REVIEW ARTICLE

The Role of Gap Junction Proteins in Infertility

[1]Piyush Tripathi, [2]Manorama Tripathi

[1]Postdoctoral Research Associate, Department of Internal Medicine, Washington University School of Medicine, St. Louis, USA

[2]Postdoctoral Research Associate, Department of Medicine, Washington University School of Medicine, St. Louis, USA

Correspondence: Piyush Tripathi, Postdoctoral Research Associate, Department of Internal Medicine, Campus Box 8126 Washington University School of Medicine, St. Louis, MO 63110, USA, Phone: (314) 362-4309, Fax: (314) 362-8237 e-mail: ptripath@dom.wustl.edu

ABSTRACT

Testis and ovary serve an important role of producing male and female gametes. Their normal functioning is very important for the proper formation of sperm and ovum and thus has a critical role in the successful fertility outcome. Synchronized activity of various cells in the gonads is needed to provide favorable niche for the growth and development of the germ cells. Among various ways of cellular communication, intercellular communication is mediated by gap junctions, which provides open but selective exchange of ion and molecules of restricted size between two adjoining cells. The basic unit of gap junction is connexins. Their important role has been speculated in the maintenance of homeostasis, morphogenesis, cell differentiation, and growth control in higher organisms. The expression of gap junction proteins in reproductive tissues has drawn the attention and interest of researcher to investigate their role in the reproductive outcome. The reports about the correlation of gap junction protein expression pattern in infertility patients and in animal models have suggested their implication in fertility. Some of these gap junction proteins seem to have redundant functions, whereas some could be very critical in the normal fertility and could not be dispensable for the successful outcome of the reproduction.

Keywords: Gap junction, Connexin, Fertility.

INTRODUCTION

Various kinds of cellular interactions exist for anchorage, support and communication between cells. Gap junction (GJ), adherens junction, and tight junction (TJ) are three major types of junctions responsible for intercellular adhesions and communications among different cell types. Communications among cells are very essential for synchronized functioning of different cells as unit and also to convey signals for terminal actions. Most cells in animal tissues communicate with surrounding cells via gap junctions, with few exception like terminally differentiated skeletal muscle cells and blood cells. The basic unit of gap junction is a transmembrane channel called connexon, which itself is made up of six connexins subunits. As the name suggests, unlike other intercellular junctions, gap junction does not seal the cell membrane but connect both communicating cells by the lumen of junction. These gap junctions provide physical connection between two cells to pass inorganic ions and small water-soluble molecules from the cytoplasm of one cell to other. Apart from metabolic coupling, gap junctions also serve the purpose of electrical coupling at greater speed, which is very essential for synchronized contractions of heart muscle cells and the smooth muscle cells during peristaltic movements.[1]

Date of Received: 27-08-10
Date of Acceptance: 29-09-10
Date of Publication: Sep. 2010

The role of the gap junctional intercellular communication is confirmed in the homeostasis and in various developmental processes, including morphogenesis, cell differentiation, and growth. Apart from its expression in various parts of the body, gap junctions are widely distributed in male and female reproductive organs and in tissues directly involved in germ cell development. It is expression in Sertoli cells, which functions as mother cells for developing male germ cells, and in somatic layer around developing oocyte in the ovary is quite intriguing and indicates toward their specific role during the development of germ cells.[2,3] Various fertility defects were witnessed in connexin (Cx) knockout animal models, which confirm the role of connexins in the reproduction.[4,5] Animal model of connexin mutation also showed defective oogenesis.[6] The information available so far about the expression of Cx in reproductive tissues and correlation of their functionality with the reproductive outcome has indicated their role in fertility. The present review is focused on the recent developments on the role of Cxs in the germ cell development and infertility.

GAP JUNCTIONS: OPEN BUT SELECTIVE WAY OF CELLULAR COMMUNICATION

For the body to work as system, it needs all the cells of different tissues to work in coordinated fashion. During the evolution different ways of cellular communication between adjoining and distance cells have established. For the communication between distant cells, various signaling molecules are needed

to carry the message from the source to the target tissues. They further have to undergo a cascade of events from recognition of those signals on the cell surface by different receptors to their internalization and bringing the message to effect at cellular level. However, this communication could be more direct by cellular contact with adjoining cells. The cellular integration and the coordination of diverse biological functions in most of tissues are established by the contact between adjacent cells by three major types of cell junctions—adherens junction, tight junction and gap junction.

Gap junctions (GJ) are widely distributed and formed by specially organized channels. Each channel is formed when two half channels or connexon present in surface of plasma membrane of one cell joins the connexon on plasma membrane of another cell via noncovalent interaction.[7] Tens to thousands of GJ channels cluster together forming GJ plaques. The intercytoplasmic connections created by GJ channels allow the exchange of ions and metabolites up to 1kDa in size and secondary messengers like IP3 and cAMP from cytosol of one cell to the other.[8] GJs are very peculiar in sense that they have very high turnover rate compared to most integral membrane proteins. Half lives of most of them ranges from 1 to 3 hours and this provides rapid and highly coordinated trafficking system. The complete GJ life cycle consists of connexin synthesis and oligomerization, hemichannel translocation to the plasma membrane for incorporation into GJs, and finally removal from the plasma membrane and degradation.[9]

Each individual connexons are made up of six protein subunits called connexins, surrounding a central hydrophilic core (Fig. 1). The connexon can be homomeric or heteromeric depending on whether it consists of one type or multiple types of connexins. Moreover, the intercellular channel between adjacent cells can be homotypic or heterotypic, if composed of identical or different connexons. In humans, 21 and in mouse 20 connexins (Cxs) has been identified. Connexins are named according to their molecular weight and most of them have a wide tissue distribution pattern.[10] Structurally each connexin protein contains amino and carboxyl terminus in the cytoplasm, four transmembrane domain and two extracellular loops being highly conserved among different connexins and a single cytoplasmic loop and the C-ter tail shows the most diversity.

