ROLE OF IMMUNOGLOBULIN G (IgG) FROM THE INDUCTION OF *Escherichia coli* PILI ADHESION PROTEIN ISOLATED FROM INFERTILE MALE SEMEN WITH 32.2 KDA MOLECULAR WEIGHT AS OPSONIN AND ANTI-ADHESION AN IN VITRO *Escherichia coli* INFECTION

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ABSTRAK

Escherichia coli (E. coli), penyebab utama infeksi saluran kelamin laki-laki, asimtomatik dan dapat menyebabkan infertilitas pria. Kami telah berhasil mengisolasi dan mengkarakterisasi protein isolat E. Pili coli dari semen orang yang tidak subur yang berfungsi sebagai adhesin dengan berat molekul 32,2 kDa. Penelitian ini bertujuan untuk membuktikan kemampuan IgG hasil protein adhesi yang diinduksi pili E. MW coli 32,2 kDa sebagai opsonin untuk mengetahui nilai aktivitas dan kapasitas fagositik dan sebagai anti adhesi dengan menghitung jumlah rata-rata E. coli yang terlampir. Untuk spermatozoa manusia. Superman sperma jantan E. coli dikultur dengan menggunakan bakteriologi standar. Makrofag peritoneal diisolasi dari tikus. Spermatozoa dari donor disiapkan menggunakan Sil dengan Select Plus. IgG diperoleh dari tikus yang diimunisasi dengan (1) PBS (kontrol), (2) protein adhesi E. coli pili yang diisolasi dari semen manusia tidak subur dengan MW sebesar 32,2 kDa dan (3) E. coli yang lemah diisolasi dari air mani laki-laki yang tidak subur. Nilai aktivitas fagositik ditentukan dengan menghitung jumlah sel yang teraktivasi makrofag proses fagositosis dalam 100 sel. Nilai kapasitas fagositik ditentukan dengan menghitung jumlah bakteri yang tertelan oleh 25 makrofag. Uji anti adhesi dilakukan dengan menghitung jumlah bakteri yang menempel pada 100 spermatozoa. Hasil penelitian ini menunjukkan perbedaan (p=0,000) pada aktivitas fagositik dan kemampuan fagositik (p=0,000) antara perlakuan (1) dan (2), dan antara perlakuan (1) sampai (3). Namun, pengobatan (2) dan (3) tidak berbeda dalam aktivitas fagositik (p=0,693) atau pada kapasitas fagositosis (p=0,125). Uji anti-adhesi menghasilkan perbedaan (p=0,000) dalam jumlah E. coli yang terikat pada spermatozoa manusia antara perlakuan (1) dan perlakuan (2), dan antara perlakuan (1) dan (3). Jumlah E. coli yang melekat pada spermatozoa manusia antara perlakuan (2) dan perlakuan (3) tidak berbeda nyata (p=0,371). Kesimpulannya, IgG dari induksi protein adhesi E. coli pili isolat laki-laki mani yang tidak subur dengan MW sebesar 32,2 kDa dapat meningkatkan aktivitas dan kapasitas fagositik serta berfungsi sebagai anti adhesi. Dengan demikian, IgG dari induksi protein adhesi E. coli pili isolat manif laki-laki yang tidak subur dengan MW 32,2 kDa sangat protektif terhadap infeksi E. coli in vitro, sehingga dapat digunakan sebagai bahan untuk mencegah infeksi saluran reproduksi laki-laki karena E Coli. ***(FMI 2017;53:94-100)***

Kata kunci: *imunoglobulin, anti-adesi, opsonin, E. coli, spermatozoa, protein adesi pili E. coli dari semen pria infertil dengan BM 32.2 kDa.*

