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REVIEW

Tetraspanins in mammalian reproduction: spermatozoa, oocytes and embryos

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Abstract

It is known that tetraspanin proteins are involved in many physiological somatic cell mechanisms. Additionally, research has indicated they also have a role in various infectious diseases and cancers. This review focuses on the molecular interactions underlying the tetraspanin web formation in gametes. Primarily, tetraspanins act in the reproductive tract as organizers of membrane complexes, which include the proteins involved in the contact and association of sperm and oocyte membranes. In addition, recent data shows that tetraspanins are likely to be involved in these processes in a complex way. In mammalian fertilization, an important role is attributed to CD molecules belonging to the tetraspanin superfamily, particularly CD9, CD81, CD151, and also CD63; mostly as part of extracellular vesicles, the significance of which and their potential in reproduction is being intensively investigated. In this article, we reviewed the existing knowledge regarding the expression of tetraspanins CD9, CD81, CD151, and CD63 in mammalian spermatozoa, oocytes, and embryos and their involvement in reproductive processes, including pathological events.

Keywords CD9 · CD81 · CD151 · CD63 · Extracellular vesicles

Introduction

Key molecular processes and mechanisms of the immune system are also engaged in mammalian reproduction. Based on current knowledge, an important role in fertilization is attributed to the surface molecules of gametes and reproductive cell tissue. Cluster of differentiation (CD) molecules such as CD9, CD81, and CD151 belonging to the tetraspanin superfamily take a prominent place in gamete interaction [1, 2]. CD63, is another member of the tetraspanins superfamily, and is known to be a part of extracellular vesicles (EVs), but its significance and potential in reproduction has not been fully addressed [3].

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Tetraspanin proteins are involved in many cellular mechanisms. They can interact with a range of other proteins such as integrins, members of the immunoglobulin superfamily and proteases, and with each other creating a large network called the tetraspanin web [4]. The association of tetraspanins with proteins and lipids leads to an organization of specific microdomains located in membranes, these are the so-called tetraspanin-enriched microdomains (TEMs), and these are different from lipid rafts. Although the tetraspanin family includes distinct proteins, all of them are defined by a common structure consisting of four transmembrane domains containing conserved polar residues, small (SEL) and large (LEL) extracellular loops with four, six, seven or eight conserved cysteine residues located in the variable region, and short cytoplasmic tails. Most tetraspanins are glycosylated and also palmitoylated [5–9]. In addition, research points to the fundamental role of tetraspanins in the pathogenesis of viral, bacterial, parasitic, and fungal infections (reviewed in [10–12]), and cancer (reviewed in [13, 14]).

In this study, the participation of tetraspanins CD9, CD81, CD151, and CD63 in mammalian reproduction (summarized in Fig. 1), including certain pathological processes were addressed. It was long believed that the first recognition and primary binding of spermatozoa to the glycoprotein coat of oocytes, the *zona pellucida* (ZP), induces acrosomal exocytosis in sperm that enables them to interact with oocytes [15]. According to the real time observations made by Jin et al. [16], most fertilizing mouse spermatozoa begin their acrosome reaction before contact with the ZP. Spermatozoa undergo the acrosome reaction at the end of sperm maturation process called capacitation, occurring within the female reproductive tract [17–19], which represents a sequence of many biochemical and biophysical changes (reviewed in [20]). During the acrosome reaction, the sperm plasma membrane (PM) fuses with the underlying outer acrosomal membrane (OAM) leading to the release of acrosomal content (hydrolytic enzymes) and the exposure of the inner acrosomal membrane (IAM) proteins on the sperm head

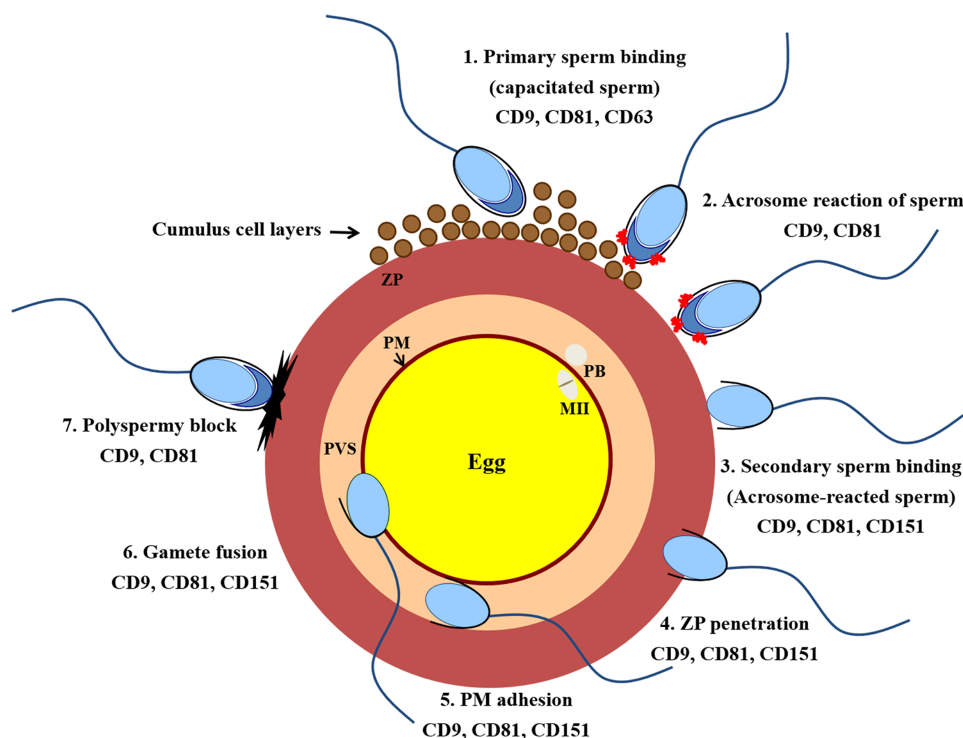

Fig. 1 The engagement of tetraspanins during the sperm and egg interaction. 1. Recognition and primary binding of capacitated sperm to the *cumulus* cells (CC) and *zona pellucida* (ZP) of the egg is assisted by CD9, CD81, and CD63 and followed by 2. the sperm acrosome reaction and CD9 and CD81 relocation (mouse) that facilitates 3. a secondary sperm binding in presence of CD9, CD81 (mouse, human), and CD151 (mouse, human, cattle). This results in 4. the sperm penetration through ZP and 5. adhesion to the oocyte plasma membrane (PM) both in assistance of CD9, CD81 and CD151. Finally, 6. in presence of CD9, CD81, and CD151, gamete membrane fusion occurs followed by pronuclei fusion and a zygote formation. 7. shortly after a sperm fuses with the oocyte, the impermeability of ZP to other sperm is ensured by the polyspermy block. (PB) polar body, (PVS) perivitelline space

surface. Only completely acrosome-reacted spermatozoa are able to penetrate the ZP and perivitelline space (PVS, the space between the oolema and ZP) and fuse with the oocyte plasma membrane (oolema) [15]. After the membrane fusion, the sperm nucleus, mitochondria, centriole, and flagellum enter the oocyte and male and female gamete nuclei merge together to form a zygote. A monospermic fertilization where an egg is fertilized by only one sperm, is ensured by the blocking of other sperm passing through the ZP and fusing with oolema by a polyspermy block [15].

