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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°C. 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

to the head with the head, the head of the tail, or the tail with the tail section.

Adult male mice, aged 8-10 weeks, strain Balb / C, as many as 24 tails fertile dissected the posterior part of the abdomen. Epididymis were taken and separated from the fat. Spermatozoa isolated by flushing method (Haila and Daulat, 2001), the syringe containing 1 ml Baker's buffer was inserted into the channel cauda epididymis. Furthermore, the needle slowly pressed so Baker's buffer can drive spermatozoa in the cauda epididymis through the vas deferens out and obtained a suspension of spermatozoa. The number count of sperm agglutination was done by putting 50 mL suspension on a glass object and a concave mirror with a light microscope under 400X magnification microscope. Observations by counting the number of agglutination between head with head, head to tail and tail to tail using a hand counter to the repeated observation of 3X.

2.3 Data Analysis

Data type of agglutination narrated. Data were analyzed with the amount of agglutination one-way ANOVA ($\alpha = 0.05$). If there was a real difference between the treatment continued test of Duncan.

3. RESULTS & DISCUSSION

3.1 Number of agglutination

Observations were made of the number of spermatozoa agglutination mice after incubation for 20 seconds with an antibody against the protein subunit memban rabbit spermatozoa. The sperm agglutination count can be seen in Table 1.

Based on the Kolmogorov-Smirnov test obtained significance level 0.478, ($p > 0.05$), the data are normally distributed. Homogeneity test significance level of 0.354 is obtained, the data have a homogeneous variance. ANOVA test obtained 0.0 significance level ($p < 0,05$). This means that there is an influence of incubation of spermatozoa in the protein subunit antibodies to rabbit sperm membrane to total agglutination.

By Duncan test showed that spermatozoa are diiinkubasi groups in the protein subunit antibodies to rabbit sperm membrane BM 46 kDa, 66 kDa and 73 kDa have increased the amount of agglutination. Mean of the control group (0 kDa), the amount of agglutination 6.70 ± 1.867 significantly different with all treatment groups. Incubation in the antibody treatment group against the 46 kDa subunit protein, the amount of agglutination 18.93 ± 2.318 significantly different from the group treated with an antibody against the 66 kDa subunit protein, but not significantly different from the group treated with the antibody against the 73 kDa subunit protein, agglutination number 21, 28 ± 3.445. Treatment groups in antibodies against the 66 kDa subunit protein, the amount of agglutination 14.13 ± 2.899 significantly different from the group treated with the antibody against the 73 kDa subunit protein

Presence of agglutination indicates no interaction antigen and antibodies. Sperm agglutination indicates that each motile sperm attached to the head by head, head to tail, or the tail with the tail section. Observations were made on the number and type of agglutination agglutination of spermatozoa of mice. Agglutination test is expected as the model of the inhibition of sperm motility.

Number of agglutination on treatment increased compared to normal conditions (control). This shows that there is an incubation effect of antibodies against membrane protein subunits on sperm agglutination which can increase the amount of agglutination. According to Naz (2004), sperm membrane proteins as candidate imunokontrasepsi have a criteria, ie a specific protein that is only expressed by spermatozoa, and cause agglutination of spermatozoa.

According to Hafez and Hafez (2005), bond sperm membrane antigens with antibodies in reproductive tract can cause agglutination and hinder the movement of spermatozoa. Calamera *et al.* (2002) stated that the presence of antibodies to sperm and cause immobilization or agglutination of sperm. According Manyonda (2006), antibodies to the surface antigens of the acrosome and the tail and causing immobilization or agglutination of spermatozoa.

Based on the research found that the three antibodies capable to agglutinate spermatozoa in vitro and all were significantly different from the control. Rabbit sperm membrane antibodies that have the highest potential to agglutinate spermatozoa of mice is an antibody against 73 kDa subunit protein. Further antibodies against 46 kDa subunit protein and fewer antibodies to agglutinate spermatozoa is 66 kDa subunit protein.

