

The Effect of Anti-VDAC3 Recombinant Antibody on the Motility, Viability, and Membrane Integrity of Ejaculated Human Sperm

Laila Chuvita¹, Nurul Hikmawati¹, Silvia Werdhya Lestari^{2,3}, Andon Hestiantoro^{3,4}, and Asmarinah^{2,3*}

¹Master's Programme in Biomedical Sciences, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia

²Department of Medical Biology, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia

³Human Research Infertility and Family Planning Cluster of IMERI, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia

⁴Department of Obstetrics and Gynecology Clinic, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia

Email: {laila.chuvita, nurul.hikmawati}@ui.ac.id, {finalylsilvia, andonhestiantoro, asmarinah.si}@gmail.com

Abstract—Voltage-Dependent Anion Channels (VDACs) is one of the ion channels in sperm that play an important role in the transport of ATP, ions, and metabolites in the membrane of cells. VDAC isoform 3 in human sperm was recognized by anti-VDAC3 recombinant antibody. Fresh ejaculated human sperm samples from 12 donors were collected and treated with rabbit's serum containing anti-VDAC3 antibodies, as well as with the preimmune serum that was produced by our self from the previous study, in a ratio of 1:1. Sperm motility parameters, i.e. general motility, Wobble (WOB), Curvilinear Velocity (VCL), Linearity (LIN), Average Path Velocity (VAP), and Straight-Line Velocity (VSL) was measured using Computer Assisted Sperm Analyzer (CASA) at 0 minutes, 30 minutes, and 60 minutes after treatment. The effect of anti-VDAC3 recombinant antibody on sperm viability was evaluated by Eosin-Y staining, while on sperm membrane integrity was evaluated by Hypo Osmotic Swelling (HOS) test. There were significant differences in motility in form of general motility of ejaculated human sperm after 30 and 60 minutes incubation with anti-VDAC3 recombinant antibody ($p=0.001$; $p=0.002$, respectively). The other parameters of the sperm motility in form of WOB, VCL, LIN, VAP, and VSL decreased after incubation with anti-VDAC3 antibodies, but it was not different significantly ($p>0.05$; respectively). Anti-VDAC3 recombinant antibody could significantly decrease the percentage of living sperm cells ($p=0.003$) and increase poor membrane integrity of ejaculated human sperm after 60 minutes of incubation ($p=0.037$). Anti-VDAC3 recombinant antibody could reduce sperm motility parameters, decrease sperm living cells, and could be increased ejaculated human sperm with poor membrane integrity. It suggests this antibody would be used as a contraceptive agent.

Index Terms—anti-VDAC3 recombinant antibody, ejaculated human sperm, motility, CASA, viability, membrane integrity

I. INTRODUCTION

The world's population continues to increase based on data from the World Bank, the average world population

growth from 2008 to 2018 reached 1.2 million and the world population in 2018 reached 7,594.3 million people [1]. Excessive population growth will affect health, social and the economy [2]. Countries with the largest population such as China reached 1,392.7 million people and India reached 1,352.6 million people tried to suppress the rate of population growth [1]. In addition, Indonesia is included in the fifth country with the largest population, also made an effort to reduce the growth of its population. The efforts made by China to suppress population growth are to advocate family planning and the use of contraception to its residents. China continues to develop tools and methods that are appropriate for men and women and they can be selected as an effective contraceptive method that can be used [3]. In 2017, the Indian government launched the Mission Parivar Vikas Program which focuses on contraception and family planning services in 145 high fertility districts in seven states [4]. In Indonesia, this is evidenced by the issuance of government regulations regarding the use of contraceptives to suppress the rate of population growth. Excessive population growth will affect health, social and the economy. Government programs in dealing with population growth include the use of contraception [2]. Thus, it is necessary to find and develop appropriate methods of contraception so that it can achieve the goal of not having an explosion in population growth.

Contraception is a method to prevent the occurrence of unwanted pregnancy, with consideration not only depending on the protective effect of the method used but also assessed in terms of consistency of use and accuracy of use [5]. In general, the workings of contraceptive methods can be shared into two, namely hormonal and non-hormonal contraception [6]. One method of non-hormonal contraception is spermicide.

Spermicide is a method of contraception that is biologically effective to prevent pregnancy and has advantages, namely in its use does not depend on personal expertise (medical personnel). Spermicide can immobilize or kill sperm. The work pathways of spermicide are changing calcium balance, changing pH,

Manuscript received November 3, 2021; revised January 24, 2022.