CD9 in oocytes and embryos

CD9 is the most studied tetraspanin in terms of its involvement in fertilization. The significance of CD9 was shown by experiments using female mice with the knockout (KO) *Cd9* gene. KO females were born and grew normally, but they were mostly infertile [21–23]. During in vitro fertilization, spermatozoa that penetrated the ZP stayed imprisoned in the perivitelline space [21–23], while oocytes directly injected with spermatozoa were fertilized [21]. Similarly, Kaji et al. [24] and Zhu et al. [25] documented the "recovery" of sperm–egg fusion after the application of CD9 mRNA into *Cd9*-deficient oocytes. The CD9 molecule in mice was located in the plasma membrane of oocytes [21–23, 26]. It was detected on the oocyte surface covered by microvilli, the place is thought to be an initial contact point for spermatozoa with the PM but was not observed in the area overlying the second meiotic spindle, thus its role in the adhesion was proposed [23]. This assumption was later supported by Runge et al. [27]. They documented that CD9 may affect the rearrangement of microvilli capturing the sperm; the microvilli of the *Cd9*-deficient oocytes were short, thick, and denser in comparison to the long and thin microvilli of natural oocytes.

CD9 has also been documented in humans, where CD9 was found in oocytes in the germinal vesicle stage (GV), in metaphase I (MI), metaphase II (MII) [28, 29], and embryos [30, 31]. In pig, CD9 was present on the whole PM of oocytes, including the area over the second meiotic spindle. The CD9 level significantly increased during oocyte maturation [32]. Similarly, an increasing fluorescent signal of CD9 was detected on the PM of sheep oocytes and embryos [33] and in the mature *cumulus*-oocyte complex of yak [34].

In bovine oocytes, CD9 was observed for the first time in the plasma membrane of ZP-free oocytes by Zhou et al. [35]. The following study of Jankovicova et al. [36] described localization of CD9 in the PM of ZP-intact oocytes in different maturation stages as well as in the vesicles released into the perivitelline space of oocytes and embryos, that were most apparent after fertilization and division of the zygote. CD9 detection was dependent on the type of antibody used; the polyclonal antibody recognized the unevenly distributed clusters on the PM surface, while staining with monoclonal antibody appeared more homogenous over the PM of oocytes except for the region over the metaphase plate. Moreover, the fluorescent signal was also observed in ZP. It should be noted that the epitopes of applied antibodies probably differ. While polyclonal anti-CD9 antibody (ABIN741015) was directed to a part of the large extracellular loop (AA120-165 peptide), the monoclonal antibody IVA-50 (used also in the study of Zhou et al. [35]) was obtained after immunization with bovine thrombocytes [37] and thereby could probably recognize more extensive conformational epitope. Moreover, it was documented that tetraspanins are organized in small nanoclusters, detectable only by STED microscopy [38]. The reaction pattern of the polyclonal antibody detected by confocal microscopy in all probability represents a portion of the CD9 molecule within the larger domain, while due to more accessible epitope, IVA-50 stained CD9 more extensively. Similarly, a different reaction pattern of two anti-CD53 antibodies was documented in B cells [38]. As we mentioned previously, filament-like structures resembling transzonal projections (TZPs) were detected by monoclonal antibody in bovine oocytes passing through the ZP in a similar way to actin filaments. CD9 could be a part of a transzonal projection via interaction with actin binding proteins [39] or it could be a part of extracellular vesicles, the presence of which was detected in the TZPs by Macaulay et al. [40]. CD9 as a component of extracellular vesicles was already documented by several authors. Barraud-Lange et al. [41] reported that the transfer of CD9-containing oocyte fragments to fertilizing spermatozoa resembled trogocytosis, the process by which lymphocytes capture the membrane fragments of antigen presenting cells. In the studies of Miyado et al. [42] and Barraud-Lange et al. [43], release of vesicles (including CD9-positive vesicles) from PM into the PVS of oocytes was related to the ability of facilitating the sperm–egg fusion. Similar findings were described in hamster [42]. Recently, in human oocytes, the CD9-positive vesicles were observed in the PVS but not within the ZP of matured (MII) oocytes [31]. This observation corresponds with the findings of Miller et al. [44] in mice. Furthermore, Vyas et al. [31] documented an abundance of CD9-positive vesicles in the ZP after fertilization of human zygotes, continuing throughout cleavage to the blastocyst stage. Interestingly, similar extensive staining of CD9-positive clusters (probably vesicles within the PVS) has been already documented in bovine and porcine embryos, but CD9-fluorescent signal within the ZP was detected only in bovine but not in porcine zygotes and

embryos [36]. It could be hypothesized that the different pattern detected in the ZP of mouse, porcine, bovine, and human oocytes is caused by antibody epitope availability or non-availability (as mentioned above), or could reflect the species-specific traits of CD9 in the processes associated with gamete adhesion and intercellular communication during fertilization.

Differing results were also observed, when the role of CD9 in the fertilization process (particularly in the sperm-egg binding and fusion) was analyzed using specific anti-CD9 antibodies in in vitro fertilization assays, whereas Chen et al. [26] and Takahashi et al. [45] observed significant inhibition of sperm–egg binding and fusion in mice [26], Miyado et al. [21] and Miller et al. [44] recorded the inhibitory effect of the same antibody on fusion without a decrease in sperm binding. In human, the same anti-CD9 antibody that inhibited the fusion in mice did not affect the fusion of sperm with ZP-free oocytes [29]. However, when human intact oocytes were maintained in the presence of the antibody during ZP removal and gamete fusion, a strong inhibitory effect on gamete fusion was recorded [29].

In non-rodent species, the antibody treatment significantly reduced the sperm binding and fusion with ZP-free oocytes in pig [32], sheep [33], and cattle [35]. In contrast, the antibody treatment of ZP-intact bovine oocytes did not cause any decrease in the number of fertilized oocytes [36]. The diversity of the findings probably reflects (a) the differing experimental design (at least the use of ZP-free vs. ZP-intact oocytes), (b) interspecies variability, or (c) the fact, that the antibodies did not recognize the crucial epitopes [36]. The application of the same antibody to oocytes with or without ZP (in human, mice [29], and cattle [35, 36]) with different effects on fertilization pointed to the limitation of experiments with ZP-free oocytes. The chemical or enzymatic removal of ZP may result in impairment or loss of function of the egg proteins critical to sperm–egg interaction [46, 47]. Moreover, in experiments using ZP-free oocytes, it is not clear whether sperm binds to the PM, or ZP residues [48]. Furthermore, the results could depend on the method of removing loosely bound spermatozoa and also the fact that the acrosome reaction is not induced by ZP [49]. So far, it seems impossible to reliably distinguish between binding/adhesion and fusion defect after oocyte antibody treatment of ZP-free oocytes [49].

