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Life begins after successful accomplishment of fertilization leading to the formation of a single cell embryo, which is characteristic of virtually all animal species and is absolutely essential in order to generate new progeny who exhibit all the attributes of the species. During fertilization two haploid gametes (the egg and the spermatozoa) unite to produce a genetically distinct individual. The union between the spermatozoa and the egg is arguably the most important interaction in biology. The initial contact for fertilization is between the sperm and the zona pellucida (ZP), an extracellular, glycoproteinaceous coat that surrounds all vertebrate oocytes. In essence, the ZP serves as a "gate-keeper" to regulate sperm binding as it operates as a species-selective substrate during binding of spermatozoa and changes in the ZP, subsequent to completion of fertilization, eliminate binding of sperm, as part of the block to polyspermy. These and other features of the egg ZP strongly suggest that the ZP contains receptors ("sperm receptors") that need to be recognized by free-swimming sperm in order for them to bind to ovulated eggs in a species-specific manner.

The egg envelope in vertebrates has been reported to be composed of ZP glycoproteins (Primakoff and Myles, 2002; Wassarman 1988). To date, many ZP glycoproteins have been isolated and cloned, and form a large ZP glycoprotein family. The composition of the ZP glycoproteins expressed in one species shows obvious variations among vertebrates, especially non-mammalian ones. The biological significance of these variations is not understood. Until very recently, mammalian ZP was believed to be composed of only three glycoproteins of the ZP family, ZP1, ZP2 and ZP3, as first described in the mouse species (Bleil and Wassarman, 1980a). However, the description of the complete genome in some species, like human and rat has resulted in the detection of new proteins expressed in the ZP. Recent studies have revealed that some mammals present a ZP formed by four glycoproteins, e.g., rats, hamsters, monkeys and humans (Izquierdo-Rico et al., 2009; Ganguly et al., 2008; Hoodbhoy et al., 2005; Lefievre et al., 2004). These four glycoproteins have been designated as ZP1, ZP2, ZP3, and ZP4. In humans, ZP4 was first identified as an orthologue of mouse ZP1, but later studies revealed that the
true orthologue of mouse Zpl was a different gene called Zp1 (Hughes and Barratt, 1999). Further, employing molecular and proteomic approaches the four genes and the corresponding proteins were identified, respectively (Lefievre et al., 2004). The above studies contrast with other studies using a mouse model in which mass spectrometric analysis failed to identify ZP4. The orthologue of the human Zp4 gene is present in the mouse genome as a pseudogene (Lefievre et al., 2004) but a functional protein is not expressed. Thus, depending on the species analyzed, ZP is formed by three or four glycoproteins. In species like pig (Hedrick and Wardrip, 1987), cow (Noguchi et al., 1994), and dog (Goudet et al., 2008), the presence of three glycoproteins has also been described, but in these species the proteins are ZP2, ZP3 and ZP4. Zpl has been identified as a pseudogene in the dog and bovine genome (Goudet et al., 2008). In non-mammalian species, more than four genes have been detected, for example, in chicken genome (Goudet et al., 2008; Bausek et al., 2000) six genes are present (Zpl, Zp2, Zp3, Zp4 ZpAX, ZpD) and in Xenopus genome there are five genes encoding ZP proteins (Zp2, Zp3, Zp4, ZpD, ZpAX) (Goudet et al., 2008). These observations suggest that the expression of both ZP1 and ZP4 genes represents an ancestral condition present before the mammalian and avian lineages diverged. Thus, ZP1 and ZP4, previously considered orthologues, are in fact paralogues. These two genes come from an ancestral gene through duplication (Goudet et al., 2008; Bausek et al., 2000; Hughes and Barratt, 1999). Their persistence across the higher vertebrates is particularly significant given that a number of proteins involved in reproduction, including ZP2 and ZP3, have been shown to be under high selective pressure, representing some of the most rapidly diverging genes (Swanson and Vacquier, 2002). Thus one might reasonably predict that the preservation of both ZP1 and ZP4 genes in the higher vertebrates implies that they have functional importance.

