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Deficiency in Sperm–Egg Protein Interaction as a Major Cause of Fertilization Failure

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Abstract Complete elucidation of fertilization process at molecular level is one of the unresolved challenges in sexual reproduction studies, and understanding the molecular mechanism is crucial in overcoming difficulties in infertility and unsuccessful in vitro fertilization. Sperm–oocyte interaction is one of the most remarkable events in fertilization process, and deficiency in protein–protein interactions which mediate this interaction is a major cause of unexplained infertility. Due to detection of how the various defects of sperm–oocyte interaction can affect fertilization failure, different experimental methods have been applied. This review summarizes the current understanding of sperm–egg interaction mechanism during fertilization and also accumulates the different types of sperm–egg interaction abnormalities and their association with infertility. Several detection approaches regarding sperm–egg protein interactions and the associated defects are reviewed in this paper.

Keywords Infertility · Sperm–egg interaction · Interaction defects

Abbreviations

PPI	Protein–protein interaction
−/−	Deletion gene in knockout genome
IVF	In vitro fertilization
ZP	Zona pellucida
AR	Acrosome reaction
ART	Assisted reproductive technology
ZPIAR	ZP-induced AR
2D	Two-dimension
TMEM190	Transmembrane protein 190
SPESP1	Sperm equatorial segment protein 1
SPACA	Sperm acrosome-associated proteins
SAMP	Sperm acrosomal membrane-associated protein
SLLP1	Sperm lysosomal-like protein 1
ADAMs	Disintegrin and metalloproteinase domain
IgSF	Immunoglobulin superfamily
Itg	Integrin
Y2H	Yeast two-hybrid

Introduction

Sperm–egg interaction is a unique cell–cell connection process in sexual reproduction that involves two gametes recognizing, binding, and eventually fusing with each other (Wortzman et al. 2006). The potential intermediary of molecular process in sperm–oocyte fusion and binding has been studied over the past 20 years and is still poorly understood (Kaji and Kudo 2004; Primakoff and Myles 2002; Stein et al. 2004). During this process, many molecular interactions in the form of protein–protein interactions will mediate the sperm–egg binding process. The elucidation of sperm–egg interaction at the molecular level is crucial in solving problems in infertility and in vitro fertilization (IVF) failure (Evans 2012). IVF is implemented for couples with no sperm dysfunction and no female infertility elements. Nevertheless, it is surprising that complete fertilization failure is still a prevalent event in the process

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of IVF (Brewis et al. 2005). This suggests that the sperm and egg dysfunctions are not certain even with common analyzing, while their protein defects are as considerable cause of fertilization failure (Stein et al. 2004). This phenomenon is called unexplained infertility and remains an unknown syndrome and researchers have limited information regarding the clinical nature of the sperm and oocyte dysfunctions (Brewis et al. 2005; Hamada et al. 2012). Recent studies have represented that protein deficiencies in membrane interaction such as zona binding or the zona-induced AR (Acrosome Reaction) are significant causes of reduced fertilization and total fertilization fail in assisted reproductive technologies (Gadella 2008). They suggested the major causes of fertilization failure in conventional IVF of unexplained male infertile is due to abnormalities of protein–protein interaction in sperm–oocyte membrane interaction (Brewis et al. 2005; Hamada et al. 2012; Liu and Baker 2000). Due to detection of the molecules that mediate human membrane sperm–oocyte interaction, different experimental methods have been applied (Evans 2012). This review reflects on current understanding of sperm–egg interaction mechanism during fertilization, with particular focus on the effects of sperm and oocyte proteins on fertility status. This review also discusses the applied experimental techniques regarding identification of protein–protein interactions. Because of the difficulties in studying membrane protein–protein interactions and the inadequacy of materials, many efforts have failed to achieve comprehensive data about human sperm–egg interaction. Computational methods also can explain protein–protein interactions at various levels as moving forward regarding study on sperm–egg membrane interaction.

Fertilization

Fertilization is a distinctive cell–cell interaction occurrence encompassing two structurally dissimilar gametes that recognize, bind, and eventually fuse with each other (Sato 2014). The fertilization process has been divided into five steps in order for the eventual entry of the sperm nucleus into the ovum cytoplasm. During this process, the fertilizing spermatozoon, after capacitation, must initially interpenetrate the neighboring cumulus layer of oocyte composed of follicular cells disseminated in a polymerized matrix constituted primarily of hyaluronic acid (Wortzman et al. 2006; Yu 2008). In order to experience a specific gamete recognition process, acrosome of spermatozoa makes contact with the oocyte in most laboratory animals (Abou-haila and Tulsiani 2009). This is facilitated by the sperm plasma membrane covering the acrosomal vesicle and complementary molecules allied with the zona pellucida (ZP) of the oocyte. Later on, the spermatozoon goes through the acrosome reaction (AR) after primary sperm–zona binding. The AR makes multiple interactions between the overlying plasma membrane and the outer membrane of acrosome, which discloses the acrosomal substances of the spermatozoa and its persistent inner acrosome membrane. During the acrosome reaction, the contents of the acrosome are released outwardly and the cell membrane of the spermatozoon fuses with the outer membrane of the acrosome. When the acrosome reaction has been completed, the spermatozoon is now covered at its upper end only by the former inner membrane of the acrosome (Yanagimachi 2011). Moreover, the fertilizing spermatozoa undertakes a more determined secondary binding after induction of the AR between the ZP and the inner acrosomal membrane; this is followed by ZP penetration (Tokuhiro et al. 2012; Tulsiani and Abou-Haila 2012).