ABSTRACT

Escherichia coli (E. coli), a major cause of male genital tract infections, is asymptomatic and may result in male infertility. We have succeeded in isolating and characterizing proteins of E. Pili coli isolates from semen of infertile men who function as adhesin with a molecular weight (MW) 32.2 kDa. This study aims to prove the ability of IgG results adhesion proteins induced pili of E. MW coli 32.2 kDa as opsonin to determine the value of the activity and phagocytic capacity and as an anti- adhesion by calculating the average number of E. coli that attached to human spermatozoa. E. coli infertile men's semen were cultured using standard bacteriology. Peritoneal macrophages were isolated from mice. Spermatozoa from donors were prepared using Sil with Select Plus. IgG was obtained from mice immunized with (1) PBS (control), (2) E. coli pili adhesion protein isolated from infertile men semen with MW of 32.2 kDa and (3) weakened E. coli isolated from infertile men's semen. Phagocytic activity value was determined by counting the number of cells activated macrophage phagocytosis process in 100 cells. Phagocytic capacity value was determined by counting the number of bacteria ingested by 25 macrophages. Anti-adhesion test was done by counting the number of bacteria attached to 100 spermatozoa. The results of this study showed difference (p=0.000) in phagocytic activity and phagocytic capacity (p=0.000) between treatment (1) and (2), and between treatment (1) to (3). However, treatment (2) and (3) did not differ neither in phagocytic activity (p=0.693) nor in phagocytosis capacity (p=0.125). Anti-adhesion test produces difference (p=0.000) in the number of E. coli that attached to human spermatozoa between treatments (1) and treatment (2), and between treatments (1) and (3). The number of E. coli that attached to human spermatozoa between treatment (2) and treatment (3) was not significantly different (p=0.371). In conclusion, IgG from the induction of E. coli pili adhesion protein of infertile men semen isolates with MW of 32.2 kDa can increase phagocytic activity and capacity as well as serve as an anti- adhesion. Thus, IgG from the induction of E. coli pili adhesion protein of infertile men semen isolates with MW of 32.2 kDa is protective against in vitro E. coli infection, so that it can be used as material to prevent male reproductive tract infections due to E. coli. ***(FMI 2017;53:94-100)***

Keywords: *immunoglobulin, anti-adhesion, opsonin, E. coli, spermatozoa, E. coli pili adhesion protein isolated from infertile male semen with MW of 32.2 kDa.*

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INTRODUCTION

Infertility is a problem in the marriage. Most people assume that the woman is the cause of the inability to provide offspring. According to Khanna et al (1992) 10% of infertility cases are caused by infection of male genital tract. The results of studies on infertility clinics in Surabaya found that from 1727 of semen samples cultured from 1991 to 1997 were infected with *S. epidermidis, E. aerogenes, S. faecalis, S. aureus, S. viridans, Pseudomonas, E. coli* and *S. pyogenic. E. coli* has effect on motility (Sukarjati 2002), human spermatozoa vitality (Sukarjati 2001) and damages the integrity of the human sperm membrane (Sukarjati 2009).

Semen contaminated with *E. coli*, both in vivo and in vitro, have higher levels of Reactive Oxygen Species (ROS) and Malondialdehyde (MDA) than uncontaminated semen (Sukarjati, 2009). Thus, it has been proved, both cellularly and molecularly, that *E. coli* has negative effect on human spermatozoa quality. *E. coli* can degrade spermatozoa quality since it has the ability to attach to spermatozoa plasma membrane by using pili. *E. coli* pili protein isolated from infertile male's semen have been isolated and characterized. Th protein had Molecular Weight (MW) of 32.2 kDa. It has been proved that the pili protein is able to block the attachment of *E. coli* to human spermatozoa in vitro (Sukarjati 2013). In addition, it has been demonstrated that *E. coli* pili adhesion protein with 32.2 kDa MW is not toxic to spermatozoa (Sukarjati 2010). It has been proved that *E. coli* pili adhesion protein with 32.2 kDa MW is immunogenic (Sukarjati, 2011). The remaining question was whether IgG resulting from the induction of *E. coli* pili adhesion protein with 32.2 kDa MW is protective? The ability of projectivity was tested using phagocytosis test and adhesion inhibition test. If the IgG is immunogenic, non-toxic and protective, it can be used for the prevention of male genital tract infection.

This study is important because in general infection is contracted through sexual intercourse with alternating partner who grouped into STD (Sexual Transmitted Disease). STD is a matter of concern to the experts, not only in Indonesia but also throughout the world. STD that causes typical symptoms, such as gonorrhoe, is not a problem anymore today because it can be treated completely. The remaining problems are asymptomatic infections of genital tract and male acessory sexual glands, including prostatitis, epididymitis, vesikulitis, and orchitis, with the result of male infertility. The main cause of genital tract and male accessory sex glands infection is *E. coli*. Therefore, its prevention is necessary. The purpose of this study was to describe the protectivity of IgG resulting from *E. coli* pili adhesion protein induction from isolates of infertile male semen with 32.2 kDa MW in in vitro *E. coli* infection. The protectivity was measured by identifying phagocytic capacity and activity of peritoneal macrophages in mice ncubated with *E. coli* in vitro and anti-adhesion test on human spermatozoa.

