ADHESION MOLECULES IN HUMAN SPERM-OOCYTE INTERACTION: RELEVANCE TO INFERTILITY

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1. ABSTRACT

Fertilization involves cell-cell fusion of a sperm with the oocyte. This fusion restores the diploid genome, activates the oocyte, and initiates embryonic development. The identification of proteins mediating the fusion of sperm with oocyte plasma membrane (oolemma) is important to a deeper knowledge of fertilization. Defects in sperm-oocyte fusion may account for some form of human infertility. The hypothesis that sperm plasma membrane and oolemma carry complementary molecules involved in multistep fusion process has been validated by studies of cell adhesion molecules (integrins) in sperm-oocyte interaction in a number of animal models and human *in vitro* fertilization assays. Integrins or integrin-like molecules and complement proteins present on the surface of mammalian gametes, might be involved in the interaction between oocyte and sperm at fertilization. This review will provide an overview of the interaction of human sperm membrane with the oolemma, the nature of cell adhesion molecules, their expression profiles and their possible involvement in adhesive and fusogenic events in human fertilization. Unraveling the unique molecules involved in human sperm plasma membrane-oolemma fusion will be an important component for the development of a new set of contraceptive vaccines.

2. INTRODUCTION

Physiologic fertilization in humans results from a highly ordered sequence of events. The major events include gamete maturation, regulated sperm transport, sperm-oocyte recognition, sperm-zona penetration, and sperm-oocyte fusion. Sperm maturation involves capacitation and acquisition of a

hyperactive state, and expression of cell-cell adhesive ligands. Binding of capacitated sperm to zona pellucida (ZP) glycoproteins, which is a species-specific event, results in sperm activation and induction of acrosome reaction - an exocytotic event that releases hydrolytic enzymes from the acrosome. Proteolytic and hydrolytic enzymes released during the course of the acrosome reaction then facilitate the penetration of the fertilizing sperm through the ZP and into the perivitelline space. Finally, acrosome-reacted sperm bind to and fuse with the oocyte plasma membrane (oolemma) during fertilization. Following fusion of the sperm head and oolemma at discrete sites, sperm and egg pronuclei are formed, and fusion of both pronuclei results in the completion of fertilization, activation of the oocyte, and the initiation of embryonic development (1-2).

Despite four decades of research on the biology of fertilization carried out in a variety of laboratory animal models and more recently in human *in vitro* fertilization (IVF) systems, much of the biochemical and molecular aspects of this recognition process remains unknown. It is now thought that several complementary mechanisms may be involved in sperm-oocyte recognition and fusion and that the recognition signals on the oocyte reside on the ZP as well as on the oolemma (3-4).

3. SPERM-OOCYTE FUSION

Membrane fusion is a key event in a variety of cellular phenomena such as transport between organelles, endo-and exocytosis, myogenesis, viral infection, and fertilization (5). The specificity of membrane fusion suggests the involvement of specific modulators regulating recognition of fusion partners. A number of specific proteins have been identified as candidates for the control of membrane fusion in several model systems (6). It is thought that similar principles may apply to membrane fusion events leading to sperm plasma membrane-oolemma adhesion and fusion. The responses of the oocyte following sperm-oocyte binding and fusion are strikingly similar to the responses that occur within lymphocytes following their activation by an antigen presenting cell (7). Based on this premise, numerous investigators have begun to dissect the relative contributions of various cell adhesion molecules in sperm-oocyte fusion as well as to implicate these molecules in specific defects, such as failure in IVF systems. Similar to fusion in somatic cells, sperm-oocyte adhesion and fusion appear to involve receptor/counter-receptor binding and to be under the influence of the mechanical forces exerted by sperm on the oocyte. Furthermore, metabolic changes that lead to increased affinity (receptor clustering, phosphorylation induced by cell signaling) for sperm-oocyte fusion may also be operative. Reproductive

biologists have begun to search for the adhesion molecules on the sperm plasma membrane and oolemma, and to cell signaling events in gametes during the onset of fertilization in an attempt to find an analogy with the well-characterized membrane fusion model systems such as cell-cell differentiation and immune-microbe interactions. The best characterized membrane fusion proteins are found in viruses (6). Virus binding and fusion are mediated by either separate proteins or these separate attributes remain on a single protein but at topographically distinct sites. A conformational change in the fusion protein leads to exposure of a "fusion peptide", which then recognize counter-receptors on the target cell surface.

The hypothesis that sperm plasma membrane and oocyte membranes carry complementary molecules involved in a multistep fusion process has been validated by studies of adhesion molecules in sperm-oocyte interaction in a number of animal models (8-9). The species-specificity of sperm-oocyte recognition and binding primarily resides at the level of ZP (2). Human sperm bind and penetrate hamster oocytes denuded of ZP (10). Sperm binding and fusion with oocyte occurs at discrete regions on both the sperm membrane and oolemma. The equatorial segment of the sperm head, which constitutes the post-acrosomal region toward the posterior of the sperm head, appears to be the region of the sperm membrane that is involved in binding and fusion with the microvillar region of the oolemma (11).

In somatic cells, cell adhesion receptors have been grouped into distinct families based upon their structure and binding. The major families of membrane-anchored adhesion molecules include integrins, the immunoglobulin supergene family, selectins, cadherins, syndecans, and ADAMs (a disintegrin and metalloprotease domain) (12). Members of the ADAM family possess a potential adhesion domain as well as a potential protease domain (13). As a group, adhesion molecules are characterized by an extracellular domain anchored to the membrane of the cell by a short, hydrophobic transmembrane domain that is followed by a cytoplasmic tail of variable length. They act as "molecular bridges" connecting molecules on the outside of the cell with cytoskeletal and signal-transducing machinery within the cell (14). The primary mediators of cell-cell interaction appear to be adhesion molecules of the integrin family. This family is composed of non-covalently associated α and β subunits and classified into subfamilies based on their β subunits (called $\beta 1$, $\beta 2$, $\beta 3$, etc.) (15). At present, at least 15 α subunits and 8 β subunits have been characterized. The α and β subunits in various combinations are known to form at least 19 integrins.