However, only one sperm usually transpires into the perivitelline space situated between the egg and the ZP, and interacts with the oolemma (egg membrane) in a sperm–egg binding function, which is immediately continued by sperm–egg fusion. After sperm entry into the oocyte, different events happen, for instance, the quick supply of a sperm factor (phospholipase zeta) in inducing calcium oscillations and the role of the fertilizing spermatozoon (Tokmakov et al. 2014). The egg activation is the resultant of these oscillations, which bring about the ultimate development of the female pronucleus (Gadella 2008; Lee et al. 2010; Nomikos et al. 2012). Also, the development of the male pronucleus and Sperm chromatin decondensation takes place. The two pronuclei ultimately fuse at syngamy from the different gametes; thus, fertilization is believed to be completed at this point (van der Heijden et al. 2008).

Deficiency in cell–cell adhesion event between sperm and egg contributes to unsuccessful fertilization and assisted reproductive technology (ART) failure (Swain and Pool 2008).

Infertility

Infertility is a common medical problem having an impact on 10–15% of couples around the globe. The prevalence varies throughout developed and underdeveloped countries, being greater in the latter where inadequate resources are available for treatment and diagnosis (Hamada et al. 2011; Hotaling et al. 2011). Reports indicate that there are 37–58% of infertile couples. These cases remain infertile even past ART (Jungwirth et al. 2012; Rowe and Comhaire 2000).

Fertilization Defects

In conventional IVF, the main cause of fertilization failure of unexplained male infertility is because of abnormalities of sperm–oocyte penetration and interaction (Liu and Baker 2000). Even though most penetration defects and sperm–oocyte binding defect are owing to apparent sperm abnormalities, for instance teratozoospermia and asthenozoospermia, many patients have regular semen parameters and elusive sperm weaknesses that influence sperm–oocyte interaction. A routine semen analysis cannot show these defects, but they are obvious with sperm–ZP interaction examinations (Hamada et al. 2011).

Sperm–ZP Binding Defect

The existence of complementary binding receptors or sites on the gamete surface are prerequired for sperm adhesion to the ZP of oocyte; usually, these receptors are associated with a high rate of species specificity (Sinowatz et al. 2001). Human ZP (hZP) consists of four key glycoproteins (hZP4, hZP3, hZP2, and hZP1) where the ZP3 of human oocytes is supposed to be the main ZP receptor for capacitated acrosome-intact sperm adhesion. Although the precise sperm receptors of humans for the hZP have not been identified, numerous candidate proteins of sperm have been reported to be capable of interacting with either intact or solubilized ZP. On the other hand, it is not clear whether the reported sperm receptors are the main ones for sperm interaction with the ZP or not (Lefievre et al. 2004; Liu et al. 2009; van Gestel et al. 2007). A signal transduction cascade inside the spermatozoa is encouraged by the sperm binding to ZP3, as shown in Fig. 1, comprising various proteins and other aspects such as protein kinases A and C pathways that results in the acrosome reaction (AR). ZP2 is supposed to be bound to spermatozoa, which enables the progression into the perivitelline layer and the penetration to the zona matrix (Baldi et al. 2000; Sun and Nagai 2003).

In about 25 and 15% of subfertile men with abnormal and normal semen analysis, respectively, the defective ZP-bound sperm are present. Thus, after undergoing IVF, such individuals have a decreasing chance of attaining effective fertilization (Liu et al. 2007). It has been stated that with unexplained infertility, two out of 18 men indicated a dearth of sperm binding to the ZP in spite of having sperm parameters such as morphology or count like fertile men. However, the defective signaling pathways of protein kinases C and A could lead to the existence of imperfect sperm binding to ZP in infertile individuals with regular semen analysis. On the other hand, a lot of imperfect sperm–ZP adhesion of infertile men with normal semen analysis and those with acute teratozoospermia are

