SYNTHESIS OF BETA FERTILIN PROTEIN POLYCLONAL ANTIBODY OF HUMAN SPERM MEMBRANE AS A CANDIDATE FOR IMMUNOCONTRACEPTIVE MATERIAL

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ABSTRAK

*Peningkatan peran pria dalam kontrasepsi, merupakan pencapaian kesetaraan gender yang lebih baik. Menyikapi masalah tersebut, perlu diupayakan pengembangan metode kontrasepsi untuk pilihan alternatif, yang sampai saat ini sangat terbatas karena pembinaan teknik kontrasepsi pria sangat rendah. Imunokontrasepsi merupakan suatu metode kontrasepsi yang menggunakan prinsip induksi respon imun, dengan target hambatan terhadap ikatan reseptor dan ligand yang terdapat pada sel telur dan spermatozoa. Metode kontrasepsi pria yang telah dilakukan selama ini hanya ada 2 macam, yaitu penggunaan kondom dan vasektomi. Namun kondom efektif mencegah kehamilan sebesar 75 – 80%, sedangkan vasektomi bersifat permanen. Untuk itu perlu pengembangan kontrasepsi baru yang harus berkhasiat jangka lama, tetapi bersifat reversibel sebagai penyebab azoospermia. Tujuan penelitian ini untuk memperoleh antibodi polyclonal protein fertilin beta membran spermatozoa manusia sebagai kandidat bahan imunokontrasepsi pria terutama pengembangan vaksin rekombinan. Metode penelitian yang digunakan adalah eksploratif-laboratoris. Prosedur penelitian sebagai berikut: Melakukan isolasi protein membran spermatozoa dengan teknik elektroelusi. Selanjutnya melakukan uji aktivitas protein fertilin beta berdasarkan pH, suhu dan waktu inkubasi. Isolat protein hasil elektroelusi digunakan untuk konfirmasi protein fertilin beta dengan teknik Western Blot, imunisasi isolat protein fertilin beta manusia pada kelinci betina. Koleksi antibodi polyclonal fertilin beta manusia, pengukuran titer antibodi fertilin beta. Diperoleh hasil bahwa protein fertilin beta membran spermatozoa manusia memiliki profil dengan BM 75 kDa. Pita yang terdapat pada elektroforesis SDS-PAGE adalah pita molekul fertilin beta sebesar 75 kDa. Imunisasi isolat protein fertilin beta 75 kDa membran spermatozoa manusia pada kelinci betina juga menghasilkan antibodi polyclonal fertilin beta.**(FMI 2012;48:6-11)***

Kata kunci: *antibodi polyclonal fertilin beta, imunokontrasepsi, protein membran spermatozoa*

ABSTRACT

*Increasing role in male contraception is to achieve better gender equality. Therefore, it is necessary to integrate contraceptive methods for developing alternatives, which until now is very limited because the development of male contraceptive is very low. As a contraceptive method, immunocontraceptive used the principles of immune response induction, with the target of inhibiting receptors binding and ligand that was found in egg and sperm. Male contraceptive methods that had been carried out so far has only 2 types, the use of condom and vasectomy. However, condom effectively prevents pregnancy of only 75-80 percent, while vasectomy will be permanent. So, it is necessary to develop a new contraception that has a long-term efficacy, but reversible as the main cause of azoospermia. This study was to obtain fertilin beta protein polyclonal antibodies from male sperm cell membrane as candidate for immunocontraceptive material, especially for recombinant vaccine development. The method used was explorative-laboratories. The procedures were sperm membrane proteins were isolated with electroelution technique. Then, tests were carried out to the proteins beta fertilin based on pH, temperature and incubation period. Protein isolate resulting from the electroelution was used for confirmation of fertilin beta protein techniques in Western Blot, and immunization of protein isolate beta fertilin to female rabbit, the collection of male beta fertilin polyclonal antibodie, and measurements of beta fertilin antibodies titer. The result showed that male beta fertilin protein from cell sperm membrane had a MW as high as 75 kda. The band found in gel electrophoresis SDS-PAGE was beta fertilin molecular band of 75 kda. Immunization of fertilin beta protein isolate from sperm cell membrane of kda 75 to female rabbits also produced fertilin beta polyclonal antibodies.**(FMI 2012;48:6-11)***

Keyword: *the antibodies polyclonal fertilin beta, immunocontraception, proteins sperm membrane*

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INTRODUCTION

The rapid growth of global population is the attention of family planning that is associated with reproductive health programs and a safe and easy method to limit the number of family members. The human population is estimated to cause a phenomenon which amounted to 10 billion by 2050. In Indonesia, the total population in 2004 has exceeded 200 million and its population control be implemented in a Family Planning (KB). Men KB participants only reached 1.3% of total 58.3% of all KB participants. Various studies have focused on male contraceptive immunocontraception methods. Immunocontraception method is expected to be safe,

effective, practical, side effects are smaller and have a chance to be accepted by users of the previous contraceptive techniques. Immunocontraception an immunological contraception with the principles given by injection using a material that is both antigenic and aims to prevent a meeting between spermatozoa and oocytes. One of the materials that have potential as a contraceptive vaccine is a sperm membrane protein (Naz 2006, Griffin 2003).