MATERIALS AND METHODS

Protein adhesion pili of *E. coli* isolates semen of infertile men MW 32.2 kDa. *E. coli*, mice, mouse peritoneal macrophages, IgG Protein induction results adhesion pili of *E. coli* isolates semen of infertile men MW 32.2 kDa, human spermatozoa.

Antigen Preparation

Antigen protein adhesion pili of *E. coli* isolates semen of infertile men MW 32.2 kDa regular). The isolation of *E. coli* pili adhesion protein MW 32.2 kDa was performed in the following steps:

Propagation *E. coli* and Enriching Fimbrae (Pili)

E. coli from rejuvenated stock prior to making culture in Mc Conkey agar medium temperature of 370 C for 24 hours. Then the culture of the medium in the inoculation into 500 ml Erlenmeyer flask containing BHI medium, and incubated 24 hours, then the bacteria was poured into 250 ml 20 bottles already containing 25 ml of TGC medium (medium to enrich fimbrae bacteria), each of 10 ml. Do incubation 370 C for 48 hours. Furthermore, cultures of *E. coli* were collected together in a 1000 ml Erlenmeyer flask and prepared to do the cutting pili.

Pili Cutting

Suspension of *E. coli* ready for slaughter in omnimixer put in sterile tubes and set on omnimixer tool, then use the tool cutting pili omnimixer at temperatures 40C, 3000 rpm for 30 seconds. Once in omnimixer, then the samples were centrifuged at 40C, 6000 rpm for 15 minutes. Supernatant was collected in tubes and labeled supernatant 1 (the results of the first piece) and the pellet was suspended with PBS pH 7.4 as much as 1:1. Then cutting again with omnimixer at 40C, 3000 rpm for 30 seconds. Then centrifuged at 40C, 6000 rpm for 15 minutes. Supernatant was collected in tubes and labeled supernatant 2 (the result of cuts to two) and the

pellets were suspended in sterile PBS pH 7.4 as much as 1:1. The cutting process is repeated up to 5 times. Subsequently the supernatant 1, supernatant 2, 3 supernatant, the supernatant 4 and supernatant 5 respectively were respectively moved to at 1.5 ml eppendorf microtube and centrifuged 12,000 rpm, 40^{o}C for 15 minutes. Obtained supernatant and pellet. Super-natant and a pellet fraction pili are bacterial cells without pili (Whole cell supernatant). Furthermore, on each pili pili labeled 1 (pili results first cutting), 2 pili (pili results to two cuts), 3 pili (pili cutting results to three), 4 pili (pili cutting results to four) and 5 pili (pili cutting results to five). Similarly, the pellets also are labeled according to the results of cutting. The material is then stored at-20^{o}C until used for subsequent examination.

Pili Fraction Dialysis

Pili fractions are obtained, the dialysis process performed using PBS solution of pH 7.4 at a temperature of 40C for 2x24 hours to remove residual TCA. Furthermore, the dialysate was precipitated with 35% ammonium sulfate, centrifuged 6000 rpm 40C. Supernatant was discarded, pellet was suspended with PBS sufficiently and do dialysis again. Results dialysis is pili proteins and stored at-200C until used for further examination.

Pili fraction pieces I made using SDS PAGE electrophoresis. Electrophoresis was performed by preparing samples of work steps, making a gel plate comprising around 12.5% gel and stacking gel and running buffer made. Separation process (running) is stopped after the blue color of the marker located approximately 0.5 cm from the lower limit of the gel plate (± 90 minutes).

Staining of proteins made by soaking the gel in a solution of 0.25% Comassie brilliant blue for 30 minutes. Results of SDS-PAGE gel of 12.5% contained as many as 15 pieces of ribbon with MW 32.2 kDa protein was cut horizontally at the top and bottom of the ribbon. Then cut pieces of ribbon collected included dialysis membrane to do electroelution using a horizontal electrophoresis apparatus, with a buffer solution electrophoresis running buffer, 125 volt electricity for 2 hours. Results electroelution dialysis with PBS solution for 2x24 hours, the solution was replaced every 24 hours. Eluates in endapkan with cold absolute ethanol solution overnight, in order to obtain pure MW 32.2 kDa protein that is ready for use.