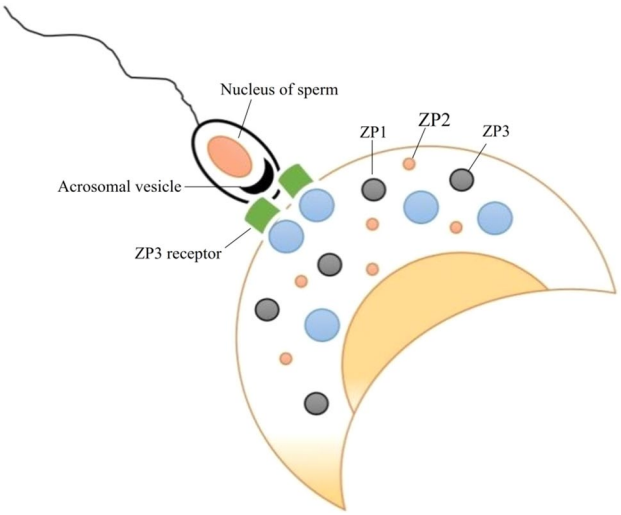

Fig. 1 Acrosome–Zp interaction: A signal transduction cascade inside the spermatozoa is encouraged by the sperm binding to ZP3, comprising various proteins and other aspects such as protein kinases A and C pathways that result in the acrosome reaction (AR)

expected to possess structural deficiencies, or lack of sperm receptors for interaction with the ZP (Liu et al. 2009). CaP_ZP3 protein is primarily detected in stage III oocytes, and the protein accumulates as oocytes that develop into stage IV oocytes and the transcription of the CaP_ZP3 protein occurs prior to its translation in studied triploid fish and it has been indicated that the transcription and translation of the ZP3 gene in this special triploid fish are asynchronous (Shi et al. 2013).

Acrosome Reaction Defect

Acrosome reaction (AR) is the interaction event of plasma membrane with the outer acrosome membrane in sperm that occurs by secreting of exocytotic proteolytic enzymes (hyaluronidase and acrosine) in reaction to sperm–ZP adhesion (Tulsiani and Abou-Haila 2001). In human sperm, the natural stimulus for the AR is ZP3 that leads to the proteolytic decomposition of the ZP and the principal binding of the ZP with intact acrosome is started. There are two types of defective AR that have clinical importance. The first is a great amount of spontaneous AR (>20% of spermatozoa presenting spontaneous AR) that is due to the prematurity of AR and the second is the reduced responsiveness to AR stimulants (when <15% of spermatozoa reacted to AR stimulants) that is the reason of insufficiency of AR. In conventional IVF treatment, both conditions are linked to weak fertilization capability (Sigman et al. 2009a).

Moreover, some unexplained infertile men presenting normal sperm–ZP adhesion possess imperfect ZP-induced

AR (ZPIAR) that is associated with weak sperm–ZP penetration and the failure of fertilization. A long period of infertility, normal semen parameters, and normal sperm–ZP adhesion have been demonstrated in patients with unexplained infertility but the patients show penetration failure of sperm to ZP. Therefore, they show low or zero rates of fertilization with conventional IVF (Liu and Baker 2003). Although the precise mechanisms of deficient AR are unidentified, imperfect ZPIAR is expected to be highly associated with structural sperm head deficiencies, for instance small or abnormal acrosomes, or disorders in the overlying plasma membrane of subfertile men (Eddy 2006). Defective ZPIAR was found in 25% of subfertile men with normal semen parameters and the rate of this deficiency was significant in subfertile men with idiopathic teratozoospermia and oligozoospermia (Liu et al. 2007).

Sperm-mediated oocyte-activating factors (SOAF) compartmentalize as part of the postacrosomal sheath of sperm perinuclear theca (PAS-PT) and trigger intracellular Ca2+-release upon fusion of spermatozoa/oocyte membranes or insemination of spermatozoa into oocyte (Anifandis et al. 2016; Sutovsky et al. 2003). Several factors have been considered as candidate for SOAFs including PLCζ (a sperm-specific phospholipase C), TR-KIT (a truncated form of the KIT receptor), PAWP (postacrosomal sheath WW domain-binding protein), and citrate synthase (Albertini 2015; Tavalaee and Nasr-Esfahani 2016; Yeste et al. 2016). Failed fertilization post-ICSI is associated with the lack or deficiency of SOAF(s) (Amdani et al. 2015). Absence of SOAF(s) in globozoospermic individuals because of the absence of acrosome and PAS-PT and during acrosome biogenesis in these individuals may be considered as one reason for failed fertilization in globozoospermic men (Tavalaee and Nasr-Esfahani 2016).

Fusion Defect of the Acrosome-Reacted Sperm with the Oocyte Plasma Membrane

The fusion capability of the acrosome-reacted human sperm equatorial region with the oocyte vitelline membrane is verified utilizing the sperm penetration assay (SPA) (Sigman et al. 2009b). This test calculates the ability of the spermatozoon to undergo AR, capacitation, fusion, and penetration via oocyte plasma membrane. During this test, a zona-free hamster oocyte was incubated with human spermatozoa and the percent of egg's penetration; normal sperm capable of penetrating 10–30% of hamster oocyte was measured. Modern refinement of this test was carried out and showed the majority of oocyte to be penetrated if the sperm was incubated in more potent capacitating manner. It has been demonstrated that 34.1% of UMI group had less than 10% egg penetration compared to 0% of fertile men group (Aitken et al. 1982). Also, in order to predict the failure or success of IVF, the ability of the SPA has been evaluated by numerous studies. Some investigators have claimed 100% predictability, while others have revealed no association with an abnormal test. In addition, a usual SPA might have 70% predictability of IVF by taking an average from diverse studies. However, semen samples that are unable to fertilize hamster oocyte typically fail to fertilize human oocyte. Therefore, the SPA is regarded as a research device, and it can be used for checking the medical level of fertility potential of patients with UMI suffering from negligible fertilization value of IVF (Hamada et al. 2011; Hamada et al. 2012; Sigman et al. 2009b).

