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# Application of recombinant antibody library for screening specific antigens in a bovine sperm cell subpopulation

Sandro Gomes Soares [a,1], Jose Elpidio Barbosa [b]

[a] Basic and Applied Immunology Postgraduate Program, School of Medicine of Ribeirao Preto, University of Sao Paulo, 14049-900 Ribeirao Preto SP, Brazil
[b] Department of Biochemistry and Immunology, School of Medicine of Ribeirao Preto, University of Sao Paulo, 14049-900 Ribeirao Preto SP, Brazil

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**Abstract**

Antibody phage display libraries are a useful tool in proteomic analyses. This study evaluated an antibody recombinant library for identification of sex-specific proteins on the sperm cell surface. The Griffin.1 library was used to produce phage antibodies capable of recognizing membrane proteins from Nelore sperm cells. After producing soluble monoclonal scFv, clones were screened on Simental sperm cells by flow cytometry and those that bound to 40–60% of cells were selected. These clones were re-analyzed using Nelore sperm cells and all clones bound to 40–60% of cells. Positive clones were submitted to a binding assay against male and female bovine leukocytes by flow cytometry and one clone preferentially bound to male cells. The results indicate that phage display antibodies are an alternative method for identification of molecules markers on sperm cells.


*Keywords:* Phage display; scFv; Nelore; Sexing

## 1. Introduction

Predetermining the sex of offspring is crucial in preventing X-linked disease transmission in humans and in increasing the rate of genetic progress in livestock. Separation of X- and Y-chromosome-bearing sperm for gender preselection is the obvious preference since sex determination takes place before fertilization (Hendriksen et al., 1996). Many attempts to separate X and Y sperm have been described using physical parameters, such as swimming velocity, density, surface charge or sex-specific antigens.

Sperms from many mammalian species can be sexed by flow cytometry/cell sorting at ~90% accuracy without damaging them unduly. However, because sperm are evaluated one at a time, in series, the number of sexed sperm produced per unit time is limited. Furthermore, the equipment required currently is expensive, in the order of 300 000 US dollars per machine (Seidel, 2003).

Antibody phage display is a new technology for the identification of novel target molecules (Geuijen et al., 2005). Antibody synthetic libraries are useful for the identification of self-antigens or phylogenetically conserved antigens in comparison to other methods based on immunization. With the development of new strategies for

*E-mail addresses:* soares@fmrp.usp.br (S.G. Soares), jebarbos@fmrp.usp.br (J.E. Barbosa).
[1] Fax: +55 16 3602 4590.

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selection, antibodies have been made against problematical antigens, such as MHC-peptide complex (Andersen et al., 1996; Chames et al., 2000; Cohen et al., 2003) and human erythrocyte antigens (Marks et al., 1993).

The present report describes efforts to identify plasma membrane proteins specific for either male or female cells on bovine sperm cells through an antibody phage display library.

## 2. Materials and methods

### 2.1. Library and antibodies

The Griffin.1 library, a human scFv phagemid library made from synthetic *V*-gene segments, was generously provided by Dr. G. Winter (Centre for Protein Engineering, Medical Research Council, Cambridge, UK). Goat anti-rabbit IgG FITC was purchased from Sigma Chemical (St. Louis, USA). Rabbit anti-VCSM13 horseradish peroxidase conjugate and rabbit anti-scFv were produced following standard immunization procedures and the reactivity was tested in our laboratory. Rabbit anti-VCSM13 antibodies were purified using affinity column chromatography, as described by Bethell et al. (1979).

### 2.2. Preparation of membrane sperm proteins

Bovine semen (*Bos indicus*, Nelore) was collected by electroejaculation and resuspended in 20 mM Tris–HCl buffer (pH 7.3) containing 150 mM NaCl, 1 mM EDTA, 1 mM PMSF and 1 mM benzamidine. The semen was centrifuged (300×*g* for 10 min) and resuspended with the same buffer. This procedure was repeated twice. Finally, the pellet was resuspended using the same buffer, now containing 0.1% of Triton X-100 and sonicated for 10 cycles of 1 min, setting 1 min in ice. Afterwards, the suspension was centrifuged at 13 750×*g* and the supernatant dialyzed against 20 mM Tris–HCl buffer containing protease inhibitors. Protein concentrations were determined by Lowry et al.'s (1951) method.

### 2.3. Selection of phage antibodies on membrane sperm proteins

The Griffin.1 library was rescued with helper phage VCSM13 and the phages were purified and concentrated from the bacterial culture supernatant by polyethylene glycol (PEG) precipitation, using the recommended protocol (see http://www.mrc-cpe.cam.ac.uk). Panning of phage antibodies was performed in immunotubes (NUNC MaxiSorp, NUNC, Roskilde, Denmark) coated with 80 μg of membrane sperm proteins, as described previously (Marks et al., 1991). Briefly, 1 ml of the phage antibodies solution (approximately $10^{13}$ phages) was added to the tubes and incubated for 30 min with continuous rotation on an under-and-over turntable and allowed to stand for at least a further 90 min at 20 °C. Non-binding phage antibodies were eliminated by sequential washing in PBS containing 0.05% Tween 20 followed by PBS. Binding phage antibodies were then eluted from immunotubes by adding 1 ml of 100 mM triethylamine, incubating for 10 min at 20 °C, transferring the solution to a new tube and neutralizing with 0.5 ml 1 M Tris–HCl, pH 7.4. Half of the eluted phage antibodies solution was used to amplify infecting *Escherichia coli* TG1, phage antibodies rescued, concentrated and used for the next round of selection. The selection process was repeated for a total of three rounds. Finally, 20 μg/well of sperm membrane proteins was immobilized onto 96-well polystyrene plate (Costar®, Corning Inc., New York, NY, USA) and polyclonal phage ELISA was performed using standard protocol (Marks et al., 1991). The binding of phage antibodies was detected with affinity-purified rabbit polyclonal anti-VCSM13 HRP conjugate antibodies.

### 2.4. Production of soluble monoclonal scFv

Soluble scFV were prepared from the *E. coli* non-suppressor strain HB2151, as described by Marks et al. (1991). Firstly, 10 μl of eluted phage antibodies was used to infect 200 μl of exponentially growing HB2151 bacteria for 30 min at 37 °C. Serial dilutions were plated on LB agar containing 100 μg/ml ampicillin and 1% glucose. These plates were incubated overnight at 37 °C and individual colonies were picked out into 100 μl LB containing 100 μg/ml ampicillin and 1% glucose in 96-well plate (Costar®, Corning). This plate was incubated in a shaker (300 rpm) overnight at 37 °C. Using a 96-well transfer device, a small inoculum (about 2 μl) was transferred from this plate to a second 96-well plate containing 200 μl LB with 100 μg/ml ampicillin and 0.1% glucose per well. The first plate was stocked by adding glycerol (15 μl/well) and storing at −80 °C. The second plate was incubated in a shaker (300 rpm) at 37 °C until the OD at 600 nm was ~0.9. Once the required OD was reached, each well received 25 μl LB containing 100 μg/ml ampicillin and 9 mM IPTG with continuous shaking at 30 °C for 20 h. The supernatant of this culture was used for the flow cytometry assay.

### 2.5. Screening of monoclonal scFv by flow cytometry

The scFv clones were selected by flow cytometry using Nelore (*B. indicus*) and Simental (*Bos taurus*) sperm cells