Molecular similarities between spermatozoa and bacteria: A fluorescent microscopy study

Vijay Prabha and **Harpreet Vander**

Department of Microbiology, Panjab University, Chandigarh- 160014, India.

The origin of natural anti-sperm antibodies in the serum of human virgin pre-pubertal girls and boys has been linked to the existence of inflammatory gastrointestinal entities; as a result of disease, cross reactive antibodies produced against gastrointestinal flora binds spermatozoa. Also, nonspecific bacterial vaccination (bacterial cell lysates), which are widely used to stop chronic childhood respiratory tract infections, may result in persistent infertility in some individuals, suggesting some form of molecular similarities between bacteria and sperm. These observations suggest that common antigenic determinants are shared by infectious organisms and reproductive system components.

In the present study we have also observed that fluorescent labelled sperm immobilization factor (SIF) isolated from *Staphylococcus aureus* not only binds to spermatozoa but to various gram positive and gram negative bacteria viz. *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica typhi*, *Shigella flexneri*, *Enterococcus faecalis*, *Bacillus cereus* and *Proteus mirabilis* indicating the presence of common SIF binding conformation on spermatozoa and bacteria. Coincubation with receptor binding SIF extracted and purified from spermatozoa completely inhibited SIF binding to all the bacteria tested. These results further provide evidence for molecular similarities between bacteria and spermatozoa.

Keywords Bacteria; spermatozoa

1.Introduction

The presence of 'natural' anti-sperm antibodies in fertile humans, virgin girls [1] and boys before puberty [2] has raised questions about the origin of these antibodies. Since the age-dependent pattern of 'natural' sperm antibody levels follows changes similar to those established for antibodies against exogenous antigens, rather than patterns typical for autoantigens [3], one hypothesis explaining their occurrence states that these may be cross reacting antibodies produced against exogenous antigens (bacteria, viruses, fungi, allergens). Cross-reactivity between certain epitopes on the bacterial surface and spermatozoa, particularly involving carbohydrate determinants, might be one potential triggering mechanism for the induction of ASA in males and females [4]. Several reports suggested the presence of cross-reactive antigens between spermatozoa and bacteria *Escherichia coli*, *Salmonella typhi* [4,5] and *Helicobacter pylori*[6]. Also, increased anti-sperm antibody levels have been reported among patients suffering from ulcerative colitis [7]. Witkin and Toth [8] explored the relationships between *Ureoplasma urealyticum* infection, antisperm antibodies, and infertility and reported that the incidence of antisperm antibodies in men with *U. urealyticum* infection was significantly higher than that in the control subjects.

In an earlier work done in our laboratory it was observed that SIF not only immobilizes spermatozoa but bacteria also indicating that the receptor for SIF might be shared between bacteria and spermatozoa. The aim of present work was to further evaluate the molecular similarities between the two.

2. Materials and Methods

2.1 Microorganisms

The bacterial isolate *Staphylococcus aureus* used in the present study was taken from the cervix of infertile woman suffering from unexplained infertility, attending the Department of Obstetrics and Gynecology, General Hospital, Sector-16, Chandigarh.

The standard bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica typhi*, *Shigella flexneri*, *Enterococcus faecalis*, *Bacillus cereus* and *Proteus mirabilis* used in the present study were procured from Microbial Type Culture Centre, IMTECH, Sector-39, Chandigarh, India.

2.2 Collection of semen sample

Human semen samples were obtained from males undergoing semen analysis by masturbation into sterile wide mouth containers. The ejaculates were collected from the clinical laboratory of General Hospital, Sector-16, and Department of Urology, PGIMER, Chandigarh. Human sperm ejaculates that satisfied the WHO criteria [9] were selected and used for further studies

2.3 Isolation and purification of Sperm Immobilization Factor (SIF)

SIF was isolated and purified from *S. aureus* by the protocol standardized earlier in our laboratory [10]. Briefly, 72 h old *S. aureus* culture filtrate was subjected to ammonium sulfate precipitation and then dialyzed against distilled water. SIF was further purified by molecular sieve chromatography (G-100) and DEAE cellulose chromatography.