The importance of the CD9 molecule in the pre-fusional adhesion steps was later proved by Jegou et al. [50]. The authors documented that CD9 generates a strong adhesion site in contact with gamete membranes that was necessary for successful fusion. This suggestion was later supported by Chalbi et al. [51], who showed that this accumulation of CD9 on the oocyte membrane was controlled by adhesion of Juno, an essential egg molecule interacting with sperm essential protein Izumo [52]. Furthermore, Ravaux et al. [53] documented that recruitment of egg CD9 to the egg–sperm interface is initiated by sperm oscillations (flagellum beating) after the contact of acrosome-reacted sperm with the egg membrane. Immediately after gamete fusion, CD9 together with associated proteins leave the PM and thus, it probably participates in the prevention of polyspermy.

The association of oocyte CD9 with other proteins was already proposed by Chen et al. [26]. According to their model, which did not take into account CD9 in sperm, CD9 through direct or non-direct association with integrin $\alpha_6\beta_1$ on the mouse oocyte surface could assist the integrin $\alpha_6\beta_1$ in binding to fertilin β located on the sperm head. However, the role of integrin $\alpha_6\beta_1$ is controversial, because another study in mice did not confirm the participation of the integrin $\alpha_6\beta_1$ in this binding process. The disagreement could likewise be caused by modification of plasma membrane proteins after ZP removal [44]. On the other hand, the study of Ziyyat et al. [29] confirmed that redistribution of $\alpha_6\beta_1$ within the PM of human oocyte is controlled by CD9. Based on the findings that anti-CD9 antibody added prior to the ZP removal inhibited the formation of $\alpha_6\beta_1$ patches formed in the absence of antibody, authors hypothesized that CD9 controls the lateral mobility of $\alpha_6\beta_1$ and maintains the tetraspanin web, to which it linked. As described above, CD9 was detected on the surface of mouse oocyte, which was covered by microvilli. The microvilli rearrangement may be related to the functional connection of CD9 with EWI-2 and EWI-F [54], immunoglobulins that are able to bind ezrin, radixin, moesin (ERM proteins), connecting to the actin filaments of cytoskeleton [55]. The CD9/EWI-2/ERM complex on the oocyte plasma membrane is able to regulate the structure and dynamics of microvilli optimal for the sperm–egg contact [27]. In very recent study based on the crystal structure of CD9 and the cryo-electron microscopic structure of human CD9, Umeda et al. [56] showed the interaction between CD9 and EWI-2, mediated by small residues in the transmembrane region and protein/lipid interaction. Authors suggested the possible role of other (not only flexible) LEL regions of CD9 in fertilization and moreover the role of EWI-2 as a bridge between tetraspanins and other proteins, important for remodeling of membrane resulting in the formation of complex protein network and vesicles. Tetraspanins clustering can directly induce the membrane curvature and thus facilitate the exosome budding or control the vesicular cargo sorting through the association with other partner proteins [56].

Taken all together, CD9 on the oocyte plasma membrane could probably participate in the reorganization and curvature of the membrane, which presumably facilitates the mutual protein–protein communication leading to successful fertilization. This ability to control the lateral mobility of protein within the tetraspanin web, connects to actin filaments and thus, reorganize the membrane applied in particular processes like microvilli rearrangement, adhesion

site generation or releasing of vesicles that could prevent polyspermy. Moreover, it could enable the intercellular communication during oocyte and embryonic development, and embryo-endometrial cross-talk [30, 57, 58].

CD9 in spermatozoa

In contrast with the negative results in mice [26] and pig sperm [32], CD9 was detected in rat and mouse spermatogonia [59, 60], and later also in mouse spermatozoa [61]. CD9 was found in the cytoplasm of mouse testicular germ cells, in spermatogonia, spermatocytes, and round spermatids. In mature spermatozoa with permeabilized membrane and spermatozoa in the initial stage of acrosome reaction, CD9 was present in the inner acrosomal membrane, mainly in the marginal region of the anterior acrosome, extended to the equatorial region with advancing acrosomal reaction [61]. In contrast, Barraud-Lange et al. [43] observed only a low portion (10%) of mature or capacitated spermatozoa positive for CD9, which appear mainly as a thin line in the acrosomal region. Conversely, 60–75% of acrosome-reacted sperm showed bright CD9 fluorescent dots. Frolikova et al. [62] described the presence of tetraspanin CD9 in both the inner and outer acrosomal membrane. During the acrosome reaction, CD9 relocated to the equatorial segment and partially over the post-acrosomal region [62]. The different pattern of CD9 localization on mouse spermatozoa might have been caused by dissimilar methods of sample fixation. Sperm possess both the outer and inner acrosomal membranes and these are unavailable for antibody staining without permeabilization treatment. While Ito et al. [61] detected CD9 on frozen-thawed spermatozoa, Barraud-Lange et al. [43] found this molecule on fresh sperm fixed with paraformaldehyde (non-permeabilizing agent). Obviously, freezing can cause drastic changes in the sperm membranes such as deterioration in integrity but it may also expose the inner acrosomal membrane, which is normally revealed after the acrosomal exocytosis. The CD9-staining in acrosome-reacted sperm was revealed by both research teams using epifluorescence microscopy. An additional signal of CD9 on the outer acrosomal membrane was detected by Frolikova et al. [62] probably due to the use of super-resolution microscopy.

In non-rodent species, Li et al. [32] did not detect CD9 in frozen-thawed pig spermatozoa, probably due to the fact that boar semen is more sensitive to cryo-damage than other species. In contrast, CD9 was detected in frozen-thawed as well as freshly ejaculated bull spermatozoa, where CD9 was localized in the apical part or through the entire anterior region of the plasma membrane of ejaculated and capacitated spermatozoa. The molecule was lost from the sperm after acrosome reaction [63]. A similar pattern was observed in human, when CD9 was localized in the apical region within the acrosomal cap of ejaculated and capacitated spermatozoa [62]. After the acrosome reaction, CD9 was located only over the equatorial segment. It seems that the localization of CD9 in bull spermatozoa is more similar to human than mouse sperm. This may be due to the different shape of mouse sperm head in comparison with bull and human spermatozoa, which probably effects spatial protein arrangement and distribution.

The authors [62] also suggested the involvement of CD9 in the tetraspanin web formation in the sperm membrane. The results of immunoprecipitation, western blot analysis, and molecular modeling identified a possible presence of CD9 dimers in human spermatozoa. The large extracellular domain of CD9 would be involved in the dimer formation and could potentially mediate the *trans*-interaction [62]. The post-translational modification by palmitoylation of CD9 [6] could participate in the CD9 web stabilization during the sperm maturation and acrosome reaction [62]. Moreover, the knowledge that CD9 is able to associate with EWI-2 and EWI-F [64] and these proteins are major partners of CD9 and CD81 acting as linkers connecting the tetraspanin microdomains to the actin cytoskeleton through their direct interaction with ERM proteins (ezrin/radixin/moesin) [65], supports the possible role of the tetraspanin web in the stabilization of the sperm acrosome. It has been previously suggested that ezrin is involved in the activation of the Rho GTPase family members followed by actin polymerization during human sperm capacitation [66].

The significance of CD9 (and also ezrin, F-actin, cdc42, and β-tubulin) in the maintenance of sperm function can be illustrated by a lower expression of these proteins in asthenozoospermic semen (sperm with reduced motility), compared to normospermic semen. It was hypothesized to be probably related to cytoskeletal reorganization and disturbance of sperm plasma membrane [67].