As demonstrated above, the defection or mutation of different molecules which play some role in sperm–egg interaction process is a major cause of unexplained infertility.

Sperm–Oocyte Interactions

One of the most remarkable processes in sexual reproduction is sperm–egg interaction. Over the past 20 years, the molecular events associated with sperm–oocyte binding and fusion have been the focus of various and numerous researches, with various sperm proteins associated as prospective intermediaries (Kaji and Kudo 2004; Primakoff and Myles 2002; Stein et al. 2004). Jan Frayne's attempt and other previous studies have introduced the sperm–oocyte relations and defined both binding and fusion events (Frayne and Hall 1999). The molecular interactions that mediate sperm–oocyte membrane adhesion are still poorly described. However, up till now, no certain candidate proteins have been described and only a small number of sperm proteins have been suggested to have a fusogenic role (Brewis et al. 2005). Figure 2 presents the molecules proposed to participate in sperm–egg membrane interactions.

Experimental Methodologies to Identify Protein Candidates in Sperm–Oocyte Interaction

In order to study the mammalian molecular candidate for sperm–egg interaction, the original and unbiased method was the utilizing of antigamete monoclonal antibody techniques (typically against sperm) that were implemented in IVF test for assessment of their stopping action and also to inspect antigen localization. Some sperm proteins including ADAM1, ADAM2, and IZUMO1 have been detected applying this technique (Evans 2012). A more recent and relevant technique has been applied to determine the sperm proteome such as proteins in definite subcellular segments, glycosylated proteins, or proteins that separate into

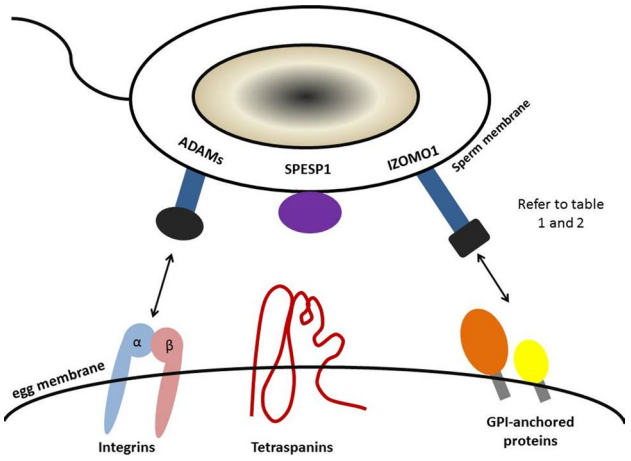

Fig. 2 Proteins participated in sperm–egg membrane interaction: GPI-anchored protein as known Juno is identified as a receptor for IZUMO1 on mouse eggs. ADAMs in sperm membrane interact with integrins in egg via their integrin ligand-like disintegrin domain. Several tetraspanins are probably involved in the regulation of membrane through associating with and/or assisting the function of other membrane proteins including different integrins and other interaction molecules. *Spesp1*$^{-/-}$ sperms have represented the reduced capability for sperm–oocyte fusion. Other sperm and egg proteins not pictured here are summarized in Tables 1 and 2

a Triton X-114 detergent phase (Hao et al. 2002; Wolkowicz et al. 2003). The proteomic methods such as 2D electrophoresis followed by mass spectrometry have identified some sperm proteins including transmembrane protein 190 (TMEM190), sperm equatorial segment protein 1 (SPESP1), and four sperm acrosome-associated (SPACA) proteins: SPACA1 [sperm acrosomal membrane-associated 32 (SAMP32)], SPACA4 (sperm acrosomal membrane-associated 14 (SAMP14)), and SPACA3 (sperm lysosomal-like protein 1 (SLLP1)) and SPACA6 (Sperm acrosome membrane-associated protein 6) (Evans 2012). It has been proposed that SPACA6 together with IZUMO1 my mediate sperm fusion by binding an as yet unknown egg membrane receptor (Lorenzetti et al. 2014).

A grouping of candidate methods and unbiased approaches such as advanced proteomic technique linked to the mass spectrometric methods have been also useful for oocyte proteomics. Applying these methods, some oocyte proteins have been identified that mediate sperm–egg interaction, for example, integrins on eggs come to mind with the detection of an integrin ligand-like domain in a sperm protein of mammalian (Blobel et al. 1992). A significant assessment is by evaluating the impacts of genetic mutation or deletion on reproductive function. A knockout mouse with a failure to generate any offset reveals a crucial task for molecular level of reproductive process for the candidate molecule. In contrast, if a knockout mouse is fertile and produces an offspring, the molecule in question is not so critical or may have been provided with a supplementary molecule that acts in multiple pathways.

