



Universitas Airlangga
Surabaya, Indonesia



UTM
UNIVERSITI TEKNOLOGI MALAYSIA

Fakulti Sains

UNIVERSITI PENYELIDIKAN



Salahaddin University
Erbil, Iraq Kurdistan

Certificate of Participation

This certificate is awarded to

SRI PUDJI ASTUTI WAHYUNINGSIH

in recognition of participation as an poster presenter in the

4th International Conference and Workshop on Basic
and Applied Sciences

&

11th Regional Annual Fundamental Science Symposium

ICOWOBAS-RAFSS 2013

on 3-5 September 2013

at Le Grandeur Palm Resort, Senai, Johor, Malaysia



Chairman ICOWOBAS-RAFSS 2013
Professor Dr Shaharuddin Salleh





UTM
UNIVERSITI TEKNOLOGI MALAYSIA



ICOWOBAS RAFSS 2013 PROCEEDINGS

*"Science and mathematics as proponents
for sustainable future"*

**The Fourth International Conference and
Workshops on Basic and Applied Sciences
(4th ICOWOBAS)**

&

**Regional Annual Fundamental Science
Symposium 2013 (11th RAFSS)**

**Le Grandeur Palm Resort
Johor, Malaysia
3-5 September 2013**

PROCEEDING OF THE
“4TH INTERNATIONAL CONFERENCE &
WORKSHOP ON BASIC AND APPLIED
SCIENCES”

&

“11TH REGIONAL ANNUAL FUNDAMENTAL
SCIENCE SYMPOSIUM 2013”

(ICOWOBAS-RAFSS 2013)

“Science and mathematics as proponents for sustainable future”

© IBNU SINA INSTITUTE FOR FUNDAMENTAL SCIENCE STUDIES 2013

All rights reserved. This proceeding, or parts thereof, may not be reproduced in any form or by any means, electronic or mechanical, including photocopying, recording or any information storage and retrieval system now known to be invented, without written permission from Ibnu Sina Institute for Fundamental Science Studies, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, and Johor Darul Ta'zim.

Typeset by:

Sugeng Triwahyono, Sabariah Ajis, Siti Nur Sakinah Ahmad, Zarina Junet
Ibnu Sina Institute for Fundamental Science Studies
Universiti Teknologi Malaysia
81310 UTM Johor Bahru
Johor Darul Ta'zim

Published in Malaysia by:

Ibnu Sina Institute for Fundamental Science Studies
Universiti Teknologi Malaysia
81310 UTM Johor Bahru
Johor Darul Ta'zim
Tel: 07-5536060
Fax: 07-5536080

COMMITTEE

| | |
|-------------------------------|---|
| Patrons | Y.Bhg. Dato'Seri Ir Dr. Zaini Ujang, Position : Secretary General II, Ministry of Education Malaysia Y.Bhg.Prof. Ir. Dr. Azraai Mohd Kassim Position : Acting Vice Chancellor, Universiti Teknologi Malaysia |
| Chair | Prof. Dr. Shaharuddin Salleh (General Chair) Prof. Dr. Sugeng Triwahyono (Deputy Chair) |
| General Co-Chair | Prof. Dr. Mustafa Shamsuddin (IIS, UTM, Malaysia) Dr. Hery Purnobasuki (UNAIR, Indonesia) Dr. Hewa Y. Abdullah (SUH, Iraqi, Kurdistan) |
| International Advisory | Prof. Dr. F. Fujita (Fujita Laboratory) Prof. Dr. S.A. Miller (University of Florida) Prof. Dr. M. Iwamoto (Tokyo Institute of Technology) Prof. Dr. Ismunandar (Institut Teknologi Bandung) Prof. Dr. E. Tahir (Salahaddin University) Prof. Dr. S. Olariu (Old Dominion University) Prof. Dr. N. J. Ismaiel (Salahaddin University) |
| Secretary/Secretariat | Dr. Khairil Juhanni Abd Karim Dr. Syarifah Zyurina Nordin Dr. Fazilah Abd Manan Dr. Suhaili Zakaria |
| Treasurer | Assoc. Prof. Dr. Umi Kalthom Ahmad |
| Assistant Treasurer | Dr. Chong Chun Shiong |
| Publicity | Prof. Dr. Noor Aini Abdul Rashid Dr. Norahim Ibrahim Dr. Mohd Firdaus Abdul Wahab |
| Website | Mr. Mohd Khalid Jasmin Mrs. Zarina Junet |
| Sponsorship | Assoc. Prof. Dr. Norma Alias Dr. Hendrik Oktendy Lintang |
| Scientific | Assoc. Prof. Dr. Farediah Ahmad Prof. Dr. Sugeng Triwahyono Assoc. Prof. Dr. Robiah Adnan Assoc. Prof. Dr. Hazri Bakhtiar Dr. Amiruddin Shaari Dr. Norhayati Abdullah |
| Logistic and Technical | Mrs. Sabariah Ajis Dr. Raja Kamarulzaman Raja Ibrahim Dr. Mohd Bakri Bakar Dr. Suhairul Hashim |
| Hospitality | Assoc. Prof. Hazimah Abdul Hamid Dr. Amidora Idris Dr. Roswanira Abdul Wahab Dr. Ani Shabri Dr. Joazaizulfazli Jamalis |
| Workshop | Dr. Roswanira Abdul Wahab Dr. Raja Kamarulzaman Raja Ibrahim Dr. Mohd Firdaus Abdul Wahab Mr. Mohd Khalid Kassim |