GERM CELLS IN THE TESTIS AND OVARY

The main function of testis is to carry out the production of sperms, the male gametes. The process includes germ cell proliferation and their final differentiation into mature spermatozoa. The testis is made up of tubular structures called seminiferous tubules, where the spermatogenesis takes places and interstitium or intertubular tissues, which contains Leydig cells, an important source of hormones needed for proper spermato and spermiogenesis.[11] The seminiferous tubule is compartmented into germinal epithelium and the peritubular tissues called lamina propria.[12] The germinal epithelium houses cells of different stages of spermatogenesis, including spermatogonia, primary and secondary spermatocytes and spermatids. Sertoli cells are also present in the germinal epithelium and serve to nurture sperm cells of different stages of spermatogenesis. Sertoli cells are interconnected by tight junctions of cellular membranes constituting 'blood testis barrier' (BTB), which partitions the seminiferous epithelium into the basal compartment and the adluminal compartment. The spermatogonia are initially present in the basal compartment and their more developed forms like primary and secondary spermatocytes and spermatids move to the adluminal compartment during spermatogenesis.[13]

Spermatogenesis begins with the division of stem cells (type A spermatogonia) to produce spermatocytes, which undergoes meiotic processing of spermatocytes to spermatids with several

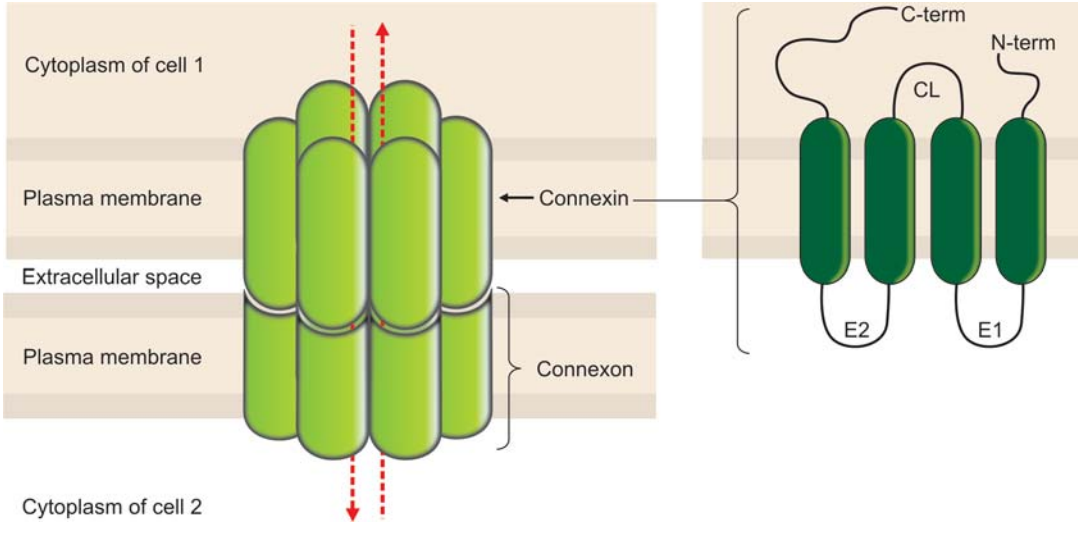

Fig. 1: The arrangement of gap junction. Each gap junction is composed of two connexins contributed by each cell. Individual connexins are made up of six protein subunits called connexins. Each connexin protein contains amino and carboxyl terminus in the cytoplasm. Four transmembrane domains in connexin are connected by two extracellular loops (E1 and E2) and one cytoplasmic loop (CL)

intermediate stages, including leptotene spermatocytes, pachytene spermatocytes, round spermatids, and finally elongated spermatids. These spermatids undergo differentiation and morphological transformation into spermatozoa and are released in the lumens of seminiferous tubule.[13] For the normal development and differentiation of spermatocytes into mature form of spermatids, their migration across BTB is essential during which continuous formation of cellular junction and their remodeling takes place between germ cells and Sertoli cells. Recent evidences suggest the important role of gap junction proteins for the successful spermatogenesis.[14]

Similar to testis, the main function of ovary is to generate female gametes. During early stages of the development, the oocyte is enclosed in somatic cells and called primordial follicle. During further follicular development, maturation of the oocyte takes place, which is surrounded by granulosa cells and enveloped by theca cells.[15] During folliculogenesis, granulosa cells give physical support and mediate signaling between different types of follicular cells and hormones.[16] The size of oocyte also increases with the increase of follicular size and antral cavity is formed near the completion of follicular growth.[17]

The primary oocyte, which is destined to undergo meiotic division to produce female haploid gamete, halts at meiosis I during early embryonic development, and resumes back with the LH (Luteinizing hormone) surge during menstrual cycle.[18] After meiosis I, primary oocyte develops into the secondary oocyte and the first polar body. Secondary oocyte undergoes meiosis II but stops at the metaphase II, and ovulated into the fallopian tube. During fertilization with a spermatozoon, meiosis resumes and with its completion, release of the second polar body takes place.[18]

