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The Human Polyclonal 116kDa Antibody Specificity Resulted from Induction of Human Spermatozoa Membrane Non-Kinase Protein in Somatic Tissue and Human Reproductive Tissue

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ABSTRACT

Immunocontraception is one of the alternative methods of contraception using the principle of induction of immune response, aimed at inhibiting receptor and ligand binding in the oocyte and sperm. Spermatozoa membrane protein used has a characteristic of non-kinase proteins found in spermatozoa membrane, believed to be specific only in spermatozoa, not found in other tissues. This study is an exploratory study to obtain the character of non-kinase protein exists only in the membrane of human spermatozoa using Antibody policlonal human 116kDa. The tissues used to determine the location and distribution of non-kinase protein are brain, heart, blood vessels, liver tissues, and prostate gland incubated with AbhP-116kDa emlopying immunohistochemistry method. The result is the brain, heart, blood vessels tissues, and the prostate gland showed the non existence of 116 kDa non-kinase protein, for there is no brown stain to appear, whereas in liver tissue specifically in the cytoplasm, non-kinase protein were detected, however, the liver tissue used for the negative control, tissue that was not given Ab-hP116kDa, also show the same visualization.

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INTRODUCTION

Increasing the participation of men in family planning program has been a national policy. The survey conducted by the National Population and Family Planning Agency (BKKBN) in 2003 showed that male contraception users remain low at around 0.9% while in 2007 increased to 1.4% in 2009 is reaching 4.5%. Therefore, the development of alternative methods for male contraception needs to be done. Therefore, the quest for alternative contraception is imminent through the development of male contraceptive methods, which to date is very limited because of the lower intensity of coaching techniques of male contraception. In developing countries, especially the one with over population problem, technique or method of male immunocontraception is being developed through the effort of finding the candidate substance for contraception. This alternative method is declared to be more secured, effective, practical, less side effects, having high specificity, and may have a chance to be accepted by users than other contraceptive techniques (Naz, R.K., P.B. Rajesh, 2004). Researches suggested that spermatozoa membrane proteins have functions related to the events in fertilization, such as, the role in sperm motility, adhesion of sperm membrane to the zona pellucida, the initiation of signal transduction (Patrat, C., 2000; Evans, J.P., 2002).

The development of immunocontraception substance required exploration and characterization of specific spermatozoa membrane protein that acts primarily as an antigen with immunogenic, specific characteristics, involved in the interaction with the zona pellucida (ZP3), and fusion with the oocyte membrane (Bhadway, D., 1999; Yamagata, K.A., 1999). Lestari (2006) successfully isolated 116 kDa male sperm membrane protein which is thought to function to communicate with the oocyte and the initiation of the acrosome reaction. Protein with a molecular weight of 116 kDa is membrane proteins grouped in protein having no phosphorylation activity. Human polyclonal116kDa antibody is specific that it only recognizes the protein at the surface of the head of human spermatozoa and spermatids located in the lumen of the testis. Human polyclonal 116kDa antibody did not recognize the protein in somatic tissues such as spleen, pancreas, and human kidney, however it has not been tested to other somatic tissues. This study is an exploratory study to obtain characters of Human polyclonal

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116kDa antibody isolated from the human sperm membrane proteins to goat sperm membrane proteins.

Substance and Methods:

This study employs an exploratory laboratory design and descriptive analysis. The indicator for recognition of the Human polyclonal 116kDa antibody to the protein in the cell is a dark brown color in the cytoplasm. Observations were made using a light microscope with 1000x magnification. Immunohistochemical method is done through the immersion of somatic tissue Preparat such as the liver, heart, pancreas, brain and reproductive tissues such as prostate gland in xilol I, II and xilol and alcohol series (100%, 90%, 70% and 30%) for five minutes each and then washed using distilled water. Preparats are washed with PBS three times – with five minutes each washing. Next, hydrogen peroxide in PBS is dripped in for ten minutes and then is dripped with 1% BSA for thirty minutes. The primary antibody, Human polyclonal 116kDa antibody, is put into the preparat, and incubated overnight at 4 ºC. Then, the preparat are given secondary antibody in the form of anti-Rat IgG-labeled biotin and incubated for one hour at room temperature. As for the comparison, the other preparat is prepared without the use of primary antibodies, but directly with the use of the anti-rabbit IgG antibody and incubated overnight at 4ºC. After incubation, preparat is set aside for 30 minutes at room temperature. SA-HRP (Strep-Avidin Horseraddish Peroxidase) is added later and visualized using DAB Chromogen (Goers, J., 1993). Preparat is mounted using *entellan* then covered with a cover glass. It is then ready to be observed under a light microscope with 400x magnification.

RESULTS AND DISCUSSION

The visualization comparison of somatic tissue on the brain, heart, liver, blood vessels and the prostate gland given 116 kDa protein polyclonal antibody with the control appears similar, meaning that it does not indicate any visualization of chromogen in the cytoplasm of the tissue, which was revealed by the emergence of brown color. This suggests that the 116 kDa protein polyclonal antibody does not recognize the protein found in spleen, kidney and pancreas tissue. And the writer believes that the all three tissues do not contain the 116 kDa protein. However, the liver tissue showed brown color in the cytoplasm, and non-kinase protein was detected specifically in the cytoplasm indicated by the emergence of brown color. The liver tissue used for negative control, tissue that was not given Human polyclonal 116kDa, also shows the same visualization.