CD81 in oocytes and embryos

Another tetraspanin studied in relation to reproduction, human and mouse CD81 shares about 43% amino acid sequence identity with CD9 [68]. Deletion of the *Cd81* gene in mice showed that the fertility of *Cd81*-deficient females decreased by 40% [69]. The reduction of fertility was also apparent when the ZP-free oocytes were incubated with anti-CD81 antibody [45], while fertilization of ZP-intact mouse oocytes in the presence of antibody did not affect sperm penetration or the rate of two-cell embryos [70]. Similarly, pre-treatment of ZP-intact bovine oocytes with the anti-CD81 antibody did not decrease the fertilization rate [36]. Its detection and localization (similar to CD9) varies in dependence on application of the antibody, using ZP-free/ZP-intact oocytes; additionally, species specificity

could not be excluded. CD81 was first observed on the surface of mouse ZP-free oocytes [45], but later the molecule was found only in the inner area of ZP [70]. The fact that CD81 production is predicted to be predominantly by wild-type *cumulus* cells and then re-localized to ZP is in contrast with the later detection of CD81 clusters on the surface of ZP-free *Cd81*-deficient oocytes after injection of mRNA encoding CD81 [70]. This discrepancy can be explained by the detection of tetraspanin within larger or smaller domains depending on the antibody epitope [38]. In human, CD81 has been localized over the whole PM of unfertilized ZP-intact oocytes with the formation of patches after ZP removal [29]. Recently, the presence of CD81 in the intact bovine oocytes (GV, MI, and MII) was detected on the PM and moreover after fertilization in PVS of zygotes and embryos [71]. In porcine, besides the oocyte PM, CD81 was also observed in the inner part of the ZP, and additionally released within vesicles concentrated into several clusters in the PVS of zygotes and embryos [36].

Initially, it was proposed that CD81 plays a complementary role with CD9, because *Cd81* and *Cd9* double knockout female mice were completely infertile and microinjection of CD81 mRNA partially compensated CD9 in *Cd9*-deficient oocytes [1]. Ohnami et al. [70] later suggested that in mice both CD81 and CD9 work independently as extracellular components in gamete fusion, whereas microinjection of CD9 mRNA reversed a fusion defect in *Cd81*- and *Cd9*-deficient oocytes, however injection of CD81 mRNA failed. It seems that the expression and co-localization of both tetraspanins might be species-specific or their detection is dependent on the antibody used. Rubinstein et al. [69] supposed that on the surface of mouse oocytes, CD81 is associated with tetraspanin enriched microdomains. According to Ohnami et al. [70], the association of CD81 and CD9 in mouse oocytes may be limited, because the localization of these two proteins has been detected in different areas of the oocytes. CD81 localized at the inner part of ZP may help to form a complex with CD9 released within the vesicles to the PVS of mouse oocytes [42] and thus help transfer the CD9 to the sperm [70]. The above mentioned localization of these two tetraspanins on plasma membrane of human, bovine, and porcine oocytes seems to be a prerequisite for the complex formation and possible cooperation of these tetraspanins not only within the vesicles, but also within the oocyte plasma membrane.

CD81 in spermatozoa

CD81 was detected in rat spermatogonia [60] and by western blot analysis of protein extracts from mouse capacitated spermatozoa [71]. The precise localization in spermatozoa has been newly described in mouse [71], cattle [71], and human [62]. In the mouse, CD81 was localized on the PM covering the apical acrosome of the *epididymal* spermatozoa and relocated during the acrosome reaction across the equatorial segment over the whole sperm head. In the bull, CD81 was apparent on the apical part and partially in the equatorial region of the *epididymal* and ejaculated spermatozoa, disappearing from the sperm head after the acrosome reaction [71]. In human, the localization of CD81 has been observed in the apical acrosomal area and partially in the post-acrosomal area of ejaculated and capacitated spermatozoa. After acrosome reaction, it disappeared from the apical part of the acrosome, but stayed detectable in the post-acrosomal region.

The high rate of CD81 and CD9 co-localization without the co-localization occurring after the acrosome reaction suggested that CD81 may interact and form complexes with CD9 on the membrane of ejaculated human spermatozoa [62]. The probable cooperation of these two tetraspanins was predicted by molecular modeling based on the open/closed (cholesterol-free/bound) conformation of CD81 that was previously proven by molecular dynamics simulation using the crystal structure model of full-length human CD81 [72] and from the data obtained on human spermatozoa [62]. The dynamics of the CD9 and CD81 interaction network is directly related to the presence or absence of cholesterol in the CD81 cavity. The CD81 cholesterol-free molecule has a more compact transmembrane region as well as a change from conical to cylindrical conformation. This change in the membrane curvature may possibly play a role in sperm–egg fusion because the convex part of the equatorial segment has been considered as a site of the sperm–egg membrane interaction. The cholesterol efflux during sperm capacitation possibly influences CD81-cholesterol binding and might lead to a more compact transmembrane region and greater positive curvature to adapt at the moment of fusion [62]. It appears that cholesterol binding modulates the activity of CD81 in cells that suggest a potential mechanism for the regulation of tetraspanin function. The assumption that CD9 and CD81 participate in sperm–egg interaction seems to be in conflict with the fact that $Cd81^{-/-}$ and $Cd9^{-/-}$ and even double knockout $Cd9^{-/-}$ $Cd81^{-/-}$ mouse males were normally fertile [69]. The cooperation of these two tetraspanins is estimated on the basis of data obtained from the human model, where the mutual co-localization detected by immunofluorescent staining was supported by co-immunoprecipitation experiments. These findings, however, were in contrast to the mouse model where both methods failed to reveal any mutual interaction of CD9 and CD81 [62]. According to Miyado et al. [42], the fertility of deficient mice could be rescued by transfer of CD9-containing vesicles from wild-type oocytes; however, these suggestions were later challenged by Gupta et al. [73] and Barraud-Lange et al. [43]. To

ensure reproduction, it appears therefore that some species have developed unique mechanisms of fertilization that may also include several "backup" mechanisms, for example; the replacement of tetraspanin function by another protein.

CD151 in oocytes

Research has shown that *Cd151* knockout mice were capable of reproduction [74]; however, the inhibition of sperm–egg fusion (up to 50%), by the anti-CD151 antibody suggested a significant but not essential role of CD151 in the gamete interaction in human [29]. The CD151 molecule was detected on ZP-intact human oocytes, where it was evenly distributed in PM. However, on ZP-free oocytes, CD151 formed patches together with $\alpha_6\beta_1$ integrin. Interestingly, the addition of anti-CD9 antibody during ZP removal inhibited the formation of these patches involved in gamete fusion in human [29]. In mice, the CD9 antibody affects the formation of $\alpha_6\beta_1$ but not CD151 patches. The authors [29] hypothesized that a well-developed hyaluronan-containing matrix in the PVS of human but not mouse oocytes [75] could attach the plasma membrane of oocyte via hyaluronan receptor CD44 [76]. Association of CD44 with tetraspanins [77] may have a stabilizing effect which is disturbed by ZP removal resulting in the elimination of proteins and vesicles from the PVS [29]. Tetraspanin CD151 is associated with various tetraspanins, integrins, or other molecules [78] within plasma membrane domains, and tetraspanin-enriched microdomains, the composition of which differ in particular cell types. Based on chemical crosslinking and stability in strong detergents, it seems that within TEMs CD151 directly associates with laminin-binding integrins [79–82]. On the cell surface, CD151 links the laminin-binding integrins to the other tetraspanins, including CD9 and CD81 which interact with their partners. CD9 and CD81 can interact with immunoglobulin superfamily proteins EWI-2 and EWI-F and link them via CD151 to $\alpha_3\beta_1$ integrin [82, 83].

