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Unraveling the Molecular Events of Human Fertilization: A Systematic Review of Human Gamete Studies

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ABSTRACT

The interaction between molecules of gametes is intricate, as fertilization is a highly regulated process in humans. With recent breakthroughs in molecular biology, it has become possible to investigate these events in unprecedented detail, though some mechanistic steps remain unclear. This systematic review followed PRISMA rules. Peer-reviewed literature was searched through major scientific databases between 2015 and 2025, limited to human studies. The initial search yielded 89 records. After removing duplicates and applying inclusion/exclusion criteria, 53 full-text articles were assessed, with 35 deemed eligible. Molecular mechanisms, experimental methods, and translational implications were highlighted in data extraction and synthesis. The included studies shed light on sperm–oocyte recognition, interaction with the zona pellucida, signaling pathways in the acrosome reaction, and cortical reaction dynamics after fertilization. New methods, including CRISPR/Cas9 genome editing, transcriptomic screening, and human embryoid models, have enhanced the understanding of gamete processes. However, unanswered questions remain on the role of sperm protein SPACA6, heterogeneity of species in zona pellucida binding, and ethical restrictions in human fertilization studies. Implementation of advanced molecular techniques has significantly increased understanding of human fertilization, but essential information is still lacking. Addressing these gaps will require ethically sound advances to translate fundamental research into clinical applications.

Keywords: Human fertilization, PRISMA, CRISPR/Cas9, transcriptomics, zona pellucida, SPACA6, embryoid models, cortical reaction**.**

INTRODUCTION

Fertilization is the bedrock of human reproduction, which means the union of male and female gametes to form a diploid zygote. This process is influenced by a cascade of complex molecular interactions and strictly controlled biological processes, which ensure reproductive success[1]. The molecular events surrounding human fertilization are an emerging field of study that taps into the advances in cell biology, reproductive physiology, genomics, and assisted reproductive technologies (ART), thereby offering an understanding of both natural conception and assisted reproduction.

Among the important events are sperm capacitation, the acrosome reaction, attaching to the Zona Pellucida (ZP) glycoproteins on the oocytes, fusion of sperm and egg membranes, and activation of the egg[2]. However, for more clarification, this review will slightly delve into the formation of human gametes, that is, gametogenesis. Previous research has established that CD9 tetraspanin from the oocyte, Izumo1 from the sperm, and the oocyte's Juno receptor are required for fertilization. Studies have found that there are CD9 proteins in oocytes and IZUMO1 proteins in sperm in many mammals, and these interactions are largely similar among species[3]. The discovery of phospholipase C zeta 1 (PLCZ1 or PLCzeta) as the sperm oocyte-activating factor (SOAF) in recent studies has begun to clarify these events[4], but findings are scattered across diverse studies.

One or many disruptions of these molecular events may lead to infertility or unsuccessful fertilization in the case of ART processes. Enhanced knowledge of the molecular process of fertilization not only helps improve the diagnosis and treatment of infertile couples but also creates new opportunities in the field of reproductive medicine: non-hormonal contraceptives, genetic counseling, and genome editing[5].

Thus, we conducted a systematic review to consolidate the molecular mechanisms of human fertilization recorded in the high-quality literature over the period of 2015 to 2025, which is supported by the use of human gametes or cells. According to PRISMA recommendations, we used inclusion/exclusion criteria to evaluate bias assessment in studies, and synthesis of the existing insights into capacitation of spermatazoa, acrosome reaction, binding of spermatozoa to the zona pellucida, fusion of gametes, intracellular signaling, and activation of oocytes.