*Corresponding author: asmarinah.si@gmail.com

becoming a sulfhydryl binding agent, as a surfactant, inhibiting acrosin, damaging membranes, lipid peroxidation, and ion channels [7], [8]. The ion channel plays a crucial role in regulating the physiological processes of sperm. Ion channels form a wide variety of transmembrane proteins. One of the sperm ion channels is the Voltage-Dependent Anion Channel (VDAC). VDAC is an ion channel having a molecular weight of 30-35 kDa that can be found abundant in the outer mitochondrial membrane as well as in the cell membrane [9]-[11]. The permeability of VDACS is influenced by Ca^{2+} ions which indicate that VDACS play a very important role in Ca^{2+} homeostasis in mitochondria [12]. This channel is a porin that affects the fluctuations of ions and metabolites, including ATP mitochondrial membrane. It has been known, there are three isoforms of VDAC, i.e. VDAC1, VDAC2, and VDAC3. A study using the VDAC3-deficient mice technique showed male mutant mice are in healthy but infertile conditions. The sperm concentration of the male mice is normal but has decreased in terms of sperm motility [12], [13]. In addition, other studies have shown that anti-VDAC3 antibodies can reduce human sperm motility as indicated by the parameters that can reduce sperm motility and increase the number of immovable sperm [14]. Based on previous studies, it suggests that anti-VDAC3 antibodies could be developed as male contraceptive substances. Furthermore, for the development of effective and applicable male contraceptive substance with real conditions, in this study, we generated the antibody in rabbit against VDAC3 recombinant protein that was obtained from the previous study, then evaluated the effect of this anti-VDAC3 recombinant antibody and preimmune serum on the motility parameters, viability and membrane integrity of ejaculated human sperm.

II. METHODS

A. Anti-VDAC3 Recombinant Antibody Production in Rabbit

The human VDAC3 recombinant protein produced in the *E. coli* BL21 strain from a previous study was used as an antigen to generate anti-VDAC3 antibody recombinant [14]. This recombinant protein at a dose of 300 μg was emulsified with 700 μL of Freund's Adjuvant Complete, then injected into three rabbits with a period of once a week until the first serial of 5 weeks. The total immunization duration was 14 weeks. Rabbits were immunized with recombinant protein subcutaneously in 3 places in the rabbit's back area, namely the position of the front legs, the middle of the back, and the back legs. Immunizations were carried out in 2 serial injections. The first series was done for 5 weeks with a time interval of 1 week after each injection, interspersed with a washout period of 4 weeks with a time interval of 1 week. In subsequent serial immunizations, the protein was emulsified with Freund's Adjuvant Incomplete. Rabbit serum was collected at weeks 6, 7, 8, 9, 12, and 14. Rabbit serum was collected by sticking a needle into the marginal vein in the ear and dripping blood was collected

in the 15 mL falcon. The collected blood was left at room temperature for 1 hour, then centrifuged at a speed of 5000 rpm for 5 minutes.

Before immunization, rabbit serum was taken to obtain a pre-immune serum which will be used later as a control of the experiments conducted. The serum was stored at -20°C . The serum obtained was then characterized and measured for concentration by the ELISA method, using a 96 well plate, as much as 4 μg recombinant protein was put into the well, then added with an ELISA coating and incubated at 4°C overnight. Then blotting with a blotto solution for 1 hour at 37°C . The blotto solution was removed, and the plate was washed 3 times with PBST at room temperature, then made a series of primary antibodies resulting from the immunization of recombinant VDAC3 protein in rabbits (1/100, 1/200, 1/400, 1/800, 1/1600, 1/3200, 0) in a blotto solution. Then the primary antibody was inserted into each well and incubated at 4°C overnight. After incubation, the sample was washed three times with PBST. Then make a secondary antibody dilution in a blotto solution, then incubated at room temperature for 1 hour or at 4°C overnight. After incubation, the sample was washed 3 times with PBST. Then TMB substrate was added to each well, then covered with aluminum foil to avoid light, then shaken at 100 rpm for 15 minutes, the substrate reaction was stopped with 1N HCl, then optical density was readings with ELISA reader with a wavelength of 450 nm.

B. Evaluation the Effect of Anti-VDAC3 Recombinant Antibody on Motility Parameter of Ejaculated Human Sperm

The samples were from pure semen of 12 fertile men aged 25-40 years who have children. The ejaculate sperm samples were allowed at room temperature for 30 minutes for total liquefaction. Then, the samples were added to anti-VDAC3 antibodies and preimmune serum with a ratio of 1: 1, then evaluated motility using CASA (Computer Assisted Sperm Analysis) Hamilton Thorne Human Sperm Analysis under a light microscope. Observations were made on the number of sperm that move within 0 minutes, 30 minutes, and 60 minutes after administration of antibodies and preimmune serum in a ratio of 1: 1. Then, 5 μL of this mixture was put into each room on the Leja slide (in this study using a four rooms Leja slide). This test was done in duplicate and the parameters used include the percentage of Wobble (WOB), Curvilinear Velocity (VCL), Linearity (LIN), Average Path Velocity (VAP), and Average Path Velocity (VSL).