The participation of the CD151 in the fertilization process has also been demonstrated using a protein interaction network approach. This network described a set of possible candidates involved in human sperm–egg interaction. In addition, the network also proposed the interaction of CD9 and CD151 in the PM of the egg with integrin α3 (CD49C) in the sperm membrane and interaction of integrin α4 (CD49D) of sperm with CD81 in the oocyte [84]. These data have relevance in relation to the recently documented localization of integrins in mouse spermatozoa [85]. This could suggest that CD151 (and also CD9) could interact with integrins not only within the oocyte (*cis* interaction) but also in *trans* interaction with integrins in sperm.

CD151 in spermatozoa

In our very recent study, we report for the first time, the presence of CD151 in the sperm of three species. This protein is expressed in germ cells during spermatogenesis and remains during *epididymal* transport and ejaculation of mouse and bull spermatozoa, and in human sperm a similar feature is assumed. CD151 is located in the equatorial segment, the initial fusion region of sperm that is exposed after the acrosomal exocytosis [86]. Based on the recently obtained data confirming the presence of the $\alpha_6\beta_1$ and $\alpha_6\beta_4$ heterodimers on mouse sperm [85] and the knowledge that CD151 share the same location in the equatorial segment with $\alpha_6\beta_4$ but not with $\alpha_3\beta_1$ integrin, it is possible that CD151 could stabilize the equatorial domain via $\alpha_6\beta_4$ and plectin [86] surrounding the sperm nucleus [87] at least in mouse spermatozoa. Although, the pattern of CD9 and CD81 differs between mouse, bull, and human spermatozoa [62, 63, 71], CD151's location was consistent among all the three species (Fig. 2).

CD63 in extracellular vesicles

CD63 is one of the most prominent tetraspanins present on the surface of late endosomes, multivesicular bodies (MVBs), late lysosomes, and extracellular vesicles. It was also documented in the cell membrane in smaller quantities [88].

CD63 has also been found incorporated in tetraspanin-enriched microdomains, typically consisting of tetraspanins and other molecules, such as cholesterol, integrins (β_2, $\alpha_4\beta_1$, $\alpha_3\beta_1$, $\alpha_6\beta_1$, LFA-1), and other molecules, forming an active tetraspanin web [88]. Latysheva et al. [89] identified Syntenin-1, a PDZ-domain-containing protein, as a new component of TEMs, direct partner of CD63, and referred PDZ-domain of Syntenin-1 specifically binding to C-terminus of CD63. It is supposed that the other tetraspanins also possess the potential PDZ-domain binding sites like CD81, whose C-terminus binds the proteins with PDZ-domain EBP50 (SLC9A3R1) and Sap97 (DLG1) [90]. In general, PDZ-domains containing proteins are very abundant in the cells and participate in many cellular and biological functions, especially mediating the interaction of signal transduction complexes [91].

Seeing that some of the integrins mentioned above have recently been localized in the mouse spermatozoa [85], CD63 could potentially be involved in the sperm–egg interaction by association and may possibly also cooperate with integrins.

Although *Cd63* knockout mice appeared viable and fertile with no observable morphological abnormalities in the majority of tissues, this does not completely exclude the role of CD63 in fertilization processes [92]. The same authors

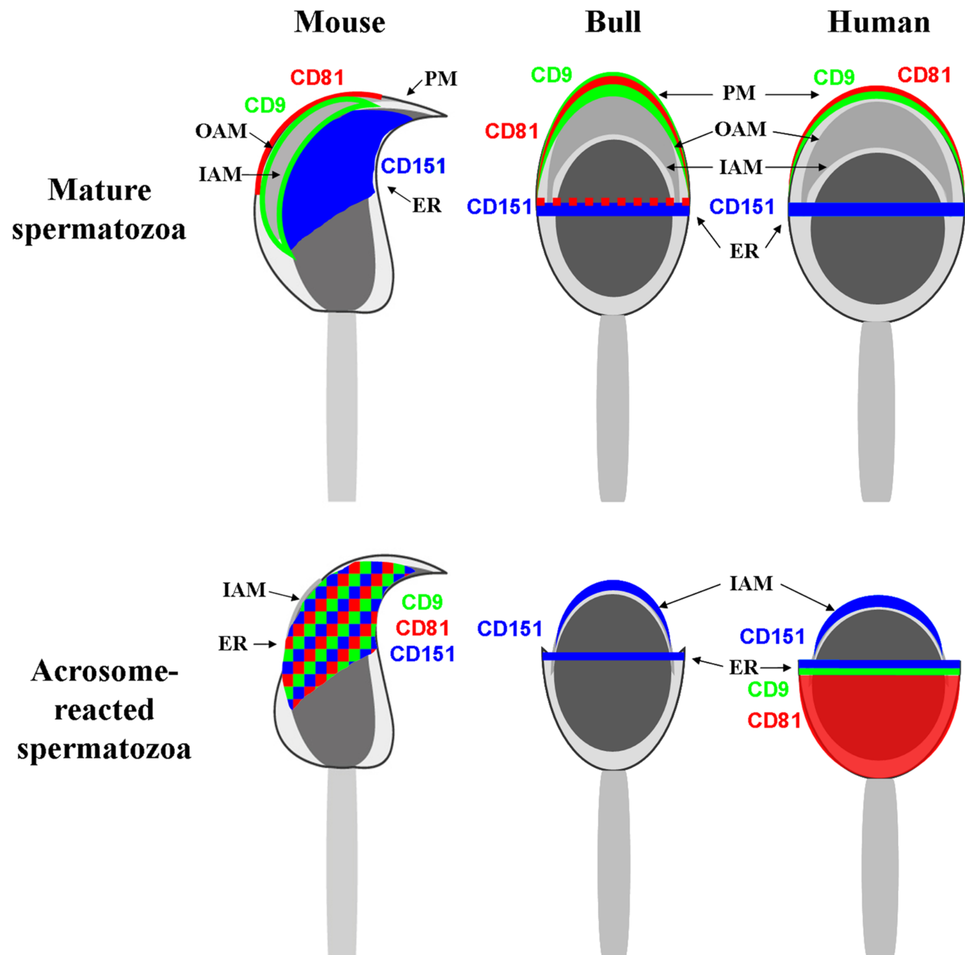

Fig. 2 Localization of tetraspanins in mouse, bull, and human spermatozoa. The diagram presents the localization of CD9 (green), CD81 (red), and CD151 (blue) in mouse, bull, and human spermatozoa before and after the acrosome reaction. Plasma membrane (PM), outer acrosomal membrane (OAM), inner acrosomal membrane (IAM), and equatorial region (ER).

suggested that the loss of the CD63 protein could possibly be compensated for, probably by other tetraspanins.

