Introduction
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Development of an effective and safer contraceptive vaccine is one of the possibilities to curtail the burgeoning human global population. Achieving contraception by means of vaccine is a novel approach, which entails generation of specific antibody response against antigens critically involved in the process of mammalian reproduction. Zona pellucida (ZP) glycoproteins have been considered to be the attractive target antigens for designing an immunocontraceptive vaccine by virtue of their critical involvement during fertilization.

ZP, an acellular translucent envelope surrounding the oocyte serves as the docking site for the species-specific recognition and binding of the spermatozoa to the oocyte, induces acrosome-reaction in the zona bound spermatozoa, affects avoidance of polyspermy and protects the preimplantation blastocyst. ZP comprises of three biochemically and immunologically distinct glycoproteins, which have been classified as ZP1, ZP2 and ZP3 on the basis of their mobility on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). In the murine model, ZP3 (~83 kDa) acts as the primary receptor for sperm binding to the oocyte and induces acrosome-reaction in the spermatozoa bound to ZP (Bleil and Wassarman, 1980a; 1983). ZP2 (~120 kDa) acts as a secondary receptor and maintains the binding of the acrosome-reacted spermatozoa to the ZP (Bleil et al., 1988). ZP1 (~200 kDa; dimer) has been postulated to cross-link ZP2-ZP3 heterodimer filaments (Greve and Wassarman, 1985). However, it is becoming increasingly apparent that the molecular mechanisms of the sperm-oocyte interaction vary among the mammalian species. In porcine system, ZP3 fails to bind to the spermatozoa. Instead, ZP1-ZP3 heterocomplexes have been shown to bind with high affinity to boar sperm-associated zona receptors (Yurewicz et al., 1998). In the rabbit
model, baculovirus-expressed ZP1 has been shown to bind to the sperm in a dose-dependent manner (Prasad et al., 1996). These observations suggest that ZP1 may also possess sperm-receptor activity, and information available from mouse model may not necessarily hold true in other species. Further, in murine model it was established that glycosylation of ZP3 is critical for its binding to the spermatozoa (Florman et al., 1984; Florman and Wassarman, 1985). However, recent experiments demonstrating the binding of recombinant (r) human ZP3, expressed in E. coli, suggest that polypeptide backbone is sufficient for binding to the human spermatozoa (Chapman et al., 1998). The involvement of more than one ZP glycoproteins in the initial binding of spermatozoa to the oocyte and ability of the non-glycosylated ZP3 in humans to bind to the sperm and trigger acrosome-reaction, demands for more careful investigations with respect to the role of the ZP glycoproteins and their glycosylation in sperm-oocyte interaction. Availability of cDNA from various species has made it possible to obtain ZP glycoproteins by employing heterologous expression system, thus providing a homogenous molecular population free from other ovarian contaminants. The recombinant proteins will be useful in understanding the molecular mechanisms of gamete interaction.

It has been known for quite some time that antibodies against ZP glycoproteins can successfully inhibit sperm-egg recognition and binding in vitro (Sacco et al., 1981; Bagavant et al., 1993; Gupta et al., 1996; Afzalpurkar et al., 1997a). Studies in several animal models have demonstrated that infertility can be achieved following active immunization either with native or recombinant ZP proteins (Skinner et al., 1984; Dunbar et al., 1989; Sacco et al., 1989; Jones et al., 1992; Paterson et al., 1992; Bagavant et al.,
1994; Martinez and Harris, 2000). However, it is invariably associated with either transient or irreversible changes in cyclicity, alteration of the sex steroids hormonal profile and disrupted follicular development in the ovary (Skinner et al., 1984; Dunbar et al., 1989; Paterson et al., 1992). This ovarian dysfunction has been attributed to various factors such as the purity and form of the antigen used, susceptibility of the animal model to immunization with the self proteins, the adjuvants employed and the presence of oophoritogenic T-cell epitopes on the immunogen. Another factor that can influence the outcome of such an immunization approach for fertility regulation is the expression of the ZP glycoproteins at various stages of follicular development and other ovarian cells such as granulosa cells.