MATERIALS AND METHODS

Articles published 2015-2025 were selected in PubMed, Google Scholar, Research Gate, and Web of Science, using search terms such as human sperm capacitation, acrosome reaction, binding to the zona pellucida, IZUMO JUNO human, PLC zeta fertilization, and related keywords. We restricted ourselves to human original peer-reviewed studies with human oocytes or spermatozoa, or cell lines made outside the human body. Inclusion criteria: (1) Publications in English within 2015-2025; (2) experimental work on human gametes, reproductive tissues, or any human cell lines; (3) combining molecular or biochemical mechanisms during fertilization stages. Exclusion criteria: (1) Non-human studies; (2) reviews, case reports that do not have mechanistic data, or articles that do not involve the molecular focus; (3) studies of fertilization treatments such as IVF protocols that do not include mechanistic endpoints. The titles/abstracts were screened by two reviewers, and subsequently the full text was reviewed, and differences were resolved by discussion. Extracted data were types of study design, samples, methodology, and significant molecular outputs. Qualitative determination of risk of bias was done by assessing the size of the sample and controls, along with validating the assay used. Studies were mapped in the case of key terms and processes.

Study selection and quality

The literature search (2015–2025) identified 89 records. After the removal of 36 duplicates, 53 unique records remained for screening. All 53 records were screened at the title/abstract level and progressed to full-text assessment. Following application of the predefined inclusion and exclusion criteria, 35 studies were included in the final review. Included studies comprised in vitro human sperm/egg experiments, genetic analyses, and clinical case series. Most studies were small exploratory investigations (N<50 donors or samples) due to ethical and logistical constraints; few were randomized trials. Common limitations included variability in sample preparation and lack of in vivo confirmation. Overall bias risk was moderate, reflecting consistent replication of key findings across independent groups.

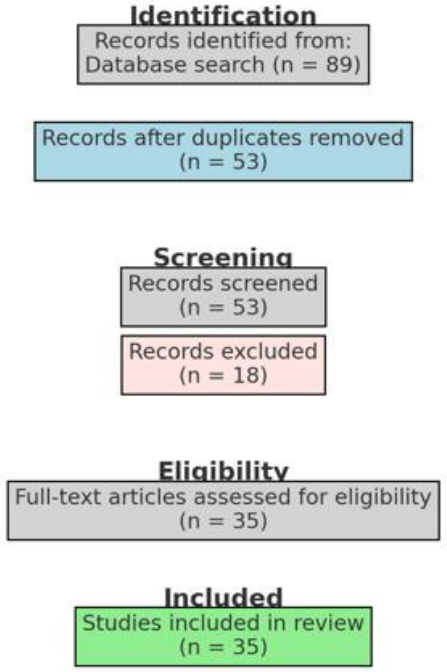

Figure 1: PRISMA flow diagram of study selection. The diagram illustrates the flow of records through identification, screening, eligibility, and inclusion, showing the number of records identified, excluded, and included. After duplicate removal, records were screened by title/abstract, and full texts were assessed against inclusion/exclusion criteria.

Sperm capacitation

With capacitation, human sperm are prepared to move at increased speed and release the contents of the acrosome. Capacitation means that there is a change in the plasma membrane, and signal transmission occurs in the sperm. In particular, albumin working in vivo boosts the ability of cholesterol to leave cell membranes, thereby increasing their fluidity[6,7] and signaling platforms. Simultaneously, bicarbonate (HCO^{3-}) and Ca^{2+} influx into the sperm cell activate soluble adenylyl cyclase, which increases cAMP and subsets protein kinase A (PKA). Buffone *et al*. demonstrated that human sperm capacitation depends on cAMP-and bicarbonate-based signaling cascade: cAMP-activation of PKA results in a high rate of protein tyrosine phosphorylation, a characteristic of capacitation[8,9]. Capacitation requires this conversational pathway, cAMP/PKA. The other targets that cAMP can bind to besides PKA and exert its influence on are the exchange protein (EPAC), which is directly activated by cAMP. Sperm cells of several species, including humans, express EPAC1 and EPAC2[10]. Thus, sperm capacitation is driven by intracellular alkalinization and cAMP-mediated signaling, resulting in protein phosphorylation and membrane changes that enable downstream events.