The important role of cellular junctions between oocyte and somatic cells has been discovered for the normal progression of oocyte growth and maturation. The recent study by Vaccari et al have shown that the disruption of junctional interaction between oocyte and somatic cells motivates the resumption of meiosis in mature oocytes.[19] Further another group has also shown important role of gap junctions in communication between the different follicular cell types.[20,21]

DISTRIBUTION OF GAP JUNCTION PROTEINS IN TESTIS AND OVARY

Along with the germ cells, gap junction proteins are expressed in various locations of male and female reproductive organs, including, seminiferous tubules, leydig cells, sertoli cells, endothelial cells, peritubular cells in the testis and in granulosa cells of ovarian follicles in the ovary. Till date, 21 connexins (Cxs) have been identified in human and 20 in mouse genome, among which 19 has corresponding orthologs in human and mouse.[10] In rodent testis, the expression of 12 connexins has been recognized, including, Cx26, Cx30.2, Cx30.3, Cx31, Cx31.1, Cx32, Cx33, Cx37, Cx40, Cx43, Cx45, Cx46 and Cx50.[22,23] Further Cx26, Cx32, Cx37, Cx43, Cx45 and Cx60 have been identified in ovaries of different species.[3,24,25]

Cx26 protein has been identified in seminiferous epithelium.[22] Cx 31 was only detected in spermatogonia and early spermatocytes but not in Sertoli cells,[26] similar to Cx50 expression detected in spermatocytes and round spermatids,[22] but contrary to Cx 32, which was indentified in sertoli cells but not in germ cells.[27]

In contrast to various other Cxs, Cx33 is exclusively expressed to the testis, specifically in seminiferous tubules and Sertoli cells.[28,29] It was further identified that the heteromeric complex of Cx33 with other Cx are less stable and thus have an inhibitory effect on gap junction channel function by changing the junctional conductance.[30] *In vivo* study confirmed that the Cx33 makes unstable heterocomplex with Cx43 at cell surface and are rapidly internalized by endocytosis.[29] The same group has later on shown that the instability of the Cx33/Cx43 complex at the cell surface is affected by the Cx43 phosphorylation status and its further interaction with the c-Src kinase.[31]

One of the most abundant connexins in the testis is Cx43, which is expressed in the Leydig cells, and in the seminiferous tubules between Sertoli cells and germ cells like spermatogonia and spermatocytes.[32,33] Colocalization and other biochemical experiments have shown that the physically interaction of Cx 43 with cortactin is essential for the formation of cellular junctions between Sertoli cells and germ cells.[34] Recent study about the Sertoli cell specific Cx43 knockout study has shown that Cx43 plays an important role in communication between Sertoli cell and germ cells and in turn the spermatogenesis.[2]

Connexin 43, along with the connexin 37 is also the most abundant connexin in the ovary. Cx43 is expressed in the somatic compartment of the ovarian follicle and may be involved in cellular communication between granulosa cells.[3] However, the expression specificities of Cx43 in ovary are further debated by various groups. Some studies have detected the Cx43 expression in the oocytes of rodent and other species,[35,36] which could not be confirmed in some recent studies.[37,38] It is further demonstrated that the Cx37 is exclusively expressed in the oocyte but not in the somatic compartment of the ovary.[39] These findings indicate that the heterotypic gap junctions constituted by Cx37 and Cx43 could be involved in cellular communication between the oocytes and surrounding somatic cells.

Cx32 was detected in granulosa cells of small antral follicles but not healthy follicles of cattle.[40] Cx26 was detected in the oocyte and in the granulosa and thecal cell layers of preovulatory follicles indicating their possible role in folliculogenesis.[41] The low level of RNA expression of Cx57 has been detected in murine ovary tissue,[42] however its porcine ortholog, Cx60, is expressed in cumulus and theca cells.[43]

REGULATION OF CONNEXIN EXPRESSION IN TESTIS AND OVARY

The study of normal and defective spermatogenesis mice has revealed a stage specific relation of connexin expression in the testis.[44] It is evident that various physiological effectors that intend to modulate the functioning of gonads may also influence the expression of connexins in these tissues.[45]

In the culture experiments, it was demonstrated that the gap junction mediated cellular communication is increased in response to follicle stimulating hormone (FSH).[46] Other studies have indicated that the FSH and its second messenger, cAMP regulate the electrical and functional coupling of gap junctions[46,47] and Cx43 distribution in Sertoli cells.[48] It was further shown that the dibutyryl cAMP in a Ca2C-free medium can regulate the electrical coupling in intact tubule and in cultures indicating that Ca2C is involved in the response to cAMP.[47] A recent study has further demonstrated that FSH, cAMP, DHT, and 17β-E2 can significantly change Cx43 distribution and gap junction coupling.[49]

Apart from nongenomic effect of steroids hormones on connexins, the presence of oestrogen response elements on the Cx43 promoter is indicative of the fact that 17b-E2 can increase Cx43 through a genomic pathway mediated by the nuclear oestrogen receptor.[50] It is also observed that in Sertoli cells, 17b-E2 can induce a Src-mediated translocation of estrogen receptors to the cell surface.[51] The available evidences indicate that 17b-E2 can control Cxs and thus the Sertoli cell gap junctions through both genomic and nongenomic pathways. The cell surface distribution of Cx43 in the testis can also be influenced by thyroid hormone, which is known for its role in testicular function and development.[52] However, its role in the regulation of Cx43 expression in the testis could not be recognized.[53]

