

## COMPARISON OF ZP3 PROTEIN SEQUENCES AMONG VERTEBRATE SPECIES: TO OBTAIN A CONSENSUS SEQUENCE FOR IMMUNOCONTRACEPTION

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### 1. ABSTRACT

The deduced ZP3 amino acid (aa) sequences of 13 vertebrate species namely mouse, hamster, rabbit, pig, porcine, cow, dog, cat, human, bonnet, marmoset, carp, and frog were compared using the PILEUP and PRETTY alignment programs (GCG, Wisconsin, USA). The published aa sequences obtained from 13 vertebrate species indicated the overall evolutionary conservation in the N-terminus, central region, and C-terminus of the ZP3 polypeptide. More variations of ZP3 polypeptide sequences were seen in the alignments of carp and frog from the 11 mammalian species making the leader sequence more prominent. The canonical furin proteolytic processing signal at the C-terminus was found in all the ZP3 polypeptide sequences except of carp and frog. In the central region, the ZP3 deduced aa sequences of all the 13 vertebrate species aligned well, and six relatively conserved sequences were found. There are 11 conserved cysteine residues in the central region across all species including carp and frog, indicating that these residues have longer evolutionary history. The ZP3 aa sequence similarities were examined using the GAP program (GCG). The highest aa similarities are observed between the members of the same order within the class mammalia, and also (95.4%) between pig (ungulata) and rabbit (lagomorpha). The deduced ZP3 aa sequences per se may not be enough to build a phylogenetic tree.

### 2. INTRODUCTION

The zona pellucida (ZP) forms an extracellular matrix around the developing oocyte and the preimplantation embryo (1). ZP is involved in several functions during fertilization, including mediation of species-specific binding of sperm to egg, induction of acrosome reaction of the bound sperm, and prevention of polyspermy. ZP also serves to protect the embryo prior to implantation in the uterine wall.

Studies in the mouse have suggested that ZP is composed of three sulfated glycoproteins, referred to as ZP1, ZP2, and ZP3, respectively (2, 3) and specific functions have been ascribed to each. ZP3 induces the sperm acrosome reaction and mediates the initial binding of sperm to the egg via O-linked oligosaccharide side chains. ZP2 acts as a secondary sperm receptor, and along with ZP3, is biochemically modified after fertilization to provide a block to polyspermy. An  $\alpha$ -linked oligosaccharide of the ZP3 protein has been shown to be essential for sperm binding (4). The O-linked oligosaccharide on one or more of the serine residues of murine ZP3 (from position 331 to 335) are critical for sperm receptor activity (5). ZP2 and ZP3 exist as dimers in long filaments that appear to be cross-linked by ZP1 (6).

The importance of ZP in the fertilization process has long been recognized, and therefore it has been the focus of extensive research for more than three decades (7). During the last 10 years, ZP3 cDNAs of several species have been cloned and sequenced, that include mouse (8, 9), hamster (10), rabbit (11), pig (12), cow, dog, cat, and porcine (13), human (14), bonnet (15), marmoset monkey (16), carp (17), and frog (18). The human recombinant ZP3 protein has been expressed in Chinese hamster ovary (CHO) cells and the glycosylated recombinant protein induces sperm acrosome reaction (19).

Because active immunization with the native and recombinant ZP proteins induces infertility in primates (20, 21), attempts have been made to utilize ZP3 as an immunogen for contraceptive vaccine development. However, immunization with whole ZP also leads to transient or complete loss of ovarian function (22, 23) and in primates, a gradual loss of the primordial oocyte pool results in a state akin to premature menopause (24).

## ZP3 protein sequences among vertebrate species

Since the cell-mediated immunity (CMI) is primarily responsible for oophoritis, it is thought that by eliminating T-cell epitopes of ZP3 in vaccine formulation, one might be able to avoid ovarian failure and still able to retain the antibody-mediated reversible inhibition of fertility. During last five years, the research has focussed on obtaining minimal murine B-cell epitope sequence(s) of ZP3 that can produce specific antibody response (25, 26). In these studies, typically a T-cell epitope from a non-ZP/ovarian source such as from *Plasmodium falciparum*/tetanus toxoid/diphtheria toxoid was conjugated to ZP3-derived B-cell epitope (synthetic peptide) to produce target-specific antibodies and an irrelevant T-cell response. The aim of the present study was to search for a consensus sequence(s) among the ZP3 sequences of 13 vertebrate species (mouse, hamster, rabbit, pig, porcine, cow, dog, cat, human, bonnet, marmoset, carp and frog) that could be used for the development of a contraceptive vaccine applicable to various species of animals.

