follicles: effects of luteinizing hormone and follicle-stimulating hormone. Endocrine, 2002. 18(3): p. 261-70.
52. Joseph A.; Shur, B. D.; Ko, C.; Chambon, P.; Hess, R.A. Epididymal hypo-osmolality induces abnormal sperm morphology and function in estrogen receptor alpha knockout mouse. Biol. Reprod. 2010, 82, 958-967. doi: 10.1095/biolreprod.109.080366
53. Juneja, S.C.; Barr, K.J.; Enders, G.C.; Kidder, G.M. Defects in the germ line and gonads of mice lacking connexin 43. Biol. Reprod. 1999, 60, 1263-1270. doi: 10.1095/biolreprod60.5.1263
54. Kahiri, C.N.; Khalil, M.W.; Tekpetey, F.; Kidder, G.M. Leydig cell function in mice lacking connexin43. Reproduction 2006, 132, 607-616. doi: 10.1530/rep.1.01234
55. Kalma, Y.; Granot, I.; Galiani, D.; Barash, A.; Dekel, N. Luteinizing hormone-induced connexnin 43 down-regulation: inhibition of translation. Endocrinology 2004, 145, 1617-1624. doi: 10.1210/en.2003-1051
56. Kidder, G.M. and A.A. Mhawi, Gap junctions and ovarian folliculogenesis. Reproduction, 2002. 123(5): p. 613-20.
57. Kimura, N.; Totsukawa, K.; Sato, E. Significance of mammalian cumulus-oocyte complex matrix in oocyte meiotic maturation: review of the synthetic control and possible roles of hyaluronan (HA) and HA-binding protein. J. Mammal. Ova Res. 2006, 23, 36-51. doi.org/10.1274/jmor.23.36
58. Kordowitzki P, Hamdi M, Derevyanko A, Rizos D, Blasco M. The effect of rapamycin on bovine oocyte maturation success and metaphase telomere length maintenance. Aging (Albany NY). 2020 Apr 27;12(8):7576-7584. doi: 10.18632/aging.103126.
59. Kotula-Balak M.; Hejmej, A.; Sadowska, J.; Bilinska, B. Connexin 43 expression in human and mouse testes with impaired spermatogenesis. Eur. J. Histochem. 2007, 51, 261-268. PMID: 18162455
60. Kruger, O., et al., Defective vascular development in connexin 45-deficient mice. Development, 2000. 127(19): p. 4179-93.
61. Kumar, T.R., et al., Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. Nat Genet, 1997. 15(2): p. 201-4.
62. Lee, K.-H. Differential expression of multiple connexins in rat corpus and cauda epididymis at various postnatal stages. J. Anim. Sci. Tech. 2013, 55, 521-530. doi.org/10.5187/JAST.2013.55.6.521
63. Lee, K.-H. Modification of gene expression of connexins in the rat corpus epididymis by estradiol benzoate or flutamide exposure at the early neonatal age. Dev. Reprod. 2015, 19, 69-77. doi: 10.12717/DR.2015.19.2.069
64. Lee, V.R., et al., Pannexin 1 regulates adipose stromal cell differentiation and fat accumulation. Sci Rep, 2018. 8(1): p. 16166.
65. Leybaert, L., et al., Connexin channels, connexin mimetic peptides and ATP release. Cell Commun Adhes, 2003. 10(4-6): p. 251-7.