The expression of Cx43 in Sertoli cells could be influenced by retinoids, which are well-known for their role in the maintenance of spermatogenesis. The decrease in testicular Cx43 expression was observed in vitamin A-deficient-rat model, associated with impaired spermatogenesis, where vitamin A supplement was able to restore Cx43 expression.[54]

Cx43 expression in the ovary is regulated by gonadotropins like FSH and lutinizing hormone (LH). The initial indication came from stage specific expression level of Cx43 during estrous cycle, which was correlated with the level of FSH and LH. Increase in the Cx43 expression in the large antral follicles was observed where the FSH was relatively elevated.[55] Contrary to FSH, the high concentration of LH during preovulatory surge of gonadotropins was correlated with the decreased expression of Cx43.[55] *In vitro* experiment has demonstrated that in the presence of LH, Cx43 mRNA level was only decreased, whereas Cx43 protein was completely eliminated in rat ovarian follicles, indicating its role in additional layer of regulation by controlling the translation of connexin protein.[56] It was further confirmed that LH inhibits Cx43 translation in rat ovaries.[57]

It is further reported that human chorionic gonadotropin (hCG) can induced down regulation of Cx32 in mouse cumulus–oocyte complexes.[58] It is also reported that Cx32 is less stringently regulated by LH compared to cx43. The levels of Cx32 may remain high for up to 5 h after the LH surge.[37]

FUNCTION OF CONNEXINS IN THE TESTIS AND OVARY

The evidences gathered so far about the specific and stage dependent expressions of connexins in the gonads indicate that they have important role during gonad development and gamete formation in testis and ovary. Some of the most compelling evidences came from the knockout studies, where specific connexins were deleted, and their correlation with fertility problems. Development of techniques to generate conditional knockouts using Cre-loxP system has enabled researchers to study the relevance of particular Cxs of interest in specific tissues, where complete knockouts were embryonic lethal.

Using Cre-loxP conditional knockout technique Cx43 was specifically deleted only in Sertoli cells of the testis, whereas it was normally expressed in other places inside and outside the testis.[4,5] The choice of Sertoli cell specific Cx43 knockout has avoided the neonatal lethality of the Cx43 complete knockout and enabled to study the role of Cx43 in reproductive tissues.

Inside the seminiferous tubules of testis, Cx43 is involved in the formation of intercellular contacts between Sertoli cells and proliferating germ cells.[33] These gap junctions were observed in a stage-dependent manner indicating an important relationship between the germ cells and Cx43.[32] Analysis of homozygous Cx43 null allele mice revealed reduction in primordial germ cells (PGCs) during embryonic development.[59] Later on, it was discovered that the lack of Cx43 affects the PGC motility during mid gestation in mice embryo. It is also evident that the lack of Cx43 may induce abnormal p53 activation leading to the apoptotic loss of PGCs at the later stages in the Cx43 knockout mouse embryos.[60]

Apart from the role of Cx43 during embryonic testis development, its role in Sertoli cell proliferation has been observed during the neonatal period. Gilleron et al have shown nongenomic effect of tri-iodothyronine (T3) on Cx43 Sertoli cells in *in vitro* experiments and suggested that Cx43 could be an intermediate target for T3 mediated regulation of neonatal Sertoli cell proliferation.[61] It was further demonstrated that the Cx43 gap junctions between Sertoli cells is involved in regulation of Sertoli cell proliferation whereas Cx43 gap junctions between Sertoli cells and spermatogonia influence germ cell survival.[27]

Further studies have provided a direct correlation between Cx43 and spermatogenesis. Disrupting the Cx43 gene function by substituting Cx43 gene by the coding sequences of Cx32 and Cx40 resulted in mature animals with severe spermatogenesis defects and giving rise to seminiferous tubules with 'Sertoli cell only', SCO phenotype.[62] This indicates that Cx32 and Cx40 cannot replace the role of Cx43 in testis. There are further evidences that not all the Cx knockouts result in fertility defects. Cx31, Cx32, Cx40, Cx46 and Cx50 knockouts show normal fertility.[63] It is possible that the lack of phenotype in some Cx knockouts could be due to overlapping functioning of these genes. In non-rodent animal system also, stage dependent expression of Cx43 was observed with higher levels when spermatogenesis is at maximum level.[64] Expression of Cx43 in human patients with spermatogenesis defects like Klinefelter's syndrome and in mice models was investigated. Strong correlation was found between spermatogenic defects and absence of Cx43 expression within the seminiferous tubules.[65]

Similar to studies in testis, various evidences came up in recent years establishing important role of Cxs in ovary and female gamete formation. Cx43 and Cx37 have been specifically recognized for their role in follicle development and oocyte growth.