Antigen of *Escherichia coli*

E. coli from rejuvenated stock prior to making culture in Mc Conkey agar medium temperature of 370 C for 24 hours. Then the culture of the medium in the inoculation into Erlenmeyer containing 50 ml of BHI medium, and incubated 24 hours. Inoculum was centrifuged at 10 000 rpm for 10 minutes. Pellet was resuspended with 5 ml of PBS and then centrifuged at 10 000 rpm 10 minutes. Pellets were dissolved in 5 ml of PBS, homogenized with vortex, measured on OD 620 nm with 0.1 to determine the bacterial content of 108 cells/ml. Further suspension of *E. coli* is inactivated in a water bath at a temperature of 56 degrees Celsius for 60 minutes, then cooled and ready to be used as an antigen to produce antibodies.

Antibody Production

Immunization and harvest serum containing IgG

Experimental animals used were mice, are male. Before use in the prior acclimatization for 2 weeks. Mice were divided into 3 groups, each group of 10 mice. The first group, mice were immunized with a protein antigen adhesin pili of *E. coli* isolates semen of infertile men MW 32.2 kDa. Immunization procedure was as follows: 100 mg of antigen was suspended with 100 mL PBS, plus complete Freund adjuvant 100 mL, were mixed to form a white emulsion. Then injected sub-cutaneous at point 5, where the parts to be injected in the first disinfection with 70% alcohol. Came one week later repeat antigen injected with Freund's adjuvant mixed incomplete in the sub-cutaneous in 5 points. Immunization followed each week in the same way until the end of week five. Three days after the last immunization mice in surgery. The surgery for two purposes, namely for the isolation of murine peritoneal macrophages and taking blood from the heart to obtain IgG. Blood was collected in tubes that have been labeled and stored in cupboards ice. Blood stored in cupboards ice will break up into sections containing blood cells and serum. Then in each tube was taken and the serum was transferred to another tube. The second group, mice immunized with inactivated *E. coli* bacteria in advance as described above. Immunization procedure is similar to the first. The third group, the control mice injected with PBS only were mixed with Freund ajuvant alone.

Antibody Purification

SAS serum plus 50% with a volume ratio of 1:1, in the vortex, allowed to stand 30 minutes in the cupboard ice, then centrifuged 6000 rpm, temperature of 40^{o}C for 10 minutes. SAS pellets plus 50% by volume of 10 x pellet volume, in the vortex, allowed to stand in an ice cupboard 30 minutes then centrifuged at 1000 rpm, temperature of 40C for 10 minutes. Furthermore pellet plus 0.2 M phosphate buffer pH 8 with a ratio of 1:1, in the vortex. Dialysis in phosphate buffer pH 0.1 alamari 7 in ice overnight. Further coupled with cold ethanol volume ratio of 1:1 and stored in cupboards ice 30

minutes. Then centrifuged 6000 rpm 10 minutes and put the freezer 5 minutes. Last pellets plus Tris Cl 200 mL and stored at-200ºC.

Phagocytosis Test

Macrophages Preparation

Mice were anesthetized with chloroform turned off. Abdominal skin of mice was opened and the peritoneum of mice sheath cleaned with 70% alcohol. Furthermore, 10 cc of a solution of RPMI (Rosewell Park Memorial Institute) cold injected into the peritoneal cavity. Peritoneal macrophages massaged gently to get quite a lot. Then the aspirated fluid back and put in a falcon tube 15 cc. The fluid is then centrifuged at a speed of 1200 rpm at 4°C for 10 minutes. Then washed with PBS 3 times. After the supernatant was discarded, then added 3 ml of RPMI complete medium consisting of RPMI 1640, FBS (Fetal Bovine Serum) 10% of the pellets were obtained. Cells were counted with a hemocytometer and then diresuspensikan again with complete RPMI medium to obtain a cell suspension with a density of 2.5 x 106 cells/ml. After the cells were cultured in complete medium in 24-well microplate is a flat bottom and basically given a glass object (coverslip), each pitting 200 uL (density of 5 x 10^5 cells/ml), then incubated in a CO2 incubator at 37°C for 30 minutes., then added 1 ml of complete RPMI medium pitting each and incubated again for 2 hours.