Another kind of significant phenotype that has long been studied is synthetic lethality, which occurs if a mutation in a single gene has poor to no efficacy on livability but joining with a mutation in other gene(s) may result in a lethal phenotype. On the other hand, a defect in a single gene may have little efficacy on fertilization, while the integration of the defect with other genetic imperfection eventuates in total infertility. For example, *Cd81*$^{-/-}$ females have a slight loss of fertilization, knockout female mice with *Cd9*$^{-/-}$ are barely subfertile, whereas *Cd9*$^{-/-}$/*Cd81*$^{-/-}$females are completely infertile (Rubinstein et al. 2006). Mice ovule has more oocytes per cycle than the ovulation in human females; therefore, a genetic imperfection accounts for only a moderate fertilization deficiency in mice. In order to make developments related to reproduction, especially in humans in spite of the experimental challenges in analysis and identification, the evaluation of fertility status is very important, both in vivo subfertility and those that revealed infertility via in vitro approaches (Ola et al. 2001; Tournaye et al. 2002).

Candidate Sperm Proteins in Sperm–Oocyte Interaction

To date, some proteins in sperm have been identified that mediate sperm–egg binding and fusion, using described techniques.

IZUMO1

IZUMO1 is one of the sperm proteins and is a member of the immunoglobulin superfamily (IgSF) of proteins. This protein was recognized using antisperm monoclonal antibodies by liquid chromatography tandem–mass spectrometry (Anifandis et al. 2014). Mouse IZUMO1 is a 56-kDa protein which contains one immunoglobulin-like domain including an N-glycosylation site that seems to be testis-specific. Sperm–egg adhesion with knockout ZP eggs was prevented by monoclonal antibody OBF13, against IZUMO1, and similarly, the antibodies to the recognized human IZUMO1 inhibit the fusion of human sperm to ZP knockout hamster eggs (Inoue et al. 2005).

However, the possible relationship between human infertility and IZUMO1 abnormality has been considered in the previous research and the most significant knowledge about this relationship has been provided by the knockout mouse. In fact, *Izumo1*$^{-/-}$ females act healthy and possess normal fertility. Conversely, in spite of the fact that *Izumo1*$^{-/-}$ males have normal ejaculation and mating behavior and have regular sperm migration and motility

into the oviduct, these males are infertile (Granados-Gonzalez et al. 2008; Hayasaka et al. 2007; Inoue et al. 2005).

Izumo1-null sperm was able to enter the ZP and then penetrates to the perivitelline layer in IVF assays but the fertilization failed. Therefore, *Izumo*$^{-/-}$ sperm has been found with deficient interaction through the egg plasma membrane. Despite the exact function of IZUMO1 is poorly understood and it is not definite that IZUMO1 act as an adhesion molecule, as a fusogen, and/or as a fusogen regulator, the evidence shows that IZUMO1 is critical for sperm–oocyte fusion. IZUMO1 with the immunoglobulin-like domain probably interacts with other proteins (Brümmendorf and Lemmon 2001). Structure function analysis of IZUMO1 is still demanding, as the most interesting assessment on knockout sperm with the *Izumo1*$^{-/-}$ background. Thus far, researchers have revealed that *Izumo*-null males represented significantly decreased value of sperm–egg fusion in IVF (Inoue et al. 2008).

ADAMs

The ADAM (contain disintegrin and metalloproteinase domain) family was an interesting protein family in reproductive study, where fertilization was blocked using antibody against several members. ADAM2 (fertilin β) is one of the members that act in fertilization process is sperm. Overall, ADAMs in sperm make interaction with numerous members of the integrin family. Many of the integrin members are expressed in the egg and may be involved in sperm–oocyte interaction. The assessment of the relationship between ADAMs in sperm and the integrin pairs in the egg have showed that the integrin α9β1 in the egg specifically interact with ADAM2 as its binding partner (Desiderio et al. 2010; Eto et al. 2002; Tomczuk et al. 2003). Despite previous research on the role of ADAMs family in sperm–oocyte interaction, the function of several members of ADAMs in mammalian fertilization is still poorly determined. In order to study the function of several ADAMs, multiple *Adam*-null mice have been produced (Kim et al. 2006; Nishimura et al. 2004). In several of these *Adam* knockouts, the sperm showed abnormalities in its surface proteins with lack of various ADAMs and reduced penetration to the zona matrix and/or decreased binding and fusion to plasma membrane of the egg. Investigation of gamete membrane interactions showed that the *Adam2*$^{-/-}$ knockout relates to functional defects of sperm, while other *Adam* knockouts possess low or no obvious effect on male fertilization (Desiderio et al. 2010; Horiuchi et al. 2003).