Membrane proteins of human spermatozoa have been found and have been tested as immunogenic proteins and the specific function in fertilization. These proteins include 17 kDa (Gritzzi et al 2003), 55 kDa and 95 kDa (Naz 2006), 57 kDa (Rajeev & Reddy 2004), 80 kDa (Bandivdekar et al 2005), PH -20 (Toshimori 2000), SPAG9 (Jagadish et al 2005). However, some researchers claim that the human sperm membrane proteins that have been found, did not meet the requirement of immunocontraception material.

Proteins that have been found are still conserved in other cell types, it is necessary to look for a membrane protein that is thought to exist only in specific spermatozoa. So that the membrane protein immunogenic the antibody induction outcome is not expected to inhibit the activity of other cellular networks. One of the sperm membrane proteins that have the potential was fertilin ß. Fertilin beta is only found in mature spermatozoa and ejaculated that function as adhesion molecules. It is necessary to prove whether fertilin beta human sperm membrane identified immunogenic and can provide responses in antibody formation.

MATERIALS AND METHODS

This research was an exploratory – laboratoris experimental, to obtain location protein fertilin beta on the human sperm membrane and produce antibodies polyclonall fertilin beta cell membrane sperm of human. Semen sampels from the fertil man and willing to participate in research will be isolated their membrane proteins spermatozoa. Man protein Isolate fertilin beta sperm cell membrane is a subject that will be used to antibodies polyclonal and developed further to material immunocontraseption. In the initial setup, the subjects received explanation about the aim of the research and approve participate with signing the letter appearing a greater willingness has been approved and tested by the Commission of Ethics Airlangga University Medical School. Samples taking Semen will be done at the clinic Andrology Dr Soetomo Hospital, then an investigation analysis of semen.

Samples Semen obtained from the fertil man (normozoospermia) that has been evaluated in dr. Soetomo General Hospital Clinic Andrology Surabaya. Ejaculate obtained from masturbation after abstinensia sexual 2-5 days. After liquifaction (37 0 C, 30 minutes) will be done analysis of Semen based on the instructions World Health Organization (1999). Sperm samples were placed in a tube ependorf plus PBS, divortek and disentrifuse 3000 room temperature, rpms (10 minutes), process is done twice. The result is pelet, plus detergents NOG (1N-Octyl- ? -D-Glycopyranoside) (enhances its standing) (Rajeev & Reddy 2004) or 2 percent CHAPS (Brimming with loss innocence Hexyl acids-1Propane-Sulphonic acid (Bohring et al 2001), it was carried out vortek for 10 minutes, sonificationed by using ultrasonic cleaner for 20 minutes and centrifugated, 6000 rpm for 5 minutes. Supernatan that is obtained then is added the ethanol with a comparison 1:1 and put in refrigerator compressors for 60 minutes (if not yet there is a mass and white, time to be added). If it has been seen a mass white sperm cell membrane hence a protein was centrifugate 10,000 room temperature, rpms (10-minute) so that a mass settle, then putting it in to refrigerator compressors at high temperature -20 o C for 5 minutes. Ethanol in extracting was removed and be wounded in order to lost smell of ethanol. Extracting diluted with buffer Tris-Cl with comparison between 1:1 for the volume of sediment extract membrane proteins sperm. Extracting stored in refrigerator compressors at temperature -20 o C

Immunization will be done in doe as sub cutan using protein isolate elution result as many as 200 µl with Complete Freunds Adjuvant (CFA) with a comparison 1:1 and be vortexed until formed emulsion. Immunization will be done two days after taking blood pre-immune response and in the fourth week experimental animals were immunized 4 all re-(booster) by using 200 µl protein isolate elution result that added Incomplete Freunds Adjuvant (IFA) with a comparison 1:1 for increasing an immune response to experimental animals. Booster II will be done two days after taking 5th blood (week 7) with the dose same with first immunization. As a comparison used rabbits without treatment (controls). Harvesting serum will be done in the 4th, 5 th, 6 th, 7 th and 8 th week of first immunization and the first taking done a week after second booster, and rabbit's blood taken from a vein in the ears rabbit (veins auricularis). Blood rabbit is incubated in the room temperature ? 10 minutes then centrifugated 1500 rpm for 15 minutes and then taken it's supernatan and then put him in cryotube for further saved in freezer with the temperature -70 o C or used for IgG purification.