2.4 Binding of FITC labelled SIF to human spermatozoa

2.4.1 Fluorescent labeling of ligand

An FITC Protein Labeling Kit (Bangalore Genei, Pvt. Ltd, India) was used to label SIF (1 mg/mL). 20µl of 1M sodium carbonate bicarbonate buffer was added to 200µl of SIF. 1 vial of Fluoroscein isothiocyanate (FITC) was reconstituted into 200µl of solvent and was immediately added in required amount according to F/p ratio. Reaction vial was completely covered with aluminium foil and was incubated for 2 h at room temperature. At the end of incubation 1/20 the volume of 1M ammonium chloride was added and the reaction mixture was incubated for 1 h.

2.4.2 Separation of labeled ligand from free dye

The separation of labeled ligand was done using 2ml sephadex G-25 column and was eluted with 3ml of PBS (50mM, pH 7.2) and 250µl fractions were collected and absorbance of each was monitored at 280 nm. The first fraction was used as reference (Blank).

Two bands were visible during elution and the conjugate was present in first band, the main fractions were pooled. The column was washed with 30 ml PBS (50mM, pH 7.2) to remove unbound fluorophore. The conjugate was stored by adding 1%w/v BSA and 1%w/v sodium azide at 4ºC, protected from light.

2.4.3 Reaction of labeled SIF with spermatozoa

Semen sample was allowed to liquefy at room temperature for 30 min. The sample was washed twice with PBS (50mM, pH 7.2) and was finally suspended in 500µl of PBS (50mM, pH 7.2). To 100µl of sperm suspension 200µl of conjugated ligand was added and incubated at 37˚C for 1h. Then 150µl of 3% formaldehyde was added and the reaction mixture was incubated at 37˚C for 1h. After incubation the reaction mixture was washed twice with PBS and the pellet was finally suspended in 50µl of PBS (50mM, pH 7.2). A wet mount was prepared and observed under fluorescent microscope (Nikon, Japan). Similarly, two controls were set up (a) spermatozoa/bacteria and unlabelled SIF (b) PBS and unlabeled SIF.

2.4.4 Reaction of labeled SIF with bacterial strains

Bacterial cultures grown for 6-8 h in nutrient broth were pelleted and washed twice in PBS (50mM, pH 7.2) and finally suspended in 1ml of PBS (50mM, pH 7.2). From these suspensions, 50 µl was taken and mixed with 50µl of FITC labeled SIF and incubated for 2 h. Then reaction mixture was fixed with 50µl of 3% formaldehyde in PBS and incubated at 37˚C for 1h. After washing twice with PBS (50mM, pH 7.2), it was finally suspended in 100µl of PBS. A wet mount was prepared and observed under fluorescent microscope (Nikon, Japan). Similarly, controls were set up along with bacterial cultures and unlabelled SIF.

2.5 Isolation and purification of SIF receptor from spermatozoa

SIF receptor from spermatozoa was isolated and purified according to the protocol standardized in our laboratory earlier. Briefly, salt extraction of the receptor from washed human spermatozoa sample was done by treating sperm sample with 3 M NaCl for 4 h and further purified by gel filtration chromatography [11].

2.6 Blocking studies of SIF binding by SIF receptor from spermatozoa

Purified SIF receptor and FITC labeled SIF were coincubated with washed spermatozoa/bacteria. After 2 h of incubation at 37ºC the blockage of binding was evaluated using fluorescent microscopy.

3. Results

3.1 Isolation and purification of Sperm Immobilization Factor (SIF)

The SIF was isolated from 72 h culture filtrate at 60-80% ammonium sulfate precipitation. Further it was purified by gel permeation and DEAE chromatography with peak values in fraction 14 and 49, respectively.

3.2 Binding of FITC labelled SIF to human spermatozoa and bacteria

The results of test sample appeared as a bright green fluorescence on spermatozoa and bacteria which depicts the binding of SIF with spermatozoa and bacteria. This binding of spermatozoa with SIF showed the presence of receptor for SIF on spermatozoa and bacteria **(Fig. 1)** whereas the controls in all the cases showed no fluorescence **(Fig. 2)**.

