Sperm Proteins IZUMO1 and TMEM95 in Mammalian Fertilization

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Gamete membrane fusion is a critical cellular event in sexual reproduction. In addition, the generation of knockout models has provided a powerful tool for testing the functional relevance of proteins thought to be involved in mammalian fertilization, suggesting IZUMO1 and TMEM95 (transmembrane protein 95) as essential proteins.

Keywords: fertilization ; gamete fusion ; IZUMO1 ; mammals ; reproduction ; spermatozoa ; TMEM95 (transmembrane protein 95)

1. Introduction

The fertilization process is a vital step in sexual reproduction that entails a series of synchronized events to produce a zygote that is genetically unique. After ejaculation, millions of sperm are deposited in the female reproductive tract. However, only a few of these cells will reach the ampulla of the oviduct and meet the oocyte. Sperm acquire the ability to fertilize oocytes during this transit through a physiological and molecular changes known as capacitation [1]. As a consequence of capacitation, spermatozoa exhibit three fertility-related modifications: changes in sperm motility patterns; regulation of signal transduction pathways that allow them to respond to chemoattractants; and the ability of sperm to undergo the AR (acrosome reaction) [2][3][4]. Upon ovulation, only one spermatozoon successfully fuses with the oocyte.

It is known that fertilization occurs in four successive steps. First, the sperm must undergo the AR to release the enzymes and ligands necessary for the fertilization [5]. Sperm-reacted can fuse with the oocyte plasma membrane through a remnant of the sperm plasma membrane lying over the equatorial segment (EQ) [6]. As a second step, sperm need to penetrate the zona pellucida (ZP) in order to reach the perivitelline space, which is an extensive gap between the oocyte and the ZP [7]. Thirdly, the acrosome-reacted sperm must adhere to the oolemma; this step is highly specific to mammalians [8][9].

Cellular adhesion is determined by known or putative molecular interactions between sperm proteins and the oolemma. Following gamete adhesion, the formation of fusion pores allows cytoplasmic continuity and gamete fusion [9]. Fusogen proteins are responsible for facilitating membrane fusion during cell interactions [6]. The generation of knockout models has provided a powerful tool for testing the functional relevance of proteins proposed to have a role in mammalian fertilization contributing to the construction of a new scheme of fertilization mechanism.

In this way, through using loss-of-function experiments in transgenic or mutant mice, researchers have proven that two sperm proteins are essential for the sperm to adhere to the oolemma during fertilization: IZUMO1 [10][11] and SPACA6 (sperm acrosome membrane-associated protein 6) [12]. Similarly, oocyte proteins, such as the tetraspanins CD9 [13] and CD81 [14] and JUNO [15], are also required. Females lacking these proteins have a marked phenotype; despite exhibiting a normal behaviour and being able to produce oocytes that are normal in appearance, their fertilisation fails at the final adhesion and fusion steps.

Specifically, IZUMO1 protein is a testis-specific cell-surface protein belonging to the class of immunoglobulin type-I cell superfamily, characterized by a cytoplasmic C-terminal tail, a transmembrane region and a conserved ‘Izumo domain’, which is linked to an extracellular immunoglobulin-like (Ig-like) C2-type domain. It has been demonstrated that each of these domains plays a critical role in gamete adhesion [10][16][17]. After AR in mammalians, IZUMO1 is localized in the EQ [18] to mediate gamete adhesion with the oocyte plasma membrane. The absence of IZUMO1 leads to the impairment of gamete adhesion and to an accumulation of sperm in the perivitelline space [10]. However, despite being a protein necessary to carry out membrane fusion, it lacks a fusogenic peptide or SNARE-like structure, and thus IZUMO1 could be one of the components that form the fusogenic machinery in spermatozoa, acting at the level of organization or stabilization of a multiprotein complex [19].

Recently, three new sperm proteins that are essential for mammalian fertilization have been identified through the use of CRISPR technologies: TMEM95 (transmembrane protein 95), SOF1 (Sperm-egg fusion protein LLCFC1) [20] and FIMP (Fertilization-influencing membrane protein) [21]; these are small proteins that are expressed highly in the testis. Male mice lacking any one of these proteins phenocopied IZUMO1-deficient males; they produced sperm with normal morphology and motility, and their passage of the ZP and binding to oocytes were comparable with that of wild-type sperm. Nevertheless, this final step was unsuccessful, and the sperm was unable to fuse with the oocyte. These articles propose that one of these three proteins functions as a fusogen during fertilization.

