



# SPERM-ZONA BINDING INTERACTION: AN IN SILICO BLUEPRINT TO IDENTIFY MARKER MOLECULES FOR EFFECTIVE FERTILIZATION

**Rishika Sharma, Monica Thakur, Linz-Buoy George  
and Hyacinth Highland**

Department of Zoology, Biomedical Technology and Human Genetics, School of Sciences,  
Gujarat University, Ahmedabad-380009

E-mail: Corresponding Author: hnhighland@gujaratuniversity.ac.in

## ABSTRACT

*Precise binding interaction between sperm surface-specific molecules and the ovum zona-pellucida is the key to a positive fertilization outcome. An in-silico approach was therefore adopted to understand the intricate molecular docking between areceptor molecule of the Zona-pellucida (ZP3) and effective sperm ligands selected viz., IZUMO1, ADAM2 and ERp57. The study aims at identifying the possible molecular biomarkers on the sperm plasma membrane which could efficiently initiate the acrosome reaction, recognize and bind to barrier membrane molecules and mediate fusion of sperm with ovum, thus culminating in successful fertilization. The in-silico identification of sperm-oocyte interaction biomarkers would serve a dual purpose in aiding to recognise potential targets for fertility regulation and infertility research.*

**Keywords:** ZP3 receptors, sperm ligands, fertilization, binding interaction, protein- protein docking.

## INTRODUCTION

The fusion of a sperm cell with the ovum is an exceptionally co-ordinated series of events (Rajender et al., 2011). For successful fertilization, the binding of spermatozoa with the zona pellucida of an ovum is achieved through the intervention of several molecules associated with both the gametes. Spermatozoa are endowed with numerous surface membrane proteins that function as ligands and bind to specific receptors on the ovum zona-pellucida, ensuring successful entry into the ovum (Ashrafzadeh, 2013). Any discrepancy in this binding could directly alter the cascade of events in fertilization and subsequently result in possible unexplained infertility. Avella et al. (2019) have presented an overview of the molecular interactions leading to fertilization; however definite markers in the process still remain obscure. In the present study therefore, an *in-silico* approach was adopted to understand the molecular interactions in the ligand-receptor binding mechanism of fertilization in human gametes, to identify potential markers for a positive fertilization.

The ovum zona pellucida barrier comprises of sulfated glycoproteins termed ZP1, ZP2 and ZP3 which are produced by oocyte granulosa cells. The main role of ZP3 has been demonstrated by Nixon et al. (2007) to be that of a receptor to sperm specific proteins. Various protein-protein and protein-glycoprotein interactions are involved in sperm-zona pellucida recognition and there are multiple proteins associated with binding to zona-pellucida glycoproteins (Zita et al., 2006; Chiu et al., 2010) of which three specific proteins were selected as ligands in this investigation viz. IZUMO1, ADAM2 and ERp57. Literature survey indicates the plausible role of these three proteins in bringing about a positive fertilization outcome. IZUMO1 is an essential sperm cell- surface protein required for fertilization as it acts as a ligand for the IZUMO1R/JUNO receptor on the ovum. The IZUMO1:IZUMO1R/JUNO interaction is a necessary adhesion event between sperm and ovum that is an essential prerequisite for fertilization. In humans, the



ligand ADAM2 is a 100-kDa protein and a member of the ADAM (A Disintegrin And Metalloprotease) family proteins which are membrane-anchored multi-domain proteins that play defining roles in male reproduction. The protein ERp57 is a stress-response protein and a component of the protein disulfide isomerase family, crucial for the signal transduction process involved in recognition and binding of the gametes.

Therefore, an *in-silico* study was undertaken to elucidate the precise protein-protein interaction characteristic of normal fertilization and elucidate the binding interaction at the molecular level. The findings of this study would aid in understanding failure of *in-vivo* and *in-vitro* fertilization in the investigation of infertility, as well as provide a platform for developing inhibitors to halt the fertilization process in contraceptive research.

## MATERIALS AND METHODS

*In-silico* interaction analysis was carried out to determine binding interactions between zona-pellucida binding protein receptor ZP3 with the sperm plasma membrane protein ligands IZUMO1, ADAM 2 and ERp57. Protein-protein docking of the receptor ZP3 and the above selected protein ligands was achieved using ClusPro server. The structure of receptor and ligand was derived from UniProt and based on the structure the binding interaction sites were identified.