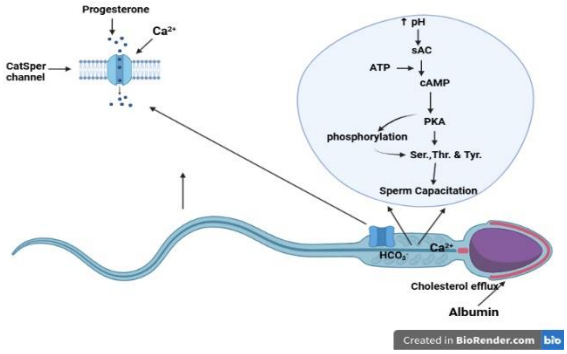

Figure 2: Schematic diagram showing the molecular mechanism of sperm capacitation, indicating how Albumin acts on the outer membrane of sperm cells by removing the cholesterol, CatSper channel opening regulation, triggering influx of Ca^{2+} & HCO_3^- into the sperm cell, which in turn increases the

pH, that unlocks the signaling cascade that leads to sperm capacitation. (Created using BioRender App).

Acrosome reaction and zona pellucida binding

While going through capacitation, sperm become receptive to the signals sent by an ovum. The acrosome reaction (AR) is the release of Ca^{2+}-dependent enzymes that let sperm pass through the zona pellucida. The fluid produced by the cumulus-oocyte complex in humans has progesterone and other substances that start AR. After progesterone binds ABHD2 on the sperm's surface, it releases inhibition of the CatSper Ca^{2+} channel[11,12]. Activation of ABHD2 by progesterone leads to the degradation of the endocannabinoid 2-arachidonoylglycerol (2AG) that opens CatSper and creates a rapid influx of Ca^{2+}. This increase of Ca^{2+} drives hyperactivation of flagella and the priming of the acrosome reaction. Moreover, it has been observed that the progesterone sensitivity of CatSper in human sperm is special since it is not the case in mouse CatSper. Prostaglandin E1 (PGE1) enhances human CatSper activity similarly to progesterone, but it appears to do so via a distinct binding site[13].

The glycoproteins of the egg have to be bound by the sperm to permit a successful fertilization process. There are four ZP proteins in the human zona pellucida (ZP1 to ZP4). By using human ZP protein in investigations and transgenic eggs of mice, it is observed that only special receptor molecules can bind human sperm. There is evidence that human ZP2 is involved in early sperm attachment: mouse eggs that only express human ZP2 (not ZP1, ZP3, or ZP4) can bind to the capacitated state of human sperm, which may suggest that it is the N-terminal region of human ZP2 that performs this recognition. However, ZP1, ZP3, and ZP4 also bind acrosome-intact human sperm and have been shown to be able to induce AR. Purified human ZP1, ZP3, and ZP4 (not ZP2) induce AR in capacitated sperm, and these indicate a role of many ZP glycoproteins acting together in sperm communication[14]. These findings underscore that human sperm-egg recognition involves both protein-protein and glycan interactions: sperm surface molecules interact with the ZP matrix and transduce Ca^{2+} signaling to initiate AR.

Sperm-Egg membrane fusion

After getting through the zona pellucida, sperm move toward the oolemma for them to fuse. Humans use sperm IZUMO1, oocyte JUNO (IZUMO1 receptor), and oocyte CD9 for the process of membrane fusion. They proved that JUNO is present on the outside layer of human oocytes, and blocking JUNO using antibodies completely stops sperm and egg fusion. Similarly, CD9 on the oocyte is needed for fusion, as first found in mice, and has proven to be present in human eggs as well. The main part of the fusion/adhesion complex is made up of IZUMO1 from the sperm binding to GPI-anchored JUNO on the egg. It seems that the work of CD9 helps shape membrane microdomains, making it easier for fusion to happen. If these (for example, IZUMO1 deficiency or missing CD9) are interfered with in human cells, gamete membrane fusion is also prevented, just like in mammals. No other sperm-egg binding proteins found so far are definitely essential for pregnancy in humans; however, SPACA6 is being closely looked at[3].

Intracellular signaling and oocyte activation

After sperm fuses with the oocyte, there is a series of small chemical changes that complete egg activation inside the cell. Plasma membrane of the egg contains phospholipase C zeta (PLCζ), which transforms the sperm fusion into IP_3 by hydrolyzing the egg's phosphatidylinositol 4,5-bisphosphate and inducing Ca^{2+} fluctuations[15].