TMEM95 is another essential protein for performing fertilization and is highly conserved in mammalians; therefore, its alterations could induce male subfertility [22]. After AR, TMEM95 localizes in the EQ as IZUMO1. However, in bovines, other localization patterns have been observed, including in the neck, thereby, indicating an additional role during the first cell divisions of the embryo [23] and its disappearance after AR implying a different function from IZUMO1 [24]. Due to the localization of IZUMO1 and TMEM95 in the EQ after AR and their co-immunoprecipitation, this suggests a possible interaction between them. Therefore, TMEM95 could act as a fusogen or mask the real fusogen.

2. Analysis of TMEM95

TMEM95 encodes a highly conserved single pass type I transmembrane protein consisting of 176 amino acids. The presence of a Pfam:IZUMO domain—database of protein families and domains—and a leucine rich repeat C-terminal domain (LRRCT) has been predicted, the latter being involved in a multitude of biological functions, such as signal transduction and cell adhesion [25]. Its expression differs between species, being exclusive to the testis in rodents, while in cattle, it is also expressed in the brain. In addition, two splicing variants have been found in bovines [26]. In mature mammalian sperm, this protein relocalizes from the outer acrosomal membrane to the EQ after undergoing AR. It has also been localized to the neck of bovine spermatozoa, indicating an additional role of TMEM95 during early embryonic cell divisions [23]. This hypothesis was confirmed after observing a lower cleavage rate in embryos obtained from spermatozoa with mutated TMEM95 [24].

The protein sequence of TMEM95 is highly conserved among mammalian species, such as rodents, boars, bovines and primates, including humans [20]. Therefore, genetic variants altering TMEM95 are likely to induce male subfertility. In humans, numerous polymorphic sites have been identified, including several potential loss-of-function variants [23]. In other species (bovine and rodents), ablation or mutation of TMEM95 prevents penetration and fusion between the sperm and oocyte membranes, leading to sperm accumulation in the perivitelline space. This blockade of fusion does not generate structural defects in spermatozoa; therefore, it is suggested that TMEM95 may not play an important architectural role. TMEM95 deficiency is not detectable in a routine seminogram as it does not affect sperm motility or morphology.

Due to the normality of the seminogram, it might be advisable to develop functional assays, e.g., for the integrity of sperm-surface proteins or for effective prospective monitoring of male fertility [22][23][24].

Assays performed in HEK293T cells, noted by their adherent growth, suggest that TMEM95 requires one or more partners as, by itself, it does not promote cell fusion after IZUMO1 and JUNO binding [22]. Coimmunoprecipitation analyses suggest interactions of TMEM95, SPACA6, SOF1 and FIMP with IZUMO1 [20]. However, the nature of this cooperation is unknown as ablation of any of these genes does not affect the amount or localization of IZUMO1 [20][22][27]. It should be noted that the expression of these five proteins is not sufficient for sperm–oocyte fusion.

On the other hand, no interactions between TMEM95 and IZUMO1 or JUNO have been detected [22]. Therefore, it is suggested that TMEM95, SOF1 and SPACA6 could directly or indirectly regulate membrane fusion through an IZUMO1-independent pathway or act as fusion mediators downstream of IZUMO1 and JUNO interaction. Membrane fusion is a highly coordinated and dynamic process, involving tight timing and proper interactions between molecules to allow the sperm head to internalize into the ooplasm [20].

Interestingly, TMEM95 disappears in bovine spermatozoa that have undergone the AR [24] implying a different function than IZUMO1. It is possible that TMEM95 does not act as a fusogenic protein that directly mediates membrane fusion between spermatozoa and oocytes. Rather, it could mask the actual fusogens that mediate fusion prior to AR; the release of TMEM95 could facilitate exposure of the functional domain of the fusogenic protein to the cell surface, which is necessary for fusion to occur. Alternatively, it is possible that sperm and oocyte membrane fusion is a bilateral fusion process, in which the fusogen must be present on both membranes; during acrosomal exocytosis, TMEM95 may be released and transferred to the oocyte plasma membrane, thus, ensuring that fusion occurs bilaterally [20].