Protein- Protein docking was done using ClusPro online server and the binding energy values were analyzed for each docked conformation. Binding energies of the protein-protein interactions are important to describe how fit the protein bind to the target macromolecule. Low binding energy indicates the effectiveness and strength of protein-protein binding interaction. A strong binding interaction can be considered as the amount of energy exhibits negative value. Therefore, conformations having low energy and exhibit favourable hydrogen bonding with the amino acid side chains were considered.

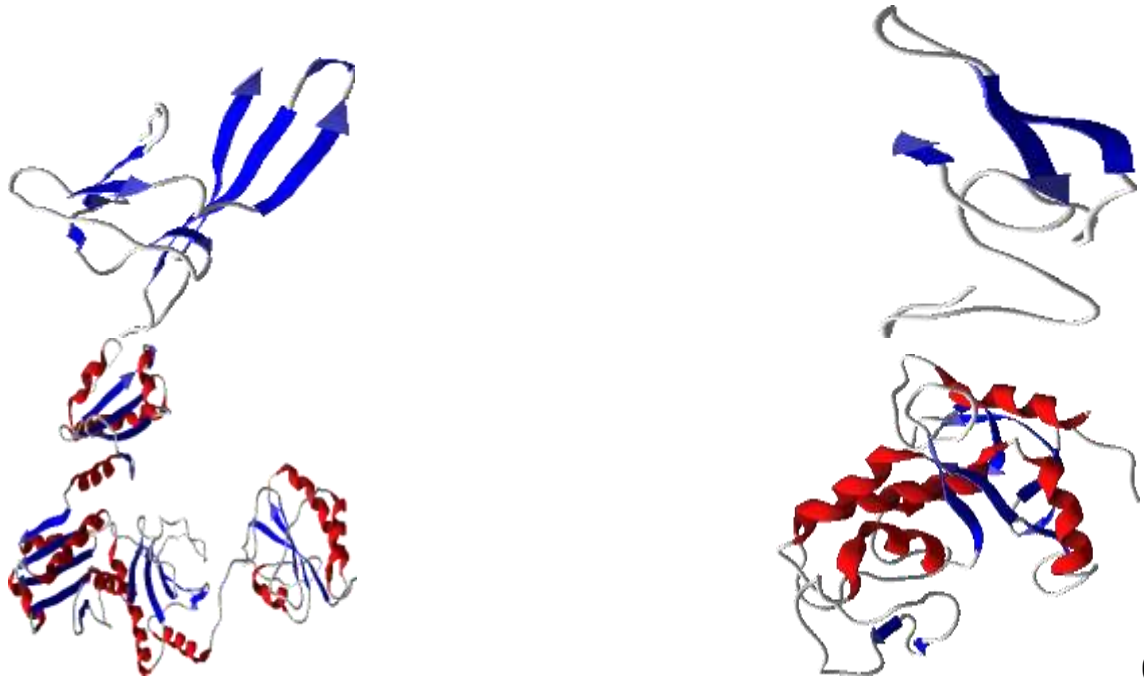
## RESULTS

The computational analysis effectively evaluated the potency of the selected sperm surface-protein ligand markers based upon the interaction with the specific recognition and binding sites with minimal binding energy (Table-1). As shown in Figure 1, the structure of receptor and ligand was derived from UniProt and based on the structure the binding interaction sites were identified as shown in Figure 2. Observations of the study revealed that the interaction energy estimate of the ligands within the active site of protein indicated that the sperm specific ligands IZUMO1, ADAM2 and ERp57 were selective towards the target ovum receptor protein ZP3 on the basis of their docking score shown in Table (1). The binding energy score of the three selected ligands were comparable and similar towards the selected receptor, with the lowest value (higher binding energy) manifest by the ERp57 ligand and a comparatively less binding energy score by the ADAM 2 protein. It was observed that the amino acid proline143 was present consistently in the interaction sequences of all three ligands. Ligands IZUMO1 and ADAM2 showed remarkable sequence identity with many common acids at the interaction site with ZP3 receptor. ERp57 depicts the highest binding score with the receptor ZP3 through only two amino-acids (Thr-106 and Pro-143) interaction. Table 1: Binding energy interaction profile of ZP3 with the selected ligands.