C. Evaluation the Effect of Anti-VDAC3 Recombinant Antibody on Viability and Membrane Integrity Parameter of Ejaculated Human Sperm

Samples were collected from 8 male donors of fertile normozoospermic who had at least one child and aged 25-40 years old. Inclusion criteria of donors should abstain from three until five days of sexual intercourse. The samples were allowed at room temperature for 30 minutes for total liquefaction. Makler® counting chamber was used to analyze sperm concentration, with exclusion

criteria included sperm concentration <15 million/ml. The samples were separated into two treatment groups. One group was added with anti-VDAC3 recombinant antibody, and another group was added with preimmune serum as a control, with a dilution ratio of 1:1 between serum and sperm suspension. Eosin-Y staining was used to evaluate the effect of VDAC3 antibody on sperm viability. The living sperm was determined by clear sperm whereas dead sperm was marked by pink color in sperm. The percentage of living sperm cells and dead sperm cells were determined from 200 cells under a light microscope at 400x magnification, with an incubation time of 0, 30, 60 minutes after the addition of anti-VDAC3 recombinant antibody and preimmune serum.

Sperm membrane integrity was evaluated using Hypo Osmotic Swelling (HOS) Test. The solution for the HOS test contains 0.005 M fructose (SIGMA Aldrich, USA) and 0.002 M sodium citrate (SIGMA Aldrich, USA) in a total volume of 100 ml distilled water. Sperm samples were incubated with preimmune serum and anti-VDAC3 recombinant antibody in the ratio 1:1 for 0, 30, 60 minutes. After incubation, these mixtures were added by HOS solution with the same volume of the mixture, then incubated for 30 minutes at 37°C. Sperm samples were then observed by a light microscope with a magnification of 400x. Sperm with coiled tails were determined sperm have good membrane integrity. While sperm with uncoiled tails were defined as sperm having poor membrane integrity. The percentage of good and poor membrane integrity was calculated in 200 sperm cells under a light microscope. In this study, two observers blindly assessed sperm viability and membrane integrity.

D. Statistical Analysis and Ethical Clearance of This Research

The effect of anti-VDAC3 recombinant antibody on motility, viability, and membrane integrity of ejaculated human sperm compared to preimmune serum were analyzed statistically by using a T-Test Paired, and the significance value was considered at $P < 0.05$. Data were analyzed using SPSS 23 Version. This research was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia with the number: KET. 814/UN2.F1.ETIK/PPM.00.02/2019.

III. RESULTS

The VDAC3 recombinant protein could induce an immune response in rabbits leading to generate the antibody. There was an increase in the absorbance value of these polyclonal antibody titers (Fig. 1).

Anti-VDAC3 antibodies can reduce human sperm motility as indicated by the parameters that can reduce sperm speed and increase the number of immovable sperm. In this study, there were significant differences in motility in form of general motility of ejaculated human sperm after 30 and 60 minutes incubation with anti-VDAC3 recombinant antibody compared to the preimmune serum ($p = 0.001$; $p = 0.002$, respectively). The evaluation of the effect of anti-VDAC3 antibody to

ejaculate human sperm general motility can be seen in Fig. 2.

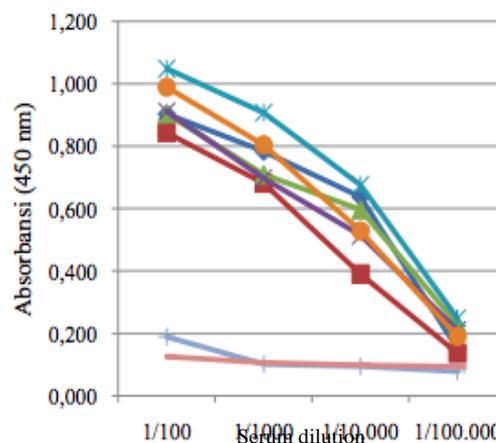


Figure 1. The result of the absorbance value of anti-VDAC3 antibody indicates the absorbance value increases with increasing anti-VDAC3 antibody titer.

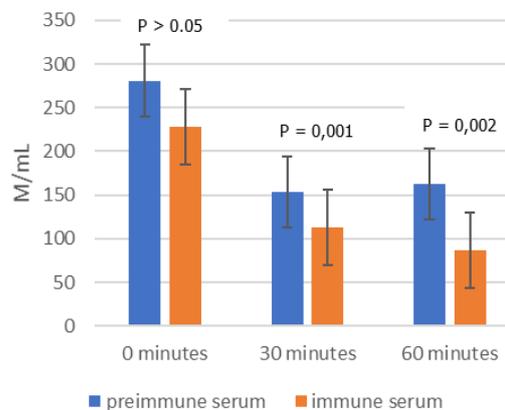


Figure 2. There were significant differences in motility in form of general motility of ejaculated human sperm after 30 and 60 minutes incubation with anti-VDAC3 recombinant antibody compared to the preimmune serum.