3. Analysis of IZUMO1

3.1. Characterization and Localization of IZUMO1

IZUMO1 encodes a single-pass type I transmembrane protein consisting of 350 amino acids with a 21 amino acid extracellular N-terminal signal peptide, a 20 amino acid transmembrane domain (amino acid position 293 to 313) and a 36 amino acid intracellular C-terminal domain (human Uniprot IZUMO1) containing several potential phosphorylation sites [28]. It belongs to the immunoglobulin superfamily with an IZUMO domain and an extracellular immunoglobulin domain [29]. The IZUMO domain includes a receptor-binding platform IZUMO1 to JUNO, which is a glycosylphosphatidylinositol (GPI)-anchored oolemma protein [15][16][17]. An isoform of IZUMO1 (IZUMO1_v2) encoded by a different exon (exon 1b) of the Izumo1 through alternative splicing has been observed in mice. This isoform exhibits functional properties identical to the original protein [30].

The IZUMO family consists of four proteins: IZUMO1, IZUMO2, IZUMO3 and IZUMO4. All of them share a region of residues of about 150 amino acids of high homology between them called IZUMO domain, located between the signal peptide and the immunoglobulin domain. Experiments with native and recombinant IZUMO proteins suggest that the IZUMO domain is involved in homodimer formation. These proteins, originally discovered in the mouse, have homologues in several mammalian species, thus it is a conserved family [28]. Western blotting has detected IZUMO1 as a 56.4 kDa protein in mice, whereas in human weights 37.2 kDa [10].

All members of the IZUMO family show a pattern of eight conserved cysteines and a similar predicted secondary structure consisting of four α-helices between cysteine motifs. Notably, IZUMO4 lacks a transmembrane domain [28]. IZUMO1, a stable monomeric protein with extensive mixed α-β secondary structural features, was characterized in 2016. The overall structure consists of two domains: a four-helical N-terminal rod-shaped bundle of four helices (4HB; residues 22–134) and an immunoglobulin-like domain (Ig-like; residues 167–254). Two antiparallel β-strands (β1 and β2) function as a hinge between the 4HB and Ig-like domains. The four helices in the IZUMO1 4HB domain (α1, α2, α3 and α4) vary from 14 to 30 residues in length. The helices are amphipathic in character with a solvent-exposed polar surface and hydrophobic residues packed in a core [16].

Generally, in immunolocalization studies, the samples were divided in capacitated or reacted (spontaneous or induced) spermatozoa and fresh spermatozoa as control group. Comparison between this groups shows the relocation of IZUMO1 during the AR. A few articles [29][31] compare cryopreserved and fresh spermatozoa (control). These studies indicate the effects of the cryopreservation, such as changes in localization and density of IZUMO1.

There are slight differences in the localization of IZUMO1 between species. In mouse spermatozoa IZUMO1 is originally localized in the acrosomal membrane (inner and outer acrosomal membranes) [18]. While in bull spermatozoa it is detected along the border between the principal and EQ of the acrosomal region [29]. During the AR IZUMO1 gradually moves with the help of the actin cytoskeleton and testis-specific serine kinase (TSSK6) towards the EQ [32][33]. It has been observed in rodents that the beginning of IZUMO1 relocation does not depend on the beginning of the AR, however, later stages of relocation correlate positively with the status of the AR. The complete translocation in the EQ coincides with the completion of the AR [32].

IZUMO1 relocation in individual sperm populations during the spontaneous AR correlates with species-specific promiscuity behaviour, exhibiting faster relocation. Notably, in rodents, induction of the AR by calcium ionophore provides comparable or identical results to the spontaneous AR, whereas induction by progesterone completes relocation 20 min faster than in the spontaneous AR [32]. Therefore, it is suggested that IZUMO1 relocation is independent of external or internal calcium ion triggering the AR. While progesterone, a hormone secreted by cumulus oophorus cells responsible for the induction of the AR, is able to initiate IZUMO1 relocation.

The location of IZUMO1 can be affected by several factors. One of them is the loss of sperm equatorial segment protein 1 (SPESP1), an acrosomal protein that localizes to the EQ after the AR. Its loss in both Spesp1$^{-/-}$ and Spesp1$^{-/+}$ mice results in an altered localization of IZUMO1, being distributed over a wider area and in an irregular manner. This event results in an inhibition of sperm–oocyte fusion, observed in both mouse and human sperm [34].

The effect of cryopreservation on IZUMO1 location has been observed in bull spermatozoa [29]. Cryopreservation generates severe damage to the acrosome, resulting in aberrant translocation of IZUMO1. After induction of the AR, a higher loss of acrosomes is observed in cryopreserved spermatozoa compared to freshly ejaculated samples. Consequently, the percentage of cryopreserved spermatozoa with normal or abnormal acrosome and a normal IZUMO1 distribution pattern decreases about a 9 ± 5%. Although the detailed mechanism for IZUMO1 translocation in

cryopreserved sperm is still unclear, Fukuda et al. suggest that cryopreserved sperm undergo changes, such as the capacitation process induced by the freezing and thawing process and by exposure to components such as egg yolk [29].