In general, integrins are subclassified into: (a) β1 integrins, sharing the β1 chain and functioning in both cell-cell and cell-substratum adhesion, (b) β2 integrins, sharing the β2 chain and mainly participating in cell-cell interactions; and (c) β3 integrins, which share the β3 chain and have variable adhesive functions. The integrin expression profile of individual cells varies greatly among different cell types and determines the ability of cells to recognize different adhesive substrates (16). In addition, different β integrins may recognize different peptide sequences of the same protein or the same structural element presented by a particular ligand (17). Integrins are involved in a variety of biological processes including platelet aggregation, leukocyte recognition, and adhesion during immune response and cell migration during embryonic development (18-20). The ability of ligands to be recognized by integrins is often associated with the presence of the tripeptide sequence Arg-Gly-Asp (RGD) in the substrate (14, 19). Not all substrates for integrins contain this sequence, however, and other sequences are clearly important for recognition by integrins.

Integrin-ligand interactions also trigger specific organizational and physiologic events (21). In general, ligand binding leads to receptor clustering within the surface membrane, the organization of cytoskeletal elements around the occupied receptors, and the generation of an intracellular signal (22). Integrin engagement also stimulates phosphoprotein kinases known to be required for information transduction and gene activation (23). Various lines of evidence suggests that integrins, or integrin-like molecules, may be present on the surface of mammalian gametes, and might be involved in the interactions between oolemma and sperm membrane at fertilization (8-9, 24-25). The molecular mechanisms by which this occurs are currently the topic of intense investigation by reproductive biologists.

4. ASSESSMENT OF SPERM-OOCYTE INTERACTION

The process of sperm-oocyte interaction and defects manifested at the level of sperm membrane-oolemma fusion, pronuclear decondensation, and oocyte anomalies that lead to IVF failure are studied by indirect and direct bioassays using light microscopic approach. These bioassays include, hamster oocyte-human sperm penetration assay (26), human oocyte-human sperm fusion test (27), subzonal sperm insertion technique (28), and intracytoplasmic sperm injection assay (29). These methods are currently used to predict fertilization as well to delineate functional defects at the level of sperm membrane-oolemma interaction observed in a subset of infertile couples.

4.1. Human sperm-zona-free hamster oocyte penetration assay (HPA)

The fusion of zona-free hamster oocytes with acrosome-reacted human sperm is the most widely used bioassay for physiological sperm-oocyte fusion. HPA has been shown to exhibit excellent correlation with the fertility of human sperm *in vivo* and *in vitro* (30). In this procedure, the hamster oocytes are stripped of their ZP by treatment with 0.1% trypsin and then exposed to suspensions of capacitated human sperm. The criterion for penetration is conventionally the percentage of oocytes possessing decondensed sperm nucleus (-ei) after a 3 hour incubation period and 25-50 oocytes are normally used to assess each sperm sample. This test is useful to predict fertilizability of human sperm or intrinsic oocyte problems in patients with total fertilization failure after IVF.

4.2. Human sperm-zona-free human oocyte fusion test

This test is used to evaluate the fusion potential of nonfertilizing human sperm. In this procedure, unfertilized human oocytes are treated with 0.5% pronase to remove ZP, loaded with DNA-specific fluorochrome, and then exposed to suspensions of capacitated human sperm. The criterion for fertilization is the presence of pronuclei (2 or more) after 15 to 20 hour incubation period and 48 hour later for cleavage. Direct examination of fertilization rates of unfertilized human oocytes inseminated with fertile donor sperm have suggested that one cause of fertilization failure may be due to intrinsic oocyte problem confined to the oolemma.

4.3. The subzonal sperm insertion (SUZI) technique

This technique is used to assess the potential of motile sperm to form pronuclei. In this method, the frequency of sperm fusion, is calculated based on the total number of male pronuclei formed in relation to the total number of sperm inserted subzonally. At least 30% to 60% of normal sperm inserted subzonally are able to fuse with the oolemma and decondense within the ooplasm. Subzonal insertion of sperm from men with abnormal semen analyses show significantly low sperm fusion with oolemma and with variable ability to form a pronucleus. Thus, SUZI allows one to distinguish defects on ZP from those of oolemma fusion.

4.4. Intracytoplasmic sperm injection (ICSI)

ICSI of unfertilized human eggs after IVF are used to evaluate the ability of sperm to activate human oocyte. In this method, oocytes are harvested after superovulation with GnRH agonist and gonadotropins. After removing the cumulus cells, a single sperm is injected directly into the cytoplasm of

metaphase II oocytes, and sperm decondensation and the number of pronuclei formed are enumerated.

4.5. Hybridoma/molecular cloning approach

Murine monoclonal antibody (mAb) library raised against human sperm head proteins and zona-free human oocytes are valuable tools to investigate the various steps in sperm-oocyte interactions. The acrosome is known to contain more than 20 hydrolyzing enzymes, such as hyaluronidase, acid phosphatase, acrosin, and a number of antigens. Several mAbs directed against the acrosomal antigens (FA-1, HS-63, SP-10, CD46, acrosin) of human sperm or to oolemma have been shown to inhibit heterologous and/or homologous gamete-interaction *in vitro* (31-34). Therefore, antigens localized on the acrosomal region and equatorial band that interfere with sperm-oocyte fusion have been extensively investigated for their contraceptive potential (4, 35). However, some of these mAbs also display extensive crossreactivity with antigens of somatic cells and placental cells.