It is desirable to generate humoral immune response that can inhibit fertilization without any deleterious effect on ovarian functions. To achieve this, efforts have been directed to identify and characterize the B cell epitopes on ZP that may elicit the appropriate immune response enough to regulate fertility without concomitant ovarian dysfunction. Segregation of the oophoritogenic T cell epitope and immunization of the female mice with a chimeric peptide comprising of a "promiscuous" foreign T cell epitope and modified peptide corresponding to B cell epitope of mouse ZP3 conferred infertility without concomitant ovarian pathology, which demonstrated the feasibility of such an approach for development of the immunocontraceptive vaccine (Lou et al., 1995a).

In order to critically evaluate the immunocontraceptive potential of a candidate ZP based antigen meant for human purpose, there is a requirement to evolve a suitable animal model that is closely related to humans. In this direction, in our laboratory, we have cloned and sequenced the bonnet monkey (bm; *Macaca radiata*) ZP1 (bmZP1). ZP2
The deduced amino acid (aa) sequence of the bmZP1, bmZP2 and bmZP3 revealed sequence identity of 92%, 94.2% and 93.9% with their respective homologues in human. The high sequence identity of ZP glycoproteins from bonnet monkey with human reiterates the suitability of this animal model not only to assess the potential of the candidate antigen to regulate fertility but also for the scrupulous analysis of any ovarian dysfunction subsequent to immunization with the self protein.

In this thesis, the bmZP1 cDNA was cloned and expressed in prokaryotic expression system. Initial attempts to express and purify r-bmZP1 using pQE30 vector revealed presence of lower molecular weight fragments. These lower molecular weight fragments might represent premature termination products during translation or may arise due to proteolytic degradation (specific or non-specific). Moreover, the purified r-bmZP1 required presence of 4 M urea to maintain it in soluble form. To address these issues, bmZP1 was expressed in E. coli strain deficient in certain proteases (ompT and lon) and as a fusion protein with histidine-tag at C-terminal end. An alternate purification regimen has been established in order to purify the r-bmZP1 in soluble (devoid of chaotropic agents) and biologically active form. Having accomplished this, the binding characteristics of r-bmZP1 to the bonnet monkey spermatozoa were also investigated to assess the role of bmZP1 during fertilization.

In order to evaluate the contraceptive efficacy of r-bmZP1 to regulate fertility, the polyclonal antibodies generated in rabbit against r-bmZP1 conjugated to diphtheria toxoid (DT) were assessed for their ability to recognize homologous (bonnet monkey) and heterologous (human and baboon) ZP. Subsequently, contraceptive efficacy studies
were carried out \textit{in vivo} in female bonnet monkeys and baboons. A group of 4 female baboons was immunized with the r-bmZP1 (purified from pQE30-bmZP1 clone) conjugated to DT. Similarly, 5 female bonnet monkeys were immunized with r-bmZP1 (purified from pRSET-bmZP1 clone) conjugated to DT. The animals were followed for the antibody titers against r-bmZP1 and DT, menstrual cyclicity, perineal sex skin swelling profile (for baboons), progesterone profile, and fertility status.

In order to determine antigenic domains on bmZP1, monoclonal antibodies (MAbs) capable of inhibiting human-sperm interaction \textit{in vitro} were generated against r-bmZP1. Using multipin peptide synthesis approach (Geysen et al., 1985), a panel of dodecamer peptides with hexameric overlap corresponding to the deduced aa sequence of bmZP1 (excluding the N-terminal signal sequence and C-terminus transmembrane-like domain) were screened in ELISA for binding with MAbs. The minimum binding domain for these MAbs was further identified using octamer peptides synthesized with an increment of one aa at a time. Identification and characterization of the pertinent epitopes, and utilization of these domains for the development of contraceptive vaccine, will help in accentuating the appropriate immune response necessary for induction of infertility and eliminating the motifs responsible for the ovarian dysfunctions. Some of these issues have been addressed in the present thesis.