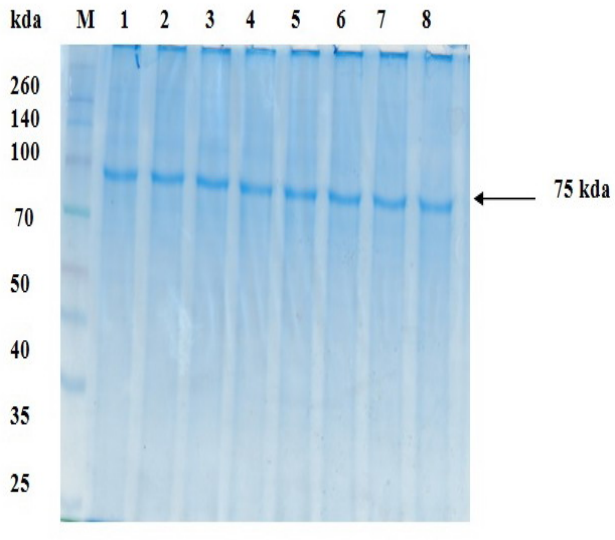

Figure 1. Ribbon profile protein fertilin beta man membrane sperm

Isolation of Immunoglobulin (antibodies) will be done with precipitating ammonium sulfate technique. A serum, purified with additional Ammonium Sulfate saturated (SAS 50%) with ratio volume between serum with SAS 50 percent, is homogenated for 1 minutes, resided for 10 minutes at high temperature as 5 0 C. Serum was then centrifugated with 3000 rpm speed for 30 minutes at high temperature as 4 0 C. Supernatan thrown away, precipitate added SAS 50% about ten times volume, serum homogenated then centrifugated again with a speed period for 30 minutes. Supernatan thrown away, precipitate added 0.2 M of buffer phosfat PH 7 with the same volume, was placed in selofan bags, then done dialysis in 0.01 M of buffer phosphate PH 7 on the temperature 5 0 C for 24 hours, end of dialysis was preceded by testing buffer dialysis outside selofan bags with BaCl2 solution (dialysis is stopped when it has not been formed white sediment BaSo4).

In the titer measurement membrane proteins anti sperm used methods indirect ELISA. Mikroplate 96 well coated with proteins adhesion as many as 100 ? l, then incubated 4 0 C for 24 hours. After that was carried washing with 0.05 percent PBS-Tween 20 as much as 6 times and then blocked by BSA grad 5 with a concentration 1% after that it was being washed and finally added substrate pNPP and if colors on the controls have been turned into a yellow, then reaction stopped. Titer result anti-protein adhesion read at ELISHA reader BIO-RAD system.

RESULTS

Man sperm cell membrane had been isolated by using detergent Tween-20 from the fertil man (has been clear feasibility of ethics of the Committee of Ethics or Medical Faculty Airlangga University). Protein isolate profile result sperm cell membrane confirmed with SDS PAGE in the Picture 1 .

SDS-PAGE result

From picture 1 sperm membrane proteins showed that result human SDS PAGE have ribbon profile protein as much as 1 band with molecular weight 75. Specifitas antibodies polyclonal protein result fertilin beta. The success induction ability of proteins fertilin beta that produced by anti- body polyclonal fertilin beta 75 kda, tested by using the method Western blot and dot blot. Western blot result showed the blue ribbon protein purplish patches with BM 75 kda, can be seen in the Picture 2 . This shows that protein fertilin beta that had been isolated from the man spermatozoa membrane identified by the antibodies polyclonal fertilin beta protein.