Molecular cloning offers an alternative approach for identifying the putative antigens involved in sperm-oocyte interactions. Antisperm antibodies that block gamete interaction *in vitro* have been used to screen human testis lambda gt11 cDNA expression library. By this method, several recombinant proteins have been identified (36-38). Fusion proteins characterized thus far, include a serine protease inhibitor (serpin), and cytokeratins localized to the acrosomal region of sperm head (39-40).

5. MOLECULES IMPLICATED IN SPERM-OOCYTE INTERACTION

Because fertilization essentially involves cell-to-cell fusion of a sperm with the oocyte, it is believed that a number of integrin molecules may be involved in this process. Integrins mediating adhesion of cells are present in an active or inactive conformation, and in addition to adhesive events, they transfer signals to the cell inducing changes in gene expression (21-23, 41). Mammalian sperm membrane-oolemma fusion results in tyrosine phosphorylation of egg proteins, increased production of phosphoinositol lipids, and substantial cytoskeletal reorganization thereby suggesting receptor-mediated signal transduction across the egg plasma membrane (42-43).

A number of recent studies also suggest a causal relationship between the complement (C) system and reproduction, particularly the expression of cell membrane-associated C regulatory proteins, C3b receptors and C inhibitors (34, 44-45). C3 fragments and C3 receptors are involved in cell-cell

adhesion reactions in both natural and inducible immunity and in membrane apposition before fusion and entry of eukaryotic cells by bacteria, virus, protozoa, and yeast cells (46). Not surprisingly, a role for C3 fragments and C3 receptors in sperm-oocyte apposition and fusion has been invoked (44).

At least three cell surface C-binding regulatory proteins are expressed on human sperm: Membrane cofactor protein (MCP or CD46), decay accelerating factor (DAF or CD55), and membrane inhibitor of reactive lysis (MIRL or CD59) have been demonstrated by biochemical and immunolocalization studies (47-53). Both CD55 and CD59 are also expressed on the plasma membrane, whereas, CD46 is only expressed as an unusual alternatively spliced product on the inner acrosomal membrane (49-50). In addition to their probable role in local C binding and inactivation, these proteins have also been implicated in human reproductive events (34, 45). In somatic cells, CD46, CD55, and CD59 in addition to their primary role in protecting cells from C-mediated damage, also participate in cell adhesion and both CD55 and CD59 contribute to cell signaling (54-56). *In vitro* gamete interaction studies using a panel of mAbs has led to the hypothesis that these C proteins may also participate in sperm-oocyte interaction or in signaling induction and protect sperm from C-mediated damage in the reproductive tract.

5.1. Adhesion molecules on the sperm

5.1.1. Fertilins

Members of a recently discovered gene family called ADAMs have been strongly implicated in integrin-mediated sperm-oocyte binding and fusion in the guinea-pig and mouse model systems (8-9, 13). Fertilins possess both a potential adhesion domain and a potential protease domain (57). The PH-30, a transmembrane protein, isolated from the guinea-pig sperm plasma membrane by Primakoff and colleagues appears to have sperm-oolemma fusion, and metalloprotease properties (58) and may be considered as a potential oocyte receptor ligand. The complex is expressed on the posterior surface of the mature sperm head, at the appropriate location for molecules involved in oocyte fusion (59). mAbs to PH-30 disrupts the fusion of acrosome-reacted guinea-pig sperm with the vitelline membrane of the oocyte (59). Antibody to PH-30 precipitates two tightly coupled immunologically distinct subunits (α and β) that behave as a single integral membrane protein (8). Both α and β subunits are generated as large precursor proteins that seem to be modified by proteolytic cleavage during testicular differentiation and subsequent epididymal maturation of the sperm (60).

The mature PH-30 α/β complex, being involved in sperm-oocyte fusion, resembles certain viral fusion proteins in membrane topology and predicted binding and fusion functions (8). Cloning of PH-30 and sequence analysis suggests that it may be responsible for both recognition and fusion with the guinea-pig oolemma. The α subunit is composed of a peptide core of 289 amino acids, with a single membrane-spanning domain towards the C terminus, a large extracellular domain and a short cytoplasmic tail. The putative fusion peptide has been identified on the α subunit, and it appears to be somewhat similar in sequence to a potential fusion peptide on the rubella virus (61). A feature of the fusion protein motif on PH-30 α is its relative hydrophobicity which, in conjunction with the transmembrane anchoring segment on the same subunit, is thought to permit the molecule to interact simultaneously with the plasma membranes of both sperm and the oocyte (62).

The sequence data on the β subunit indicate that it contains a 353-amino acid peptide core, with two potential sites for N-glycosylation and a single membrane spanning domain. The β subunit contains a putative oocyte recognition domain characteristic of a family of small, soluble (about 50 amino acids) integrin-binding peptide ligands called disintegrins (57-58). Disintegrins, which are present in many snake venoms, contain RGD, or related sequences, in the context of extended loop structure (63). They recognize sites on integrins that normally interact with the RGD-based cell recognition sequences in many matrix-associated glycoproteins (*e.g.*, fibronectin, fibrinogen). These soluble disintegrins block platelet aggregation and promote uncontrolled bleeding by competitively inhibiting the function of the platelet integrin GPIIb/IIIa (αIIbβ3). In the case of the sperm, the disintegrin domain is immobilized as part of a larger transmembrane glycoprotein and therefore it has the potential to act as a cell surface-bound ligand (counter receptor) to promote a cell-cell adhesion event (64).