**4th INTERNATIONAL CONFERENCE AND WORKSHOP ON BASIC AND
APPLIED SCIENCES**

&

**11TH REGIONAL ANNUAL FUNDAMENTAL SCIENCE SYMPOSIUM 2013
(ICOWOBAS-RAFSS 2013)**

3-5 September 2013

Le Grandeur Palm Resort Senai, Johor

Organized by

Faculty of Science, Universiti Teknologi Malaysia, MALAYSIA

In Collaboration with

Ibnu Sina Institute for Fundamental Science Studies, Universiti Teknologi Malaysia,
MALAYSIA

Universitas Airlangga, Surabaya, INDONESIA

Salahaddin University Erbil, IRAQI KURDISTAN

| <u>CONTENT</u> | <u>PAGE</u> |
|---|-------------|
| QSAR Modeling for Corrosion Inhibition of Steel using Pyrimidine Derivatives <i>Abdo M. Al-Fakh, Madzlan Aziz, Hassan H. Abdallah, Hasmerya Maarof, Bishir Usman</i> | 1 |
| Studies on Indonesian Essential Oils <i>Alfinda Novi Kristanti, Nanik Siti Aminah, Hery Suwito</i> | 7 |
| Determination of Lead and Copper concentration in some brands of tea sold in Gharian-Libya <i>Dr. Fathi Al Harari, Dr. Mohamed Elazumi, Dr. Abdunnaser Magrahi, Dr. Al Nageh Al Taib</i> | 9 |
| Stimulatory effect of Vitamin A on the genotoxicity of nicotine in Male of albino mice <i>Mus musculus</i> <i>Galawezh Obaid Othman and Nadhum Jalal Ismael</i> | 11 |
| Synthesizes and Characterization Studies of some metal ion complexes with 2,2'-(5,5'-(ethane-1,2-diyl)bis(1,3,4-oxadiazole-5,2-diyl))bis(sulfanediyl)dibenzeneamine (EOSBE) and 2,2'-(5,5'-methylenebis(1,3,4-oxadiazole-5,2-diyl)bis (sulfanediyl)) dibenzeneamine (MOSBE) <i>Hikmat Ali Mohamad and Media Kurshid</i> | 17 |
| Novel Supramolecular Amphiphilic Benzene for Nanosensors of Nitro Anions <i>Juan Matmin, Leny Yuliati and Hendrik O. Lintang</i> | 22 |
| Antifungal Activity Of Secondary Metabolites Produced by <i>Dumortiera Hirsuta</i> <i>Junairiah, Sukarti Moeljopawiro, Endang Semiarti, Ni'matuzahroh</i> | 27 |
| First record of Potamon magnum (Decapoda: Crustacea) in Greater Zab river Kurdistan region-Iraq <i>Luay A. Ali and Hayfa J. Jawair</i> | 33 |
| Synthesis Of Coconut Shell Porous Carbons By Chemical Activation With Novel Potassium Acetate <i>Mohammed Jibril, Noraini Jaafar Noor Shawal Nasri and Farid Nasir Haji Ani</i> | 36 |
| Characterization of Cetylpyridinium Bromide (CPB) Loaded Kaolinite with FTIR Spectroscopy: Effect of CPB Loading <i>Nur Isti'annah Ramli, Mashitah Mad Salim, Siti Aishah Mohd Hanim and Nik Ahmad Nizam Nik Malek</i> | 40 |
| PREPARATION & CHARACTERIZATION OF ACTIVATED CARBON FROM SUGAR CANE HUSK <i>Nurul' Ain binti Jamion, Siti Mazleena binti Mohamed</i> | 45 |
| Method Validation of Home-Made Portable Micro Solid Phase Extraction Approached For Determination of Polycyclic Aromatic Hydrocarbons in Water Samples <i>Nurul Nabilah Zainal Abidin, Mohd Marsin Sanagi, Wan Aini Wan Ibrahim,, Salasiah Endud</i> | 50 |
| POTENCY OF SARGASSUM SP FROM MADURA STRAIT AS IRREVERSIBLE HYDROCOLLOID IMPRESSION MATERIAL <i>Prihartini Widiyanti, Siswanto</i> | 53 |
| Homology Modeling of Human Sweet Taste Receptors using multi templates <i>Ragheed HussamYousif and NurulBahiyah Ahmad Khairudin</i> | 57 |
| Toxicity of Furadan 3G Insecticides and Neem Leaves Extract On Fission Duration and Reproduction Rate of <i>Colpoda cucullus</i>,M <i>Saikhu Akhmad Husen</i> | 60 |