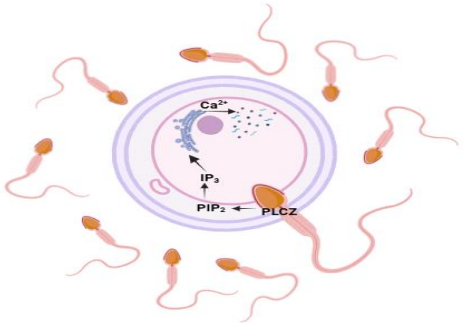

Figure. 2: Intracellular signaling cascade: After sperm-oocyte fusion, PLCζ in the egg's membrane triggers calcium oscillations by hydrolyzing PIP_2 into IP_3, activating the oocyte by releasing cortical granules to prevent polyspermy, and resuming meiosis. (Created using BioRenderApp)

For Oocyte activation, these Ca^{2+} rises are necessary everywhere: they cause cortical granules to be released (modifying the ZP to stop multiple sperm from entering), and help the oocyte come out of metaphase II and form pronuclei. Studies describe PLCζ as the critical activator between human sperm and eggs; they also point out that men with PLCZ1 gene mutations experience abnormal PLCζ placement in sperm, leading to other fertilization problems[15;16]. This finding confirms that defective PLCζ signaling underlies some cases of human fertilization failure. Furthermore, studies also note that absence or abnormal expression of PLCζ causes oocyte activation deficiency[17]. Thus, sperm PLCζ-induced Ca^{2+} changes are critical for normal human fertilization[17,18]. High-frequency and strong Ca^{2+} waves start the activation cascade, whereas weaker or fewer waves cause it to be incomplete. Besides, the release of calcium leads to the release of granules by the egg and changes in the egg membrane, which prevent further sperm from entering[19]. In Summary, the last important step, intracellular signaling in the oocyte with PLCζ/IP_3/Ca^{2+} is needed, and changes at this level, which is PLCZ1, cause infertility in humans.

Recent advances in human gamete studies

New, state-of-the-art tools make it possible to explore human gametes and embryos in high resolution down to a level never before achieved. As an example, specific genome editing (CRISPR/Cas9), whole transcriptomic interrogation, and embryo models produced in stem cells (blastoids or gastruloids) are providing new windows on gamete recognition, gamete fusion, and early zygotic differentiation. These methods supplement traditional in vitro fertilization (IVF) experiments and assist in bypassing some drawbacks of research on humans only[20,21].

Below, we discuss each advance and highlight remaining questions, including the roles of SPACA6 and species-specific zona pellucida (ZP) interactions.

CRISPR/Cas9 and genetic approaches in gamete research

Functional studies in reproduction have also been transformed by the CRISPR/Cas9 Technique of genome editing. Key fertilization genes have been validated by CRISPR-induced knockouts in animal models. As an example, mouse zygotes that had been edited to lack an activity-competent domain of the Astl gene verified that ovastacin indeed cleaved ZP proteins and established protection against polyspermy[22]. Like in vitro germline editing approaches, similar editing approaches in human gametes or embryoid models could be possible, in theory, but are technically and practically at a more exploratory and ethically limited stage in humans. First-generation editing of CRISPR entire human zygotes, such as nonviable tripronuclear embryos, was lacking in efficiency and mosaicism, confirming challenges related to technical difficulties[23,24]. Importantly, every study emphasizes that CRISPR applications must overcome off-target and mosaic editing effects, and that stringent ethical guidelines such as the 14-day rule, bans on heritable editing, etc., constrain human embryo experiments[25;26]. Nevertheless, gene-

editing tools continue to evolve; even catalytically dead Cas9 (dCas9) fusions allow transcriptional modulation of gamete genes. Together, these genetic tools promise to test gene function in fertilization, for example, by knocking out candidate sperm or egg surface proteins, provided safety and ethical issues are resolved.