66. Li, M.W.; Mruk, D.D.; Lee, W.M.; Cheng, C.Y. Connexin 43 and plakophilin-2 as a protein complex that regulates blood-testis barrier dynamics. Proc. Natl. Acad. Sci. 2009, 106, 10213-10218. doi: 10.1073/pnas.0901700106
67. Li, M.W.; Mruk, D.D.; Lee, W.M.; Cheng, C.Y. Connexin 43 is critical to maintain the homeostasis of the blood-testis barrier via its effects on tight junction reassembly. Proc. Natl. Acad. Sci. 2010, 107, 17998-18003. doi: 10.1073/pnas.1007047107
68. Li, S.-H.; Lin, M.-H.; Hwu, Y.-M.; Lu, C.-H.; Yeh, L.-Y.; Chen, Y.-J.; Lee, R.K.-K. Correlation of cumulus gene expression of GJA1 , PRSS35, PTX3 and SERPINE2 with oocyte maturation, fertilization, and embryo development. Reprod. Biol. Endocrinol. 2015, 13, 93. doi: 10.1186/s12958-015-0091-3
69. Liu, Q.; Kong, L.; Zhang, J.; Xu, Q.; Wang, J.; Xue, Z.; Wang, J. Involvement of GJA1 and gap junctional intercellular communication between cumulus cells and oocytes from women with PCOS. Biomed Res. Int. 2020, 28, 5403904. doi: 10.1155/2020/5403904
70. Liu, Q.; Li, Y.; Feng, Y.; Liu, C.; Ma, J.; Li, Y.; Xiang, H.; Ji, Y.; Cao, Y.; Tong, X.; Xue, Z. Single-cell analysis of differences in transcriptomic profiles of oocytes and cumulus cells at GV, MI, MII stages from PCOS patients. Sci. Rep. 2016, 6, 39638. doi: 10.1038/srep39638
71. Locovei, S.; Wang, J.; Dahl, G. Activation of pannexin 1 channels by ATP through P2Y receptors and by cytoplasmic calcium. FEBS Lett. 2006, 580, 239-44. doi: 10.1016/j.febslet.2005.12.004

72. Lohman, A.W.; Leskov, I.L.; Butcher, J.T.; Johnstone, S.R.; Stokes, T.A.; Begandt, D.; DeLalio, L.J.; Best, A.K.; Penuela, S.; Leitinger, N.; Ravichandran, K.S. Pannexin 1 channels regulate leukocyte emigration through the venous endothelium during acute inflammation. Nat. Commun. 2015, 6, 7965. doi: 10.1038/ncomms8965
73. Lydka, M.; Kopera-Sobota, I.; Kotula-Balak, M.; Chojnacka, K.; Zak, D.; Bilinska B. Morphological and functional alterations in adult boar epididymis: Effects of prenatal and postnatal administration of flutamide. Acta Vet. Scand. 2011, 22, 12. doi: 10.1186/1751-0147-53-12
74. male rat treated with estradiol benzoate or flutamide. Dev. Reprod. 2014, 18, 293-300. doi: 10.12717/devrep.2014.18.4.293
75. Nelles, E., et al., Defective propagation of signals generated by sympathetic nerve stimulation in the liver of connexin32-deficient mice. Proc Natl Acad Sci U S A, 1996. 93(18): p. 9565-70.
76. Nitta, M., et al., Identification and expression analysis of connexin-45 and connexin-60 as major connexins in porcine oocytes. J Anim Sci, 2010. 88(10): p. 3269-79.
77. Norris, R.P., et al., Cyclic GMP from the surrounding somatic cells regulates cyclic AMP and meiosis in the mouse oocyte. Development, 2009. 136(11): p. 1869-78.
78. Orellana, J.A.; Velasquez, S.; Williams, D.W.; Saez, J.C.; Berman, J.W.; Eugenin, E.A. Pannexin1 hemichannels are critical for HIV infection of human primary CD4+ T lymphocytes. J. Leukoc. Biol. 2013, 94, 399-407. doi: 10.1189/jlb.0512249
79. Panchin, Y., et al., A ubiquitous family of putative gap junction molecules. Curr Biol, 2000. 10(13): p. R473-4.
80. Penuela, S., et al., Pannexin 1 and pannexin 3 are glycoproteins that exhibit many distinct characteristics from the connexin family of gap junction proteins. J Cell Sci, 2007. 120(Pt 21): p. 3772-83.
81. Penuela, S.; Gehi, R.; Laird, D.W. The biochemistry and function of pannexin channels. Biochim. Biophys. Acta 2013, 1828, 15-22. doi: 10.1016/j.bbamem.2012.01.017
82. Pfenniger, A.; Wohlwend, A.; Kwak, B.R. Mutations in connexin genes and disease. Eur. J. Clin. Invest. 2011, 41, 103-116. doi: 10.1111/j.1365-2362.2010.02378.x
83. Pfenniger, A.; Wohlwend, A.; Kwak, B.R. Mutations in connexin genes and disease. Eur. J. Clin. Invest. 2010, 41, 103-116. doi: 10.1111/j.1365-2362.2010.02378
84. Phelan, P. Innexins: members of an evolutionarily conserved family of gap-junction proteins. Biochim. Biophys. Acta 2005, 1711, 225-245. doi: 10.1016/j.bbamem.2004.10.004
85. Pointis, G; Segretain, D. Role of connexin-based gap junction channels in testis. Trends Endocrinol. Metab. 2005, 16, 300-306. doi: 10.1016/j.tem.2005.07.001
86. Qu, Y., et al., Pannexin-1 is required for ATP release during apoptosis but not for inflammasome activation. J Immunol, 2011. 186(11): p. 6553-61.
