Antisperm antibodies (ASA) may be an important cause of infertility, but current tests for the detection of ASA have poor diagnostic value. The inadequacy of current tests, in part, reflects their inability to define the antigenic specificity of the sperm proteins that the ASA react with. Identification of the sperm proteins that ASA bind to is a necessary preliminary step to the development of more useful diagnostic tests for ASA. Identification of sperm proteins that are the antigens for ASA may also lead to a greater understanding of the basic biology of fertility. We have used two-dimensional electrophoresis and western blots to identify a 16 kDa sperm protein as the antigen for ASA from infertile men. Amino acid sequencing by mass spectrometry of tryptic peptides from the protein identified it a previously uncharacterised protein of the α-lactalbumin/c-type lysozyme family which we have named SPRASA. A polyclonal antiserum reactive with SPRASA indicated that SPRASA is localised to the acrosome of human, bovine, ovine and cervine sperm. SPRASA and a murine orthologue of SPRASA appear to be expressed primarily in the testis. We conclude that SPRASA is a highly conserved sperm protein that is the antigen for ASA from infertile men and that it is likely to be important in the fertility of humans and other species.