Transcriptomic profiling of human gametes and embryos

The high-throughput "omics" technology has contributed to the abundant atlas of gene expression in human germ cells and early embryos. Single-cell RNA sequencing (scRNA-seq) has been used to profile human oocytes and preimplantation embryos, which identified developmental gene dynamics and candidate molecules. In one example, Zhang et al. also used scRNA-seq to achieve high-resolution transcriptome analysis of human oocytes of different health status with normal or abnormal (agar-like) zona pellucida (ZP). They found more than 1,300 differentially expressed genes, with many extracellular matrix (ECM)-related genes being repressed significantly and DNA damage-response genes up-regulated significantly in oocytes with defective ZP[27]. Gene ontology and pathway analysis confirmed that ECM-receptor interaction and focal-adhesion pathways were among the most affected, implicating ZP structural components in abnormal eggs. Hub genes such as TLR4, CCNA2, & ITGB3, relevance in the network with ZP and cell-cycle were pointed out[27]. Such results provide evidence of transcriptomic ability to identify molecular signatures of gamete quality. Others have also profiled cumulus oocyte complexes, sperm RNAs, and early human embryos at the single-cell level, tabulating the waves of transcription and epigenetic reprogramming following fertilization. These datasets are used both to inform on typical fertilization programs and infertility syndromes like ZP gene mutations. In summary, transcriptome profiling of human gametes provides a global view of the molecular players at each stage and identifies novel candidates for functional study.

Human embryoid (blastoid) models as research tools

Since natural human embryos are in limited supply and are ethically questionable, researchers have come up with embryos of stem cells. The production of three-dimensional aggregates of human pluripotent cells, self-organizing into blastocyst-like structures, known as blastoids, is one of the breakthroughs in the development of the relevant tissue. Such blastoids efficiently repeat the structure of a ~day-6 human blastocyst (with both inner cell mass and trophectoderm spheres) and could even implant in vitro[20]. This has led to human blastoids being relatively easy to study in terms of peri-implantation development, lineage decisions, and probably even the early fertilization cues. They are amenable to greater experimental manipulation because they are generated in vitro: for example, stem cells can be labeled or edited before blastoid formation to test their functions. Simpler 2D embryo-like models can also be used to study some aspects of post-fertilization development in addition to blastoids[28]. By circumventing issues of embryo scarcity and ethical restrictions, embryoid models serve as an indispensable tool for investigating early human developmental events[20;29;30], in the supply of human embryos that would allow multiple experiments and live imaging. They do not exactly recapitulate fertilization, but they could now allow indirect experiments, i.e., determination of the effects of various sperm proteins on the development of a fertilized-like structure, which would be impractical otherwise.

Gamete recognition: Zona pellucida binding and species specificity

As explained earlier, one of the first molecular events in fertilization is sperm binding to the zona pellucida (ZP) of the oocyte. Human ZP differs from many lab animals: it contains four glycoproteins (ZP1–ZP4) compared to three in

mice[14,31]. ZP proteins form long filaments (heterodimers of ZP2–ZP3 or ZP2–ZP4 bridged by ZP1)[32]. Species-specific differences in ZP composition and sperm surface lectins underlie strict cross-species barriers. For example, human sperm bind best to human ZP3/ZP4 glycan patterns and largely ignore rodent ZP. The precise sperm receptor(s) in humans remain debated: some models emphasize carbohydrate ligands on ZP3 (as in mouse), while others highlight peptide motifs on ZP2/ZP4. These "competing models" of ZP recognition reflect unresolved questions. Recent studies on infertile patients with mutated ZP proteins (e.g., ZP2 or ZP1 variants) confirm that even small changes in ZP structure can abrogate human fertilization. Transcriptomic analyses of oocytes with abnormal ZP (as above) also suggest that ECM-cytoskeleton genes affecting ZP integrity are crucial[32]. In short, species-specific nuances of sperm–ZP binding remain an active area of investigation; new molecular data are needed to reconcile disparate models of how the human ZP engages sperm.