Preparation of *E. coli* isolates semen of infertile men

E. coli from rejuvenated stock prior to making culture in Mc Conkey agar medium temperature of 370 C for 24 hours. Then the culture of the medium in the inoculation into Erlenmeyer containing 50 ml of BHI medium, and incubated 24 hours. Inoculum was centrifuged at 10,000 rpm for 10 minutes. Diresuspensikan pellet with 5 ml of PBS and then centrifuged at 10 000 rpm 10 minutes. Pellets were dissolved in 5 ml of PBS, homogenized with vortek, measured on OD 620 nm with 0.1 to determine the bacterial content of 108 cells/ml.

Phagocytosis Test

IgG induction of adhesion proteins results pili of *E. coli* isolates MW semen of infertile men and 32.2 kDa IgG result of induction of *E. coli* bacteria that has been attenuated each number 100 ug/ml and 0.5 x 10^8 cells/ml mixed bacterial micro tubes were incubated at 30 minutes on seker. Furthermore, the suspension in a centrifuge at 1000 rpm for 10 min and pellets dipeoleh in RPMI medium added. The suspension containing the bacteria and IgG is then added to the macrophages that had been prepared, and then incubated for 2 hours at 37 degrees C in CO2 incubator. After incubation for 2 hours at the top of the liquid waste in a manner microplate pipette. Then preparations in fixation with absolute alcohol and stained with Gram stain.

Values determined according to the number of phagocytic activity of macrophage cells that are actively engaged in the process of phagocytosis in 100 cells and expressed in percent. Meanwhile, the phagocytic capacity value is determined by counting the number of bacteria in ingested by macrophages 25 are still active.

Anti-adhesion Test

Spermatozoa Preparation

Spermatozoa from 10 male donors who have normal spermatozoa according to WHO criteria (1999) is a motility of 25% or $a^3 + b^3$ 50%, 30%³ normal morphology, sperm concentration of 20 million/ml, the volume of 3 ml or more. When it is normal spermatozoa obtained with these criteria, then the sperm prepared by Sil Select Plus with the following steps: Medium upper tube is put in through the walls of the tube, then put the medium with the lower bottom of the tube with the needle position carefully so as not to mix, then cement is placed through the tube wall. Centrifuged for 20 min at 2500 rpm, the supernatant was discarded, the pellet plus 2 ml of washing medium, then centrifuged for 10 marry at 2500 rpm, the supernatant was discarded and the pellet is left 0.5 ml. Furthermore, concentrations calculated using Neuber haemositometer and carried out observations of sperm motility.

Spermatozoa with IgG coating

Each microtube containing specific IgG concentration plus 100 uL spermatozoa were prepared. Then, in rocking with a shaking water bath at 37°C for 60 minutes

Incubation of spermatozoa coated with *E. coli* IgG

After spermatozoa coated with IgG and then added 100 uL of *E. coli* that has been prepared. Furthermore shaken again by shaking water bath at 37°C for 60 minutes. After 60 minutes eppendorf microtube taken from the shaking water bath and centrifuge made 1500 rpm for 10 min at room temperature. The supernatant containing the bacteria are not attached to the spermatozoa in the exhaust, and the pellets are left around 100 uL homogenized by means pipetted. Fur-thermore, the objects created on glass and smear staining performed. Carried out observations of the number of *E. coli* attached to spermatozoa using a microscope with a magnification of 1000 times. Obser-vations were made on 100 spermatozoa on each preparation.

RESULTS

Phagocytic activity data

After phagocytosis test, then obtained data on activity and phagocytic capacity. The data presented in the graph in Figure 1.

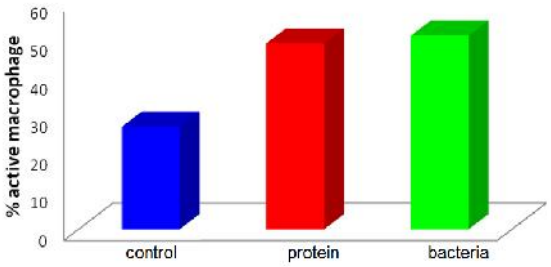

Fig. 1. Phagocytic activity of peritoneal macrophages of mice with treatment (1) controls. (2) Protein (IgG result of induction of protein adhesion pili of *E. coli* isolates semen of infertile men MW 32.2 kDa), (3) bacteria (IgG result of induction of *E. coli* bacteria were inactivated in advance).