| <u>CONTENT</u> | <u>PAGE</u> |
|--|-------------|
| Chemical components and Antimicrobial Activities of Methanol extract from the Leaves of <i>Pellacalyx saccardianus</i> (Rhizophoraceae) <i>Salam Ahmed Abed, Hasnah Mohd Sirat</i> | 64 |
| The effect of aqueous and ethanol extract of <i>Teucrium polium</i> L on some microbial agents and elimination of antimicrobial resistance genes <i>Sawsan Mohammed Abdullah Sorchee, Luma Abdal Hady Zwain, Sundus Jamel Yaseen</i> | 68 |
| Antioxidant activity of Ethanol Extract and Ethyl Acetate Fraction of Reeds (<i>Imperata cylindrica</i>) towards level of Malondialdehyde (MDA) on <i>Rattus norvegicus</i> Hypercholesterolemia <i>Siti Khaerunnisa, Sutji Kuswarini, Gwenny IP, Andira Bulan Nandinanti, Suhartati</i> | 73 |
| Properties of Curcumin: Assay of Tyrosinase Activities <i>Sugiharto Arbakariya Ariff, Syahida Ahmad, Muhajir Hamid</i> | 80 |
| Free Radical Scavenging Activity and Phytochemical Analysis of <i>Matricaria recutita</i> <i>Suhailah Wasman Qader</i> | 83 |
| Development of Bio-plastic Material Blending Konjac Glucomanan Flour and Chitosan Using Single Screw Extruder <i>Suyanto, Handoko Darmo Koesoemo, Humaira</i> | 87 |
| Application Of Ionic Liquid-Microextraction Based On Green Chemistry To The Analysis Of Carcinogenic Nitrosamine <i>Yanuardi, Raharjo, Aning Purwaningsih</i> | 89 |
| Cr Doped TiO₂ Supported on TUD-1 Photocatalyst for Dye Removal <i>Yee Khai Ooi, Leny Yuliati and Siew Ling Lee</i> | 93 |
| Potentiometric Determination of Melamine in Milk Using Nanoporous Carbon Paste/Molecularly Imprinted Polymer Electrode <i>Muji Harsini, Suyanto, Bambang Suprijanto, Asri Zulchana Sari</i> | 98 |
| Microwave-assisted Synthesis and Characterization of Poly(methyl methacrylate)-grafted Agar <i>Neda Pourmand, Mohd Marsin Sanagi, Ahmedy Abu Naim Wan Aini Wan Ibrahim</i> | 105 |
| Inorganic solid waste economic value of Science and Technology Faculty of Airlangga University (FST UA) <i>Nita Citrasari and Dian Andri S. Purba</i> | 111 |
| Determination Of Azole Antifungal Drugs In Biological Samples By Dispersive-Micro Solid Phase Extraction Coupled With High-Performance Liquid Chromatography-Tandem Mass Spectrometry <i>Noorfatimah Yahaya, Mohd Marsin Sanagi, Noorizan Abd Aziz, Hadi Nur, Wan Aini Wan Ibrahim</i> | 114 |
| Study of Antibiotic Resistance by Plasmid DNA in Uropathogenic <i>E. coli</i> <i>Zhiangazi N. and Zirak F.A. Abdulrahman</i> | 117 |
| Ecological Evaluation Shoreline and Mangrove on the East Java North Coast (Madura Strait) Using Remote Sensing <i>Thin Soedarti, Bambang Irawan, Sucipto Hariyanto, Noer Moehammadi, and Moch. Affandi</i> | 121 |