The fusogenic properties of sperm protein have been investigated using liposomes. A synthetic peptide representing the putative fusion domain of PH-30 binds to vesicles composed of both neutral and acidic lipids (64). In the intervesicular lipid mixing assay, the synthetic peptide undergoes conformational transition to a β-structure and induces fusion of large unilamellar vesicles supporting the hypothesis that PH-30 mediates sperm fusion with oocyte (64). The role of the sperm surface protein PH-30 in sperm-oocyte fusion has been further tested by using peptide analogues of a potential integrin binding site in the fertilin β subunit. Peptide analogues that induce a QDE sequence (Thr-Asp-Glu) from the disintegrin region of fertilin β have been shown to bind to the oolemma and strongly inhibit sperm-oocyte fusion (58). Even though, the disintegrin motif of PH-30 β does not contain an RGD sequence, there are ample examples of integrin ligands in which this particular tripeptide is absent.

A mouse analogue of PH-30, has recently been identified on mouse sperm (65). Mouse sperm fertilin has a QDE tripeptide (instead of RGD) in its cell recognition region and synthetic peptides containing QDE inhibit sperm-oocyte fusion in the mouse bioassay.

A human fertilin β has recently been identified using cDNA cloning, deduced amino acid sequence analysis, Northern blot analysis, and chromosomal localization (66). Human fertilin β is encoded by a 2205 nucleotide (735 amino acids) open reading frame; its deduced amino acid sequence contains pro-metalloproteinase-like, disintegrin-like, and cysteine-rich domains, which are structurally homologous to P-III snake venom metalloproteinases. Human fertilin β shares a 90% amino acid identity to monkey, 56% to guinea pig, and 55% to mouse fertilins. A FEE binding tripeptide is located within the disintegrin loop of human fertilin β and could be the site competing for recognition by integrin and other receptors on the oolemma. Northern blot analysis of poly $(A)^+$ RNA from 16 human tissues revealed human fertilin β's 2.9 kb message only in the testis (66).

5.1.2. β1-integrins

Presence of disintegrin domain on guinea pig, mouse, and human sperm suggests that an oocyte integrin might serve as a receptor for sperm. Integrins, through their property to recognize the RGD tripeptide sequence, also mediate cell-cell and cell-extracellular matrix recognition and adhesion in reproductive tissues. The recent discovery of β1-integrins on human sperm (67), human endometrium (68), and human trophoblast (69) has expanded the role of these molecules in the biology of reproduction. Indirect evidence suggest that β1 integrins are involved in sperm membrane-oolemma recognition and fusion as well. The most relevent receptors are the family of integrins which bind to fibronectin, vitronectin, and laminin. β1-integrins are represented by the very-late-activation antigen (VLA) molecules. Each VLA molecule contains a common β1 subunit in association with one of at least nine different α subunits (70). Glandner and Schaller described the binding patterns of β1-integrins (α3, α4, α5, α6) and their matrix proteins, fibronectin and laminin, on human sperm by flow cytometry (67, 71). They compared the expression of β1 integrin with the fertilizing ability of sperm from men of couples who underwent IVF for either unexplained, tubal or male factor infertility. When compared with normal sperm,

sperm from patients with teratozoospermia showed a significant decrease in expression of these adhesion molecules. Compared with semen samples from men with unexplained or male factor infertility patients, samples from patients with tubal infertility had significantly higher percentage of sperm expressing β1 integrins. Also, higher IVF success rate and pregnancy rates were obtained when compared with couples suffering from male or unexplained infertility (71). Immunolocalization techniques using a panel of mAbs against the β1-integrin cell adhesion molecules indicated that the α chains 4, 5, and 6 are expressed by a small percentage of sperm from fertile individuals (67, 71). After the loss of the acrosome a significantly higher expression of α4, α5, and α6 chains of integrins was detected in the fertile semen group (72). These findings suggest that certain β1 integrin cell adhesion molecules, through their ability to recognize the RGD sequence, may be involved in the early stages of sperm-oocyte recognition and interaction.

The 3 integrin complexes detected on human sperm are known to be expressed on several mesenchymal and epithelial cells types where they function as receptors for extracellular matrix proteins such as fibronectin, laminin, and collagens (73). Two β1 integrins (α4/β1, α5/β1) that bind fibronectin utilize the variable region (CS-1) and RGD binding domains, respectively (73-74). α3/β1 is a receptor for several extracellular matrix proteins including fibronectin and laminin (74). Its binding to fibronectin involves the RGD site while its binding to laminin is RGD-independent. α6/β1 is a receptor for laminin and it does not recognize the RGD sequence (74). In addition to being extracellular matrix receptors, α5/β1 and α3/β1 can be involved in dynamic cell to cell adhesion. In somatic cells, these integrins are localized in areas of cell to cell contact (75). The integrins expressed on sperm might therefore function in cell-cell and/or cell-matrix interaction during sperm-oocyte fusion.

