520 Analysis of 3-0-sulfogalactose residue as an epitope of sperm immobilizing antibodies in sterile women's sera. <u>H.Fukuda</u>, <u>Y.Tsuji</u>, S.Isojima, Dept.Obst.and Gynec., Hyogo Medical College, Hyogo.

A new sperm immobilizing monoclonal antibody (SI-MAb) which was produced by immunizing mouse with human choriocarcinoma. The carbohydrate epitope of MAb 2H12 was identified as 3-0-sulfogalactose residue which located on terminal site of sulfoglycolipids such as sulfatide and seminolipid by using high performance thin layer chromatography immunostaining. Seminolipid is a specific sulfoglycolipid on the mammalian sperm (1973 Ishizuka et al., J. Biochem. 73,77). The sperm immobilizing activity of MAb 2H12 was inhibited by sulfatide and seminolipid but not by galactocerebroside. This result suggests that MAb 2H12 causes SI activity by binding to 3-0-sulfogalactose residue of seminolipid on the sperm surface. We examined whether SI-Abs of sterile women react to seminolipid on sperm as well as MAb 2H12. SI positive sera of sterile women were treated with same above, then examine their SI activities. The result was that SI activities were diminished by sulfatide and seminolipid, but not by galactocerebroside. These results suggest that 3-0-sulfogalactose residue of seminolipid may be important structure on human sperm to which SI-Abs could bind to cause sperm immobilization.

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Human sperm were sequentially extracted with PBS, NaClO4, deoxycholate (DOC) and sodium dodecyl sulfate (SDS) solutions. Two antigens were identified that interacted with the anti-sperm antibodies present in a serum obtained from an infertile woman by immunoblot. Their estimated Mr are 33 and 55 kD. Control serum from a healthy multigravida woman did not react with these sperm antigens. The PBS extract contained the 33 and 50 kD protein; the NaClO4, the 33-kD protein, DOC and SDS extracts contained no specific interacting protein. The 33- and 50-kD proteins were purified by Pharmacia FPLC system, hydroxylapatite chromatography, electroelution and reverse phase chromatography. Each sperm antigen migrated as a single homogenous band on analysis by SDS-PAGE. Both antigens did not bind to Con A and were not denatured on heating at 80% for 30 min., or by treatment with trypsin. The present findings of several anti-sperm antibodies in the serum from an infertile woman suggest that the cause of the infertility may be multifactorial rather than to the production of antibodies against a single specific sperm component.

522 The effects of sperm immobilizing antibodies in sera of infertile women on Japanese monkey sperm. K.Kameda, H.Fukuda, Y.Tsuji, K.Koyama,

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In order to study the interspecies cross-reactivity of sperm immobilizing antibodies (SI-Abs) found in sera of unexplained sterile women, the effects of patients' sera on monkey sperm were examined by using sperm immobilization test (SIT). Among 17 SI-Abs positive sera, 13 showed SI activity on monkey sperm as well, while among 14 SI-Abs negative sera, only 2 showed the activity but other 12 did not show any activity or very weak. All patients' sera showed a strong sperm agglutinating activity to monkey sperm, regardless of the results in SIT to human sperm. SI activity to human or monkey sperm in patients' sera could be absorbed out only with sperm of each corresponding species. None of human and mouse monoclonal antibodies with SI activities for human sperm showed SI and SA activities to monkey sperm. The treatment of human and monkey sperm with periodate strongly diminished their absorbing capabilities of SI-Abs in patients' sera. These results indicated that there might be two kinds of SI-Abs in patients: one to human sperm antigens and the other to monkey sperm, and these two SI-Abs might recognize different antigen epitopes which are possibly composed of carbohydrate moieties.