Ligands	Docking Score (Kcal/mol)	Interaction Sequence
<b>IZUMO 1</b>	-951.5	Val 42, Leu 43, Val 44, Gly 45, Cys 46, Gln 47, Met 52, Met 54, Phe 60, Ala 134, Ile 136, Pro 137, Ile 138, Gln 139, Cys 140, Arg 141, Pro 143
<b>ADAM 2</b>	-822.2	Val 42, Leu 43, Val 44, Gly 45, Cys 46, Gln 47, Met 52, Met 54, Phe 60, Thr 106, Ala 134, Ile 136, Pro 137, Ile 138, Gln 139, Cys 140, Arg 141, Pro 143

ERp57	-961.3	Thr 106, Pro 143
-------	--------	------------------

**Fig: 1** Structure of target protein and ligands retrieved from UniProt  
(1) ZP3 (UniProt ID-P21754), (2) IZUMO 1 (A6ND01)  
(3) ADAM 2 Structure (Q99965) (4) ERp57Structure (P30101)



**Fig: 2** Protein-Protein Docking and binding poses of ZP3 and ligands



(a) ZP3+IZUMO 1 (b) ZP3+ ADAM 2 (c) ZP3+ERp57

## DISCUSSION

There are several complex pathways involving the interaction of specific molecules between the sperm and the ovum for the successful culmination of fertilization (Elder and Dale, 2020). Despite the intensity of research in this field there is little consensus in identifying the specific interaction that confirms a positive interaction at fertilization. The main purpose of the *in-silico* evaluation of the binding interaction of the specific sperm surface ligands to the ZP3 receptor on the ovum zona pellucida was to identify and confirm their role in the sperm-ovum interaction at fertilization since there is much ambiguity in this context based on the molecular analysis alone.

IZUMO1 is an integral sperm membrane protein which is located in the inner acrosomal membrane and equatorial region of sperm (Nixon et al., 2007). It is a specific member of the male germ line that belongs to immunoglobulin super-family. CD9 and CD81 are the cell adhesion molecules to which IZUMO1 can interact and bring about effective binding of sperm with the zona pellucida. CD9 and CD81 are members of the hydrophobic membrane protein family called tetraspanins which have four transmembranes and two extra cellular loops.



These proteins are in the high cholesterol domain of the oolemma and make a network with kinase and integrins in the lipid rafts to control sperm-oolemma interaction and penetration. (Sutovsky et al., 2009). ADAM family members (A Disintegrin And Metalloprotease epidermal growth factor) have cysteine rich domains and are integral sperm membrane proteins. They belong to the integrin family which helps in cell adhesion by favouring cell-cell interaction at the molecular level (Evans, 2002). These proteins function in sperm-oocyte penetration and also in migration of sperm in the oviduct (Marcello et al., 2010). ADAM2 on the human sperm membrane binds to the integrin receptor of oolemma (integrin  $\alpha 6\beta 1$ ) thereby enhancing a positive binding interaction between sperm and oocyte (Nixon et al., 2007). ERp57 is a protein present in the apical region of non-acrosome reacted sperm, in tail region and after the acrosome reaction it is re-located to the equatorial segment of human spermatozoa where it contribute to sperm-oocyte penetration (Zhang et al., 2007). It is a 57kDa protein which has been reported to be missing in 80% of infertile patients and is dramatically down-regulated in another 20% of male infertility cases (Rajeev et al., 2004).

The present study revealed that the interaction energy estimate of the ligands within the active site of protein reflected the binding energy of the target proteins and indicated that the sperm specific ligands IZUMO1, ADAM2 and ERp57 were selective towards the target ovum receptor protein ZP3. The binding energy score of the three selected ligands were comparable indicating strong binding affinity towards the selected receptor. ERp57 showing the lowest value would consequently manifest higher binding energy to its target receptor. The amino acid sequence detected at the interaction locus revealed that ligands IZUMO1 and ADAM2 showed remarkable sequence identity, having many common acids at the interaction site with the ZP3 receptor. In addition to having the highest binding score, ERp57 was found to interact with the receptor ZP3 mainly through two key amino-acids (Thr-106 and Pro-143).

