

ABSTRACT

POTENCY ANALYSIS OF SUB-UNIT PROTEIN OF RABBIT SPERM MEMBRANE AS A CANDIDATE OF IMMUNOCONTRACEPTIVE MATERIAL

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These objectives of this research were to identify the rabbit sperm membrane proteins, to analyze the sub-unit protein of rabbit sperm membrane, and to test the potential of sub-unit proteins of rabbit sperm membrane via binding assay, both in-vitro and in-vivo fertilization methods.

Sperm membrane proteins were isolated from male rabbits of local strain. The other experimental animal was mice (*Mus musculus*), Balb/C strain. Rabbit's sperm membrane protein was electrophoresed in SDS-PAGE. Protein was immunized in mice. Serum was isolated from blood. This serum was analyzed for antibody titer, type of immunoglobulin, and antigen determinant. The sub-unit protein with molecular weight (MW) 46, 66, and 73 kDa was purified by electroelution, then followed by 2D electrophoresis. Each sub-unit protein was immunized in mice. The serum was collected. Then, the serum was used for the analysis of the strength of immune response, immunolocation, and agglutination test. Serum was used to test the potential contraceptive effect by using binding assay and in-vitro fertilization methods. Male mice was immunized with the sub-unit protein of MW 46, 66, and 73 kDa. Male mice was mated with female mice. After 15 days of gestation, the number of fetus was calculated.

The results of this research showed that: (1) The rabbit sperm membrane protein was composed of 7 sub-unit protein fraction with MW 16, 28, 34, 46, 66, 73 and 250 kDa. These had caused a cross-reaction in mice with variety of the immunoglobulin classes of IgG, IgM, and IgA. Protein that had MW 46, 66, and 73 kDa contained antigen determinant. (2) The sub-unit protein of rabbit sperm membrane with MW 46, 66, and 73 kDa had isoelectric points between pH 4.3 to 6.5. These were located on the sperm head in acrosome and tail of the middle piece. The protein with MW 46, 66, and 73 kDa had immunogenic character. Antibodies could increase the sperm agglutination of mice with most types of agglutination between the heads. (3) The anti sperm antibody against sub-unit protein that had MW 46, 66, and 73 kDa could prevent interaction of spermatozoa with oocytes which could decrease the binding rate about 93.45 to 97.04 %. This antibody could also decrease fertilization rate about 91.67 to 98.33 % in in-vitro fertilization. The sub-unit protein of rabbit sperm membrane that had been immunized into the mice male could inhibit the ability of sperm to fertilize the oocytes, thereby reducing the number of fetuses about 67.4 to 100 % in in-vivo fertilization.

Keywords: rabbit, sperm membrane, immunoconceptive, agglutination, fertilization