| <u>CONTENT</u> | <u>PAGE</u> |
|--|-------------|
| The Potential of Sapindus rarak Fruit as Spermicides: Female Mice as Animal Model <i>Alfiah Hayati, Doni Alfian, and Listijani Suhargo</i> | 126 |
| Effect Growth Regulator Substance NAA and BAP on Axillary Bud Aglaonema rotundum N.E. Brown Induction <i>Dwi Kusuma Wahyuni, Junairiah, Ahmad Syaqui, Hery Purnobasuki</i> | 130 |
| Magnetic Solid Phase Extraction of Selected Chlorinated Pesticides Using Gas Chromatography-Microelectron Capture Detection <i>Wan Aini Wan Ibrahim, Hamid Rashidi Nodeh, Mohd Marsin Sanagi</i> | 134 |
| Bifunctionalised Silica-Based Sol-Gel Organic-Inorganic Hybrid Sorbent Materials For Solid Phase Extraction Of Methylene Blue and Methyl Orange Dyes <i>Wan Aini Wan Ibrahim, Norfazilah Muhamad and Mohd Marsin Sanagi</i> | 138 |
| Study on Prevalence and Risk Factor Associated with Schistosoma haematobium Infections among Children's and Adults in Sokoto State, Nigeria. <i>Kabiru Mohammed, Ikeh Eugene. I., Aziah Ismail., Julia Omar, Fabiyi Joseph P, Mohamed Rusli A.</i> | 142 |
| Comparison Study Between Clinical and Environmental Isolates of Legionella pneumophila in Erbil City/Kurdistan Region <i>Khadija Kh. M. Barzani and Amara M. M. Al-Rawi</i> | 147 |
| Visualization of The Shell Morphological Characters of The Unionid Mussels From Brantas River, East Java, Indonesia <i>Moch. Affandi, Bambang Irawan, Agoes Soegianto</i> | 152 |
| Studies to investigate the effects of nitrogen sources of media to androgenesis response of aromatic and non-aromatic cultivars in indica rice (Oryza Sativa L). <i>Rebaz Abdulwahab Baqra, Muhammad Arshad Javeda, Karwan Talaat Mohammeda</i> | 157 |
| Antimicrobial Resistant Patterns of Bacterial Isolates from Patients Attending Erbil Hospitals <i>Suhaila N. Darogha, Rebwar M. Hamasalish and Mahmoud K. Nuri</i> | 162 |
| Effect Of Biofertilizer and Planting Media To The Growth And Crop Production Of Cayenne Paper (Capsicum Frutescens L.) In Polybag <i>Tini Surtiningsih, Ni Putu Ayu Fergyantini, Fita Khoiril Umah, Edy Setiti Wida Utami, Agus Supriyanto</i> | 167 |
| COMBINATION OF Acinetobacter Sp. P2 (1) BIOSURFACTANT AND CRUDE LIPASES ON THE OIL SLUDGE SOLUBILITY <i>Ni'matuzahroh, Intan Ayu Pratiwi, Isnaini Septi Irmayanti, Tini Surtiningsih, Fatimah, Sri Sumarsih</i> | 172 |
| The Use Of Sea Cucumber Paracaudina australis Extract To Improve Glucose Tolerance In Diabetes Mellitus <i>Dwi Winarni, Saikhu Akhmad Husen, Alfinda Novi Kristanti</i> | 178 |
| Optimization of Biosurfactant Production from Exiguobacterium sp. P2(1) Using Glucose Substrate <i>Fatimah, Suharjono, Ni'matuzahroh, Tri Ardyati, Afaf Baktir, Ahmad Thontowi</i> | 182 |
| Agglutination of Mice Sperm in Antibody of 46, 66, and 73 KDa Protein from Rabbit Sperm Membrane <i>Sri Puji Astuti Wahyuningsih</i> | 187 |