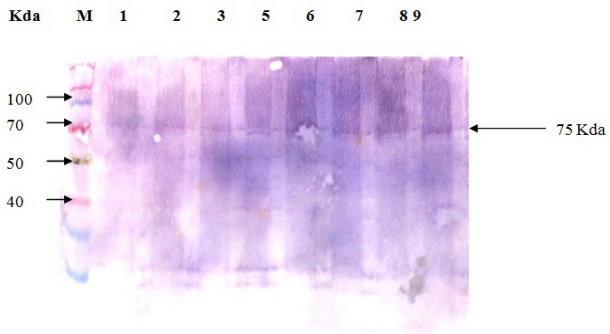

Figure 2 . Trial specifitas antibodies fertilin beta using western blot . Note: M = protein marker, 1-9 = fertilin beta samples

A qualitative, specifitation test is based on the dot blot methode using a pacifier blotter (Bio-Rad), and produced by data visualization of the occurrence of specific reaction between the antigen or protein isolate fertilin beta with its antibody or the introduction to the antibodies protein fertilin beta protein to fertilin beta which are seen as spot purplish patches, such as in the Picture 3 . By using antibodies polyclonal fertilin beta on bleeding 1-10 and with dilution of 1/20, shows that there is a blue color gradation purplish patches. Color gradation shows low and higher values titer antibodies fertilin beta. Based on color gradation in qualitative research shows that 8th bleeding give color darkest landscape, which means the bleeding was titer antibodies fertilin beta shows the highest.

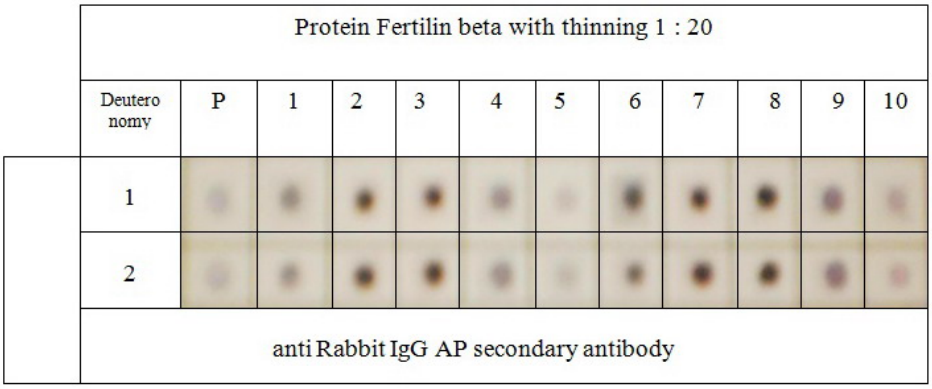

Figure 3. Specification test antibodies proteins fertilin beta using the dot blot . Positive reactions between antibodies fertilin beta with protein beta fertilin assembly with blue purplish patches.

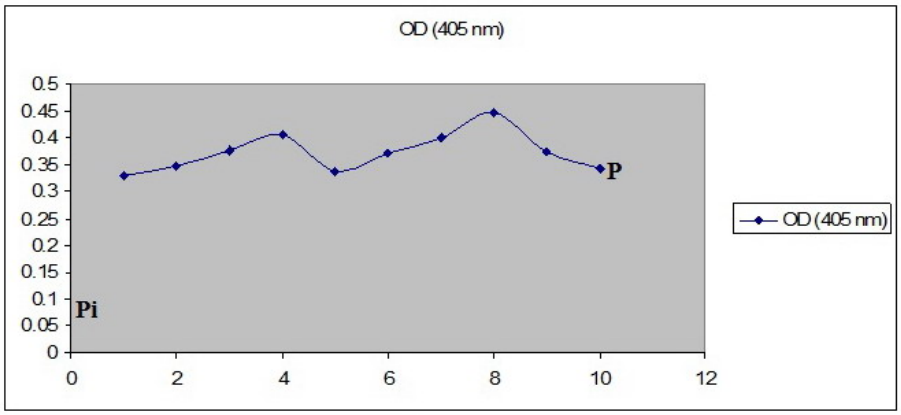

Figure 4 . Titer profile antibody protein fertilin beta 1/20 in rabbit (Oryctolagus cunniculus)
Note: Pi = control (pre-immune response), P = treatment immunization.

Conformational result with the data that is measured in qualitative research using the indirect ELISA, can be shown in picture 3 . Look at titer 8th4r bleeding.

The titer measurement polyclonal antibodies fertilin beta protein

The ability of protein fertilin beta to induct antibodies polyclonal fertilin beta in numerical terms can be measured by the method indirect Elisa (Enzyme linked immunoabsorbant assay). The antibodies polyclonal existence fertilin beta measured based on 405 nm absorbantion at it's titer result against protein fertilin beta. Picture 4 , is the result titer antibodies polyclonal fertilin beta in rabbits with thinning 1/20.
Result antibodies polyclonal fertilin beta titer shows highest 8th bleeding or the 79th day after second booster. The highest in 8th bleeding was also indicated by trial qualitative research based on dot blot method, which produces darkest color. In general, the data which is produced from the method indirect Elisha shows that fertilin beta were able to induces antibodies polyclonal fertilin beta.