Membrane fusion factors and unresolved candidates

Other than IZUMO1/JUNO, other sperm proteins have recently been discovered as fusion factors. It is worthwhile to mention that SPACA6 (sperm acrosome-associated 6) has been deemed a pivotal fusion protein. Recent structural biology showed that human SPACA6 contains a bipartite fold (four-helix bundle and an Ig-like domain) quite homologous to IZUMO1, suggesting that it belongs to an IST superfamily of fusion proteins[33,34]. Functional studies in mice and humans indicate SPACA6 is indeed required: SPACA6 is normally localized on sperm and relocalizes to the equatorial segment after the acrosome reaction, and antibodies against SPACA6 block human IVF in vitro[35,36]. Nonetheless, in contrast to IZUMO1/JUNO (again, whose binding and knockout phenotype is well delineated), the molecular partners and precise role of SPACA6 in human fusion are unknown. Other gamete proteins searching for similar answers include TMEM95, FIMP, SOF1, and DCST1/2: all are needed to fuse sperm and egg in animals but do not have specific equivalents in eggs. These unresolved factors represent major open questions. For example, does SPACA6 bind a specific egg ligand or act by clustering membranes? Clarifying these unknowns will likely require creative use of human gametes or embryo models and sensitive biophysical assays[37].

Ethically, as Baumann notes, "research on human embryos is limited by their low availability and by ethical concerns". Even powerful tools like CRISPR/Cas9 face additional barriers: current guidelines prohibit clinical germline editing, and any laboratory editing of human zygotes can only be done under strict oversight and typically with non-viable embryos[38]. In this way, IVF-derived cells or synthetic models will have to provide much of our insight. However, using new technologies and tactful ethical paradigms, scientists can gradually provide the molecular choreography of human fertilization.

DISCUSSION

This systematic review gathers current research results on how fertilization happens in people. Much of how capacitation, AR, ZP binding, membrane fusion, and egg activation are related is now known at the molecular level. For cellular capacitation, this review affirms that the key roles belong to cAMP/PKA and cholesterol, leaving the membrane[39]. The correct role of steroid hormones in the function of sperm CatSper is demonstrated, and progesterone from cumulus cells directly operates through ABHD2 to quickly increase calcium ions and trigger final movement of human sperm, or hyperactivation, which includes the undisputable requirement for IZUMO1–JUNO and CD9 in the process of human gamete fusion[3,11]. The last detail to mention is that the sperm PLCζ-Ca^{2+} reaction is known to always trigger egg activation[15]; similarly, research has shown that mutations in PLCZ1 can cause polyspermy during in vitro fertilization[40-42].

The findings of these studies can be applied in practice to identify the cause of some total fertilization failures and to search for promising new ways to develop contraceptives. Some issues are still open and need to be solved. Even though human ZP2 is key to the initial binding of sperm to ZP glycoproteins, the other sperm receptors for these glycoproteins remain unclear. In the same way, other fusion factors found in model organisms, such as SPACA6 and DCST1/2, should be studied in humans.

The strength of this review lies in its adherence to PRISMA guidelines, ensuring a transparent and systematic synthesis of evidence drawn exclusively from human studies. By restricting the analysis to human data, this review maximizes clinical relevance, though it also highlights the scarcity of large-scale human trials in this area. Future investigations should aim to identify the complete repertoire of sperm receptor complexes interacting with the zona pellucida and to validate these molecular pathways in clinical settings. Such efforts are essential to translate laboratory findings into tangible benefits for infertility treatment and contraceptive innovation.

The majority of the available data relies on experiments with isolated gametes under laboratory conditions, while direct experiments in humans remain constrained by ethical guidelines. Additional limitations include small sample sizes and methodological variability across studies, which may introduce bias. Despite these challenges, consistent replication of key findings across independent groups provides moderate confidence in the robustness of current models. By collating current evidence, this review provides a resource for reproductive biology researchers and clinicians seeking to understand the molecular events underpinning human fertilization, while also pointing to the outstanding gaps that require resolution in future work.

CONCLUSION

Human-focused studies over the past decade have clarified key molecular mechanisms of fertilization, from capacitation signaling to gamete membrane fusion and oocyte activation. These insights not only enhance understanding of infertility but also hold promise for advancing assisted reproductive technologies and contraceptive development.

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