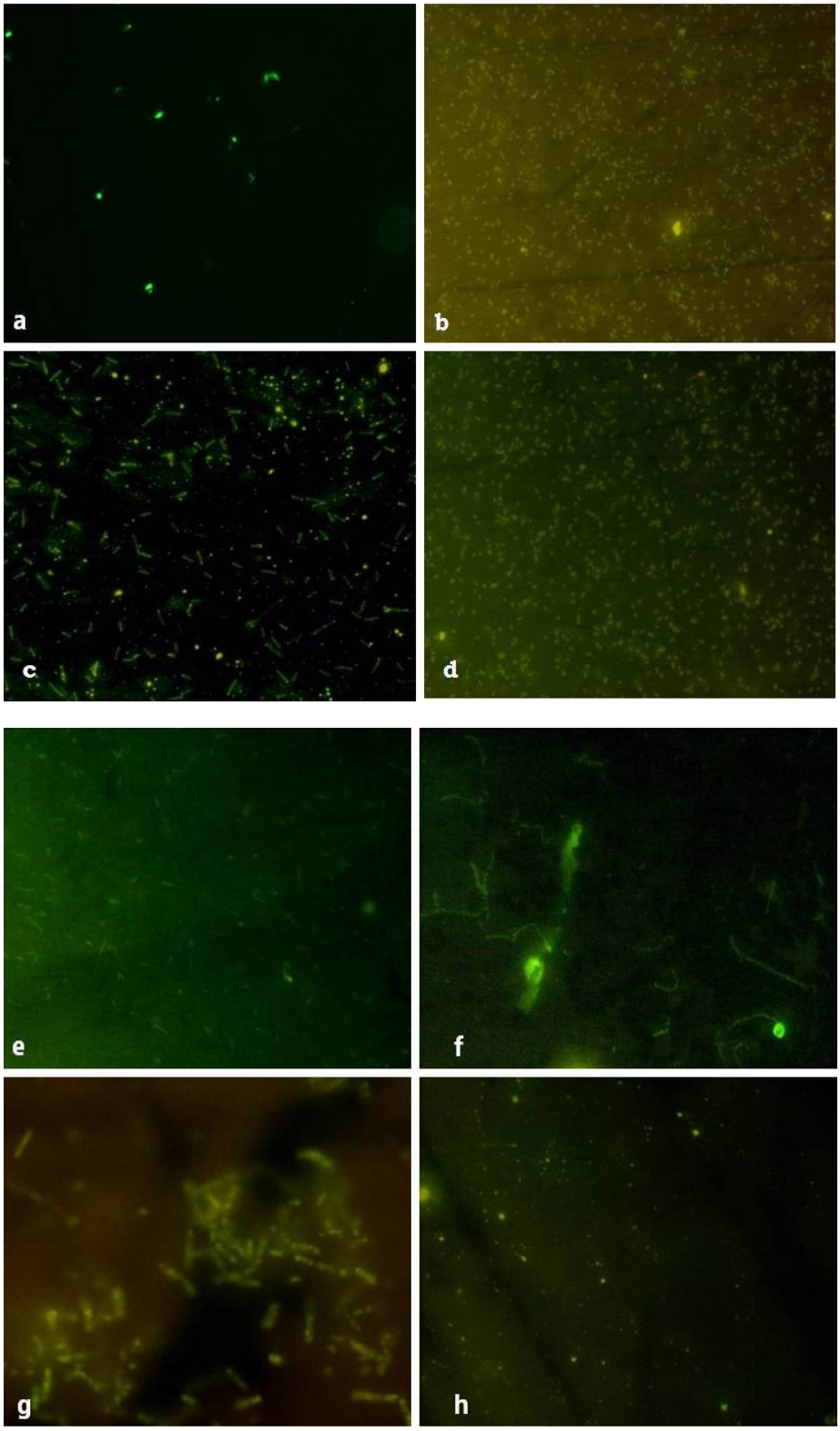


Fig 1.Fluorescent microscopy of FITC labeled SIF incubated with (a) human spermatozoa (b) *S. flexneri* (c) *E. coli* (d) *S. enterica typhi* (e) *P. aeruginosa* (f) *B. cereus* (g) *E. faecalis* (h) *P. mirabilis*