The other parameters in form of WOB, VCL, LIN, VAP, and VSL decreased but did not differ significantly between serum-containing anti-VDAC3 antibodies and the preimmune serum ($p > 0.05$; respectively). The evaluation of the effect of anti-VDAC3 antibody to ejaculate human sperm in some derivate parameters (WOB, VCL, LIN, VAP, and VSL) can be seen in Fig. 3.

The anti-VDAC3 recombinant antibody was evaluated on ejaculated human sperm viability by Eosin-Y staining. The colored sperm cells due to the Eosin-Y substance are identified as dead sperm cells. While the colorless sperm is a living sperm cell (Fig. 4). Anti-VDAC3 recombinant antibody evaluation on ejaculated human sperm viability indicates that the length of time this antibody treatment incubation can affect the percentage decrease in human sperm viability significantly after 30 minutes and 60 minutes incubation ($p = 0.005$ and $p = 0.003$; respectively) compared to preimmune serum. Sperm incubation with this antibody treatment at 0 minutes may not significantly decrease sperm viability, statistically ($p = 0.053$). Percentage of sperm viability after 0, 30, and 60 minutes

anti-VDAC3 antibody treatment presented in Fig. 5. Anti-VDAC3 recombinant antibody recognized VDAC3 protein in ejaculated human sperm, especially in the midpiece region of the sperm tail

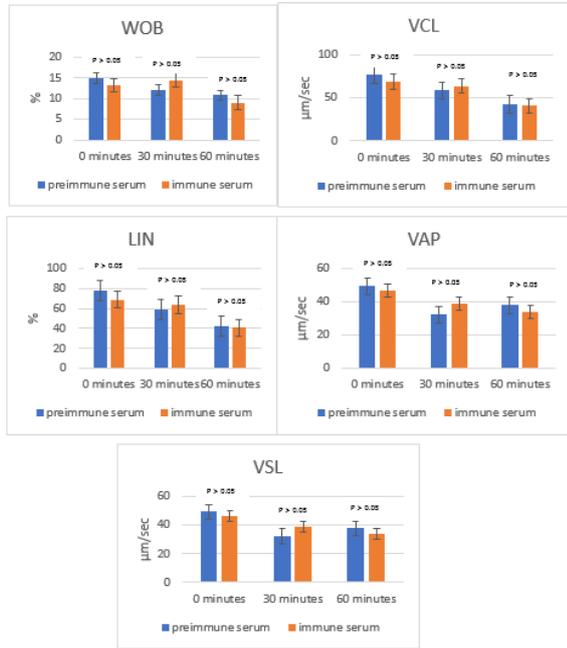


Figure 3. The parameters in form of WOB, VCL, LIN, VAP, and VSL did not differ significantly between serum containing anti-VDAC3 antibodies and preimmune serum ($p > 0.005$).

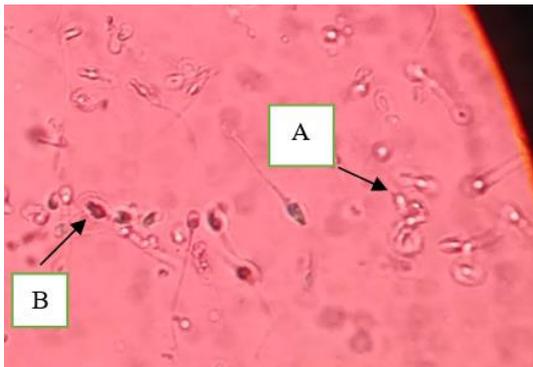


Figure 4. Viability test of ejaculated human sperm by Eosin-Y staining. The living sperm was marked by colorless sperm (A). Whereas a dead sperm was marked by pink color in sperm (B).

The membrane integrity on the sperm tail was evaluated by a hypo-osmotic swelling test. A good sperm showed that the tail makes a coiled or “swell” while the sperm with poor membrane integrity showed uncoiled or straight tail. It was indicated that the cell's membrane plasma has been damaged by anti-VDAC3 recombinant antibody (Fig. 6). Anti-VDAC3 recombinant antibody could increase the percentage of ejaculated sperm with poor membrane integrity significantly ($p=0.037$) after 60 minutes incubation compared to the preimmune serum. Incubation ejaculated sperm in time of 0 minutes and 30 minutes could not yet increase poor membrane integrity significantly statistically ($p=0.215$; $p=0.769$), respectively (Fig. 7).