87. Ratchford, A.M.; Esguerra, C.R. Moley, K.H. Decreased oocyte-granulosa cell gap junction communication and connexin expression in a type 1 diabetic mouse model. Mol. Endocrinol. 2008, 22, 2643-2654. doi: 10.1210/me.2007-0495
88. Read, C.C.; Willhelm, G.; Dyce, P.W. Connexin 43 coupling in bovine cumulus cells, during the follicular growth phase, and its relationship to in vitro embryo outcomes. Mol. Reprod. Dev. 2018, 85, 579-589. doi: 10.1002/mrd.22993
89. Risley, M.S.; Tan, I.P.; Roy, C.; Saez, J.C. Cell-, age- and stage-dependent distribution of connexin 43 gap junctions in testes. J. Cell Sci. 1992, 103, 81-96. PMID: 1331136
90. Ruan, Y.C.; Shum, W.W.C.; Belleannée, C.; Da Silva, N.; Breton, S. ATP secretion in the male reproductive tract: essential role of CFTR. J. Physiol. 2012, 590, 4209-4222. doi: 10.1113/jphysiol.2012.230581
91. Sampaio da Silva, A.M.; Bruno, J.B.; Lima, L.F.; Ribeiro de Sá, N.A.; Osmarini Lunardi, F.; Accioly Ferreira, A.C.; Vieira Correia, H.H.; Nascimentode Aguiar, F.L.; Rocha Araújo, V.; Lobo, C.H.; de Alencar Araripe Moura, A.; Cabral Campello, C.; Smitz, J.; de Figueiredo, J.R.; Ribeiro Rodrigues, A.P. Connexin 37 and 43 gene and protein expression and developmental competence of isolated ovine secondary follicles cultured in vitro after vitrification of ovarian tissue. Theriogenology 2016, 85, 1457-1467. doi: 10.1016/j.theriogenology.2016.01.001
92. Sanchez, F. and J. Smitz, Molecular control of oogenesis. Biochim Biophys Acta, 2012. 1822(12): p. 1896-912.
93. Sang, Q.; Zhang, Z.; Shi, J.; Sun, X.; Li, B.; Yan, Z.; Xue, S.; Ai, A.; Lyu, Q.; Li, W.; Zhang, J.; Wu, L.; Mao, X.; Chen, B.; Mu, J.; Li, Q.; Du, J.; Sun, Q.; Jin, L.; He, L.; Zhu, S.; Kuang, Y.; Wang, L. A pannexin 1 channelopathy causes human oocyte death. Sci. Transl. Med. 2019, 27, eaav8731. doi: 10.1126/scitranslmed.aav8731
94. Santiquet, N., C. Robert, and F.J. Richard, The dynamics of connexin expression, degradation and localisation are regulated by gonadotropins during the early stages of in vitro maturation of swine oocytes. PLoS One, 2013. 8(7): p. e68456.
95. Santiquet, N.W., et al., Regulation of gap-junctional communication between cumulus cells during in vitro maturation in swine, a gap-FRAP study. Biol Reprod, 2012. 87(2): p. 46.
96. Sasseville, M.; Gagnon, M.-C.; Guillemette, C.; Sullivan, R.; Gilchrist, R.B.; Richard, F.J. Regulation of gap junctions in porcine cumulus-oocyte complexes: contributions of granulosa cell contact, gonadotropins, and lipid rafts. Mol. Endocrinol. 2009, 23, 700-710. doi: 10.1210/me.2008-0320
97. Sato, E.; Yokoo, M. Morphological and biochemical dynamics of porcine cumulus-oocyte complexes: role of cumulus expansion in oocyte maturation. Ital. J. Anat. Embryol. 2005, 110, 205-217. PMID: 16101040
98. Shimada, M.; Maeda, T.; Terada, T. Dynamic changes of connexin-43, gap junctional protein, in outer layers of cumulus cells are regulated by PKC and PI 3-kinase during meiotic resumption in porcine oocytes. Biol. Reprod. 2001, 64, 1255-1263. doi: 10.1095/biolreprod64.4.1255

99. Shin, K.T.; Nie, Z.-W.; Zhou, W.; Zhou, D.; Kim, J.-Y.; Ock, S.A.; Niu, Y.J.; Cui, X.-S. Connexin 43 knockdown induces mitochondrial dysfunction and affects early developmental competence in porcine embryos. Microsc. Microanal. 2020, 26, 287-296. doi: 10.1017/S1431927620000033
100. Shinohara, T., et al., Treatment with the MAPK kinase inhibitor U0126 during the first two hours of in vitro maturation improves bovine oocyte developmental competence. Reprod Domest Anim, 2018. 53(1): p. 270-273.
