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Role of zona pellucida glycoproteins during fertilization in humans

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ABSTRACT

In the last decade, scientific investigations pertaining to the role of zona pellucida (ZP) glycoproteins during fertilization in humans have led to new insights. This has been achieved using purified native/recombinant human zona proteins and transgenic mice expressing human ZP glycoproteins. The proposed model in mice of ZP glycoprotein-3 (ZP3) acting as primary sperm receptor and ZP glycoprotein-2 (ZP2) as secondary sperm receptor has been modified for sperm–egg binding in humans. ZP glycoprotein-1 (ZP1), ZP3, and ZP glycoprotein-4 (ZP4) have been shown to bind to the capacitated human sperm. ZP2 binds to the acrosome-reacted human spermatozoa. Further, the eggs obtained from transgenic mice expressing human ZP2 alone or in conjunction with other human instead of mouse zona proteins showed binding of human sperm, suggesting that ZP2 might also play a role in sperm–egg binding. This function has been mapped to a domain corresponding to amino acid residues 51–144 of ZP2. In contrast to mice, where ZP3 is the primary agonist for inducing the acrosome reaction, in humans, the acrosome reaction can be mediated by ZP1, ZP3, and ZP4. The effect of mutations in the genes encoding zona proteins on the ZP morphology and infertility has not been established. Further, the role of autoantibodies against ZP in women with 'unexplained infertility' leading to poor outcome of in vitro fertilization is currently controversial and needs further investigations. Understanding the role of ZP glycoproteins during human fertilization facilitates the development of new contraceptives and strategies to overcome the problem of infertility.

1. Introduction

Fertilization involves the fusion of two highly differentiated haploid gametes (the egg and the spermatozoon) leading to the formation of a single cell embryo, which is essential for generating new progeny. During fertilization, spermatozoa initially bind to the zona pellucida (ZP). The ZP is an extracellular, glycoproteinaceous coat that surrounds mammalian oocytes. Under physiological conditions, ZP matrix plays an important role in taxon-specific binding of the spermatozoa to the oocyte and induction of the acrosome reaction in the spermatozoa bound to ZP. It also plays a role in the prevention of polyspermy, which leads to the formation of non-viable polyploid embryos subsequent to the fusion of sperm membrane with oolemma, and protection of the growing embryo till implantation. Hence, it is critical to understand the composition of ZP matrix and the role of its constituents during fertilization, which will facilitate development of new treatment regimens to overcome infertility. These investigations will also help in the development of novel contraceptives including ZP-based contraceptive vaccines. It may be prudent to mention here that intracytoplasmic sperm injection (ICSI) commonly used in the infertility clinics to overcome the

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problem of infertility, bypass the essential physiological role of ZP glycoproteins in sperm–egg binding and the ZP-induced acrosome reaction. In this manuscript, the current status pertaining to the role of ZP glycoproteins during fertilization in humans will be reviewed. How their role differs during fertilization in humans compared with the well-studied mouse model will also be highlighted. The relevance of autoantibodies against the ZP in women with 'unexplained infertility' and the outcome of in vitro fertilization (IVF) will also be described.