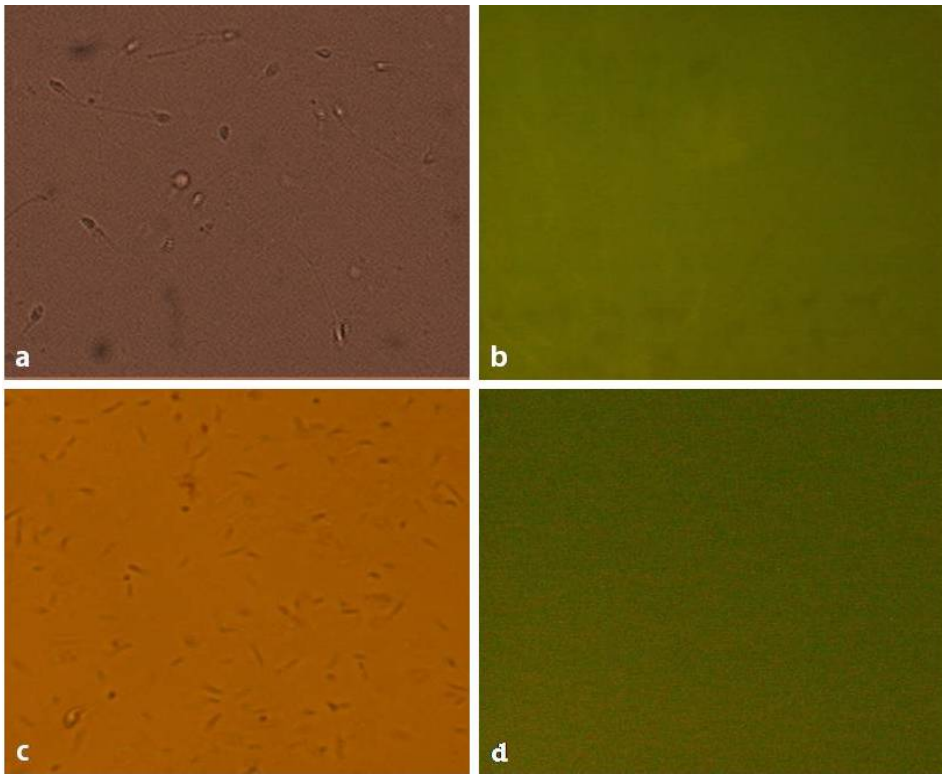
Fig 2. Fluorescent microscopy of unlabeled SIF incubated with spermatozoa/ bacteria/ PBS