The *in-silico* analysis carried out in the present study further indicated that the ligands IZUMO1 and ADAM2 both being integral membrane proteins, show remarkable amino acid sequence similarity at the interaction site which possibly favours the interface with the receptor ZP3. This suggests their specific molecular interaction related to their role in sperm-ovum adhesion and membrane fusion in humans (Sutovsky et al., 2009). Inoue et al. (2005) have described a putative glycosylation site on the extracellular immunoglobulin domain of IZUMO 1 and a disulfide bridge that is thought to be formed by the two cysteine residues. Therefore in correlation with the amino acids detected, the presence of a disulfide bond raises the possibility that it is cleaved during sperm-ovum fusion, initiating a series of conformational changes that promotes this process.

Tetraspanins as mentioned earlier have an important role in membrane organisation (Hemler, 2003) and also helps in mediating protein interactions at primary, secondary and tertiary levels (Hemler, 2005). Therefore, tetraspanins IZUMO1R can well establish their interactions with integrins (ADAM proteins) as well as with membrane anchored growth factors (ERp57) (Hemler, 2005). Ellgard and Frickel (2003) had earlier speculated that the presence of ERp57 is important for sperm-zona recognition and oocyte membrane interaction, an observation that is substantiated by the results obtained in this study. It regulates the glycoprotein folding and also acts as a chaperone for calcium regulation by co-binding with lectin chaperones calnexin and calreticulin (Vangheluwe et al., 2005). Similarly Wiwanitkit et al. (2010) have highlighted the role of ERp57 in post-translational modification in bringing about phosphorylation of amino acid moieties at the target site. Our studies also indicate that ERp57 manifests efficient binding interaction specifically employing two amino acids on the surface of the target receptor. Since ERp57 is a component of surface acrosomal region of the sperm, it brings about sperm activation through its oxido-reductase activity. This is the trigger which possibly further mediates the action of IZUMO1 and ADAM2, both being integral sperm membrane proteins, vital in achieving the positive fusion of sperm with the zona pellucida barrier. Thus, from the binding energy score obtained in this study it could be stated that the protein-protein interaction establishes an efficient cell-cell adhesion between the sperm and the ovum. The significance of these sperm specific ligands in achieving successful binding and fusion of the gametes could therefore validate their role as effective markers of positive fertilization.

## CONCLUSION

It can be concluded that the binding sites and the molecular interactions for successful fertilization could be evaluated and verified through *in-silico* analysis. Observations of the



study revealed that the interaction energy estimate of the ligands within the active site of protein indicated that the sperm specific ligands IZUMO1, ADAM2 and ERp57 were selective towards the target ovum receptor protein ZP3. The binding energy score of the three selected ligands were comparable indicating strong binding affinity towards the selected receptor, with the lowest value (higher binding energy) manifest by the ERp57 ligand. The amino acid sequence revealed that ligands IZUMO1 and ADAM2 showed remarkable sequence identity with many common acids at the interaction site with ZP3 receptor. ERp57 depicted the highest binding score with the receptor ZP3 through only two amino-acids (Thr-106 and Pro-143) interaction.

The data from the present study indicates that the selected ligands show intrinsic molecular interaction with the zona pellucida receptor ZP3 and could be considered as potent biomarkers for male fertility and are thus pivotal in ensuring positive fertilization. This *in-silico* based model will therefore help to map the blueprint on which *in-vitro* tests may be based to identify whether the specific interaction motif is structurally viable on spermatozoa of individuals having repetitive fertilization failure. This investigation therefore lays out the scaffold upon which further studies may be designed for *in-vitro* or *in-vivo* studies in fertility regulation.