The extracellular matrix proteins, fibronectin and vitronectin have been identified on the plasma membrane of capacitated sperm (76-77). It has been demonstrated that co-incubation of human sperm and zona-free hamster oocytes in the presence of micromolar concentration of RGD-containing oligopeptides results in a significant decrease in the number of oolemma-adherent sperm as well as complete inhibition of fertilization (24, 78). The effect of these peptides is greatly reduced by changing the D residue into an E, a mutation known to abolish recognition by integrins. Beads coated with RGD-containing peptides bind to the oocyte, but not to the sperm surface indicating that the oocyte express RGD-receptors (24). These findings suggest a role forβ1 integrins in fertilization. In contrast, β2

integrins, the primary mediators of adhesive immune interaction of sperm with phagocytes are not present on the surface of sperm (79). Fibronectin has also been localized in the region where the sperm fuses first with the egg plasma membrane during fertilization (11). Sperm adhesion to the egg can be inhibited by antifibronectin antibodies. The wide variation in the fibronectin concentration in semen obtained from different donors also suggest that defects in fibronectin expression might play a role in sperm dysfunction and infertility (76-77). Fibronectin is localized on the entire sperm surface and on the equatorial segment, laminin is localized solely on the sperm tail and vitronectin is detectable primarily on the equatorial band on the sperm head (76-77, 80). Bronson and associates suggest that vitronectin and fibronectin expressed on the surface of capacitated sperm could act as a ligand for specific receptors on the oocyte, and might play a role in sperm-oolemmal adhesion (77).

β1 integrins are known to function in a dual capacity as adhesion and signaling molecules (82). Vitronectin is recognized as an adhesive substrate by cells expressing at least one of four known vitronectin receptors: integrins αvβ1, αvβ3, αvβ5, or αIIbβ3 (82). Thus, antibodies reacting with β1 integrins can lead to defective sperm-oocyte interaction. This deficiency may occur either by direct interference with the function of the integrins or by integrin cross-linking, leading to alterations in membrane functions. Therefore, integrins may find utility as prognostic markers for clinical outcome or as therapeutic targets in infertility.

5.1.3. β2-integrins

The receptors for C3, the primary mediators of phagocyte-microbe interaction (46), have also been shown to facilitate sperm-oocyte interaction during the hamster penetration test (44). Anderson and associates showed that some mAbs specific for C receptor type 1 (CD35) and type 3 (CR3) bind to the human oolemma, indicating that specific C-binding molecules may play a role in the attachment of C3 catabolites to oocytes (44). In their bioassay, subsaturating concentrations of dimeric C3b (<1 μM) promoted penetration of hamster oocytes by human sperm, whereas saturating doses (>10 μM) inhibited this process. In addition, antibodies to both CD46 and C3 significantly inhibited penetration of hamster oocytes by human sperm (44). These data suggest that regulated gamete-induced generation of C3 fragments and the binding of these fragments by selectively expressed receptors on sperm and oocytes may be an initial step in sperm-oocyte interaction, and subsequent membrane fusion and fertilization. The dimeric C3b at low levels is thought to serve as a bridge between sperm (CD46) and oocyte (CR1, CR3) C receptors, facilitating fertilization. At high

levels, however, dimeric C3b could saturate all receptor binding sites for C3 fragment and inhibited the apposition of gamete membranes. CR3, a β2-integrin, which is expressed on oocytes may bind the human sperm homologue of the PH-30 integrin and facilitate membrane fusion by the PH-30 chain homologue (44).

5.1.4. Complement inhibitors (CD35, CD46, CD55, and CD59)

Acrosome intact and acrosome-reacted human sperm express C inhibitors, CD55 and CD59, whereas, CD46 is expressed only on the sperm head region of acrosome-reacted sperm (45, 49-53). The CD46 and CD59 on sperm have C-inhibitory activity *in vitro* (50-51). However, the low potency of these proteins in protecting antisperm antibody-induced C-damage *in vitro* suggest that they have functions other than binding and regulation of C activity (49). In fact, mAbs directed against CD46 and CD59 variably inhibit the penetration of hamster oocytes by human sperm, suggesting that CD46 and perhaps C3 play a role in sperm-oocyte interaction (34, 44-45).

CD46 serves as a C3b/C4b inactivating factor for the protection of host cells from autologous C attack (83). Naniche *et al.*. have recently shown that CD46 acts as a human cellular receptor for measles virus, allowing cell binding, fusion, and viral replication (84). Okada *et al.* showed that CD46 is a keratinocyte receptor for the M protein of the group A streptococcus (85). On human sperm, CD46 is located on the inner acrosomal membrane (34). Johnson and associates showed that human sperm binding and pronuclear formation in zona-free human oocytes can be significantly inhibited by preincubation of both sperm and oocytes with anti-CD46 mAb (86). This effect was not observed when either of these gametes alone was incubated with mAb. CD46 is also expressed by zona-free human oocytes (86). The expression of CD46 by zona-free oocytes and acrosome-reacted sperm suggest a role for CD46 at the level of apposition of the inner acrsomal membrane and the oolemma.

CD46 consists of 4 short consensus repeat (SCR) regions which are the predominant extracellular structural motifs each of which is a cysteine-rich domain of approximately 60 amino acids. The cofactor activity of CD46 for C inhibition resides in SCR-3/4 while the site for human sperm-oocyte interaction resides on SCR-1, since only mAbs directed to epitopes in SCR-1 inhibit *in vitro* gamete interaction (86). Also, the sites for measles virus binding and for C3b/C4b inactivation appear to reside on different SCR. Using Chinese hamster ovary cell clones expressing each SCR deletion mutants, Iwata and associates confirmed which of the 4 SCR of CD46 contribute to its function (87). The functional domains of CD46 for the natural ligands C3b, C4b and measles virus were mapped to different SCR domains. They found that SCR1 and SCR2 were mainly involved in measles virus binding and infection and SCR2, SCR3, and SCR4 contribute to protection from C damage (88). Motifs for N-glycosylation on CD46 are present on SCR1, 2, and 4 (88). These N-linked glycans are essential for the recognition of CD46 by measles virus (89), whereas, the presence of nonglycosylated CD46 on acrosome-reacted sperm suggest a protein-protein type gametic interaction.