Proteins functioning in phosphorylation are generally owned by the somatic cells and spermatozoa. This relates to its function as a molecule involved in fundamental mechanism which include the cellular events control, in the form of cell division and growth, adhesion and migration, metabolic activity and response to environmental stimuli, cell communication, signal transduction and apoptosis (Grudzinskas, J.G. and J.L.Yovich, 1995). Kinase protein is a protein involved in the phosphorylation, a protein that has the ability to transfer phosphate from ATP to a protein substrate, so that the protein can carry out their activities. Kinase proteins generally have a group of serine, threonine and tyrosine. It is because of that kinase protein is conserved in all cell types (Kobori, H., 2000). Figure 1 shows the immunolocalization of 116 kDa protein in (A) brain, (B) heart, (C) liver, and (D) blood vessels. Normal controls were given haemotoxilin-eosin staining (left), a negative control is done by immunohistochemistry without Human polyclonal 116kDa antibody (middle), the treatment by immun histochemistry of AbhP-116 kDa (right), white arrows indicates that the core cell does not indicate the brown color; blue arrows show that there is no brown color in the intercellular cavities, while the red arrow indicates that the brown color emerges in the cell nucleus, which means Human polyclonal 116kDa antibody recognize proteins in the tissue and it can be interpreted that the tissue is subject to observations using a light microscope with 400x magnification. Figure 2 shows the immunolocalization of 116 kDa protein in the prostate gland. Normal controls were given haemotoxilin-eosin staining (left), negative control is performed through immunohistochemistry without giving Human polyclonal 116kDa antibody (center), treatment with immuno-histochemistry with the infusion of AbhP 116kDa (right); white arrows indicates that the the brown stain in the cell nucleus; blue arrows indicates that the intercellular cavities are clear from brown stain. The observations is done using the light microscope with 400x magnification.

Conclusions:

Human polyclonal 116kDa antibody is not recognized by the brain tissue, blood vessels, heart and prostate gland. The tissues used to determine the location and distribution of non-kinase protein are brain, heart, blood vessels, liver tissues, and prostate gland incubated with AbhP-116kDa emlopying immunohistochemistry method. The result is the brain, heart, blood vessels tissues, and the prostate gland showed the non existence of 116 kDa non-kinase protein, for there is no brown stain to appear, whereas in liver tissue specifically in the cytoplasm, non-kinase protein were detected, however, the liver tissue used for the negative control, tissue that was not given Ab-hP116kDa, also show the same visualization.

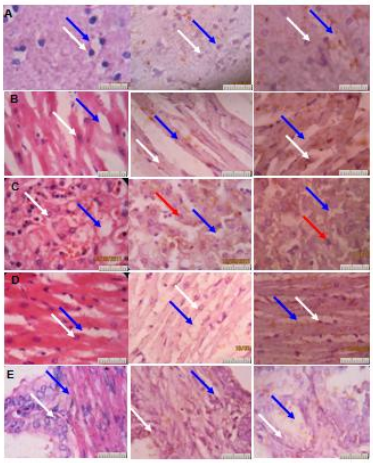

Fig. 1: Immunolocalization of protein in (A) brain, (B) heart, (C) liver, and (D) blood vessels.

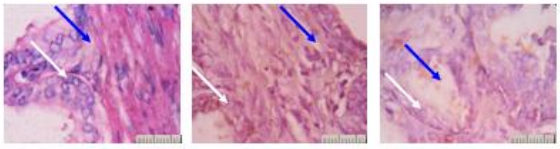

Fig. 2: Immunolocalization of 116 kDa protein in the prostate gland.

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REFERENCES

Naz, R.K., P.B. Rajesh, 2004. Role of tyrosine phosphorylation in sperm capacitation/ acrosome reaction. *Rep Biol Endol*, 2: 1-1

Patrat, C., C. Serres And P. Pierre Jouannet, 2000. The arosome reaction in human spermatozoa. *Biol of The Cell*, 92: 255-266.

Evans, J.P., 2002. The molecular basis of sperm-oocyte membrane interactions during mammalian fertilization. *Hum Reprod up*, 4: 297-311.

Bhadway, D., 1999. Electroelution of Protein from SDS PAGE for Use Immunozation. Devesh@icgeb.Res in.

Yamagata, K.A., S. Honda, Kashiwabara and T. Baba, 1999. Difference of acrosomal serine protease system between mouse and other rodent sperm. *Dev.Genet*, 25: 115-122.

Lestari, U., 2006. *Profil protein Membran Spermatozoa Manusia (Homo sapiens) dan Sapi (Bos taurus).* Seminar Nasional Basic Science. Malang

Goers, J., 1993. *Immunochemical Techniques Laboratory Manual*. Academic Press Harcount Brace Iovanovich Publisher San Diego. California.

Grudzinskas, J.G. and J.L.Yovich, 1995. *Gametes the spermatozoon*. Cambridge University Press.

Kobori, H., S. Miyasaki, Y. Kuwabara, 2000. Characterization of intracellular Ca^{2+} increase in response to progesterone and cyclic nucleotides in mouse spermatozoa. *Biol Reprod*, 63:113-120.