Phagocytic capacity data

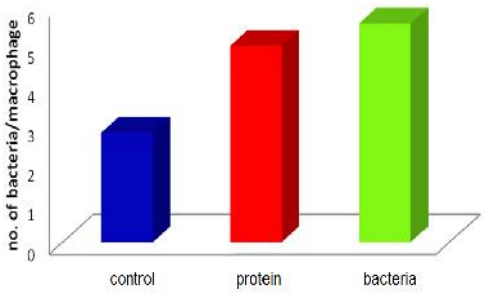

Fig. 2. Phagocytic capacity of peritoneal macrophages of mice with treatment (1) controls. (2) Protein (IgG result of induction of protein adhesion pili of *E. coli* isolates semen of infertile men MW 32.2 kDa), (3) bacteria (IgG result of induction of *E. coli* bacteria were inactivated in advance).

The results of the analysis of data obtained as follows: there are differences in the results of the induction of IgG protein and *E. coli* to phagocytic activity (p=0.000). Differences occurred between the control IgG protein induction outcome (p=0.000) and IgG control the induction of *E. coli* results (p=0.000). While the induction has IgG and IgG proteins induced *E. coli* results showed no differences in phagocytic activity (p=0.693)

The data obtained analyzed with the following results: there is a difference of IgG protein induction results and *E. coli* to phagocytosis capacity (p=0.000). The phagocytic capacity differences occurred between the control IgG protein induction outcome (p=0.000) and controls with IgG induction of *E. coli* results (p=0.000). While the induction hasi IgG and IgG proteins induced *E. coli* results do not happen phagocytic capacity difference (p=0.125)

Phagocytosis Test Results Visualization

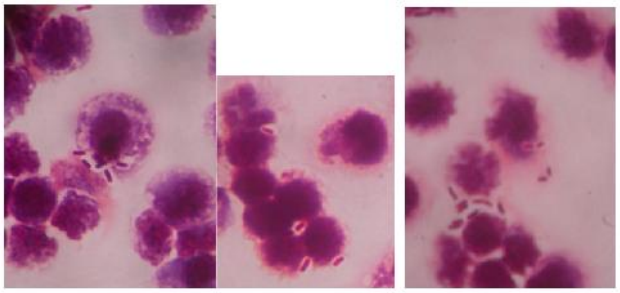

Fig. 3. Control group

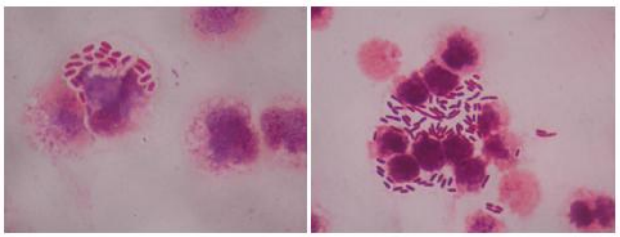

Fig. 4. Group Proteins induced IgG results

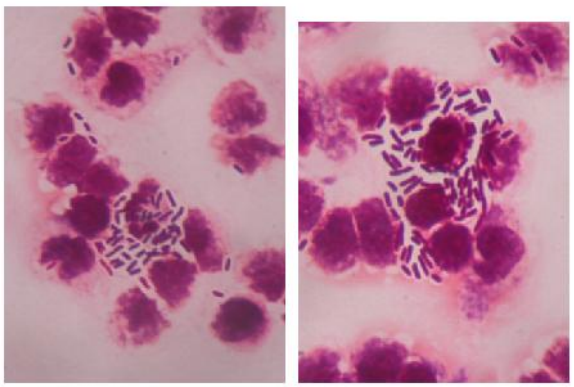

Fig. 5. Group IgG induction results inactivated *E. coli* bacteria

Shown in the picture that the number of activated macrophages and the number of bacteria in phagocytes by macrophages in control is less than the number of activated macrophages and the number of bacteria in phagocytes in *E. coli* were incubated with IgG results pili of *E. coli* protein induction Bm and the 32.2 kDa *E.*

coli were incubated with IgG results induced by *E. coli* bacteria that has been attenuated.

Anti-adhesion test results

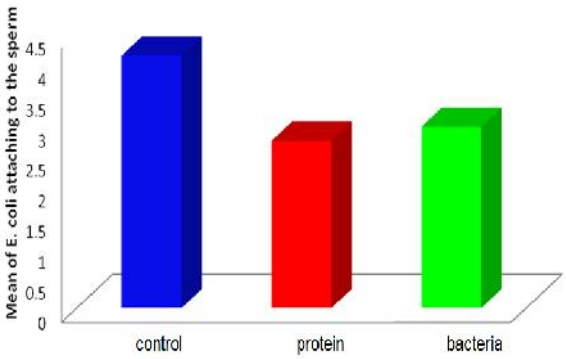

Fig. 6. Inhibition of adhesion of *E. coli* test on human spermatozoa with treatment (1) controls. (2) Protein (IgG result of induction of protein adhesion pili of *E. coli* isolates semen of infertile men MW 32.2 kDa) (3) Bacteria (IgG result of induction of *E. coli* bacteria were inactivated in advance).