CD55 blocks the C cascade by accelerating the decay of C3/C5 convertase complexes whereas CD59 interferes with the assembly of the membrane attack complex of C (90-91). Both CD55 and CD59 are glycosyl phosophatidylinositol (GPI)-anchored glycoproteins. Perturbation of GPI-anchored CD55 and CD59 molecules by cross-linking with antibodies causes activation of T cells and neutrophils (54-56). The mediators of signal transduction are likely to be the tyrosine kinases that are tightly associated with GPI-anchored molecules on GPI-rich membrane clusters (55). CD59 acts as a signal-transducing molecule (56). CD59 associates with kinases in membrane clusters and gains Ca signaling capacity (55). Nonlethal C attack induces a similar series of events, including increase in intracellular-free Ca concentration and production of reactive oxygen radicals (92). During sperm-oocyte fusion, there is a rapid membrane depolarization followed by release of internal Ca (42-43). CD59 expressed by acrosome-reacted sperm and oolemma may be involved in these events before sperm-oocyte fusion. CD55 may be involved in acrosome reaction. For example, mAbs to CD55 antigen inhibit acrosome reaction induced by the oocyte-cumulus complex but not by calcium ionophore. Thus, during the physiological sperm acrosome reaction both CD55 and CD59 complement-binding proteins may be part of the signaling machinary. In addition, the binding of sperm to oocyte and activation of ovum by hydrolysis of phosphotidylinositol 4-5-biphosphate may involve a multimolecular complex that includes CD55 and CD59. Inositol 1, 4, 5-triphosphate (IP$_3$)-gated Ca channels and upstream components (including G$_{\alpha q/11}$, PLCβ$_1$ and IP$_3$ gated Ca store) of the phosphoinositide signaling system have been identified in the acrosomal region of sperm (93). In fact, the level of IP$_3$ binding in sperm is among the highest observed in mammalian tissues (93).

There is also a report that C1q, a component of the classical C pathway accelerates the human sperm-hamster oocyte adhesion but inhibits fusion (94). C1q is one of the high molecular weight proteins that bind with fibronectin (95). C1q is a component of classical C pathway that can react with

Fc fragment of immunoglobulins and with other proteins, such as fibronectin, and laminin. Presence of C1q in the coincubation media resulted in a significant increase in sperm adhesion to oolemma but inhibited penetration of ovum (94). C1q receptor was immunolocalized both on hamster oolemma and human sperm (94). mAb to C1q variably inhibited penetration of ovum.

5.1.5. Calmodulin

Immunogold localization of ultrathin sections of human sperm before and after incorporation into hamster oocyte revealed redistribution of calmodulin on the sperm head (96). Following entry of sperm head into egg cytoplasm, post acrosomal calmodulin disappeared whereas the subacrosomal calmodulin was unaltered (96). These data suggest a role for calmodulin in sperm-oocyte fusion and in the initial pulse of intracellular Ca occurring during fertilization.

5.2. Adhesion molecules on the oolemma

Successful fertilization requires communication between the fertilizing sperm and the oocyte. In somatic cells, expression of major histocompatibility complex (MHC) molecules participates in cell-cell interaction (97). Similarly, the expression of IgG Fc receptors provides a class of surface molecules which promote cell-cell interactions (98). Human oocytes lack class I MHC antigen (99). However, all three classes of Fc(γ) receptors have been demonstrated on unfertilized human egg oolemma but not on sperm (25, 100). Oolemmal Fc receptors promoted the adhesion of antisperm antibody labeled human sperm to zona-free hamster oocytes (100).

The finding that a disintegrin domain-containing molecule may be the sperm ligand for the oolemma implies that the oocyte receptor is an integrin. In fact, integrin subunits $\alpha 2$, $\alpha 4$, $\alpha 5$, αL, $\beta 1$, $\beta 2$, and $\beta 7$ have been immunolocalized on human oocytes (25, 99). Unfertilized mouse oocytes also express $\beta 1$ integrins both at mRNA and protein levels (101). In these cells, mRNAs for the $\beta 1$, $\alpha 5$ and $\alpha 6$ and corresponding proteins, $\alpha 5 \beta 1$ and the $\alpha 6 \beta 1$ complexes are present (101). $\alpha 3 \beta 1$ was expressed on the oocyte surface (101). Integrin subunits $\alpha 6$ and $\beta 1$ were differentially distributed on the oocyte surface. $\alpha 6$ antigen was mainly confined to the microvilli while $\beta 1$ was homogeneously distributed over the whole oolemma (101). Recent reports suggest that mouse oocyte $\alpha 6 / \beta 1$ functions as a sperm receptor (9). In addition, studies using human sperm-hamster zona-free oocyte penetration assay suggest that echistatin (a disintegrin known to block the binding of fibronectin and vitronectin to their respective integrin ligands, $\alpha 5 / \beta 1$ and $\alpha v / \beta 1$), interacts with gametic fusion events (102). The binding of sperm to oolemma was echistatin-sensitive whereas, the gamete membrane fusion was resistant to echistatin (102). Whether integrins can actually function as human sperm receptors in the human fertilization process, however, has not yet been unequivocally demonstrated.

C inhibitors and signal transducing molecules, CD55, and CD59 are also expressed on human oolemma (103-104). The human C3b/C4b receptor CR1 is not expressed on sperm or oolemma, whereas, CR3, a $\beta 2$ integrin is expressed on oolemma (44). It is thought that CR3 binds the human sperm homologue of the PH-30 integrin and facilitates membrane fusion. Anderson and associates suggest that C3 fragments (C3b/iC3b) may serve as bridging ligands between sperm CD46 and oocyte CR3 and facilitate apposition of the sperm inner acrosomal membrane with the oolemma (44).