3.2. Role of IZUMO1 in Mammalian Fertilization

The molecular mechanism of recognition between IZUMO1 and JUNO was recently discovered, in which IZUMO1 interacts with JUNO through its N-terminal domain [35]. The crystal structure indicates that the residues of the three IZUMO1 regions (4HB, hinge and Ig-like) contact JUNO 20-228 through extensive van der Waals, hydrophobic and aromatic interactions. There are also two intermolecular salt bridges and eight hydrogen bonding interactions. However, all these interactions are weak [16]. On the other hand, Inoue et al. described two types of dimer configuration in IZUMO1: open and closed. The open dimer could increase JUNO affinity to increase the fertilization rate. While the closed dimer directly participates in membrane fusion after binding to JUNO [36].

First, JUNO binds to monomeric IZUMO1, gradually accumulating at the sperm contact site to induce its dimerization, which is followed by a tight junction phase in which IZUMO1 folds the entire structure to the sperm membrane side through a thiol-disulphide exchange reaction. Following this, IZUMO1 stops binding to JUNO to bind to a second putative oocyte receptor.

Thus, the role of JUNO is to rearrange IZUMO1 so that it can generate the necessary strength to collapse the repulsion between the juxtaposing membranes through an unidentified receptor on the oocyte. However, this is not sufficient to fuse both membranes, requiring other proteins, such as CD9 in the oocyte and SPACA6 in the sperm [12][13][37][38]. CD9, IZUMO1 and JUNO are involved in gamete recognition and membrane adhesion, but do not induce membrane fusion [15]. Interestingly, CD9 has been shown to regulate the interaction between JUNO and IZUMO1 in wild-type mice [39].

Recently, the structure of the human JUNO/IZUMO1 complex has been reported and critical amino acids have been identified during the two molecules interaction [16][17]. According to structural data, mutations in W148, H157 and R160 of IZUMO1 and W52 and L81 of JUNO are essential for sperm and oocyte plasma membrane recognition [17].

After fusion, the sperm membrane comprises a continuous single membrane plane with a complicated invaginated structure. However, sperm–oocyte fusion is not completely achieved at this point, as electron microscopy images have showed, the “internalization” of the invaginated inner acrosomal membrane occurs later in the fertilization process [40].

References

1. Austin, C.R. Scientific and Clinical Aspects of Fertilization and Implantation. Proc. R. Soc. Med. 1974, 67, 925–927.
2. Bernecic, N.C.; Gadella, B.M.; Leahy, T.; de Graaf, S.P. Novel methods to detect capacitation-related changes in spermatozoa. Theriogenology 2019, 137, 56–66.
3. Florman, H.M.; Fissore, R.A. Fertilization in Mammals. In Knobil and Neill’s Physiology of Reproduction: Two-Volume Set; Elsevier Inc.: Amsterdam, The Netherlands, 2015; Volume 1, pp. 149–196. ISBN 9780123977694.
4. Kay, V.; Robertson, L. Hyperactivated motility of human spermatozoa: A review of physiological function and application in assisted reproduction. Hum. Reprod. Update 1998, 4, 776–786.
5. Hirohashi, N.; Yanagimachi, R. Sperm acrosome reaction: Its site and role in fertilization. Biol. Reprod. 2018, 99, 127–133.
6. Bhakta, H.H.; Refai, F.H.; Avella, M.A. The molecular mechanisms mediating mammalian fertilization. Development 2019, 146, dev176966.
7. Bianchi, E.; Wright, G.J. Find and fuse: Unsolved mysteries in sperm-egg recognition. PLoS Biol. 2020, 18, e3000953.
8. Bianchi, E.; Wright, G.J. Cross-species fertilization: The hamster egg receptor, Juno, binds the human sperm ligand, Izumo1. Philos. Trans. R. Soc. B Biol. Sci. 2015, 370, 20140101.
9. Yanagimachi, R. Zona-free hamster eggs: Their use in assessing fertilizing capacity and examining chromosomes of human spermatozoa. Gamete Res. 1984, 10, 187–232.
10. Inoue, N.; Ikawa, M.; Isotani, A.; Okabe, M. The immunoglobulin superfamily protein Izumo is required for sperm to fuse with eggs. Nature 2005, 434, 234–238.
11. Inoue, N. Novel insights into the molecular mechanism of sperm–egg fusion via IZUMO1. J. Plant Res. 2017, 130, 475–478.