In the absence of Cx37, folliculogenesis is arrested at the early antral stage and the oocytes could not attain their normal size.[66] To study the ovarian development in Cx43 knockouts, which has postnatal death, ovaries were removed and studied *in vitro*. Ovaries lacking Cx43 have retarded oocyte growth and halted folliculogenesis beyond the primary follicle stage.[59]

Further chimera studies involving null mutant oocytes and wild-type granulosa cells or vice versa indicated that mouse oocytes do not need to express Cx43 for its maturation and functionality, but must express Cx37 to communicate with granulosa cells, which is necessary for oogenesis.[67] Another study using Cre-loxP system to specifically delete the Cx43 in the oocyte have reported that the preimplantation development of oocyte could be normal but implantation of resultant blactocysts is affected severely.[68]

CONNEXINS AND INFERTILITY

Expression analysis of connexins in pathological testis and the reproductive phenotype of Cx knockouts have indicated their important role in overall fertility outcome. Investigations of human testis samples with impaired spermatogenesis have shown normal changes in the testicular gap junctions. One of the earliest descriptions about the abnormal gap junctions in pathological testis was presented by Nagano et al 1976 by showing no gap junctions between Sertoli cells of feminized testis.[69] Others have also demonstrated the occurrence of atypical gap junctions in the seminiferous tubules of infertile patients of azoospermia and oligospermia.[70]

Cx43, which is one of the widely expressed connexins, has been implicated in fertility defects by various reports. Cx43 protein could not be detectable in seminiferous tubules of patients with SCO syndrome,[71] however it was later discovered that the expression of Cx43 in the seminiferous tubules was controlled at transcriptional level in these patients.[72] Spermatogenesis defects were evident in Sertoli cell specific conditional Cx43 knockouts.[4,5] The possible mechanism for the reduction of germ cells in these knockout studies could be regulation of proliferation and differentiation of Sertoli cells, which serve as nurse cell for the growing germ cells. Indispensible role of Cx43 in the maintenance of Sertoli cell was further backed up by other studies, where it was shown that its loss in Sertoli cells cannot be compensated by other Cxs expressed in Sertoli cells and spermatogonia, i.e. Cx26 and Cx32.[73] The knockouts of other Cxs did not show any significant reproductive phenotype (Table 1). The possible reason for this observation could be overlapping expression and function of the Cxs expressed in the gonads, however when it comes to some critical Cx, like Cx43, their loss of function becomes indispensible.

Cx43 is encoded by *GJA1* gene. Mutations in the *GJA1* gene were associated with nonsyndromic deafness[74] and later on with oculodento digital dysplasia, ODDD (OMIM164200).[75] Various studies have investigated the mutations in the *GJA1* gene and represented correlation with ODDD and other phenotypes.[75,76] Though mouse model of ODDD has oogenesis defects and poor gestational outcome but neither animal model of ODDD nor the mutations in *GJA1* gene has documented male fertility problems.

The reasons for the disparity between the features observed in connexin 43 knockout mice and Cx mutations/mutant model of ODDD could be multifold. Cx43 complete knockout was lethal and thus could not be studied. All the information about its role in reproductive system came from conditional knockouts, like Sertoli cell specific Cx43 KO, where the gene function was completely inactivated by Cre-LoxP system. Various mutations in the connexins can have differential functional consequences and possibly that result in variable isolated or pleiotropic phenotypes. The heterozygous mutations in connexins may have milder effect than the complete deletion of Cx in conditional knockouts.

Cx43 also plays a very important role during oogenesis. Cx43 knockouts have severe oocyte deficiency and arrested

Table 1: Sites of Cxs expression in male and female gonads, knockout viability and phenotypes

Cx type	*Expression in male and female gonads*	*Viability/lethality*	*Reproductive phenotype*
Cx26	Spermatocytes, spermatids, apical compartment of seminiferous tubules[22,78]	Embryonic lethal	Unknown
Cx31	Spermatogonia, spermatocytes, spermatids and peritubular cells[22,26]	Viable	No effect on spermatogenesis[62]
Cx32	Spermatocytes, spermatids and Sertoli cells, apical compartment of seminiferous tubules[22,78]	Viable	No effect on spermatogenesis[79]
Cx37	Peritubular cells, oocytes in the ovary[22,39]	Viable	No alteration of spermatogenesis but female are sterile[66]
Cx40	Spermatocytes, spermatids and peritubular cells[22]	Viable	No alteration of spermatogenesis[80,81]
Cx43	Sertoli cells, spermatogonia, Leydig cells, peritubular cells, granulosa cells of the ovary[3,71,72,78]	Neonatal lethality	Germ cell deficiency, Sertoli cell only syndrome, retarded oocyte growth and halted folliculogenesis[59, 82, 83]
Cx45	Peritubular cells[22]	Embryonic lethal	Unknown
Cx50	Spermatocytes, spermatids[22]	Viable	No alteration of spermatogenesis[84]

folliculogenesis during development. One of the recent studies has shown that the mouse model having dominant loss-of-function Cx43 mutation, $Cx43^{G60S}$, $Gja1^{Jrt}/+$ displaying features of the ODDD has oogenesis defects and is subfertile.[6] Another study has implicated Cx43 in parturition in mouse model. Cx43 is also a major myometrial gap junction protein and is found to be upregulated just before the onset of delivery indicating an important role of Cx43-mediated gap junctional intercellular communication (GJIC) in uterine contraction during parturition. The phosphorylated form Cx43 was significantly reduced in the myometrium of the $Gja1^{Jrt}/+$ mouse and was not increased before parturition corresponding to weaker contraction of the mutant myometrium and poor gestational outcome and parturition.[77]