Agglutination is an antigen-antibody reaction in the category of secondary manifestations. Antibodies that are in the immune serum will react with the antigen determinant of ligand. The stability of this combination depends on several factors specifically pH, ionic strength and temperature. Due to this reason, the antigen-antibody reaction in vitro carried out at specific temperatures and in media buffer solution (buffered medium) containing electrolytes. The combination between antigen and antibody is done by means of non-covalent bonds (Bellanti, 1993).

3.2 Type of agglutination

Agglutination of the spermatozoa as indicators of the influence of antibodies, can be seen with the attachment between the tail with the tail, head to tail or head to head. The observation of agglutination types of spermatozoa after incubation for 20 seconds can be seen in Table 2 and Figure 1.

Table 1. The mice spermatozoa agglutination after incubation for 20 seconds in the antibody and the test statistic

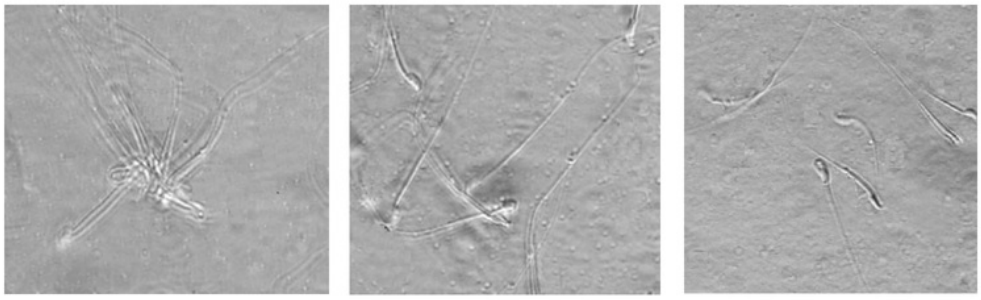
Repication	The number aglutination of mice sperm after antibody incubation ……			
	0 kDa	46 kDa	66 kDa	73 kDa
1	7,56	18,33	18,11	23,22
2	5,22	15,11	12,44	26,00
3	4,11	17,89	10,11	21,56
4	8,89	20,33	15,11	17,44
5	6,11	20,33	12,78	22,33
6	8,33	21,56	16,22	17,11
Mean ± SD	**6,70 [a] ± 1,867**	**18,93 [c] ± 2,318**	**14,13 [b] ± 2,899**	**21,28 [c] ± 3,445**

Description: The same Superscript showed no significant difference (α = 0.05).

Table 2. Types of spermatozoa agglutination after incubation with rabbit sperm membrane protein subunits for 20 seconds

Antibody	Aglutination type	The number of agutination type						Mean ± SD
		1	2	3	4	5	6	
0 kDa	HH	18.67	6.67	5.33	13.67	12.00	17.00	**12,22 ± 5,382**
	HT	2.00	5.33	3.67	5.00	5.67	6.33	**4,67 ± 1,578**
	TT	2.00	3.67	3.33	8.00	0.67	1.67	**3,22 ± 2,588**
46 kDa	HH	40.67	31.33	34.00	. 44.00	39.67	46.00	**39,28 ± 5,666**
	HT	12.33	12.33	15.33	. 12.00	14.00	13.67	**13,28 ± 1,290**
	TT	2.00	1.67	4.33	. 5.00	.7.33	5.00	**4,22 ± 2,115**
66 kDa	HH	45.00	35.00	20.67	. 34.67	28.00	40.33	**23,94 ± 8,675**
	HT	6.67	2.33	9.00	10.00	9.67	7.00	**7,44 ± 2,857**
	TT	2.67	0.00	0.67	0.67	0.67	1.33	**1,00 ± 0,919**
73 kDa	HH	57.33	53.00	43.00	.40.00	53.00	42.33	**48,11 ± 7,185**
	HT	12.33	22.00	17.33	. 11.67	13.33	9.00	**14,28 ± 4,654**
	TT	0.00	3.00	4.33	0.67	. 0.67	0.00	**1,44 ± 1,797**

Description: the aglutination type is HH (head-head), HT (head-tail), TT (tail-tail)



A B C

Fig. 1 The types of mice sperm agglutination after incubation in antibodies to rabbit sperm membrane protein subunits. Agglutination: A). heads. B). head-tail, and C). tail-tail, magnification 1000 times.