101. Simon, A.M.; Goodenough, D.A.; Li, E.; Paul, D.L. Female infertility in mice lacking connexin 37. Nature 1997, 385, 525-529. doi: 10.1038/385525a0
102. Skerget S.; Rosenow, M.A.; Petritis, K.; Karr, T.L. Sperm proteome maturation in the mouse epididymis. PLoS One 2015, 10, e0140650. doi: 10.1371/journal.pone.0140650
103. Snoeck, F.; Szymanska, K.J.; Sarrazin, S.; Ortiz-Escribano, N.; Leybaert, L.; Van Soom, A. Blocking connexin channels during vitrification of immature cat oocytes improves maturation capacity after warming. Theriogenology 2018, 122, 144-149. doi: 10.1016/j.theriogenology.2018.09.011
104. Söhl G, Willecke K. An update on connexin genes and their nomenclature in mouse and man. Cell Commun Adhes. 2003 Jul-Dec;10(4-6):173-80. doi: 10.1080/cac.10.4-6.173.180. PMID: 14681012.
105. Sommersberg, B., et al., Gap junction communication and connexin 43 gene expression in a rat granulosa cell line: regulation by follicle-stimulating hormone. Biol Reprod, 2000. 63(6): p. 1661-8.
106. Sridharan, S.; Simon, L.; Meling, D.D.; Cyr, D.G.; Gutstein, D.E.; Fishman, G.I.; Guillou, F.; Cook, P.S. Proliferation of adult Sertoli cells following conditional knockout of the gap junctional protein GJA1 (connexin 43) in mice. Biol. Reprod. 2007, 76, 804-812. doi: 10.1095/biolreprod.106.059212
107. St-Pierre, N.; Dufresne, J.; Rooney, A.A.; Cyr, D.G. Neonatal hypothyroidism alters the localization of gap junctional protein connexin 43 in the testis and messenger RNA levels in the epididymis of the rat. Biol. Reprod. 2003, 68, 1232-1240. doi: 10.1095/biolreprod.102.010504
108. Sun, F.; Bahat, A.; Gakamsky, A.; Girsh, E.; Katz, N.; Giojalas, L.C.; Tur-Kaspa, I.; Eisenbach, M. Human sperm chemotaxis: both the oocyte and its surrounding cumulus cells secrete sperm chemoattractants. Hum. Reprod. 2005, 20, 761-767. doi: 10.1093/humrep/deh657
109. Sutovský, P.; Fléchon, J.E.; Fléchon, B.; Motlik, M.; Peynot, N.; Chesné, P.; Heyman, Y. Dynamic changes of gap junctions and cytoskeleton during in vitro culture of cattle oocyte cumulus complexes. Biol. Reprod. 1993, 49, 1277-1287. doi: 10.1095/biolreprod49.6.1277
110. Talhouk, R.; Tarraf, C.; Kobrossy, L; Shaito, A.; Bazzi, S.; Bazzoun, D.; El-Sabban, M. Modulation of Cx43 and gap junctional intercellular communication by androstenedione in rat polycystic ovary and granulosa cells in vitro. J. Reprod. Infertil. 2012, 13, 21-32. PMID: 23926521
111. Tan, I.P.; Roy, C.; Saez, J.C.; Saez, C.G.; Paul, D.L.; Risley, M.S. Regulated assembly of connexin33 and connexin43 into rat Sertoli cell gap junctions. Biol. Reprod. 1996, 54, 1300-1310. doi: 10.1095/biolreprod54.6.1300
112. te Velde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update. 2002 Mar-Apr;8(2):141-54. doi: 10.1093/humupd/8.2.141. PMID: 12099629
113. Tomsig, J.L.; Usanovic, S.; Turner, T.T. Growth factor-stimulated mitogen-activated kinase (MAPK) phosphorylation in the rat epididymis is limited by segmental boundaries. Biol. Reprod. 2006, 75, 598-604. doi: 10.1095/biolreprod.106.05239
114. Tong, D., et al., In vivo analysis of undocked connexin43 gap junction hemichannels in ovarian granulosa cells. J Cell Sci, 2007. 120(Pt 22): p. 4016-24.