12. Lorenzetti, D.; Poirier, C.; Zhao, M.; Overbeek, P.A.; Harrison, W.; Bishop, C.E. A transgenic insertion on mouse chromosome 17 inactivates a novel immunoglobulin superfamily gene potentially involved in sperm-egg fusion. Mamm. Genome 2014, 25, 141–148.
13. Kaji, K.; Oda, S.; Shikano, T.; Ohnuki, T.; Uematsu, Y.; Sakagami, J.; Tada, N.; Miyazaki, S.; Kudo, A. The gamete fusion process is defective in eggs of Cd9-deficient mice. Nat. Genet. 2000, 24, 279–282.
14. Rubinstein, E.; Ziyyat, A.; Prenant, M.; Wrobel, E.; Wolf, J.P.; Levy, S.; Le Naour, F.; Boucheix, C. Reduced fertility of female mice lacking CD81. Dev. Biol. 2006, 290, 351–358.
15. Bianchi, E.; Doe, B.; Goulding, D.; Wright, G.J. Juno is the egg Izumo receptor and is essential for mammalian fertilization. Nature 2014, 508, 483–487.
16. Aydin, H.; Sultana, A.; Li, S.; Thavalingam, A.; Lee, J.E. Molecular architecture of the human sperm IZUMO1 and egg JUNO fertilization complex. Nature 2016, 534, 562–565.
17. Ohto, U.; Ishida, H.; Krayukhina, E.; Uchiyama, S.; Inoue, N.; Shimizu, T. Structure of IZUMO1–JUNO reveals sperm–oocyte recognition during mammalian fertilization. Nature 2016, 534, 566–569.
18. Satouh, Y.; Inoue, N.; Ikawa, M.; Okabe, M. Visualization of the moment of mouse sperm-egg fusion and dynamic localization of IZUMO1. J. Cell Sci. 2012, 125, 4985–4990.
19. Inoue, N.; Ikawa, M.; Okabe, M. The mechanism of sperm-egg interaction and the involvement of IZUMO1 in fusion. Asian J. Androl. 2011, 13, 81–87.
20. Noda, T.; Lu, Y.; Fujihara, Y.; Oura, S.; Koyano, T.; Kobayashi, S.; Matzuk, M.M.; Ikawa, M. Sperm proteins SOF1, TMEM95, and SPACA6 are required for sperm-oocyte fusion in mice. Proc. Natl. Acad. Sci. USA 2020, 117, 11493–11502.
21. Fujihara, Y.; Lu, Y.; Noda, T.; Oji, A.; Larasati, T.; Kojima-Kita, K.; Yu, Z.; Matzuk, R.M.; Matzuk, M.M.; Ikawa, M. Spermatozoa lacking Fertilization Influencing Membrane Protein (FIMP) fail to fuse with oocytes in mice. Dev. Biol. 2020, 117, 9393–9400.
22. Lamas-Toranzo, I.; Hamze, J.G.; Bianchi, E.; Fernández-Fuertes, B.; Pérez-Cerezales, S.; Laguna-Barraza, R.; Fernández-González, R.; Lonergan, P.; Gutiérrez-Adán, A.; Wright, G.J.; et al. TMEM95 is a sperm membrane protein essential for mammalian fertilization. eLife 2020, 9, e53913.
23. Pausch, H.; Kölle, S.; Wurmser, C.; Schwarzenbacher, H.; Emmerling, R.; Jansen, S.; Trottmann, M.; Fuerst, C.; Götz, K.U.; Fries, R. A Nonsense Mutation in TMEM95 Encoding a Nondescript Transmembrane Protein Causes Idiopathic Male Subfertility in Cattle. PLoS Genet. 2014, 10, e1004044.
24. Fernandez-Fuertes, B.; Laguna-Barraza, R.; Fernandez-Gonzalez, R.; Gutierrez-Adan, A.; Blanco-Fernandez, A.; O'Doherty, A.M.; Di Fenza, M.; Kelly, A.K.; Kölle, S.; Lonergan, P. Subfertility in bulls carrying a nonsense mutation in transmembrane protein 95 is due to failure to interact with the oocyte vestments. Biol. Reprod. 2017, 97, 50–60.
25. Kobe, B.; Deisenhofer, J. A structural basis of the interactions between leucine-rich repeats and protein ligands. Nature 1995, 374, 183–186.
26. Zhang, S.; Cai, H.; Yang, Q.; Shi, T.; Pan, C.; Lei, C.; Dang, R.; Chen, H.; Lan, X. Identification of novel alternative splicing transcript and expression analysis of bovine TMEM95 gene. Gene 2016, 575, 531–536.
27. Barbaux, S.; Ialy-Radio, C.; Chalbi, M.; Dybal, E.; Homps-Legrand, M.; Do Cruzeiro, M.; Vaiman, D.; Wolf, J.P.; Ziyyat, A. Sperm SPACA6 protein is required for mammalian Sperm-Egg Adhesion/Fusion. Sci. Rep. 2020, 10, 5535.
28. Ellerman, D.; Pei, J.; Gupta, S.; Snell, J.; Myles, D.; Primakoff, P. Izumo is part of a multiprotein family whose members form large complexes on mammalian sperm. Mol. Reprod. Dev. 2009, 76, 1188–1199.
29. Fukuda, M.; Sakase, M.; Fukushima, M.; Harayama, H. Changes of IZUMO1 in bull spermatozoa during the maturation, acrosome reaction, and cryopreservation. Theriogenology 2016, 86, 2179–2188.e3.
30. Saito, T.; Wada, I.; Inoue, N. Alternative splicing of the Izumo1 gene ensures triggering gamete fusion in mice. Sci. Rep. 2019, 9, 3151.
31. Llavanera, M.; Delgado-Bermúdez, A.; Fernandez-Fuertes, B.; Recuero, S.; Mateo, Y.; Bonet, S.; Barranco, I.; Yeste, M. GSTM3, but not IZUMO1, is a cryotolerance marker of boar sperm. J. Anim. Sci. Biotechnol. 2019, 10, 61.
32. Sebkova, N.; Ded, L.; Vesela, K.; Dvorakova-Hortova, K. Progress of sperm IZUMO1 relocation during spontaneous acrosome reaction. Reproduction 2014, 147, 231–240.
33. Sosnik, J.; Miranda, P.V.; Spiridonov, N.A.; Yoon, S.Y.; Fissore, R.A.; Johnson, G.R.; Visconti, P.E. Tssk6 is required for Izumo relocalization and gamete fusion in the mouse. J. Cell Sci. 2009, 122, 2741–2749.
34. Wolkowicz, M.J.; Digilio, L.; Klotz, K.; Shetty, J.; Flickinger, C.J.; Herr, J.C. Equatorial Segment Protein (ESP) Is a Human Alloantigen Involved in Sperm-Egg Binding and Fusion. J. Androl. 2008, 29, 272–282.