Reactions that occur affect agglutination between the heads, head-tail and tail-tail. Based on data from the study showed that incubation in antibodies against various protein subunits, the average dominance type of agglutination that occurs is between the heads, then the head-tail. Agglutination type at least the tails of spermatozoa both in the control and treatment.

Type of agglutination between the heads after incubation in antibodies against the 73 kDa subunit protein of 48.11, then the treatment incubation with antibodies against the 46 kDa subunit protein of 39.28. Last on treatment incubation with antibodies against 66 kDa subunit protein of 23.94. On the control agglutination also occurred at 12.22.

Type of agglutination that occurs between the head-tail spermatozoa average highest during incubation for 20 seconds treatment with antibodies against the 73 kDa subunit protein of 14.28, subsequent treatment incubation

with antibodies against 46 kDa subunit protein of 13.28, and at least on treatment incubation with antibodies against 66 kDa subunit protein of 7.44. On the control also occurred between the head-tail agglutination of 4.67.

Type of agglutination that occurs between the tails of spermatozoa average value is dominated by the group treated with antibodies against the 46 kDa subunit protein of 4.22. Then incubated with antibody treatment against the 73 kDa subunit protein of 1.44 and 66 kDa of 1.00. Control occurs agglutination of 3.22.

Type of agglutination on incubation of spermatozoa for 20 seconds in antibodies against 73 kDa subunit protein shows most likely to cause agglutination agglutination, especially between the heads, then the head-tail and tail-tail at least. The second highest number of agglutination caused by incubation in antibodies against the 46 kDa subunit protein with the same type of agglutination with other treatments. Agglutination fewest number if incubated in antibody against 66 kDa subunit protein. At kontrolpun agglutination also occurred, but fewer in number when compared with the treatment.

In this study, the type of agglutination agglutination distinguished between the heads, heads-tails, and tails. This occurs because the antibodies bind to a particular part of the surface membrane of spermatozoa both head and tail. The reaction between antigens with antibodies cause adhesions occur. Based on the research is that the type of agglutination in most spermatozoa were among the heads of spermatozoa. Second type of agglutination is between the head-tail of, and agglutination between tail-tail found in the least amount. Presence of agglutination indicates that the protein subunit is ligand surface. Grace *et al*. (2002); Harayama *et al*. (2000) in Cesari *et al*. (2005) stated that in some species of Mammalia, the head of the sperm agglutination was observed when the head of the sperm from the epididymis diluted or when incubated in serum.

The agglutination between the heads, because of the similarities of the protein that is causing the acrosome protein antigen-antibody reaction and cause agglutination. Agglutination that occurs between the head-tail spermatozoa because the second part is a type of protein that contained similarities there, giving rise to sperm agglutination due process of antigen-antibody interaction. While agglutination that occurs between the tail with the tail of spermatozoa, because of the similarity of proteins contained in the sperm tail so that the interaction between antigen-antibody and cause agglutination

This happened because the antigen diresponss spermatozoa in different places by individual spermatozoa and spermatozoa will be staged by forming antibodies. According to Cesari *et al*. (2005), agglutination that occurs is the result of a reaction between antibodies and antigens on the cell surface of spermatozoa.

Effect of agglutination is a decreased ability of sperm motility. Naturally, spermatozoa are allowed in certain media will undergo agglutination or clumping. Indirectly, agglutination will reduce the level of efficiency of spermatozoa in an egg (Haviz *et al*., 2008). Reduction or disappearance of the ability of spermatozoa to fertilize eggs affect the failure of fusion of sperm and ovum at fertilization. This situation will result in the individual male infertility. Representative schemes antibody that blocks the receptor spermatozoa can be seen in Figure 2.

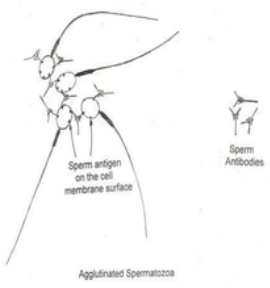


Fig. 2 Antibodies that block receptor spermatozoa (Calamera *et al*., 2002).

4. CONCLUSION

Antibodies to rabbit sperm membrane protein subunits can increase sperm agglutination mice as an animal model with most types of agglutination between head with head.

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