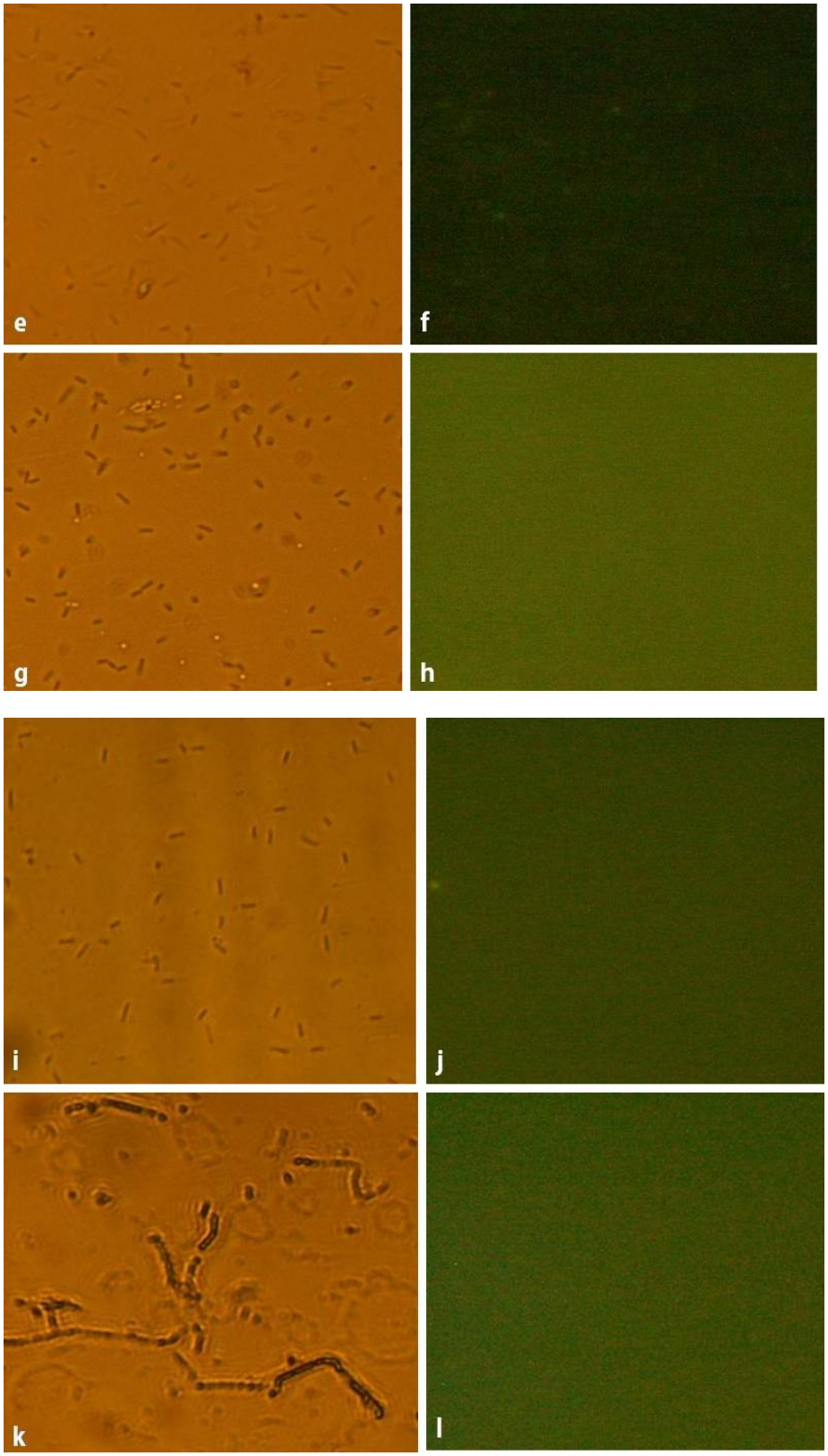
3.3 Isolation and purification of SIF receptor from spermatozoa

The receptor could be efficiently isolated and purified from human spermatozoa by salt treatment with 3M NaCl and further purified by gel permeation chromatography with peak value in fraction 3.

3.4 Blocking studies

Coincubation of human spermatozoa receptor with FITC labeled SIF could efficiently inhibit the binding of SIF to spermatozoa and bacteria **(Fig. 3)**.