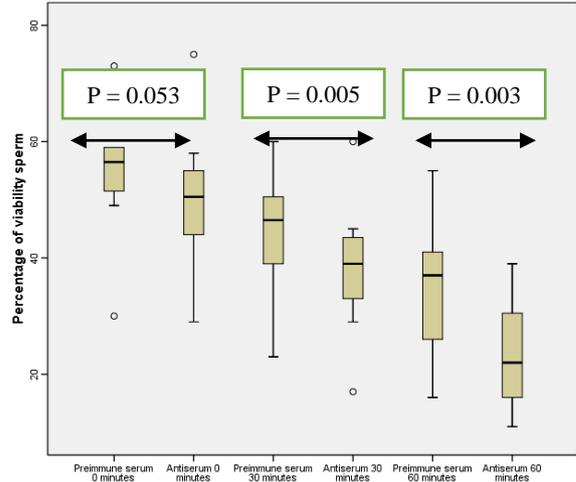


Figure 5. Evaluation of the effect of anti-VDAC3 recombinant antibody into ejaculated human sperm viability in 0, 30, and 60 minutes. The length of time this antibody treatment incubation can affect the percentage decrease in human sperm viability significantly after 30 and 60 minutes incubation ($p=0.005$ and $p=0.003$, respectively) compared to preimmune serum.

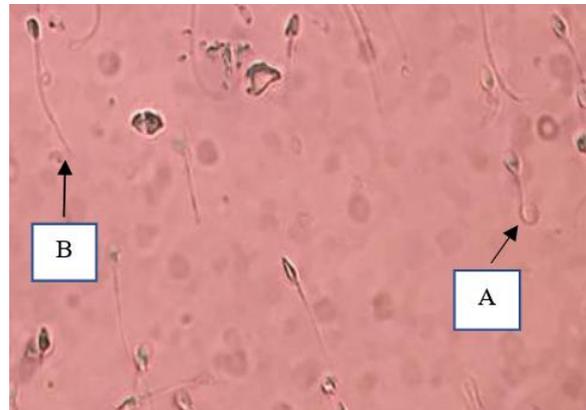


Figure 6. Membrane integrity test by Hypo-Osmotic Swelling (HOS). Sperm with good membrane integrity have coiled tail region (A), while poor sperm have uncoiled tail region (B).

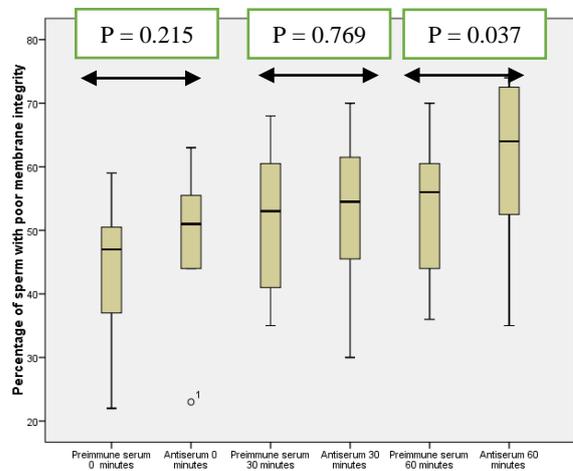


Figure 7. Evaluation of effect anti-VDAC3 recombinant antibody on ejaculated human sperm with membrane integrity test. At incubation times of 0 and 30 minutes, antibody has not increased damage to sperm cells. While at 60 minutes incubation, this antibody showed damaged sperm cell membrane, which causes poor sperm membrane integrity to increase significantly, compared with preimmune serum.

IV. DISCUSSION

Immunocontraception is a contraceptive method developed with an immunological approach, which is the bond between antigens and antibodies. VDAC3 protein is one of the ion channel proteins found in the spermatozoa cell membrane. The VDAC3 protein in sperm facilitates the flow of calcium ions and ATP molecules that are indispensable in the function of spermatozoa motility [15], [16]. Studies conducted by Sampson, et al, showed a decrease in spermatozoa motility in mutant male mice which has a deletion in the last 4 exons of the VDAC3 gene, compared with wild-type male mice [13]. This is also consistent with research conducted by Asmarinah, et al, which showed that in asthenozoospermia patients there was a mutation in exon 5, 6, 7, and 8 of the VDAC3 gene. The mutation could disrupt the function of the channel protein leading to decreased motility of the sperm [15]. The exon 5-8 VDAC3 gene is responsible for the formation of the VDAC3 protein channel. In previous studies conducted by Asmarinah, et al, the production of VDAC3 recombinant proteins has been successfully carried out through vector construction by inserting 5-8 human spermatozoa exon genes that play an important role in motility function [14].

Canal protein is needed for the entry of specific ions needed by spermatozoa. The presence of ionic changes in the spermatozoa environment can cause membrane hyperpolarization. An increase in intracellular calcium ion concentration can modulate the movement of asymmetric flagellum which can cause changes in the direction of movement of spermatozoa which then affect the movement of spermatozoa to oocyte cells [6]. Calcium ions are indispensable in the motility of spermatozoa by interacting directly with the components that form axons. It can be concluded that the exon 5-8 VDAC3 gene is associated with spermatozoa motility [17], [18]. The development of contraceptive vaccines by making anti-VDAC3 protein as an antibody against VDAC3 which in this case is considered as an antigen can inhibit spermatozoa motility to prevent fertilization. In addition, previous studies have reported that anti-VDAC can interfere with the function and morphology of spermatozoa. VDAC can induce abnormalities of the acrosome structure, volume, and membrane integrity of spermatozoa, it is suspected that VDAC located in the acrosome acts as a porin and is involved in the acrosomal reaction through the regulation of calcium ion transport [19].