| <u>CONTENT</u> | <u>PAGE</u> |
|--|-------------|
| Gen Expression of ECM and CAMs On brain GD-10, Hippocampal Cell (mHT-22 Cell Line), Human glioblastoma-astrocytoma (hLN-405), rat glioma cell (rF98) by real time PCR <i>Yulia Irnidayanti and Win Darmanto</i> | 192 |
| Response Surface Methodology Analysis of Photoreduction of CO₂ via Titania Nanoparticles Coated Mesh <i>Saeed Delavari, Nor Aishah Saidina Amin, Mohammad Reza Sazegar</i> | 201 |
| Parasitic Infections with Gyrodactylus (Monogenea) on Common Carp Cyprinus carpio from Ainkawa Fish Hatchery in Erbil City, Kurdistan Region, Iraq <i>Shamall M. A. Abdullah and Kamaran S. Mama</i> | 206 |
| Application of Extracted Pink-Red Pigment (Cyanidin-3-Glucoside) from Cherry Fruit as an Acid-Base Indicator <i>Kamal M. Mahmoud, Mohammed A. Hussain</i> | 211 |
| Optical and physical properties of Lithium Potassium Borate glass doped Eu⁺³ <i>M. M. A. Maqableh, S. Bin Hashim, Y. S. M. Alajerami, K. M. A. Maqableh, M. H. A. Mharebland R. S. Dawwud</i> | 222 |
| More Results On The Stick And Rope Problem <i>Mans Mananohas and Iwan Pranoto</i> | 226 |
| Numerical Simulation of Nanofluids for Improved Cooling Efficiency in Microchannel Heat Sink <i>Nur Hazwani Mohamad Noh, Nor Azwadi Che Sidik</i> | 230 |
| Design and Construct of Bending Plate for Fiber Optic Pressure Sensor <i>Paradorn Pakdeevanich, Pathumrat Phonamuaylap and Warangkana Thongrasmee</i> | 236 |
| QSAR Modeling of Corrosion inhibition Efficiency of Thiophene derivatives Using Interval Partial least square Analysis –Partial Least Square Regression <i>Bishir Usman, Madzlan Aziz, Hasmerya Maarof, Hassan H. Abdallah, Rosmahaida Jamaludin</i> | 241 |
| Choice of Percentage of Cumulative in Principal Component Analysis to Define Region <i>Shazlyn Milleana Shahrudin, Norhaiza Ahmad and Fadhilah Yusof</i> | 246 |
| Measurements of Mass Attenuation Coefficient of Natural Minerals <i>Siti Sarah Yusof, M A Saeed</i> | 252 |
| Performance Test of a New Pilot Plant Air Stripper for Benzene Removal from Industrial Wastewater <i>Abdullahi Mohammed Evuti, Mohd Ariffin Abu Hassan, Zainura Zainon Noor, RK Raja Ibrahim, Hamidah Kamarden</i> | 255 |
| Design and Optimization of Silicon Micropumps for Medical Application <i>Aida Fatehah Bt Mohd Shukur, Neoh Siew Chin, Norhayati Bt Sabani and Bibi Nadia Taib</i> | 261 |
| A Density Functional Theory Study Of Ground State Structural Stability And Electronic Properties of Albi Compound <i>Nor Ashwani Bt Abd Rahim, R.Ahmed, A. Shaari, Bakhtiar Ul Haq, Mazmira Bt Mohamad</i> | 267 |

| <u>CONTENT</u> | <u>PAGE</u> |
|---|-------------|
| Radioactivity Measurements of Soil samples from Soran District in Kurdistan Region-Iraq <i>Ali Hassan Ahmed and Abdulbast Ali Gafur2</i> | 271 |
| The Influence Generator Power 2450 MHz to Test in vivo Hyperthermia <i>Fadli Ama</i> | 277 |
| Effect of Germanium concentration on the lattice Thermal Conductivity for SiGeAllo Nanowires <i>HawkarTaher Taha</i> | 281 |
| Terahertz Radiation Field Regime Absorption in Cancer-Health Tissue for Medical Application <i>Muhamad Hamdi ,Yusof Munajat, Raja Kamarulzaman Raja Ibrahim ,Rosly Abd Rahman</i> | 286 |
| Design and Optimization of Microneedle for Drug Delivery System <i>Nur Afifah Ismail, Neoh Siew Chin, Norhayati Sabani and Bibi Nadia Taib</i> | 292 |