Other Sperm Proteins

Biochemical fractions and structures of numerous sperm proteins have been revealed in their functions in membrane interaction of the gametes. SPESP1 (Sperm equatorial segment protein 1) is one of the sperm proteins whose function in sperm–egg interaction has been studied with generation of a knockout mouse. *Spesp1*$^{-/-}$ sperms have represented the reduced capability for sperm–oocyte fusion and also showed delayed migration via the reproductive tract of female in comparison with sperm of wild-type controls. Generally, the fertility level of *Spesp1*$^{-/-}$ males was slightly lower than the wild-type controls (Fujihara et al. 2010). *Spesp1* deletion also affects biochemical and localization features of the protein that are probably involved in sperm–egg membrane interaction such as IZUMO1, equatorin, and another sperm proteins. Furthermore, the deletion of *Spesp1* affects membrane morphology of sperm; in these sperms, evaluation by electron microscopy shows damage of the equatorial segment membrane. The considerations of other sperm proteins which are involved in sperm–oocyte interaction, comprising SPACA1, SPACA3, SPACA4, equatorin, CRISP1 and TMEM190, and numerous enzyme activities and adhesion molecules are summarized in Table 1 (Evans 2012).

Candidate Oocyte Proteins in Sperm–Oocyte Interaction

To date, some oocyte proteins have been identified that mediate sperm–egg binding and fusion, using described techniques.

Integrins

The principle role of integrins in the oocyte in sperm–oocyte interaction event has been revealed by integrin ligand-like domain in ADAM2 which is an antigen of a function-blocking antisperm antibody (Liu et al. 2010). Knockout mouse in several integrins' backgrounds have been studied to consider the significance of integrins in fertilization. Mouse eggs express 8 of 18 integrin α subunits and 3 of 8 integrin β subunits (*Itgb1, Itgb3, Itgb5, Itga1, Itga2, Itga3, Itga5, Itga6, Itga8, Itga9*, and *Itgav*) in mouse eggs and therefore at least ten different α-β integrins integration according to identified heterodimer pairs can be expressed (Desiderio et al. 2010). Several of the integrin heterodimer pairs, especially ITGA9-ITGB1 (α9β1), interact with several ADAMs (Edwards et al., 2008).

The certain deletion of egg-expressed integrins (*Itga1, Itga2, Itgb3, Itgb5*) has shown no infertility status, while the other seven in this list are lethal for embryos or neonates. For example, the *Itga9*-defect oocytes have the clearest imperfection. Although oocytes defects in *Itgb1, Itga3* or *Itga6*, can be fertilized in vitro, *Itgb1*-null eggs display delay in time-lapse video analysis and also in modified

Table 1 Participating sperm proteins in sperm–oocyte membrane interactions

Protein	Properties and result of defective proteins
IZUMO1	Sperm-specific (at protein level). Detectable on sperm surface (Nomikos et al. 2012). *Izumo*-null males represented significantly decreased value of sperm–egg fusion in IVF (Liu et al. 2009)
ADAMs	Membrane proteins. These proteins have been represented to mediate sperm–egg adhesion by interacting their disintegrin-like domain with an integrin on the egg plasma membrane (Liu and Baker 2003). Lack of various ADAMS reduced penetration to the zona matrix and/or decreased binding and fusion to plasma membrane of the egg (Liu et al. 2010)
CRISP1	Epididymal protein. Knockouts sire litters of normal sizes in normal time frames in conventional mating trials; null sperm have moderate deficiencies in sperm–oocyte interaction in vitro assays (Ola et al. 2001)
SPACA1 (SAMP32)	Inner acrosomal membrane protein. Antibodies reduce binding and fusion of human sperm to ZP-free hamster eggs (Inoue et al. 2008)
SPACA3 (S LLP1)	Acrosomal matrix protein. Antibodies and recombinant protein reduce sperm–egg binding and fusion (Amdani et al. 2015)
SPACA4 (SAMP 14)	Inner acrosomal membrane protein. Antibodies reduce binding and fusion of human sperm to ZP-free hamster oocytes (Perkins et al. 2010)
Equatorin	Novel protein localized in the equatorial segment (Primakoff and Myles 2002)
TMEM190	Identified in a fraction of surface and vesicle proteins (Rowe and Comhaire 2000)
E-cadherin (CDH1)	Identified on human sperm. Antibodies reduce the adhesion of human sperm to ZP-free hamster oocytes (Rubinstein et al. 2006)
N-cadherin (CDH2)	Identified on human sperm. Antibodies reduce the adhesion of human sperm to ZP-free hamster oocytes (Sachs et al. 2006)
avβ3 or α6β1 integrin	Characterized in mouse sperm; antibody-based inhibition (Sato 2014)
Enzyme activities	
Zinc metalloprotease activity	Inhibitors and zinc chelators reduce sperm–oocyte fusion (Shi et al. 2013)
Protein disulfide isomerases (PDIs)	Identified in a fraction of surface and vesicle proteins (Sigman et al. 2009a); inhibitors reduce sperm–oocyte fusion (Sigman et al. 2009b; Singson et al. 2008)

assays, subtle defects have been detected with *Itga3*- and *Itga6*-imperfection oocytes. Amount of fertilized eggs and sperm–egg binding and fusion have been decreased with ITGA9 knockdown egg in comparison with controls. ITGA9-null eggs did not show a complete failure of fertilization, possibly due to only partial ITGA9 decrease on the surface of oocyte, and it is possible that other egg surface molecules have a likely role in gamete membrane interaction (Evans 2012).