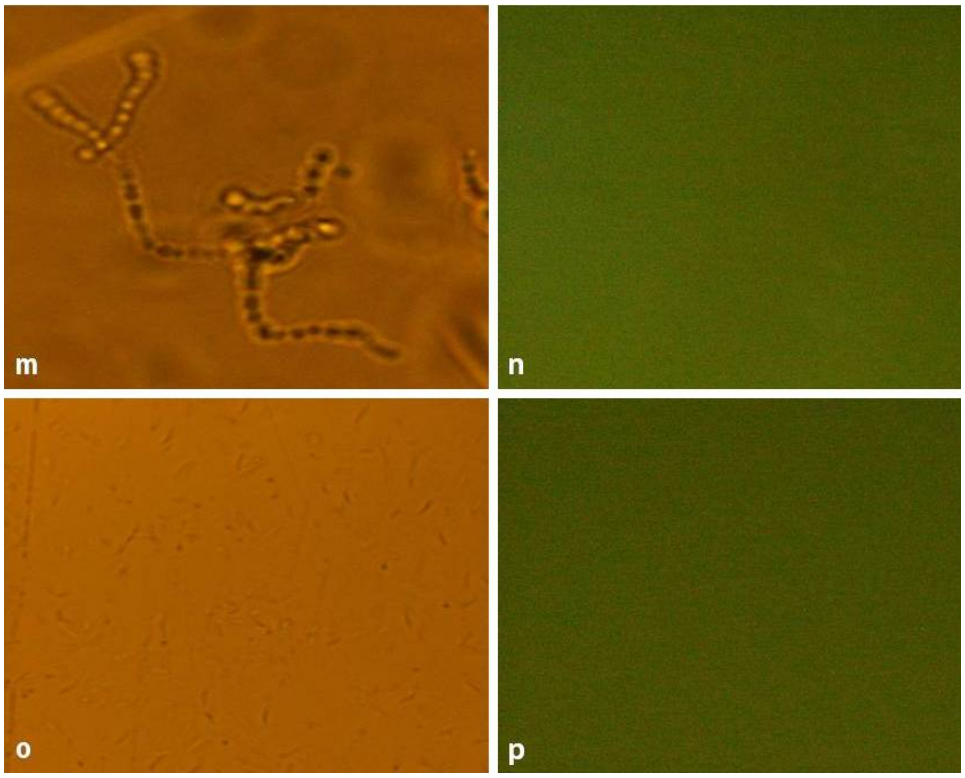


Fig 3. Bright field and Fluorescent microscopy of FITC labeled SIF incubated with human sperm receptor and (a,b) human spermatozoa (c,d) *S. flexneri* (e,f) *E. coli* (g,h) *S. enterica typhi* (i,j) *P. aeruginosa* (k,l) *B. cereus* (m,n) *E. faecalis* (o,p) *P. mirabilis*

5. Discussion

Antisperm antibodies are produced in both women and men. The possible roles of sperm-immobilizing antibodies in infertility are well known. However, the factors that affect the production of sperm-immobilizing antibodies in infertile women are not fully understood. Moreover, the reason why most women do not develop an immune response upon exposure to sperm is not yet clear. Women do not generally produce antibodies against sperm; however, some infertile women have been found to possess antisperm antibodies, which may contribute to their infertility. Therefore, the following questions arise: who produces sperm-immobilizing antibodies, what makes women produce these antibodies, and what are the corresponding antigens? Common antigenicity has been established between spermatozoa and bacteria, viruses, fungi and allergens. Recent evidence suggests that many antigens shared among unrelated cell types are surface membrane or lipid conjugate complex carbohydrates. A number of investigators have demonstrated the existence of such cross-reactive antibodies by the means of different detection techniques, utilizing both polyclonal antisera and monoclonal anti-sperm antibodies. Thus, common antigenicity has been established for spermatozoa and *P. aeruginosa*, *Klebsiella pneumoniae*, *E. coli* [5]; *Trichomonas vaginalis*, *Mycoplasma hominis*, *U. urealyticum*, *Candida albicans* [12]. An earlier report by Kalaydjiev et al.[13]that 'naturally occurring' anti-sperm antibodies may consist, at least in part, in cross-reacting antibodies produced against bacterial intestinal pathogens, such as *Shigella* and *Salmonella*.

In the current study we also observed that FITC labeled SIF not only binds spermatozoa but also to various gram positive and gram negative bacteria. Further this binding could be inhibited by SIF receptor from spermatozoa. This shows the presence of common SIF binding motif on spermatozoa and bacteria. This similarity between the sperm as well as the bacteria could prove to be a milestone in the field of infertility caused by infections and the receptor could be used for the treatment of the infertility and related problems, especially those involving the bacteria causing immobilization.

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