The various adhesion molecules that have been implicated in human sperm plasma membrane-oolemma adhesion to date are listed in table 1.

6. SPERM-OOCYTE FUSION AND TRANS-MISSION OF HIV

Sperm-oocyte fusion may serve as a vehicle for transfer of dormant viruses to oocytes. This transmission occurs by two different pathways to the ovulated ova. Viral entry may occur directly via fertilizing sperm. Alternately, virus entry into the oocyte may take place by viruses released from other sperm during acrosome reaction. Recent evidence suggests that sexual transmission of human immunodeficiency virus (HIV) can be mediated via sperm-oocyte fusion (105). HIV particles have been detected on and around organelles in mature sperm either ejaculated by AIDS patients or incubated *in vitro* with HIV. In the first case, the nucleoid of the virus can be devoid of an envelope or is enveloped by a membrane-like coat. These viral particles bud from the plasma membrane. In the *in vitro* infected sperm, only membrane enveloped nucleoids are present. Baccetti and co-workers suggest that HIV particles penetrate sperm from AIDS patients in various stages of development. During different stages of sperm development, viral particles replicate and bud through the plasma membrane. In contrast, *in vitro* infected sperm, only display penetrated viral particles and do not exhibit buddings and membrane-free nucleoids. Baccetti and co-workers using immunoelectron microscopy, *in situ* hybridization, and polymerase chain reaction technique, showed that HIV-1 binds and enters normal sperm (106). Presence of viral particles, their antigens, and nucleic acid were shown in sperm of HIV-1 infected men. These sperm could transfer HIV-1 like particles to normal human oocytes (106). It was suggested that

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Table 1. Overview of expression of various cell surface recognition molecules on human sperm and human oolemma.

CD	Receptor	Gene family	Ligand(s)	Sperm	Oolemma	Sperm/ oocyte interaction	References
	b*1 integrins*						
CD49c/CD29	α_3/β_1	Integrin	Fibronectin	+	−	+	67, 71, 72
CD49d/CD29	α_4/β_1	Integrin	Fibronectin	+	+	+	67, 71, 72
CD49e/CD29	α_5/β_1	Integrin	Laminin	+	−	+	67, 71, 72
CD49f/CD29	α_6/β_1	Integrin	Fibronectin/ Vitronectin	+	+	+	67, 71, 99
CD51/CD61	$\alpha_v\beta_1$	Integrin	Vitronectin	+	+	+	76, 99
	C proteins						
CD35		RCA	C3b/C4b	−	+	+	44
CD11b/CD18	$\alpha_m\beta_2$	Integrin	C3bi	−	+	+	44
CD46		RCA	C3b/C4b	+	−	+	34, 86, 104
CD55		RCA	C3b/C4b	+	+	+	86
CD59			C5b-8, C5b-9	+	+	+	45, 103

galactosylceramide-like compound on the sperm membrane could function as a receptor for HIV (106).

Scofield and co-workers demonstrated that sperm/leukocyte-mediated cell interactions are dependent on HLA class II molecules expressed on leukocytes (107). Sperm receptor activity requires presence of sulfated carbohydrates as carriers for HLA-DR binding ligands. Scofield and associates demonstrated that sperm can bind HIV through the mechanism related to class II MHC-CD4 molecules (106-111). A CD4-binding glycoprotein (17.5 kDa) has been purified from human seminal plasma (110). This glycoprotein may act on the sperm as a receptor for the HIV-1.

7. FACTORS IMPAIRING SPERM-OOCYTE FUSION

Based on hamster or human oocyte penetration assays it has been suggested that defects in sperm-oocyte fusion may account for some form of human infertility. These defects may arise from failure of the sperm to respond to Ca influx and proton efflux and to undergo acrosome reaction. They may also be due to structural abnormalities in the sperm particularly at the equatorial region of the sperm head where fusion with the oocyte is initiated. In addition, biochemical defects in the plasma membrane of sperm and oolemma can inhibit the initiation of exocytosis, acrosome reaction and fusogenic changes required in fertilization of egg.

7.1. Free radical-mediated lipid peroxidation

Oxidative stress, commonly defined as a disturbance in the prooxidant and antioxidant levels, may be involved in the defective sperm-oocyte fusion. Human sperm are highly susceptible to peroxidative damage because they contain high leves of longchain polyunsaturated fatty acids and because unlike somatic cells, they lack the mechanism to institute membrane repair (112). Numerous studies have shown that human sperm exhibit the capacity to generate reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, and hydroxyl radicals (112-117). Most of the hydrogen peroxide appears to be generated by the action of sperm superoxide dismutase on the superoxide radical produced by sperm. The production of ROS by human sperm is due to a membrane-bound NADPH oxidase system (112).

Under normal circumstances, this NADPH oxidase complex is quiescent. However, in certain cases of male infertility this system is extremely active, and results in a chronic elevation in the rate of production of ROS (117). In such cases, the capacity of sperm for sperm-oocyte fusion is diminished. Aitkin *et al.* suggest that high failure rate of sperm-oocyte fusion bioassay can be related to increased generation of lipoperoxides (115). In a subset of infertile patients sperm are refractory to the second messenger signal generated by Ca ionophore, excessively generate ROS and exhibit a high failure rate in sperm-oocyte fusion bioassays (115).

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The major consequence of generation of reactive oxygen species is to induce lipid peroxidation in sperm. As a consequence, the plasma membrane of sperm loses the integrity and fluidity necessary for acrosome reaction and fusion with the oolemma. Superoxide appears to be the main inducer of lipid peroxidation (114). The initiation of lipid peroxidation is thought to involve the generation of hydroxyl radicals from the hydrogen peroxide and superoxide anion by the sperm and the contaminating leukocytes in the ejaculate (113). Iron catalyzes the peroxidation of constituents of plasma membranes of sperm and results in a loss of sperm-function. This is reflected in a failure of the sperm to exhibit sperm-oocyte fusion in response to Ca ionophore.