Yoshida et al. [93] suggested that the assurance of the reproduction cycle, certainly in mice, is backed-up by the existence of more than one pathway of sperm–egg fusion (and in all probably also by other processes), that can compensate for the impact of the absence of a single gene and thereby minimize the severity of a malfunction. This could equally be the case of mouse *Cd63*-knockout, *Cd151*-knockouts [74], and other tetraspanin-knockouts as well. The significant role in these bypassing mechanisms could involve only tetraspanin proteins; this can be illustrated by recovered fusibility of *Cd81*-deficient mouse eggs via forced CD9-expression (CD9 mRNA-injection) [70].

The EVs' function involves discarding unnecessary cellular proteins; transporting proteins and lipids from the cell of origin to the recipient cell; efficient transfer of mRNA and miRNA to selective targets; activation of T cell responses through antigen transport, and suppressing the immune system and enhancing angiogenesis in tumor formation [88, 94]. To date, the functional role of EVs in mammalian reproduction has not been fully clarified. In general, it is proposed that their function is to ensure the intercellular communication in the reproductive tract and many studies documented their participation in processes related to gamete maturation, fertilization, polyspermy prevention, and embryo implantation [3, 95].

EVs (carrying the CD63 molecule) were detected in the seminal plasma of boar [96–98] and bull [99], in ovarian follicular fluid in horse [100], cattle [101], and human [102], in uterine luminal fluid of human [103] and sheep [104], in human blastocoel fluid [105] and interestingly, in non-mammalian animals, such as seminiferous tubules and *ductuli efferentes* of turtle [106, 107] and oviduct of hen [59]. The sperm storage tubules of the hen oviduct are responsible for prolonged spermatozoa storage while maintaining the vitality and fertilizing potential of the sperm. After in vitro insemination, it was observed that there was a decrease of CD63 protein in the sperm storage tubules (SST) cells containing sperm, compared to SST cells without sperm. This could indicate that the CD63-positive EVs secreted by SST cells help mediate a beneficial effect on the sperm stored in the oviduct [108]. Secretion of EVs with CD63 was also observed in human [30] and bovine [57] embryos.

CD63-positive EVs were also suggested to play a role in fertilization, egg implantation, and mother–embryo communication or endometrial embryo cross-talk [104, 109]. At the moment of ejaculation, the seminal fluid carries not only spermatozoa and soluble signaling molecules, but also EVs from the entire male reproductive tract (including epididymosomes and prostasomes) [95]. These components have a complex effect on the female reproductive tract and prime the immune response, thereby affecting the success of egg implantation and female immune response [109].

There are various criteria used to distinguish between different EVs subpopulations. The most common one (also used in the methods of isolation), is the size. Exosomes are smaller EVs of 40–120 nm, while microvesicles are considered to be larger (120–1000 nm) [98]. Regarding the mechanism of formation, microvesicles bud directly out of the plasma membrane, whereas exosomes bud inward into the late endosomes, forming multivesicular bodies inside the cell. When this multivesicular body fuses with the plasma membrane, exosomes are released in a burst [3]. Differences were described between the EVs populations in terms not only of size and mechanisms of formation but also distribution of individual tetraspanins on their surface. Various types of tetraspanin distribution were described in the subpopulations present in porcine seminal plasma by Barranco et al. [98]. While CD9 and CD63 were observed to be mostly expressed on microvesicles, CD81 was more abundant on exosomes [98]. The different expression of individual tetraspanins on EVs surface could reflect their specific roles in the reproductive tract and on fertilization, and also serve as a marker outlining the physiological and pathophysiological processes [97, 98, 110]. These findings could also have practical implications in farm animal breeding, e.g., pigs and cattle, as these EVs were found to bind to the spermatozoa [99] and promote their maturation and long-term viability [107] or to prevent early capacitation [96]. Our preliminary results showed a positive CD63 signal detected by immunohistochemistry, using a polyclonal antibody against CD63 on permeabilized smears of ejaculated frozen-thawed bull sperm. A signal for CD63 was identified in the sperm equatorial region which is commonly associated with the process of sperm–egg interaction and fusion. No significant change was detected after in vitro capacitation of the frozen-thawed sperm, but after inducing an in vitro acrosome reaction, there was either a weak signal or no signal detected. This dynamic change of the tetraspanin CD63 profile on bull sperm could indicate that CD63 plays an unidentified role in mammalian fertilization [111].

Summarized data of presence and localisation of CD9, CD81, CD151, and CD63 in germ cells, gametes, and embryos of mammals

Taken in their totality, tetraspanins were shown to be present in bovine, porcine, mouse, and human gametes and in other mammalian species. However, observations sometimes differed considerably from what could result from a distinct experimental approach and moreover, the obvious species-specific traits of tetraspanin expression and thereby their role in the fertilization process certainly exists. All available data regarding the tetraspanin expression in mammalian gametes and their supposed role is shown in Tables 1, 2.

Other tetraspanins in reproductive tract and gametes

In addition to the tetraspanins, which are known and heavily studied in the reproductive tract and gametes and were described in the previous chapters, this section focused on the tetraspanins less frequently studied in relation to reproduction.

CD82/TSPAN27 was detected in the reproductive tissue of mice, in particular on ciliated epithelia cell and epithelium of *epididymis* [120]. Risinger et al. [121] described that *Cd82*$^{-/-}$ mice were normally fertile and have an average litter size. The effect on reproduction has not been demonstrated conclusively. There is no data on the expression of this molecule on gametes. Other tetraspanins such as CD53 was found in transcriptomic profile of human spermatozoa or TSPAN12 has been classified to the transcript group of spermatozoa that were obtained from men whose partners did not achieve pregnancy after intrauterine insemination. The role of this molecule is not clarified; it may possibly be an infertility marker [122]. Expression of four proteins from the tetraspanin family TSPAN1-4 was observed in human reproductive tracts, especially in prostate (TSPAN1) and uterus (TSPAN1-4) [123]. The *TSPAN6* gene has been differentially expressed between fertile and infertile individuals. Additionally, *TSPAN6* was in the list of transcripts that were down-regulated in the asthenozoospermic infertile group compared to normozoospermic infertile men [124]. Assou et al. [125] reported the whole genome transcriptome of human *cumulus oophorus*. In addition to CD81, CD63, and CD151 molecules, the other tetraspanin, TM4SF8/Tspan3, has been found to overexpress in *cumulus oophorus* cells.