2. Composition of ZP matrix

In mammals, the ZP is composed of either three or four glycoproteins. For example, in mice, ZP matrix is composed of three glycoproteins designated zona pellucida glycoproteins-1 (ZP1), -2 (ZP2), and -3 (ZP3) (Bleil and Wassarman, 1980a). Characterization of ZP from pig oocytes revealed that it is also composed of three glycoproteins, but instead of ZP1, zona pellucida glycoprotein-4 (ZP4) is present (Hedrick and Wardrip, 1987). Bioinformatics analysis confirmed *Zp4* to be a pseudogene in the mouse genome (Goudet et al., 2008). Interestingly, the presence of all four zona glycoproteins has been documented in the ZP matrix of human oocytes (Lefièvre et al., 2004; Chakravarty et al., 2005; Ganguly et al., 2010). Molecular characterization of human ZP glycoproteins showed that the *Zp1* gene is located on chromosome 11 and encodes a polypeptide of 638 amino acids (aa) (Hughes and Barratt, 1999; Lefièvre et al., 2004; Ganguly et al., 2010). The human *Zp2* gene is located on chromosome 16 and encodes a polypeptide of 745 aa (Liang and Dean, 1993). The human *Zp3* gene is located on chromosome 7 and encodes a polypeptide of 424 aa (Chamberlin and Dean, 1990). In addition, the polymorphic form of the human *Zp3* gene, having an extra G in exon 8 and thereby encoding a truncated and probably nonfunctional protein of 372 aa residues, has also been reported (Van Duin et al., 1992). In humans, POMZP3 encoded by a novel bipartite RNA transcript derived from a gene homologous to rat *POM121* (encoding a nuclear pore membrane protein) and *Zp3* has also been reported (Kipersztok et al., 1995). The human *Zp4* gene is located on chromosome 1 and encodes a 540 aa long polypeptide (Harris et al., 1994). Human ZP glycoproteins are heavily glycosylated. Human ZP has exposed mannosyl, N-acetylglucosaminyl, and beta-galactosyl residues. After removal of terminal sialic acid residues, the presence of beta Gal-(1-3)GalNAc sugar sequences has also been shown (Maymon et al., 1994). Characterization of the glycosylation pattern of native immunoaffinity purified human ZP glycoproteins by selective removal of *N*-linked glycosylation by N-glycosidase-F and *O*-linked glycosylation by alkali hydrolysis suggests that human ZP2, ZP3, and ZP4 might have predominantly *N*-linked glycosylation (Chiu et al., 2008b). Mass spectrometry data of mammalian-expressed recombinant human ZP3 revealed that out of four potential *N*-linked oligosaccharide sites, three (Asn_{125}, Asn_{147}, and Asn_{272}) are occupied and *O*-linked glycans was observed in two clusters of 156–173 and 260–281 aa residues (Zhao et al., 2004). Ultrasensitive mass spectrometric analysis of the human ZP revealed that the sialyl lewis^X sequence [NeuAcα2-3Galβ1-4(Fucα1-3)GlcNAc] is the most abundant terminal sequence on both *N*- and *O*-linked glycans (Pang et al., 2011).

3. Role of ZP glycoproteins in sperm–egg binding

To understand the role of respective ZP glycoproteins in human sperm–egg binding, various studies have been performed employing either purified native or recombinant zona proteins. To gain further insight into the role of ZP glycoproteins in sperm–egg binding, transgenic animals have been used, in which mouse zona proteins have been replaced with human zona proteins and the ability of the humanized zonae pellucidae to bind human spermatozoa has been investigated. These investigations have led to the following observations:

i) *In humans, in addition to ZP3, ZP4 also binds to the capacitated sperm*: To investigate the role of human ZP3 and ZP4 in binding to human spermatozoa, these were expressed in *Escherichia coli* as well as in the insect cells using the baculovirus expression system (Chakravarty et al., 2005). The purified recombinant ZP3 and ZP4 were labeled with fluorescein-isothiocyanate (FITC) and used to study their binding profile with the capacitated acrosome-intact and the acrosome-reacted human sperm by direct immunofluorescence assay (Chakravarty et al., 2008). These studies revealed that both ZP3 and ZP4 showed binding to either acrosomal cap or the equatorial region of the capacitated acrosome-intact spermatozoa (Table 1; Chakravarty et al., 2008). However, differences in the binding profile of ZP3 and ZP4 to capacitated human sperm have been observed. In comparison to ZP3, a higher proportion of capacitated sperm showed binding of ZP4 to the acrosomal cap. Acrosome-reacted sperm failed to show any binding of ZP3 or ZP4 to the acrosomal cap. Both *E. coli*- and baculovirus-expressed ZP3 and ZP4 showed a similar binding profile to spermatozoa, suggesting that glycosylation of ZP3 and ZP4 might not be essential for their binding to sperm (Chakravarty et al., 2008). Interestingly, co-localization experiments using recombinant ZP3 and ZP4 labeled with different fluorescent tags showed simultaneous binding to the acrosomal cap of capacitated spermatozoa, suggesting that these proteins might bind to different ligands on spermatozoa. To confirm binding characteristics of human ZP3 and ZP4 to human sperm, these proteins have been purified from human zonae pellucidae using highly specific monoclonal antibodies (MAbs)-based immunoaffinity column (Chiu et al., 2008b). The purified native human ZP3 binds to the acrosome, equatorial region and mid-piece of the acrosome-intact capacitated spermatozoa, which was observed only in the mid-piece of the sperm after the acrosome reaction (Table 1) (Chiu et al., 2008b). Purified human ZP4 (presence of a minor contaminant of ZP1) binds to the entire head region of capacitated sperm, which was lost after the acrosome reaction (Table 1) (Chiu et al., 2008b). The observed binding of human ZP3 to the capacitated