## REFERENCES

- 1) Ashrafzadeh, A., Karsani, S.A., and Nathan, S. (2013). Mammalian sperm fertility related proteins. *Int J Med Sci*, 10(12), 1649-1657.
- 2) Avella, M. A., Milne, K. A., Dawood, S., Dawood, A. and Tucker, M. J. (2019). Assisted Hatching of Human Embryos for Successful Implantation. In *In Vitro Fertilization* (pp. 567- 579). Springer, Cham.
- 3) Chiu, P.C., Wong, B.S., Lee, C.L., Lam, K.K., W, Chung, M.K. and Lee, K.F. (2010). Zona pellucida-induced acrosome reaction in human spermatozoa is potentiated by glycodelin-A via down-regulation of extracellular signal-regulated kinases and up-regulation of zona pellucida-induced calcium influx. *Hum Reprod*, 25, 2721-33.
- 4) Comeau, S. R., Gatchell, D. W., Vajda, S., and Camacho, C. J. (2004). ClusPro: an automated docking and discrimination method for the prediction of protein complexes. *Bioinformatics*, 20(1) 45-50.
- 5) Comeau, S.R., Gatchell, D.W., Vajda, S. and Camacho, C.J., 2004. ClusPro: a fully automated algorithm for protein-protein docking. *Nucleic acids research*, 32(suppl\_2), pp.W96-W99.
- 6) Elder, K. and Dale, B. 2020. *In-vitro fertilization*. Cambridge University Press.
- 7) Ellgaard, L. and Frickel, E.M. (2003). Calnexin, calreticulin, and ERp57: teammates in glycoprotein folding. *Cell Biochem Biophys*, 39, 223-47
- 8) Evans, J. P. (2002). The molecular basis of sperm-oocyte membrane interactions during mammalian fertilization. *Hum. Reprod*, 8, 297-311.
- 9) Hemler, M. E. (2003). Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. *Annu. Rev. Cell Dev. Biol*, 19, 397- 422
- 10) Hemler, M. E. (2005). Tetraspanin functions and associated microdomains. *Nat. Rev. Mol. Cell Biol*, 6, 801-811.
- 11) Inoue, N., Ikawa, M., Isotani, A. and Okabe, M., (2005). The immunoglobulin superfamily protein Izumo is required for sperm to fuse with eggs. *Nature*, 434, 234-238.
- 12) Kozakov, D., Beglov, D., Bohnuud, T., Mottarella S, Xia B, Hall DR, Vajda, S. How good is automated protein docking? *Proteins: Structure, Function, and Bioinformatics*, 2013 Aug
- 13) Kozakov, D., Brenke, R., Comeau, S. R. and Vajda, S. PIPER: An FFT-based protein docking program with pairwise potentials. *Proteins*, 2006 Aug 24
- 14) Marcello, M.R. and Evans, J.P. (2010). Multivariate analysis of male reproductive function in *Inpp5b*<sup>-/-</sup> mice reveals heterogeneity in defects in fertility, sperm-egg membrane interaction and proteolytic cleavage of sperm ADAMs. *Mol Hum Reprod*, 16, 492-505.
- 15) Nixon, B., Aitken, R. and McLaughlin, E. (2007). New insights into the molecular mechanisms of sperm-egg interaction. *Cell Mol Life Sci*, 64, 1805-23.
- 16) Rajeev, S. and Reddy, K. (2004). Sperm membrane protein profiles of fertile and infertile men: identification and characterization of fertility-associated sperm antigen. *Hum Reprod*, 19, 234- 42.
- 17) Rajender, S., Avery, K. and Agarwal, A. (2011). Epigenetics, spermatogenesis and male



- infertility. *Mutat Res*, 727(3), 62-71.
- 18) Sutovsky, P. (2009). Sperm-egg adhesion and fusion in mammals. *Expert Rev Mol Med*, 11, 1- 12.
  - 19) Vangheluwe P., Raeymaekers, L., Dode, L. and Wuytack, F. (2005). Modulating sarco (endo) plasmic reticulum Ca<sup>2+</sup> ATPase 2 (SERCA2) activity: cell biological implications. *Cell Calcium*, 38,291–302.
  - 20) Wiwanitkit, V. (2010). Potential protein post-translational modification in ERp57: A phenotype marker for male fertility. *Journal of Human Reproductive Sciences*, 3(3), 146–147.
  - 21) Zhang, J., Wu, J., Huo, R., Mao, Y., Lu, Y. and Guo, X. (2007). ERp57is a potential biomarker for human fertilization capability. *Mol Hum Reprod*, 13, 633-9
  - 22) Zitta, K., Wertheimer, E.V. and Miranda, P.V. (2006). Sperm N-acetylglucosaminidase is involved in primary binding to the zona pellucida. *Mol Hum Reprod*, 12,