Additional evidence for the adverse effects of lipid-peroxidation on sperm-oocyte fusion has also come from studies in which several antioxidants, (α-tocopherol, superoxide dismutase, xanthine oxidase, catalase), partially rescue the function of sperm exposed to inducers of ROD (118-121).

7.2. Immature sperm surface proteins

SUZI or partial zona dissection are being employed for treatment of severe male factor infertility or standard IVF failure. These techniques allow sperm to directly access to the oolemma, by bypassing the ZP. Similar to the physiological fertilization process, only acrosome-reacted sperm bind to ZP-free oocytes. These studies indeed confirm that oolemma is strictly selective for acrosome-reacted sperm. Interestingly, direct cytoplasmic injection of sperm produces higher fertilization rates than SUZI and partial zona dissection when the same sperm preparation is used (122). Therefore, it appears that the acrosome reaction may be only required for sperm to penetrate the ZP and fuse with the oolemma. The acrosome status does not influence the ability of sperm heads to decondense in the ooplasm and form into a male pronucleus. The use of SUZI in IVF has shown that one cause of human infertility is due to incomplete processing of sperm surface proteins or immature surface proteins resulting in defective sperm-oocyte fusion.

7.3. Abnormal nuclear condensation

SUZI and ICSI have provided evidence that some fertility defects may be related to intrinsic sperm anomalies at the sperm membrane and/or chromatin (123). Such anomalies could lead to abnormal appearance of the pronuclei. Fertilizing sperm is known to trigger oocyte activation as a prelude to pronuclear formation. This is thought to occur by binding to a G-protein coupled receptor located in the oolemma. Activation of G-protein would induce the production of the second messenger, inositol triphosphate, which in turn would lead to increased level of intracellular Ca and lead to oocyte activation. A decrease of the amplitude and frequency of Ca oscillation can slow down the time course of pronuclear formation. A relationship between the organization of the sperm chromatin and the success of fertilization has been reported (123). Sperm with abnormal nuclear chromatin are more frequent in infertile men than in fertile men and may lead to the reduced capacity of sperm to fertilize an oocyte *in vitro*. One cause of this is the decrease in protamine P2 content in sperm chromatin (123). Thus, decondensation of sperm head may be hampered when chromatin organization is abnormal.

7.4. Antisperm antibodies (ASA)

Presence of ASA in the male or female have been shown to interfere with sperm-oocyte interaction (124-130). ASA are directed to multiple sperm antigens and can interfere with normal fertility by preventing the sperm transport or possibly by interfering with sperm-oocyte fusion and fertilization of oocyte (125-127). Hendry and coworkers studied infertile couples who underwent treatment by IVF (128). In their study, the fertilization rate in men with ASA was significantly reduced when compared to men without ASA. However, the pregnancy rate per embryo transfer was not significantly different in the two groups. This indicated that ASA in the male interfere with sperm-egg fusion and subsequent fertilization but once fertilization has occurred, the pregnancy rate remains the same. Sperm-associated ASA interfered with sperm binding to the ZP but not to the oolemma of the oocytes that had failed to fertilize *in vitro*, or the oolemma of the human ZP-free oocyte (129). Based on this evidence, it was suggested that ASA blockade of fertilization may be due to significant impairment of sperm binding to the ZP. These findings contrasts with studies in which ASA did impair sperm penetration and decondensation in ZP-free hamster oocytes (124). The success of IVF using SUZI of antibody-coated sperm was inversely related to the density of sperm-bound antibodies (130). Thus, ASA reduce fertilization by interfering with the regulatory mechanisms of events leading to pronuclear formation.

8. CONCLUDING REMARKS

In the past five years, our knowledge of sperm-oocyte interaction has increased considerably. Sperm-oolemma adhesion and fusion appear to be the result of coordinated expression of several of cell adhesion molecules. The sperm-oocyte adhesion appears to be initiated primarily by the β1-integrin cell adhesion molecules. Whereas, sperm-leukocyte recognition and sperm entry is initiated by the binding of β2-integrin protein, CR3, to antisperm antibody and C-coated motile sperm. On the other hand, the entry of sperm into non-phagocytic cells and genital tract epithelial cells is non-integrin mediated. Reproductive biologists are now faced with the challenge to further unravel the relevant molecules involved in sperm membrane-oolemma adhesion and fusion. Availability of antibody probes to generate a

variety of human gamete-specific recombinant proteins and the use of human IVF systems to assess their function would certainly help to unravel the molecular events that lead to human physiological fertilization. Identification of a subset of patients with specific defects at the level of sperm membrane-oolemma interaction will be quite useful in further delineating the functional significance of these adhesion molecules. This rapidly moving area of reproductive biology may provide us with important tools for contraception. Alterations of receptor/counter-receptors on gametes, leading to a delay or prevention of binding, fusion and/or oocyte activation is one possible approach for reversible contraception. Moreover, qualitative molecular changes affecting the adhesion of sperm membrane-oolemma can be used as a basis for intervention in fertilization. In principle, it should be possible to inhibit sperm-oocyte interaction by inducing antibodies to the unique adhesive receptors or by peptides reproducing integrin binding sites of sperm or the active site of putative oocyte integrin. Inhibition of fertilization at the level of sperm-oocyte fusion has been documented in the presence of auto or iso ASA and mAbs specific to sperm head antigens.

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