Two uroplakin-type proteins, tetraspanins UPIa/TSPAN21 and UPIb/TSPAN20, have been described in mouse and

Table 1 Summary on CD9, CD81, CD151 and CD63 tetraspanin localization in oogonia, oocytes, and embryos

TSPAN	Oocytes/embryos	Localization	Mammalian species	Step of fertilization/proposed role	References
CD9	GV, MI oocytes (within ovary or isolated oocytes)	PM	Mouse	Sperm-egg binding and fusion	[22, 26]
CD9	MII oocytes (ZP-intact, within ovary or isolated oocytes with or without CC)	PM, vesicles in PVS, CC membrane	Mouse	Sperm-egg binding and fusion/adhesion protein, fusion protein, formation or stabilization of multimolecular complexes	[18, 19, 23, 26, 38–41, 64, 66]
CD9	MII oocytes (ZP-free)	PM, microvilli not over second meiotic spindle	Mouse	Sperm-egg binding and fusion/adhesion protein, fusion protein, formation or stabilization of multimolecular complexes	[20–24, 48, 51, 66]
CD9	Oocytes after fusion	Vesicles in PVS	Mouse	Contribution to polyspermy block	[53]
CD9	Embryos and blastocysts	PM, vesicle in PVS	Mouse	Embryo invasion and implantation	[22, 112]
CD9	GV, MI and MII oocytes	PM	Human		[28]
CD9	MII oocytes (ZP-intact or ZP-free)	PM, EVs in PVS, EVs in ZP (only part of MII oocytes)	Human	Sperm-egg fusion/controls the redistribution of some membrane proteins	[29, 31]
CD9	Embryos (from zygote to blastocyst)	PM, EVs in PVS and ZP; secretion of EVs to medium	Human	EVs as a possible communication tool (maternal–fetal interface)	[30, 31]
CD9	GV, MI and MII oocytes (within ovary or isolated)	PM, clusters in ZP, cytoplasm	Pig	Oogenesis; sperm-egg binding and fusion/sperm-egg interaction and adhesion, participation in membrane reorganization and effects the curvature	[32, 36, 113]
CD9	GV, MI and MII oocytes (ZP-free)	PM	Pig	Oogenesis/development; sperm-egg binding and fusion/sperm-egg interaction	[32]
CD9	Embryos	PM, vesicles in PVS and ZP	Pig	Vesicles as a possible communication tool	[36, 114]
CD9	GV, MI and MII oocytes (within ovary or isolated ZP-free oocytes)	PM, CC membrane	Sheep	oogenesis; sperm-egg binding and fusion	[33]
CD9	ZP-free embryos	PM	sheep	embryo development and implantation	[33]
CD9	GV, MI and MII oocytes	PM, clusters in ZP component of TZPs in ZP	Cattle	Sperm-egg interaction and adhesion, participation in membrane reorganization and effects the curvature	[36]
CD9	MII oocytes (ZP-free)	PM,patches on PM	Cattle	Sperm-egg binding and fusion/sperm-egg interaction and adhesion, participation in membrane reorganization and effects the curvature	[35, 36, 115]
CD9	Embryos and blastocysts	PM, cluster in PVS and in ZP; secretion of EVs to medium	Cattle	Vesicles as a possible communication tool	[36, 57, 116]
CD9	GV, MI and MII oocytes with CC	CC, along the PM and ZP in PVS	Yak		[34]
CD9	Blastocysts	PM, cytoplasm	Yak		[34]
CD 81	GV, MI and MII oocytes (within ovary)	Oocyte, CC of MII oocytes	Mouse	Involvement in the acrosome reaction, prevention of polyspermy, zona hardening	[117]
CD 81	MII oocytes (ZP-intact or ZP-free)	PM, vesicles in PVS and ZP	Mouse	Sperm penetration, sperm-egg binding and fusion	[45, 69, 70]

Table 1 (continued)

TSPAN	Oocytes/embryos	Localization	Mammalian species	Step of fertilization/proposed role	References
CD 81	MII oocytes (ZP-intact or ZP-free)	PM, patches on PM	Human		[29]
CD81	blastocysts	EVs in blastocoel fluid	Human	Cell communication	[105]
CD 81	GV, MI and MII oocytes, embryos	Clusters in PM and along ZP, PVS in embryo	Pig	Sperm-egg interaction and adhesion, participation in membrane reorganization and effects the curvature; vesicles as a possible communication tool (embryo)	[36]
CD 81	GV, MI and MII oocytes (ZP-intact or ZP-free, MII with CC, embryo)	Clusters in PM, patches on PM, CC membrane, clusters in PVS of embryo	Cattle	Sperm-egg interaction and adhesion, participation in membrane reorganization and curvature; vesicles as a possible communication tool (embryo)	[71]
CD 81	GV, MI and MII oocytes with CC	CC, along PM and ZP, in PVS	Yak		[34]
CD 151	MII oocytes (ZP-intact or ZP-free)	PM, patches on PM	Human	Sperm-egg fusion/controls the redistribution of membrane proteins	[29]
CD63	Embryos	Secretion of EVs to medium	Human		[30]
CD63	Blastocysts	EVs in blastocoel fluid	Human	Cell communication	[105]
CD63	Blastocysts	Secretion of EVs to medium	Cattle		[57]

GV oocytes in the stage of germinal vesicle, *MI* oocyte in metaphase I, *MII* oocyte in metaphase II, *CC cumulus* cells, *ZP zona pellucida*, *PVS* perivitelline space, *PM* plasma membrane, *EVs* extracellular vesicles

Table 2 Summary on CD9, CD81, CD151, and CD63 tetraspanin localization in testicular germ cells and spermatozoa

TSPAN	Germ cells/spermatozoa	Localization	Mammalian species	Step of fertilization/proposed role	References
CD9	Spermatogonia, spermatocytes, round spermatids	Cytoplasm	Mouse	Spermatogenesis/regulation of cell adhesion	[59, 61]
CD9	Spermatozoa capacitated (fresh, frozen-thawed)	Inner/inner and outer acrosomal membrane	Mouse	Sperm-egg fusion/interaction, tetraspanin web stabilization	[61, 62]
CD9	Spermatozoa capacitated	Thin line in the acrosomal region (only on 10%)	Mouse		[43]
CD9	Spermatozoa acrosome-reacted	Bright dots/equatorial segment and partially over the post-acrosomal region	Mouse	Tetraspanin web stabilization	[43, 62]
CD9	Spermatogonia, spermatocytes	PM	Rat	Spermatogenesis/maintaining the spherical nature of spermatogenic cells, regulation of cell adhesion, participation in the development of apoptotic bodies in spermatogenic cells	[59, 60, 118]
CD9	Spermatozoa ejaculated, capacitated	Head and tail/apical acrosomal area	Human	Tetraspanin web stabilization	[62, 67]
CD9	Spermatozoa acrosome-reacted	Equatorial segment	Human	Sperm-egg interaction; tetraspanin web stabilization	[62]
CD9	Spermatogonia, spermatocytes, spermatids, spermatozoa	Acrosomal region, AM	Pig	Sperm development and maturation in *epididymis*	[119]
CD9	Spermatozoa ejaculated, capacitated (fresh, frozen-thawed)	PM	Cattle	Sperm-egg interaction	[63]
CD9	Spermatozoa capacitated	Whole spermatozoa	Yak		[34]
CD81	Spermatozoa capacitated	PM over the apical acrosome	Mouse		[62, 71]
CD81	Spermatozoa acrosome-reacted	The equatorial segment or whole sperm head	Mouse	A regulator of tetraspanin web	[62, 71]
CD81	Spermatogonia, spermatocytes	PM	Rat	Spermatogenesis/regulation of cell adhesion, participation in the development of apoptotic bodies in spermatogenic cells	[60]
CD81	Spermatozoa ejaculated, capacitated, acrosome-reacted	apical acrosome post-acrosomal area	Human	Sperm-egg fusion/sperm-egg interaction, participation in membrane reorganization and effects the curvature	[62]
CD81	Spermatozoa (*epididymal* within tissue or isolated) ejaculated, capacitated (fresh, frozen-thawed)	PM apical acrosome, equatorial region (only part of sperm)	Cattle	Protein organization within the sperm membrane during the events foregoing the sperm-egg fusion	[71]
CD81	Spermatozoa capacitated	Whole spermatozoa	Yak		[34]
CD151	Germ cells spermatozoa capacitated acrosome-reacted	Equatorial segment and inner AM	Mouse	Sperm-egg fusion/sperm-egg interaction, stabilization of multimolecular complexes	[86]
CD151	Spermatozoa ejaculated, capacitated, acrosome-reacted	Equatorial segment and inner AM	Human	Sperm-egg fusion/sperm-egg interaction, stabilization of multimolecular complexes	[86]
CD151	Germ cells, spermatozoa (*epididymal* within tissue or isolated) ejaculated, capacitated, acrosome-reacted	Equatorial segment and inner AM	Cattle	Sperm-egg fusion/sperm-egg interaction, stabilization of multimolecular complexes	[86]
CD63	Spermatozoa ejaculated, capacitated (frozen-thawed)	Equatorial segment	Cattle		[111]