35. Inoue, N.; Hamada, D.; Kamikubo, H.; Hirata, K.; Kataoka, M.; Yamamoto, M.; Ikawa, M.; Okabe, M.; Hagihara, Y. Molecular dissection of IZUMO1, a sperm protein essential for sperm-egg fusion. Development 2013, 140, 3221–3229.
36. Inoue, N.; Wada, I. Monitoring dimeric status of IZUMO1 during the acrosome reaction in living spermatozoon. Cell Cycle 2018, 17, 1279–1285.
37. Miyado, K.; Yamada, G.; Yamada, S.; Hasuwa, H.; Nakamura, Y.; Ryu, F.; Suzuki, K.; Kosai, K.; Inoues, K.; Ogura, A.; et al. Requirement of CD9 on the Egg Plasma Membrane for Fertilization. Science 2000, 287, 321–324.
38. Le Naour, F.; Rubinstein, E.; Jasmin, C.; Prenant, M.; Boucheix, C. Severely Reduced Female Fertility in CD9-Deficient Mice. Science 2000, 287, 319–321.
39. Chalbi, M.; Barraud-Lange, V.; Ravaux, B.; Howan, K.; Rodriguez, N.; Soule, P.; Ndzoudi, A.; Boucheix, C.; Rubinstein, E.; Wolf, J.P.; et al. Binding of sperm protein Izumo1 and its egg receptor juno drives cd9 accumulation in the intercellular contact area prior to fusion during mammalian fertilization. Development 2014, 141, 3732–3739.
40. Huang, T.T.F.; Yanagimachi, R. Inner acrosomal membrane of mammalian spermatozoa: Its properties and possible functions in fertilization. Am. J. Anat. 1985, 174, 249–268.