Tetraspanins

CD9 is a member of the tetraspanin family and *Cd9*-null mouse showed the significance of this protein in fertilization. $Cd9^{-/-}$ females are severely subfertile and create a few offspring and in some cases, no offsets. $Cd9^{-/-}$ females may be fertile but have a severe delay in pregnancy (Le Naour et al. 2000; Rubinstein et al. 2006). In IVF, very few *Cd9* knockout eggs are able to be fertile. CD9 is extensively expressed in the body and the *Cd9*-null mouse survives and is healthy but they have a serious fertility defect. Therefore, it has been identified that CD9 has a critical function only in the oocyte. More than 30 tetraspanins are expressed in mammalians and CD9 is one of the multiple tetraspanins in mouse oocytes. *Cd9* deletion results a serious decrease in fertility and the remaining tetraspanins on the oocyte cannot retaliate for the lack of CD9. The precise function(s) of CD9 in sperm–oocyte interaction is not identified, even though the significance of CD9 in mouse sperm–oocyte interaction is evidently proven (Miyado et al. 2000; Rubinstein et al. 2006).

CD81 is an associated tetraspanin that is 45% similar to CD9. The *Cd81*-null mouse also indicates deficiencies in sperm–egg interaction and female fertility with in vitro–fertilized and in vivo–fertilized eggs (Rubinstein et al. 2006). $Cd9^{-/-}/Cd81^{-/-}$ female mice are entirely infertile; therefore, the combination of these two gene disruptions results in severe infertility. In spite of information from antibody inhibition researches, the role of tetraspanin participation in human fertilization has been poorly recognized. However, there is no effect of two diverse anti-CD9 antibodies on the fusion of human ZP-free eggs with human sperm, while using antibodies to CD9 have inhibitory impacts on sperm fusion and binding with pig or mouse ZP-free oocytes. A number of tetraspanins, such as CD81 and CD9, have seemingly indirect roles in membrane fusion procedures but are still poorly described (Fanaei et al. 2011; Ziyyat et al. 2006).

CD151 is another membrane of tetraspanin and treating human eggs with an antibody against CD151 showed partial inhibition in human sperm–egg fusion. Reproductive deficiencies have not been demonstrated in *Cd151* knockout mice or in humans with mutated forms of *CD151*, but this result may be because of no wide assessment in reproductive function features. The preliminarily data increase the probability that sperm–oocyte adhesion in diverse mammalian species might depend on several members of the tetraspanin family (Sachs et al. 2006; Takeda et al. 2007; Ziyyat et al. 2006).

Several tetraspanins are probably involved in the regulation of membrane through associating with and/or assisting the function of other membrane proteins including different integrins and other interaction molecules, IgSF members, ectoenzymes, and several intracellular signaling molecules (Kovalenko et al. 2007; Le Naour et al. 2006). For example, IgSF8 coimmunoprecipitates with CD9 in oocyte lysates and is absent from the surfaces of *Cd9* knockout oocytes. An anti-IgSF8 antibody shows a slight inhibitory impact on sperm–oocyte adhesion, although *Igsf8*-null mice have not been investigated (Glazar and Evans 2009).

Glycosyl Phosphatidylinositol–Anchored Proteins

The eggs were treated with phosphatidylinositol-specific phospholipase C (PI-PLC), which splits GPI-anchored proteins, and the result showed affectedly decreased values of sperm–egg binding and fusion (Coonrod et al. 1999). Succeeding researches utilized genetic ways to produce oocytes missing GPI-anchored proteins via an oocyte-specific knockout of the phosphatidylinositol glycan anchor biosynthesis, class A (PIG-A); PIG-A is a subunit of an N-acetylglucosaminyl transferase that takes part in the initial stages of GPI synthesis. In mating trials, these PIG-A-null females showed severely decreased sperm fusion and produced no pups. Two-dimensional (2D) gel electrophoresis of proteins that were isolated from PI-PLC-treated eggs has so far revealed that one identified GPI-anchored protein, CD55, is reduced on *Piga*-defective oocytes (Alfieri et al. 2003; Tiede et al. 2000). Moreover, a GPI-anchored protein as known Juno is identified as a receptor for IZUMO1 on mouse eggs and Juno-null eggs do not fuse with normal sperm. Quick lack of Juno from the egg membrane after fertilization provides a possible mechanism for the membrane block to polyspermy in mammalian eggs (Bianchi et al. 2014). A summary of egg proteins involved in sperm–oocyte interaction is represented in Table 2.