115. Tong, D., et al., Patch-clamp study reveals that the importance of connexin43-mediated gap junctional communication for ovarian folliculogenesis is strain specific in the mouse. Am J Physiol Cell Physiol, 2006. 290(1): p. C290-7.
116. Turmel, P.; Dufresne, J.; Hermo, L.; Smith, C.E.; Penuela, S.; Laird, D.W.; Cyr, D.G. Characterization of pannexin1 and pannexin3 and their regulation by androgens in the male reproductive tract of the adult rat. Mol. Reprod. Dev. 2011, 78, 124-138. doi: 10.1002/mrd.21280
117. Van Soom, A.; Tanghe, S.; De Pauw, I.; Maes, D.; de Kruif, A. Function of the cumulus oophorus before and during mammalian fertilization. Reprod. Domest. Anim. 2002, 37, 144-151. doi: 10.1046/j.1439-0531.2002.00345.x
118. Veitch, G.I., et al., Selective assembly of connexin37 into heterocellular gap junctions at the oocyte/granulosa cell interface. J Cell Sci, 2004. 117(Pt 13): p. 2699-707.
119. Wang, H.X., et al., Connexin expression and gap junctional coupling in human cumulus cells: contribution to embryo quality. J Cell Mol Med, 2009. 13(5): p. 972-84.
120. Wang, H.X., et al., The canonical WNT2 pathway and FSH interact to regulate gap junction assembly in mouse granulosa cells. Biol Reprod, 2013. 89(2): p. 39.
121. Wang, Y.Y.; Lin, Y.H.; Wu, Y.N.; Chen, Y.L.; Lin, Y.C.; Cheng, C.Y.; Chiang, H.S.. Loss of SLC9A3 decreases CFTR protein and causes obstructed azoospermia in mice. PLoS Genet. 2017, 13, e1006715. doi: 10.1371/journal.pgen.1006715
122. Weissgerber, P.; Kriebs, U.; Tsvilovskyy, V.; Olausson, J.; Kretz, O.; Stoerger, C.; Mannebach, S.; Wissenbach, U.; Vennekens, R.; Middendorff, R.; Flockerzi, V.; Freichel, M. Excision of Trpv6 gene leads to severe defects in epididymal Ca2+ absorption and male fertility much like single D541A pore mutation. J Biol. Chem. 2012, 25, 17930-17941. doi: 10.1074/jbc.M111.328286
123. Whitten, S.J.; Miller, M. The role of gap junctions in Caenorhabditis elegans oocyte maturation and fertilization. Dev. Biol. 2007, 301, 432-446. doi: 10.1016/j.ydbio.2006.08.038

124. Wicki-Stordeur, L.E.; Swayne, L.A. The emerging pannexin 1 signalome: anew nexus revealed? Front. Cell. Neurosci. 2014, 7, 287. doi: 10.3389/fncel.2013.00287
125. Wiesen, J.F. and A.R. Midgley, Jr., Expression of connexin 43 gap junction messenger ribonucleic acid and protein during follicular atresia. Biol Reprod, 1994. 50(2): p. 336-48.
126. Wigglesworth, K., et al., Bidirectional communication between oocytes and ovarian follicular somatic cells is required for meiotic arrest of mammalian oocytes. Proc Natl Acad Sci U S A, 2013. 110(39): p. E3723-9.
127. Winterhager E, Kidder GM. Gap junction connexins in female reproductive organs: implications for women's reproductive health. Hum Reprod Update. 2015 May-Jun;21(3):340-52. doi: 10.1093/humupd/dmv007. Epub 2015 Feb 9. PMID: 25667189
128. Xu, J.; Nicholson, B.J. The role of connexins in ear and skin physiology – functional insights from disease-associated mutations. Biochim. Biophys. Acta 2013, 1828, 167-178. doi: 10.1016/j.bbamem.2012.06.024
129. Yun, S.P., et al., Mechanism of PKA-dependent and lipid-raft independent stimulation of Connexin43 expression by oxytoxin in mouse embryonic stem cells. Mol Endocrinol, 2012. 26(7): p. 1144-57.
130. Zhou, C.-J.; Wu, S.-N.; Shen, J.-P.; Wang, D.-H.; Kong, X.-W.; Lu, A.; Li, Y.-I.; Zhou, H-X.; Zhao, Y.-F.; Liang, C.-G. The beneficial effects of cumulus cells and oocyte-cumulus cell gap junctions depends on oocyte maturation and fertilization methods in mice. PeerJ 2016, 4, e1761. doi:10.7717/peerj.1761