### 3. MATERIALS AND METHODS

The deduced aa sequences were obtained from the published sequences of ZP3 cDNAs of 13 vertebrate species (discussed above) and typed into computer manually. The PILEUP and PRETTY programs (GCG, Winsconsin, USA) were employed to generate a consensus sequence. The aa sequence similarities were calculated using the GAP program (GCG).

### 4. RESULTS

#### 4.1. Alignment of ZP3 deduced amino acid sequences

The deduced amino acid sequences of ZP3 is shown in figure 1.

#### 4.2. ZP3 protein similarities

The similarities of the ZP3 proteins are shown in table 1.

### 5. DISCUSSION

The existing ZP3 deduced aa sequences of 13 vertebrate species were aligned by the PILEUP and PRETTY programs. The aa sequences of carp and frog, which are two lower vertebrate species and far distantly related to the 11 mammalian species, showed extremely divergent alignment at the N- and C-termini, but normal alignment in the central region. The consensus aa sequences from all the 13 vertebrate species came out almost the same, which indicates the overall conservation of the ZP3 protein.

Structurally, three regions of the sequence alignment could be detected. These are: 1).putative leader sequences and the first 20-25 amino acid of the mature protein (between position 1 to 129); 2).the central region (in position 130 to 401); and 3).the C-terminal region of the protein (between position 402 to 518). However, the extremely divergent alignments of carp and frog with other

11-mammalian species make the leader sequence more prominent.

All the ZP proteins showed a putative transmembrane domain (27) near the C-terminus. The canonical furin proteolytic processing signal (R-X-R/K-R) (28) occurs just prior to the transmembrane domain in all species except carp and frog. The transmembrane domain might be removed from the mature protein at the furin processing site. The higher variations in the N- and C-termini could lead to species-specificity.

An epitope of murine ZP3 (NSSSSQFIHGPR, from position 412 to 426) in the central region occurring immediately before the furin proteolytic cleavage site has been identified by a monoclonal antibody which blocks fertilization (25). A synthetic human ZP3 peptide (GTPSHSRRQPHVMS, from position 411 to 426) has also been examined for the contraceptive potential (26). However, there is very little homology among various species in this region of the ZP3 protein. This region might contribute towards the species-specificity of sperm-egg binding.

The six relatively conserved sequences in the central region were found. These include: 1). 22 amino acids from position 129 to 151: VTVSKDLFGTGKLRP ADLTLG; 2). 54 amino acids from position 189 to 242: LVYSTFLLHDPGPNLSILRTNRAEVPICRYPRQGV S SQAAILPTWVPFRRT; 3). 24 amino acids from position 275 to 298: AHLQAEVHTGSHVPLRLFVDHCVA; 4). 16 amino acids from position 309 to 324: SPYHTIVDFHG CLVDG; 5). 28 amino acids from position 331 to 358: SAFKAPRPRPDTLQFTVDVHFANDSRN; and 6). 24 amino acids from position 378 to 401: LNKACFSKSSN SWFPVEGPADIC.

There are 11 conserved cysteine residues in the central region across all the species including carp and frog, indicating that these residues have a longer evolutionary history. Also, the conservation of large numbers of cysteine residues in the central region indicates the overall better evolutionarily conservation of this region.

The highest protein similarities were observed between the members of the same order within the class mammalia. For examples, the percentage of similarity between mouse and hamster (rodenta) is 83.9%; between dog and cat (carnivora) 83.5%; between human and monkey (primate) 95.5%; and between porcine and cow (ungulata) 84.3%. However, an exception was observed, i.e., pig (ungulata) was found to have the highest similarity (95.4%) with rabbit (lagomorpha). The high similarity between the members of dissimilar orders indicate that ZP3 aa sequences alone may not be enough to build a phylogenetic tree. The morphological change and molecular divergence are quite independent events, responding to different evolutionary pressure and following different set of rules (29). Therefore, studies that incorporate both molecular and morphological data will provide much better description and interpretation of biological diversity.



## ZP3 protein sequences among vertebrate species

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