Characterization of a human spermatozoa surface antigens recognized by HS56 monoclonal antibody

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Abstract

Introduction: Surface antigens of ovum and sperm have key role in the fertilization process. In this regard, study of these molecules and their biochemical, biophysical and physiological characteristics could be helpful in understanding the mechanism of fertilization. Moreover, many cases of infertility with unknown ethiology have been revealed to have defects in these molecules. This will further emphasize on the importance of molecules involved in fertilization. The aim of this study was to characterize the sperm surface antigen recognized by the monoclonal antibody HS56.

Materials and Methods: Clone HS56 with known specificity to human sperm antigen were injected peritoneally into the balb/c mice and the produced antibody was purified over protein- G affinity chromatography column. The isotype of the monoclonal antibody (mAb) was determined by Enzyme-Linked Immunosorbent Assay (ELISA). Surface antigens extracted from human sperm by lithinm 3,5 diiodosalicylate (LIS) method were run on SDS-PAGE and the molecular weight of antigen recognized by HS56 mAb was determined by western blotting. For localization of this antigen, indirect immunofluorescence staining was performed. Finally involvement of this antigen in acrosome reaction was tested by inhibition assay. Wilcoxon test was used for statistical analysis.

Results: By LIS extraction method many sperm antigens were isolated and seen on SDS-PAGE using ELISA, It was found that LIS extraction was a useful method for isolation of antigen recognized by HS56 mAb. The subclass of the mAb HS56 was shown to be IgG1. The molecular weight of the antigen was determined to be about 56±2 KDa and the molecule included disulfide bond (s) in its structure. The antigen of interest was localized on acrosome and midpeace region of human sperm. Finally our results showed that this antigen had no effect on acrosome reaction (P=0.11).

Conclusion: Although the HS56 antigen does not play an important role in acrosome reaction, its further characterization with regard to sperm-oocyte binding may reveal a role in fertilization process.

Key Words: Sperm, Sperm surface antigen, Monoclonal antibody, Acrosome reaction, and Fertility.

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