PM plasma membrane, *AM* acrosomal membrane

human oocytes, where these proteins form a heterotetramer complex with other uroplakins, UPII and UPIIIa. The UPIa/TSPAN21 was co-localized with molecule CD9 in mouse oocytes [126]. Similarly, tetraspanin uroplakins have been previously described in ovaries of *Xenopus laevis* [127]. In mice, the rate of fertilization was reduced in the IVF assay with oocytes incubated with an antibody against UPIa and UPIb tetraspanins [126]. The same authors described UPIb/TSPAN20 in the male reproductive tract of mice, in prostate and *epididymis*. Additionally, mRNA coding of both tetraspanin uroplakins were found in the ovary in a huge amount, while in testis, *UPIa* and *UPIb* gene expression has been recorded to a much lesser extent. UPIa/TSPAN21 and UPIb/TSPAN20 were also found in the head of spermatids in mouse testis and mature spermatozoa, mainly associated with hook. The uroplankin-enriched domain is situated in a convex part of acrosome; therefore the uroplakins presumably have no effect on sperm-egg interaction. The role of uroplakins is possibly structural, generating or maintaining the hook arrangement [126].

Schuster et al. [128] created a database (https://spermbase.org/index.php), which is dedicated to the sperm RNA expression profiling for four species (rat, mouse, human, and rabbit). Sperm-borne RNA affected the male germ cell development, fertilization and early development, and epigenetic transgenerational inheritance. Tetraspanins found in the database of sperm-borne RNA sequences (Supplementary Table 1) could be a potential topic for future studies on the participation of these molecules in the reproductive process.

CD9, CD81, CD151, and CD63 in pathologies associated with the reproductive tract

Many studies describe the expression of tetraspanins in relation to tumor stage, tumor type, growth, migration, invasion, and metastasis of tumor cells [13]. These studies have reported association of CD151 with human breast cancer [129, 130] and together with CD63 with cervical cancer, caused by persistent infection with high-risk human papillomaviruses (HPV) in most cases [131]. Several tetraspanins have been studied in connection with prostate cancer [14, 132–134]. Changes in CD9 and CD151 expression were observed in relation to the tumor growth and metastasis. Simultaneously, a decrease of CD9 level and an increase of CD151 level could be considered as a prognostic marker [132, 133]. In one of the recent studies, it was observed that the expression of CD151 and CD9 on extracellular vesicles of prostate cells was related to the prostate cancer. The alteration of these molecules on prostate cells changed the proteome of their EVs. These changes resulted in an increase of the invasive and migratory capabilities of the non-tumorigenic population of prostate cells, thus promoting activation of the metastatic potential of these cells [134]. Several studies also suggest the possible involvement of CD63- and CD81-positive vesicles in prostate cancer. Logozzi et al. [135] showed an increased level of EVs expressing both CD81 and prostate-specific antigen in the plasma of prostate cancer patients. However, in a later study, Padda et al. [136] did not confirm these findings but detected CD9-positive EVs.

Tetraspanin CD151 is also associated with a serious pig disease, the porcine viral reproductive and respiratory syndrome (PRRSV). This RNA virus, as the name suggests, causes several reproductive and respiratory diseases [137]. In the case of reproduction, PRRSV is manifested by an increased number of abortions in higher stages of gravidity, with the birth of dead fetuses (50–70%) and premature farrowing birth. In boars, the disease could be manifested by a temporary deterioration of sperm quality, reduction of their number, and altering their motility [138]. CD151 was identified as a key molecule of susceptibility to PRRSV infection. CD151 facilitated virus entry into host cells due to specific interaction with the 3′-untranslated region of PRRSV RNA. The proposed role of CD151 was based on the ability to bind RNA, and possibly in the location of ribonucleoprotein complexes at the site of viral replication [139]. The mechanism of virus entry into cells via the low pH-dependent endocytic pathway [140] and the specific role of CD151 in endocytosis was presumed previously [141]. A similar mechanism of viral infection through cell entry involving CD151 during endocytosis was described for human papillomavirus or cytomegalovirus. Their virions interact with receptors in tetraspanin-enriched microdomains and penetrate cell by fusion at the PM or via endocytic vesicles [142].

CD63 is associated with various pathologies. Its expression was increased in patients with endometriosis, where it might be responsible for survival promotion of ectopic endometrial cells, leading to endometriotic lesions. The expression of CD63 was elevated only in the proliferative phase of the menstrual cycle of patients with endometriosis [143]. According to Menon et al. [144], CD63-positive EVs could serve as biomarkers for possible pregnancy complications, such as pre-term birth or premature rupture of membranes. It is interesting that the topical application of CD63-positive mesenchymal stem cell exosomes to the endometrium of rats with intrauterine adhesions (one of the important causes in human female infertility), promoted endometrial regeneration, fertility restoration, collagen remodeling, and maintained normal uterine structure. This fact can point to the therapeutic effect of EVs not originating from the reproductive tract [145]. In the male reproductive tract, CD63-positive EVs enable correct sperm maturation and prevent pathological events such as early capacitation [96].

Concluding remarks

Tetraspanins, namely, CD9, CD81, CD151, and CD63 play an indispensable, direct or indirect, role in gamete quality, their interaction and fusion during fertilization as well as early embryogenesis. Tetraspanins are also reported to be involved in illnesses such as cancer related to the male and female reproductive tract, and they can serve as potential diagnostic or possibly therapeutic markers. Our review summarizes the present knowledge on CD9, CD81, CD151, and CD63 detection and localization in spermatozoa, oocytes, embryos, as well as their possible function in the reproduction of mammals and reproductive related pathologies. Although we focused only on 4 of 33 tetraspanins expressed in mammals, the role of the other members of this superfamily cannot be excluded without thorough examination.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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