CONCLUSIONS AND FUTURE DIRECTIONS

The gap junctional intercellular communication conveyed by gap junction proteins is involved in various developmental processes. There are substantial evidences that the Cxs have important role in the male and female gonads and the development of germ cells. The Cx43, which is the predominant Cx protein, have indispensable role in both male and female germ cell development and thus its loss is indispensible, which is evident by various reproductive phenotypes in male and female (Fig. 2). The lack of phenotype in the absence of other Cxs could be due to redundant role and functioning of some Cxs in male and female reproductive system. The use of Cre-LoxP system was very useful in deciphering the role of particular Cxs in specific tissue location of testis and ovary. This technique of conditional knockout was very useful, where the study of complete knockout was not possible due to embryonic or neonatal lethality. The mutation analyzes of Cxs coding genes have presented intriguing information about the role of Cxs in poor oogenesis and poor gestational outcome. However, still some discrepancies exist between knockout studies and Cx mutation studies. Many factors, including modifier genes, extend of functional defects caused by individual mutation and mechanism by which each variant affect the physiological outcome may be involved in variable phenotypic penetrance. In future, it will be more interesting to study the effect of individual mutant variants of each Cx on germ cell development. These studies will also shed light upon the possible mechanism by which each variant is involved in causation of various phenotypes.

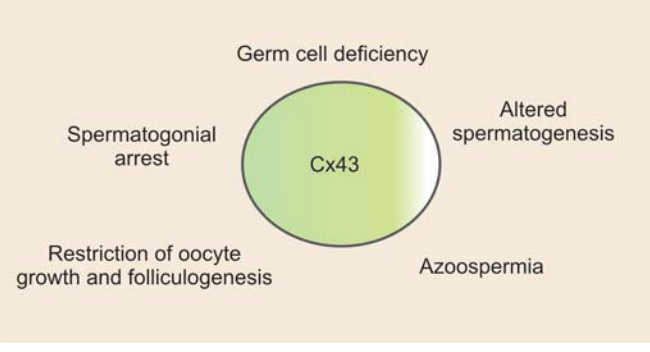

Fig. 2: Involvement of Cx43 in various male and female reproductive phenotypes. As opposed to various other Cxs, role of Cx43 is very critical and thus its loss or deficiency could not be compensated by other Cxs expressed in male and female gonads

REFERENCES

1. Herve JC, D Sarrouilhe, Connexin-made channels as pharmacological targets. Curr Pharm Des 2005;11(15):1941-58.
2. Carette D, et al. Major involvement of connexin 43 in seminiferous epithelial junction dynamics and male fertility. Dev Biol, 2010.
3. Kidder GM, AA Mhawi. Gap junctions and ovarian folliculogenesis. Reproduction 2002;123(5):613-20.
4. Sridharan S, et al. Proliferation of adult sertoli cells following conditional knockout of the gap junctional protein GJA1 (connexin 43) in mice. Biol Reprod 2007;76(5):804-12.
5. Brehm R, et al. A sertoli cell-specific knockout of connexin 43 prevents initiation of spermatogenesis. Am J Pathol 2007;171(1):19-31.
6. Tong D, et al. Oogenesis defects in a mutant mouse model of oculodentodigital dysplasia. Dis Model Mech 2009;2(3-4):157-67.
7. Maeda S, et al Structure of the connexin 26 gap junction channel at 3.5 A resolution. Nature 2009;458(7238):597-602.
8. Harris AL. Emerging issues of connexin channels: Biophysics fills the gap. Q Rev Biophys 2001;34(3):325-472.
9. Laird DW. Life cycle of connexins in health and disease. Biochem J, 2006;394(Pt 3):527-43.
10. Harris A. Connexins: A guide (1st ed) 2008, New York: Springer.
11. Cheng CY, DD Mruk. Cell junction dynamics in the testis: Sertoli-germ cell interactions and male contraceptive development. Physiol Rev 2002;82(4):825-74.
12. Davidoff MS, et al. Cellular architecture of the lamina propria of human seminiferous tubules. Cell Tissue Res 1990;262(2):253-61.
13. Mruk DD, CY Cheng. Sertoli-Sertoli and Sertoli, germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. Endocr Rev, 2004;25(5):747-806.
14. Pointis G, et al. Gap junctional communication in the male reproductive system. Biochim Biophys Acta 2005;1719(1-2):102-16.
15. Drummond AE. TGF beta signalling in the development of ovarian function. Cell Tissue Res 2005;322(1):107-15.
16. Li R, Phillips DM, Mather JP. Activin promotes ovarian follicle development in vitro. Endocrinology 1995;136(3):849-56.
17. McNatty KP, et al. Control of early ovarian follicular development. J Reprod Fertil Suppl 1999;54:3-16.
18. McNatty KP, et al. Control of ovarian follicular development to the gonadotrophin-dependent phase: A 2006 perspective. Soc Reprod Fertil Suppl 2007;64:55-68.
19. Vaccari S, et al. Generation of mouse oocytes defective in cAMP synthesis and degradation: Endogenous cyclic AMP is essential for meiotic arrest. Dev Biol, 2008;316(1):124-34.
20. Edry I, Sela-Abramovich S, Dekel N. Meiotic arrest of oocytes depends on cell-to-cell communication in the ovarian follicle. Mol Cell Endocrinol 2006;252(1-2):102-06.
21. Willingham-Rocky, LA, et al. Cloning of GJA1 (connexin43) and its expression in canine ovarian follicles throughout the estrous cycle. Gene Expr Patterns 2007;7(1-2):66-71.
22. Risley MS. Connexin gene expression in seminiferous tubules of the Sprague-Dawley rat. Biol Reprod 2000;62(3):748-54.