Taking all these data into consideration, it seems that the composition and, consequently, the structure of the mammalian ZP is more complicated than expected because, depending on the species: (1) it is formed by three or four glycoproteins; (2) in the three glycoprotein model it can be formed by ZP1, ZP2 and ZP3 or ZP2, ZP3, and ZP4; (3) the protein responsible for sperm binding is also quite varied.
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across different species. For example, in the mouse model, ZP3 binds to capacitated acrosome-intact spermatozoa and is also responsible for induction of acrosomal exocytosis, thus acting as putative primary sperm receptor (Beebe et al., 1992; Bleil and Wassarman, 1980b). Murine ZP2 binds to the acrosome-reacted spermatozoa and thus, acts as secondary sperm receptor (Bleil et al., 1988) while ZP1 cross-links the filaments formed by ZP2-ZP3 heterodimers and appears to provide mainly stability and structural integrity to the ZP matrix (Greve and Wassarman, 1985). However, the porcine ZP3β (homologue of ZP3) fails to bind to sperm receptors whereas ZP3α (homologue of ZP4)-ZP3β heterocomplexes bind with high affinity to boar sperm membrane vesicles, suggesting involvement of more than one ZP protein in sperm recognition (Yurewicz et al., 1998). In rabbits, rec55 (homologue of ZP4) binds to the spermatozoa in a dose-dependent manner (Prasad et al., 1996). The importance of rabbit ZP4 in sperm-oocyte interaction is reaffirmed by the observation that it also binds to recombinant Sp17 (a family of sperm autoantigens) (Yamasaki et al., 1995). Studies have also demonstrated that recombinant bonnet monkey (Macaca radiata) ZP4 (bmZP4) expressed in E. coli binds to the head region of capacitated spermatozoa and the binding shifts to the equatorial segment, post-acrosomal domain and mid-piece of the acrosome-reacted spermatozoa, indicating a role for ZP4 in the sperm binding (Govind et al., 2001). In humans, employing both recombinant and affinity purified native zona proteins; it has been shown that both ZP3 and ZP4 bind to capacitated acrosome- intact spermatozoa (Chakravarty et al., 2008; Chiu et al., 2008a). Interestingly, both the above proteins are able to induce acrosomal exocytosis in capacitated human spermatozoa (Chakravarty et al., 2008; Chiu et al., 2008b; Caballero-Campo et al., 2006; Chakravarty et al., 2005).

The above described observations suggest that the functional role of the individual ZP glycoproteins during the complex process of fertilization as delineated in mouse model may not be applicable to other species. As now it is known that four ZP genes, instead of three, are transcribed and translated in the human oocyte, a re-evaluation would be required to determine the structure of the ZP and the mechanism associated with sperm-zona interaction. As yet, no information is
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available on expression of human ZP1 in follicles at different stages of development or its functional significance. Hence, the present study is aimed at investigation of the presence of ZP1 protein in the ovary of non-human primate (bonnet monkey) and humans, investigating the expression pattern of ZP1 in human oocytes and follicles and to characterize its functional significance. To achieve this, the cDNA encoding human and bonnet monkey ZP1 was isolated. In the present study, attempts have also been made to express the human ZP1 glycoprotein and its fragments, in prokaryotic as well as eukaryotic expression systems, purify the recombinant proteins in the absence of chaotropic agents and evaluate their functional attributes with respect to interaction with human spermatozoa and induction of acrosomal exocytosis. The E. coli expression system would express non-glycosylated zona proteins whereas eukaryotic expression system would produce glycosylated recombinant zona proteins thus aiding in evaluating the importance of glycosylation in the above processes. In addition, highly specific polyclonal and monoclonal antibodies have been generated against, synthetic peptides corresponding to human ZP1 amino acid sequence as well as recombinant human ZP1 fragments, which are devoid of cross-reactivity with recombinant human ZP2, ZP3 and ZP4. These antibodies have been used to establish the expression profile of ZP1 in oocytes as well as ovaries.

Hence, as till date no information is available on the role of human ZPI during fertilization, the proposed studies in the present thesis unveils a new-fangled depiction of human fertilization which will lead to a better understanding of the molecular mechanism of human sperm-ZP interactions, thereby facilitating further optimization of protocols for successful fertilization in the in vitro fertilization (IVF) program and development of new contraceptive strategies.