Aglutination of Mice Sperm in Antibody of 46, 66, and 73 KDa Protein from Rabbit Sperm Membrane

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ABSTRACT

This research aimed to investigate the influence provision antibody of 46, 66, and 73 kDa protein from rabbit sperm for mice sperm agglutination. Twenty four male mice of Balb/C strain, 8-10 weeks old and 20-25 g weights were devided into four groups (six mice for control group and 18 mice for treatment group). All of mice were dissected and then taken was epididimys to get the mice mature sperm by flushing method. From the result of flushing using Baker's buffer, each sperm suspension was taken 100 µl, then added with 10 µg/ml control, 46, 66, and 73 kDa protein's antibody. Then taken 50 µl of suspension and placed on hollow glass objects, incubated for 20 second before observed using light microscopy at 400x magnification with 3x replicated observations. This research result showed that the average sperm agglutination of control group was 6.20 and treatment group was 18.92(46 kDa), 12.46 (66 kDa), and 19.60 (73 kDa). In addition types of agglutination, there are agglutination between the head with head, head to tail and tail to tail. Conclusions of this research to provision antibody 46, 66, and 73 kDa protein from the rabbit sperm membrane for mice sperm agglutination and different types of antibodies will affect the result sperm agglutination, the most potentially protein was 73 kDa protein.

Keywords : Membrane protein, Sperm agglutination, Antigen-antibody reaction

1. INTRODUCTION

On the plasma membrane of spermatozoa expressed a specific protein as sperm receptor (Suri, 2004). Sperm membrane proteins played a role in fertilization, among others, played a role in sperm motility, sperm membrane adhesion to the zona pellucida, the initiation of the signal transduction that result in exocytosis acrosome, and contributed to the egg membrane fusion (Evans, 2002; Gahmberg and Tolvanen, 1996; Patrat *et al*., 2000).

According Wahyuningsih *et al*. (2008), sperm membrane proteins were isolated from the cauda epididymis rabbit had seven protein bands. Molecular weight was 250, 73, 66, 46, 34, 28, and 16 kDa. Protein with a molecular weight of 73, 66, and 46 kDa are specific proteins to form antisperm antibodies.

Immunization with sperm membrane protein in the same or different species will lead to cross-reactions and specific immune responses. The response was the formation of antibodies against the sperm membrane proteins.

The reaction between antibodies and antigen made contraceptive effect, among other caused sperm agglutination, reduced motility, cervical mucus penetration disorders, inefficient fusion of sperm and ovum, increased phagocytosis of spermatozoa and embryo death before or after implantation. All these obstacles cause infertility (Domagala and Kurpisz, 2004). Antibodies anti sperm caused either spontaneously or induction infertile (Bohring *et al*., 2001). Antibody and spermatozoa bond in the reproductive tract can cause agglutination resulting in inhibition of the motility (Hafez and Hafez, 2005). Agglutination at reducing the ability of spermatozoa fertilization. In addition, the antibody response to antigens such as the occurrence of conception effect on male infertility (Domagala & Kurpisz, 2004). Infertility condition was utilized for the development of immunocontraceptive. Suri (2005), immunocontraceptive methode was a new strategy to solve the problems on the uncontrolled population growth Indonesia.

2. EXPERIMENTAL

2.1 Production of antibodies anti sperm membrane protein subunit

This study required 24 female mice (6 tail for the control group and 18 to be immunized with the sperm membrane protein of molecular weight of 46, 66, and 73 kDa. Dose protein was 50 µg/ml. Immunization first, antigen were emulsified with PBS to a volume of 0.05 ml and Freund's complete adjuvant (FCA) 0.05 ml (ratio 1:1). Later in the vortex for 1 hour. The mixture was injected intraperitonial. The control was immunized without protein. Immunization second, after the next 14 days with a mixture of antigens in 0.05 ml PBS and Freund's incomplete adjuvant (FICA) 0.05 ml. Immunization third, after 14 days and the next by way of the same material as the second immunization. Immunization fourth, after 7 the next day with protein in PBS without adjuvant.

One week of the last immunization, blood was taken via cardiac mice. Blood was collected in Eppendorf tubes. Blood was left at room temperature for 2 hours. Further blood in a centrifuge at 3000 rpm for 10 min temperature of 4^oC. Serum was separated from blood and was collected for the agglutination test.

2.2 Agglutination test

Incubation of mice spermatozoa of with antibodi against protein subunits (46, 66, and 73 kDa) rabbit sperm membrane. Sperm aglutination was motile sperm attached