The results of data analysis in the can there is a difference (p=0.000) the number of bacteria attached to human spermatozoa between the control-treated protein (IgG result of induction of adhesion proteins of *E. coli* isolates semen of infertile men) and bacteria treatment (IgG result of *E. coli* isolates induced cement infertile men who dilemhkan). While the number of bacteria attached to human spermatozoa between the treatment and the treatment of bacterial proteins no difference (p=0.371)

DISCUSSION

Phagocytosis is the ingestion of microbes by phagocytic cells. Phagocytic process can be divided into two stages, namely the stage of attachment and ingestion stages. During the attachment phase, occurring bacteria and powerful touch fagositosit cells. Phase of swallowing is swallowing microbes and microbes then inserted into the cytoplasm of the terinvaginasi.

The process of phagocytosis by macrophages can be increased by the presence of antibodies or immuno-globulin. Incubation of *E. coli* with the aim to coat IgG with IgG *E. coli*, in the hopes of opsonization. Known causes of bacterial opsonization by phagocytic activity of macrophages so that greater. It is evident that the results of this study phagocytic activity and phagocytic capacity was higher in *E. coli* were incubated with IgG and IgG protein induction outcome results in the induction of *E. coli* bacteria compared with controls. Not the difference between phagocytic activity and capacity of IgG and IgG protein induction results induced *E. coli* bacteria results indicate that the pili of *E. coli* adhesion protein isolate MW cement 32.2 kDa infertile men have the same ability with *E. coli* in causing the immune response and IgG role in its role as an opsonin produced. Thus the pili of *E. coli* adhesion proteins semen of infertile men isoalat MW 32.2 kDa can be developed as a candidate vaccine material in preventing infection of the reproductive tract. Development of a vaccine based on a protein adhesin is a new approach to preventing infectious diseases in which the vaccine will provide two punch in bacteria that antibodies against these proteins will block adhesion and signaling in bacteria to be destroyed by the immune system.

Research adhesin protein as a vaccine offers hope for the future. Many studies done in the search for a vaccine material based on the ability of bacteria attached to the Host. Experiments in mice immunized with antigen Fim H can decrease colonization of *E. coli* in the bladder more than 99% (Salyers and Whitt, 2002). This adhesin responsible for recognition and binding to a specific receptor structures on the host cell. Bonding that occurs may stimulate the host cell signal transduction in host defense cells that activate or inhibit cellular processes that facilitate bacterial invasion.

In adhesion inhibition test results induced IgG protein adhesion pili of *E. coli* isolates MW semen of infertile men and 32.2 kDa IgG result of induction of *E. coli* isolates were attenuated semen of infertile men by observing the number of bacteria attached to human spermatozoa. Average number of bacteria attached to the control is 4.13 per sperm and the average number of bacteria attached to the spermatozoa incubated with *E. coli* pili protein isolate MW semen of infertile men is 32.2 kDa 2.74 per sperm, while the average number of *E. coli* are attached to sperm were incubated with IgG result of induction of *E. coli* isolates were attenuated semen of infertile men is 2.97. The second treatment of the statistical analysis showed no difference, whereas if there is a difference compared with controls. This suggests that the induction of adhesion proteins IgG results pili of *E. coli* isolates cement MW 32.2 kDa infertile men have the ability to inhibit the attachment of bacteria to human spermatozoa. Thus the IgG is able to bind to the surface antigens of *E. coli* and cause a decrease in the ability of the bacteria to the plasma membrane adhesivitas spermatozoa.

The results of this study concluded there was no difference in the phagocytic activity and capacity test results

induced phagocytosis of IgG protein between pili of *E. coli* isolates MW semen of infertile men and 32.2 kDa IgG result of induction of *E. coli* bacteria that has been weakened and there is a difference when compared with control. Thus the results of the induction of IgG protein isolates of *E. coli* pili semen of infertile men MW 32.2 kDa protective mechanisms and increasing the activity of phagocytic capacity and acts as an anti-adhesion.

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