23. Juneja SC. mRNA expression pattern of multiple members of connexin gene family in normal and abnormal fetal gonads in mouse. Indian J Physiol Pharmacol 2003;47(2):147-56.
24. 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):295-302.
25. Nitta M, et al. Identification and expression analysis of CX45 and CX60 as major connexins in porcine oocytes. J Anim Sci 2010.
26. Mok BW, Yeung WS, Luk JM. Differential expression of gap-junction gene connexin 31 in seminiferous epithelium of rat testes. FEBS Lett 1999;453(3):243-48.
27. Gilleron J, et al. Connexin 43 a potential regulator of cell proliferation and apoptosis within the seminiferous epithelium. Int J Biochem Cell Biol 2009;41(6):1381-90.
28. Fischer P, et al. Connexin 33: A rodent-specific member of the gap junction protein family? J Androl 2005;26(1):75-84.
29. Carette D, et al. Connexin 33 impairs gap junction functionality by accelerating connexin 43 gap junction plaque endocytosis. Traffic 2009;10(9):1272-85.
30. Chang M, Werner R, Dahl G. A role for an inhibitory connexin in testis? Dev Biol 1996;175(1):50-56.
31. Carette D, et al. Heteromeric connexin 43/connexin 33 complex endocytosis: A connexin phosphorylation independent mechanism. Biochimie 2010;92(5):555-59.
32. Batias C, et al. Connexin43 gene expression and regulation in the rodent seminiferous epithelium. J Histochem Cytochem 2000;48(6):793-805.
33. Decrouy X, et al. Functional characterization of Cx43 based gap junctions during spermatogenesis. J Cell Physiol 2004;200(1):146-54.
34. Vitale ML, Akpovi CD, Pelletier RM. Cortactin/tyrosine-phosphorylated cortactin interaction with connexin 43 in mouse seminiferous tubules. Microsc Res Tech 2009;72(11):856-67.
35. Granot I, et al. Connexin43 in rat oocytes: Developmental modulation of its phosphorylation. Biol Reprod 2002;66(3):568-73.
36. Sutovsky P, et al. Dynamic changes of gap junctions and cytoskeleton during in vitro culture of cattle oocyte cumulus complexes. Biol Reprod 1993;49(6):1277-87.
37. Simon AM, Chen H, Jackson CL, Cx37 and Cx43 localize to zona pellucida in mouse ovarian follicles. Cell Commun Adhes 2006;13(1-2):61-77.
38. Veitch GI, et al. Selective assembly of connexin 37 into heterocellular gap junctions at the oocyte/granulosa cell interface. J Cell Sci 2004;117(Pt 13):2699-707.
39. Teilmann SC. Differential expression and localization of connexin-37 and connexin-43 in follicles of different stages in the 4-week-old mouse ovary. Mol Cell Endocrinol 2005;234 (1-2):27-35.
40. Johnson ML, et al. Expression of gap junctional proteins connexin 43, 32 and 26 throughout follicular development and atresia in cows. Endocrine 1999;10(1):43-51.
41. Borowczyk E, et al. Expression of gap junctional connexins 26, 32 and 43 mRNA in ovarian preovulatory follicles and corpora lutea in sheep. Can J Physiol Pharmacol 2006;84(10):1011-20.
42. Manthey D, et al. Molecular cloning and functional expression of the mouse gap junction gene connexin-57 in human HeLa cells. J Biol Chem 1999;274(21):14716-23.
43. Itahana K, et al. Isolation and characterization of a novel connexin gene, Cx-60, in porcine ovarian follicles. Endocrinology 1998;139(1):320-29.
44. Batias C, et al. Modified expression of testicular gap-junction connexin 43 during normal spermatogenic cycle and in altered spermatogenesis. Cell Tissue Res 1999;298(1):113-21.
45. Tramoni M, et al. Contraceptive steroids from pharmaceutical waste perturbate junctional communication in Sertoli cells. Biochimie 2009;91(11-12):1366-75.
46. Pluciennik F, Joffre M, Deleze J. Follicle-stimulating hormone increases gap junction communication in Sertoli cells from immature rat testis in primary culture. J Membr Biol 1994;139(2):81-96.
47. Grassi F, et al. Putative second messengers affect cell coupling in the seminiferous tubules. Cell Biol Int Rep 1986;10(8):631-39.
48. Lablack A, et al. Ultrastructural and biochemical evidence for gap junction and connexin 43 expression in a clonal Sertoli cell line: A potential model in the study of junctional complex formation. Cell Tissue Res 1998;294(2):279-87.
49. Gilleron J, et al. Three-dimensional analysis of connexin 43 gap junction in the ex vivo rat seminiferous tubules: Short-term effects of hormonal effectors. Microsc Res Tech 2009;72(11): 845-55.
50. Lefebvre DL, et al. Myometrial transcriptional regulation of the gap junction gene, connexin-43. Reprod Fertil Dev 1995;7(3):603-11.
51. Lucas TF, et al. 17beta-estradiol induces the translocation of the estrogen receptors ESR1 and ESR2 to the cell membrane, MAPK3/1 phosphorylation and proliferation of cultured immature rat Sertoli cells. Biol Reprod 2008;78(1):101-14.
52. Hess RA, et al. Adult testicular enlargement induced by neonatal hypothyroidism is accompanied by increased Sertoli and germ cell numbers. Endocrinology 1993;132(6):2607-13.
53. St-Pierre N, et al. 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(4):1232-40.
54. Luk JM, et al. Identification of novel genes expressed during spermatogenesis in stage-synchronized rat testes by differential display. Biochem Biophys Res Commun 2003; 307(4):782-90.
55. Granot I, Dekel N. Developmental expression and regulation of the gap junction protein and transcript in rat ovaries. Mol Reprod Dev 1997;47(3):231-39.
56. Granot I, Dekel N. Phosphorylation and expression of connexin-43 ovarian gap junction protein are regulated by luteinizing hormone. J Biol Chem, 1994;269(48):30502-09.
57. Kalma Y, et al. Luteinizing hormone-induced connexin 43 down-regulation: Inhibition of translation. Endocrinology 2004;145(4):1617-24.
58. Valdimarsson G, PA De Sousa, Kidder GM. Coexpression of gap junction proteins in the cumulus-oocyte complex. Mol Reprod Dev 1993;36(1):7-15.
59. Juneja SC, et al. Defects in the germ line and gonads of mice lacking connexin43. Biol Reprod 1999;60(5):1263-70.
60. Francis RJ, Lo CW. Primordial germ cell deficiency in the connexin 43 knockout mouse arises from apoptosis associated with abnormal p53 activation. Development 2006;133(17):3451-60.
61. Gilleron J, et al. A potential novel mechanism involving connexin 43 gap junction for control of sertoli cell proliferation by thyroid hormones. J Cell Physiol 2006;209(1):153-61.
62. Plum A, et al. Connexin31-deficiency in mice causes transient placental dysmorphogenesis but does not impair hearing and skin differentiation. Dev Biol 2001;231(2):334-47.
63. Willecke K, et al. Biological functions of connexin genes revealed by human genetic defects, dominant negative approaches and targeted deletions in the mouse. Novartis Found Symp 1999;219:76-88; discussion 88-96.
64. Izzo G, et al. Connexin 43 expression in the testis of the frog Rana esculenta. Zygote 2006;14(4):349-57.