The function of VDAC in spermatozoa motility is related to its function as a porin responsible for the flow of ions and molecules to and from the inside of the mitochondria, namely calcium ions and ATP molecules, which play a role in moving the dynein motor from axons [11]. Interaction between VDAC and the cytoskeleton results in cytoskeleton integrity flagella, where the VDAC protein in flagella mediates the binding and transport of ATP in the formation of energy needed for the motility of flagella [20], [21]. The VDAC protein which is located on the membrane of the acrosome and the mitochondrial sheath operates in the regulation of the

transport and distribution of calcium ions. VDAC in spermatozoa motility can be inhibited in the presence of anti-VDAC antibodies. Anti-VDAC that binds to the VDAC protein can cause changes in intracellular ion concentration. This condition can cause changes in cell volume, unstable cytoskeleton, and loss of acrosome cap [19].

Based on studies that have been conducted on the role of VDAC3 on spermatozoa motility, the polyclonal antibody hVDAC3 produced successfully by rabbits is expected to be a basic ingredient in the development of contraceptive vaccine-based spermicide contraception. In this study, the polyclonal anti-VDAC3 antibodies were tested on human sperm ejaculate samples and then tested for motility using CASA with parameters taken namely motility, Wobble (WOB), Curvilinear Velocity (VCL), Linearity (LIN), Average Path Velocity (VAP), and Average Path Velocity (VSL). In this study, we found that there were significant differences in sperm motility in general that had been incubated with anti-VDAC3 antibody compared to the preimmune serum, whereas the parameters of Wobble (WOB), Curvilinear Velocity (VCL), Linearity (LIN), Average Path Velocity (VAP), and Average Path Velocity (VSL), showed a decrease, but not any significant differences.

The evaluation of anti-VDAC3 antibody on sperm viability and integrity based on the addition of eosin-Y staining and hypotonic solutions as an indicator of biochemical permeability and integrity of sperm membranes. Proper assessment of sperm viability is crucial in evaluating male infertility. Determination of viability and membrane integrity of sperm cell is done by identifying the sperm cell which has a permeable cell membrane, after the addition of the dye substance or with a hypo-osmotic solution [21]. Evaluation of sperm viability is done based on the ability of cell membranes to remove Eosin-Y staining that enters the cell. The living sperm cells will appear brightly or colorless. While the dead sperm cells will appear pink due to the Eosin-Y staining [22].

Anti-VDAC3 recombinant antibody treatment on ejaculated human sperm after incubation within 60 minutes has shown that the integrity of the sperm cell membrane is poorer compared to preimmune serum. Sperm cell membranes were exposed to the hypo-osmotic solution cause sperm tails to form coils like "balloons" to achieve intracellular osmotic pressure balance. Hypo-Osmotic Solutions (HOS) testing can effectively evaluate membrane integrity in sperm. The addition of hypo-osmotic solution can affect the expansion of sperm cell membranes, so that the cells "swell" and force the circular flagellum. Sperm incubation with this antibody causes inhibited and affected mitochondrial respiration membrane integrity and permeability with inhibition ATP synthase induces loss of membrane integrity [16]. In brief, this study showed that anti-VDAC3 recombinant antibody treatment can lower the regulation of human sperm energy sources, especially in the viability and integrity of membranes compared to preimmune serum treatment. Anti-VDAC3 recombinant antibody can be

used as future male contraceptive agent candidates, such as spermicide.

The difference in this study of evaluating serum VDAC3 antibodies to human sperm ejaculate compared to similar studies that already existed previously was from the sample used, in a previous study conducted by Asmarinah, et al, using samples of human sperm that had been given swim-up treatment [17]. The swim-up treatment makes the sample conditions do not resemble the conditions in vivo where the sperm that is enter to vaginal introitus are human ejaculate sperm, which includes sperm, ejaculate fluid, and other components. Our study more closely resembled natural conditions in vivo and more closely the application of spermicide use.

V. CONCLUSION

Anti-VDAC3 recombinant antibody could reduce sperm motility, the percentage of living sperm cells ejaculate, and increase the percentage of poor sperm membrane integrity significantly. It suggests this antibody would be used as a contraceptive agent such as spermicide that could immobilize ejaculated human sperm, which is based on antigen and antibody binding. Therefore, this antibody can be a potential agent in the development of male contraception.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Prof. Asmarinah, as a coordinator, guarantor, and design maker of the research. Laila Chuvita conducted the research, analyzed the data, wrote, and edit the paper. Nurul Hikmawati conducted the research, analyzed the data, and wrote the paper. Dr. Silvia W. Lestari and Prof. Andon Hestiantoro, as a guarantor of the research. All authors had approved the final version.