In summary, the molecular mechanisms regarding sperm–egg membrane fusion and binding are still poorly understood. In order to categorize the relevant proteins and to support many of the unanswered queries concerning the essential and fascinating human sperm–egg interaction process, a substantial research is required. In addition, the focus of most of the presented studies was on utilizing animal models to further understand the related molecular mechanisms, but in order to understand human infertility and fertilization mechanisms, the emphasis must be on the human model. The study on human model in this

Table 2 Participating egg proteins in sperm–oocyte membrane interactions

Protein Family	Proteins	fertility status in result of defective proteins
Integrins	*ITGB1*	lethal for embryos or neonates, delay and decreased in sperm–egg binding (Tulsiani and Abou-Haila 2001, 2012)
	ITGB3	No infertility status (van der Heijden et al. 2008; van Gestel 2007)
	ITGB5	No infertility status (van der Heijden et al. 2008; van Gestel 2007)
	ITGA1	No infertility status (van der Heijden et al. 2008; van Gestel 2007)
	ITGA2	No infertility status (van der Heijden et al. 2008; van Gestel 2007)
	ITGA3	lethal for embryos or neonates; slight delay in sperm–*oocyte* binding (Tulsiani and Abou-Haila 2001, 2012)
	ITGA5	lethal for embryos or neonates (van der Heijden et al. 2008; van Gestel 2007)
	ITGA6	lethal for embryos or neonates; slight delay in sperm–*oocyte* binding (Tulsiani and Abou-Haila 2001, 2012)
	ITGA8	lethal for embryos or neonates (van der Heijden et al. 2008; van Gestel 2007)
	ITGA9	lethal for embryos or neonates, decreased in sperm–egg binding (Tulsiani and Abou-Haila 2001; van der Heijden et al. 2008; van Gestel 2007)
	ITGAV	lethal for embryos or neonates (van der Heijden et al. 2008; van Gestel 2007)
Tetraspanins	CD9	Severe infertility defects and decreased sperm–oocyte fusion (Kaji and Kudo 2004; Stein et al. 2004)
	CD81	Deficiency in sperm–egg interaction (Kaji and Kudo 2004)
	CD 151	Partly inhibition in human sperm–oocyte fusion (Takeda et al. 2007; Von Mering et al. 2002)
IGSF	IgSF8	Associated with CD9; slight inhibitory function on sperm–egg binding (Tokmakov et al. 2014)
GPI-anchored proteins	CD55	Severe defects in sperm–egg fusion (Tournaye et al. 2002)

conception might be a predominantly difficult job because of the ethical issues and also because of modicum of human oocytes or embryos. Semen parameter analysis is the most generally used test to diagnose male-factor infertility as a cause of infertility. Common IVF assay is now utilized for men with normal or nearly normal semen parameters (moderate male-factor infertility). It is surprising that complete fertilization failure is still a quite common occurrence at IVF with the overall exclusion of men with sperm dysfunction (Ola et al. 2001; Tournaye et al. 2002). This highlights the fact that common semen analysis, a diagnosis test to determine a main reason of failure of fertilization, cannot display sperm dysfunction; this condition is defined as 'hidden' male-factor infertility or unexplained male infertility. According to the background of such infertility, the new research context on the interactions between sperm and egg gametes in order to understand, in cellular and molecular terms, has been initiated (Conner et al. 2007).

To date, very little has been understood about the details of medical nature of sperm dysfunction in 'hidden' male-factor infertility condition. There is a massive amount of human data to display that sperm deficiencies, for instance the zona-induced AR or zona binding defect, are substantial reasons of total fertilization failure and poor fertilization in assisted conception (Barratt and Publicover 2001; Liu and Baker 2000). Presently, there is little knowledge about the molecular nature of these imperfections in spermatozoa, or whether these signify imperfections in individual proteins included in the mechanisms. There is evidence in animals that knockouts with individual protein can impact sperm–oocyte binding (Ensslin and Shur 2003), and thus it is likely that defects in the individual protein in humans may also result in failure of fertilization. Therefore, the aim of this research study is to identify the membrane proteins of human sperm and oocyte and investigate all the potential protein interactions between them.

Conclusion and Moving Forward

Recent findings of sperm–egg interaction and different aspects of various sperm–egg interaction abnormalities have been described in this review. This article also discussed how defective sperm–egg interaction can be a major cause of fertilization failure. Even though experimental approaches, for example immunoprecipitation, generated great quality outcomes and these approaches have produced large volumes of interaction data, they were extremely time- and cost-consuming and their outcomes of the high-throughput techniques contain a great number of false-negative and false-positive relationships. As discussed, the reliability of the experimental approaches utilized to identify PPIs can have extensively diverse quality as some techniques are linked with high error rates and because of the difficulties in studying membrane protein–protein interactions and the inadequacy of materials, many efforts have failed to achieve comprehensive data about human sperm–egg interaction. In addition, the focus of the most of the presented studies was on utilizing animal models to further understand the related molecular mechanisms; but in order to understand the human infertility and fertilization mechanisms, the emphasis must be on the human model. The study on human model in this conception might be a predominantly difficult job because of the modicum of human oocytes or embryos. In addition to experimental methods, computational methods can explain protein–protein interactions at various levels. In our new study, we will intend to focus on computational methods regarding investigating the protein–protein interaction in sperm–egg interaction. This review paper provides us with comprehensive current knowledge regarding sperm–egg interaction and will be helpful for future studies.

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Compliance with Ethical Standards

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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