65. Kotula-Balak M, et al. Connexin 43 expression in human and mouse testes with impaired spermatogenesis. Eur J Histochem 2007;51(4):261-68.
66. Simon AM, et al. Female infertility in mice lacking connexin 37. Nature 1997;385(6616):525-59.
67. Gittens JE, GM Kidder. Differential contributions of connexin37 and connexin43 to oogenesis revealed in chimeric reaggregated mouse ovaries. J Cell Sci 2005;118(Pt 21):5071-78.
68. Gershon E, et al. Oocyte-directed depletion of connexin 43 using the Cre-LoxP system leads to subfertility in female mice. Dev Biol 2008;313(1):1-12.
69. Nagano T, Suzuki F. Freeze-fracture observations on the intercellular junctions of Sertoli cells and of Leydig cells in the human testis. Cell Tissue Res 1976;166(1):37-48.
70. Bigliardi E, Vegni-Talluri M. Gap junctions between Sertoli cells in the infertile human testis. Fertil Steril 1977;28(7):755-58.
71. Steger K, Tetens F, Bergmann M. Expression of connexin 43 in human testis. Histochem Cell Biol 1999;112(3):215-20.
72. Defamie N, et al. Impaired gap junction connexin 43 in Sertoli cells of patients with secretory azoospermia: A marker of undifferentiated Sertoli cells. Lab Invest 2003;83(3):449-56.
73. Winterhager E, et al. Replacement of connexin 43 by connexin 26 in transgenic mice leads to dysfunctional reproductive organs and slowed ventricular conduction in the heart. BMC Dev Biol 2007;7:26.
74. Liu XZ, et al. Mutations in GJA1 (connexin 43) are associated with non-syndromic autosomal recessive deafness. Hum Mol Genet 2001;10(25):2945-51.
75. Paznekas WA, et al. Connexin 43 (GJA1) mutations cause the pleiotropic phenotype of oculodentodigital dysplasia. Am J Hum Genet 2003;72(2):408-18.
76. Paznekas WA, et al. GJA1 mutations, variants, and connexin 43 dysfunction as it relates to the oculodentodigital dysplasia phenotype. Hum Mutat 2009;30(5):724-33.
77. Tong D, et al. A dominant loss-of-function GJA1 (Cx43) mutant impairs parturition in the mouse. Biol Reprod 2009;80(6):1099-106.
78. Risley MS, et al. Cell, age and stage-dependent distribution of connexin 43 gap junctions in testes. J Cell Sci 1992;103(Pt 1):81-96.
79. Nelles E, et al. Defective propagation of signals generated by sympathetic nerve stimulation in the liver of connexin 32-deficient mice. Proc Natl Acad Sci. USA 1996;93(18):9565-70.
80. Kirchhoff S, et al. Reduced cardiac conduction velocity and predisposition to arrhythmias in connexin 40-deficient mice. Curr Biol 1998;8(5):299-302.
81. van Rijen HV, et al. Impaired conduction in the bundle branches of mouse hearts lacking the gap junction protein connexin40. Circulation 2001;103(11):1591-98.
82. Reaume AG. et al. Cardiac malformation in neonatal mice lacking connexin 43. Science 1995;267(5205):1831-34.
83. Plum A, et al. Unique and shared functions of different connexins in mice. Curr Biol 2000;10(18):1083-91.
84. White TW, Goodenough DA, Paul DL. Targeted ablation of connexin 50 in mice results in microphthalmia and zonular pulverulent cataracts. J Cell Biol 1998;143(3):815-25.