ACKNOWLEDGMENT

The presence research was supported by Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT) 2019 grant.

REFERENCES

- [1] World Bank. (2019). World Development Indicators: Population dynamics. [Online]. Available: <http://wdi.worldbank.org/table/2.1>
- [2] Embassy of the People's Republic of China in the Republic of Lithuania. Family Planning in China. [Online]. Available: <https://www.fmprc.gov.cn/ce/celt/eng/zt/zfbps/t125241.htm>
- [3] P. Muttreja and S. Singh, "Family planning in India : the way forward," *Indian J. Med. Res.*, vol. 148, no. 1, pp. S1-S9, 2018.
- [4] Health Ministry of Indonesia. (2014). Situasi dan Analisis Keluarga Berencana. [Online]. Available: http://www.depkes.go.id/resources/download/pusdatin/infodatin/info_datin-harganas.pdf
- [5] M. J. Chen, C. R. Kim, K. C. Whitehouse, E. Berry-Bibee, and M. E. Gaffield, "Development, updates, and future directions of the World Health Organizations selected practice recommendations for contraceptive use," *Int. J. Gynecol. Obstet.*, vol. 136, pp. 113-119, 2016.
- [6] WHO, *Selected Practice Recommendations for Contraceptive Use*, 3rd Ed., Geneva: WHO, 2016.
- [7] M. Banerjee, A. Hazra, Y. P. Bharitkar, and N. B. Mondal, "Insights of spermicidal research: An update," *JFIV Reproductive Medicine, Genetics and Stem Cell Biology*, vol. 1, no. 3, pp. 1-15, 2014.
- [8] M. R. Tanner and C. Beeton, "Differences in ion channel phenotype and function between humans and animal models," *Front Biosci.*, vol. 23, pp. 43-64, 2018.
- [9] Asmarinah, E. W. Bachtiar, A. Malik, and S. Rahayu, "Establishment of human sperm-specific voltage-dependent anion channel 3 recombinant vector for the production of a male contraceptive vaccine," *Med. J. Indones.*, vol. 21, no. 2, pp. 61-65, 2012.
- [10] G. M. Cooper, *The Cell: A Molecular Approach*, USA: Sinauer Associates, Inc 2000.
- [11] R. Casadio, I. Jacoboni, A. Messina, and V. D. Pinto, "3D model of the Voltage-Dependent Anion Channel (VDAC)," *FEBS Lett.*, vol. 520, pp. 1-7, 2002.
- [12] M. J. Sampson, R. S. Lovell, W. J. Craigen, "The murine voltage dependent anion channel gene family. Conserved structure and function," *J. Biol. Chem.*, vol. 272, no. 30, pp. 18966-18973, 1997.
- [13] M. J. Sampson, W. K. Decker, A. L. Beaudet, W. Ruitenbeek, D. Armstrong, M. J. Hicks, et al., "Immotile sperm and infertility in mice lacking mitochondrial voltage-dependent anion channel type 3," *J. Biol. Chem.*, vol. 276, no. 42, pp. 39206-39212, 2001.
- [14] Asmarinah, T. P. Susmiarsih, A. Shari, P. Ratri, D. A. Pujiyanto, and E. W. Bachtiar, "Anti-VDAC3 recombinant antibody decreased human sperm motility and membrane integrity: A potential spermicide for contraception," *Asian Pacific J. of Repro.*, vol. 6, no. 6, pp. 257-263, 2017.
- [15] Asmarinah, M. I. Saleh, S. I. Wanandi, V. Narita, R. Damayanti, N. H. Moeloek, H. J. Freisleben, et al., "Polyclonal VDAC3 antibody decreases human sperm motility: A novel approach to male contraception," *Med. J. Indones.*, vol. 20, no. 1, pp. 5-10, 2011.
- [16] K. D. Hinsch, V. D. Pinto, V. A. Aires, X. Schneider, A. Messina, and E. Hinsch, "Voltage dependent anion-selective channels VDAC2 and VDAC3 are abundant proteins in bovine outer dense fiber, a cytoskeletal component of the sperm flagellum," *J. Biol. Chem.*, vol. 279, no. 15, pp. 15281-15288, 2004.
- [17] Asmarinah, T. Nuraini, T. P. Susmiarsih, R. Paramita, M. I. Saleh, V. Narita, N. Moeloek, et al., "Mutations in the exons 5, 7, and 8 of human VDAC3 gene in the sperm with low motility," *Andrologia*, vol. 44, pp. 46-52, 2012.
- [18] X. Triphan, V. A. Menzel, A. M. Petrunina, M. C. Cassara, W. Wemheuer, K. D. Hinsch, et al., "Localization and function of voltage-dependent anion channel (VDAC) in bovine spermatozoa," *Pflugers Arch.*, vol. 455, no. 4, pp. 677-686, 2008.
- [19] O. T. Ramirez, R. Zamora, G. Espinosa, E. Merino, F. Bolivar, and R. Quintero, "Kinetics study of penicillin acylase production by recombinant Escherichia coli in batch cultures," *Process Biochem.*, vol. 29, pp. 197-206, 1994.
- [20] H. Florke, F. P. Thinner, H. Wilkenbach, U. Stadtmuller, G. Paetzold, C. Morry, et al., "Channel active mammalian porin, purified from crude membrane fractions of human B lymphocytes and bovine skeletal muscle, reversibly binds adenosine triphosphat (ATP)," *J. Biol. Chem.*, vol. 375, pp. 513-520, 1994.
- [21] J. K. Amory, "Male contraception," *Fertility and Sterility*, vol. 6, no. 106, pp. 1303-1309, 2016.
- [22] P. Kumar, N. Srivastava, M. Pande, J. K. Prasad, and S. Sirohi, "Evaluating sperm cell viability and membrane integrity," in *Protocols in Semen Biology (Comparing Assay)*, N. Srivastava and M. Pande, Eds., Singapore: Springer, 2017, pp. 57-71.