DISCUSSION

Specificity of antibodies polyclonal fertilin beta induction result

Test result western blot shows that the antibodies fertilin beta produce positive response to protein fertilin beta. That Western blot result was giving the impression that the molecules fertilin protein beta bound with specific antibodies polyclonal fertilin beta as primary antibodies and anti-rabbit IgG antibodies as secondary. The antibodies polyclonal fertilin beta and anti-rabbit IgG can detect the protein fertilin beta as a band brown with

MW 75 kda. So that it will be convinced that tape that appeared in gel electrophoresis SDS-PAGE is molecules fertilin beta ribbon of 75 kDa.

Dot blot is another specifitation test used in this research. Positive result marked with the formation color dark blue dot purplish patches. This can be interpreted that isolates fertilin beta could be identified by the antibodies polyclonal fertilin beta or shows that there is a specific reactions between isolates fertilin beta with antibodies fertilin beta. A dark blue color are caused by additional a secondary antibodies labeled alkaline phosphatase (AP) with substrate western blue . There is no visualization colors dot blot dark blue or the negative result is shown in blood serum pre-immune response.

Dot blot result in the figure 3, also shows that there is a color gradation. More concentration antibodies polyclonal fertilin beta, the more increased incidence in color. The intensity color blue dot blot darkest landscape only on 8th bleeding, when this result was confirmed to Elisa result test in the Picture 4, shows that the top producing antibodies happened on 8th bleeding or in the day 86. Based on this result, it is believed that the darkest color blue dot blot contains highest concentrations antibodies fertilin beta.

Titer profile antibodies fertilin beta

Rabbits immunization with isolates man sperm membrane proteins has been done by some researchers, and will be able to give a response to the formation of antibodies. In this research, immunization protein isolate fertilin beta 75 kda man spermaozoa membrane in doe also produce antibodies polyclonal fertilin beta. A comparison titer antibodies polyclonal fertilin beta from rabbits which immunized fertilin protein beta, with titer serum pre-immune system can be seen in the Picture 4. Result of the average measurement per-serum titer immune shows absorbansi of 0.038, while titer antibodies after vaccination is very high with absorbansinya ranging from 0.331-0.343. These results indicate that immunization with proteins fertilin beta responds with the formation antibodies polyclonal fertilin beta.

Titer profile antibodies polyclonal fertilin beta is shown in the Picture 4. There is a fluctuation titer antibodies polyclonal fertilin beta that there was a decline and the increase titer. In the day 36 (bleeding I), titer antibodies fertilin beta increased until the day 59 (bleeding IV) and, experienced in the day 66 (bleeding V). Titer antibodies polyclonal fertilin beta experienced an increase and experienced a peak in the day to 96 (bleeding to VIII) after immunization primary, decreased again until the day to 110 (bleeding to 10).

The declining titer antibodies as experienced by by titer antibodies polyclonal fertilin beta, there may be a regulation mechanism during antibodies formation. Mechanism is in the form a decrease in the level antigens, setting by idiotip and emphasis by T cells. The increase titer antibodies polyclonal fertilin beta can be caused by the secondary response. The characteristics of secondary response was the creation of the immunoglobulin more quickly and took place in a long time, and to achieve the most high titer. The response of the secondary because there is a immunization, which was given through booster, causing proliferation memory cells took place in a quick and formed more antibodies for a long time.
Protein fertilin beta protein concentration 75 kda have 2407 µg/ml. It is hoped that can induct antibody formation. Through immunization with proteins fertilin beta, the antibodies polyclonal fertilin beta protein that has been produced suspected to have been formed through an immune response humoral. According to Subowo (1993), the antibodies produced from an immune response humoral, generally as IgGs, the majority of which dissolved in the blood. IgG present in the blood with a long period of time (3-10 weeks). Immune response began with such an antigen presenting cell (APC) which processes antigens to cause interaction with the cells immune system. These cells work presents an antigen to lymphoid cells that sentitized. APC cell was presented with MHC (Major histocompatibility coplex) Class II to antigens are identified by lymphoid. Signal will be brought by T helper cells (CD4+) through the receptor TcR, who then gave a signal to B cells to proliferate and it differentiates to a population (clone) plasma cells that produce and deliver specific antibodies in the blood.

CONCLUSION

Protein fertilin beta can be found in male spermatozoa membrane with molecular weight of 75 kda and characteristics of imunogenisity in experimental animals. Produced titer antibodies polyclonal of the fertilin beta has the highest level in week 8 or day 79.

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