Copyright © 2022 by the authors. This is an open access article distributed under the Creative Commons Attribution License (CC BY-NC-ND 4.0), which permits use, distribution and reproduction in any medium, provided that the article is properly cited, the use is non-commercial and no modifications or adaptations are made.



Asmarinah, born in 1965, is a teaching staff of the Department of Medical Biology, Faculty of Medicine Universitas Indonesia (FMUI). Currently, she serves as Research Coordinator for the Department of Medical Biology FMUI, Head of Doctoral-Degree Program in Biomedical Sciences FMUI. She graduated from Faculty of Mathematics and Natural Sciences UI in 1989. In 1996, she received her master degree in the field of biomedical sciences from UI and graduated

from doctoral education in Faculty of Biology, Chemistry, and Geo-Sciences Justus-Liebig University of Giessen Germany in 2003.

Prof. Asmarinah active as a facilitator, lab supervisor, and lecturer at FMUI. She is also a lecturer for Master and Doctoral Program in Biomedical Sciences FMUI.



Laila Chuvita, born in 1994, is graduated master's programme in Biomedical Sciences, Faculty of Medicine Universitas Indonesia in 2020. She graduated bachelor's degree in Midwifery from Faculty of Medicine Universitas Brawijaya, Malang, Indonesia in 2017.

She has research interests in the field of molecular sciences and andrology. Laila Chuvita was awarded as the best poster presentation in the 4th International

Conference and Exhibition on Indonesian Medical Education and Research Institute (ICE on IMERI) Jakarta, Indonesia in the year 2019.



Nurul Hikmawati, born in 1990, is graduated from Master's Programme in Biomedical Sciences, Faculty of Medicine, University of Indonesia in 2021. She graduated bachelor's degree from Faculty of Mathematics and Natural Sciences, Bogor Agricultural University in 2013.

She has research interests in biotechnology and molecular genetic biomedicine. In 2013, she was awarded as the third-best oral presentation in a scientific paper organized by

The 1st IGN-TTRC International Conference on Biotechnology for human life DAAD. Nurul Hikmawati, S.Si also attended a poster presentation at the 4th International Conference and Exhibition (ICE on IMERI) in Jakarta, Indonesia in 2019.



Silvia Werdhy Lestari, born in 1976, is an andrologist and a teaching staff of the Department of Medical Biology Faculty of Medicine Universitas Indonesia (FMUI). In 2001, she graduated MD from Faculty of Medicine Universitas Indonesia then graduated from master Programme's in Biomedical Sciences from Universitas Indonesia in 2008, graduated doctoral degree in Biomedical Sciences from the same university in 2015, and graduated as an

andrologist from Faculty of Medicine Universitas Airlangga, Indonesia in 2020.



Andon Hestiantoro, born in 1960, is a teaching staff of the Department of Obstetrics and Gynecology FMUI-RSUPN Dr. Cipto Mangunkusumo and currently serves as the Venture, Cooperation, and Alumni Relations Manager of FMUI Period 2017-2021. He completed his general medical education in 1985 at FMUI. Then continued his specialist education at the Department of Obstetrics and Gynecology FKUI-RSUPN Dr. Cipto Mangunkusumo in 1994 and became a consultant in Reproductive Fertility and Endocrinology in 2003. In 2017 Prof. Andon continued his Masters in Hospital Management at Universitas Gadjah Mada together with Doctor of Medicine at the Universitas Indonesia and the Netherlands Institute for Brain Research/ Neuroendo Netherlands, the Netherlands.