Agglutination of Mice Sperm in Antibody of 46, 66, and 73 KDa Protein from Rabbit Sperm Membrane

Sri Puji Astuti Wahyuningsih

Dept. of Biology, Faculty of Science and Technology, Airlangga University
*E-mail: sri-p-a-w@fst.unair.ac.id Tel:(62)-31-5936501, Fax : (62)-31-5936502

ABSTRACT

This research aimed to investigate the influence provision antibody of 46, 66, and 73 kDa protein from rabbit sperm for mice sperm agglutination. Twenty four male mice of Balb/C strain, 8-10 weeks old and 20-25 g weights were divided into four groups (six mice for control group and 18 mice for treatment group). All of mice were dissected and then taken was epididymis to get the mice mature sperm by flushing method. From the result of flushing using Baker's buffer, each sperm suspension was taken 100 µl, then added with 10 µg/ml control, 46, 66, and 73 kDa protein's antibody. Then taken 50 µl of suspension and placed on hollow glass objects, incubated for 20 second before observed using light microscopy at 400x magnification with 3x replicated observations. This research result showed that the average sperm agglutination of control group was 6.20 and treatment group was 18.92(46 kDa), 12.46 (66 kDa), and 19.60 (73 kDa). In addition types of agglutination, there are agglutination between the head with head, head to tail and tail to tail. Conclusions of this research to provision antibody 46, 66, and 73 kDa protein from the rabbit sperm membrane for mice sperm agglutination and different types of antibodies will affect the result sperm agglutination, the most potentially protein was 73 kDa protein.

Keywords : Membrane protein, Sperm agglutination, Antigen-antibody reaction

1. INTRODUCTION

On the plasma membrane of spermatozoa expressed a specific protein as sperm receptor (Suri, 2004). Sperm membrane proteins played a role in fertilization, among others, played a role in sperm motility, sperm membrane adhesion to the zona pellucida, the initiation of the signal transduction that result in exocytosis acrosome, and contributed to the egg membrane fusion (Evans, 2002; Gahmberg and Tolvanen, 1996; Patrat *et al.*, 2000).

According Wahyuningsih *et al.* (2008), sperm membrane proteins were isolated from the cauda epididymis rabbit had seven protein bands. Molecular weight was 250, 73, 66, 46, 34, 28, and 16 kDa. Protein with a molecular weight of 73, 66, and 46 kDa are specific proteins to form antisperm antibodies.

Immunization with sperm membrane protein in the same or different species will lead to cross-reactions and specific immune responses. The response was the formation of antibodies against the sperm membrane proteins.

The reaction between antibodies and antigen made contraceptive effect, among other caused sperm agglutination, reduced motility, cervical mucus penetration disorders, inefficient fusion of sperm and ovum, increased phagocytosis of spermatozoa and embryo death before or after implantation. All these obstacles cause infertility (Domagala and Kurpisz, 2004). Antibodies anti sperm caused either spontaneously or induction infertile (Bohring *et al.*, 2001). Antibody and spermatozoa bond in the reproductive tract can cause agglutination resulting in inhibition of the motility (Hafez and Hafez, 2005). Agglutination at reducing the ability of spermatozoa fertilization. In addition, the antibody response to antigens such as the occurrence of conception effect on male infertility (Domagala & Kurpisz, 2004). Infertility condition was utilized for the development of immunocontraceptive. Suri (2005), immunocontraceptive method was a new

strategy to solve the problems on the uncontrolled population growth Indonesia.

2. EXPERIMENTAL

2.1 Production of antibodies anti sperm membrane protein subunit

This study required 24 female mice (6 tail for the control group and 18 to be immunized with the sperm membrane protein of molecular weight of 46, 66, and 73 kDa. Dose protein was 50 µg/ml. Immunization first, antigen were emulsified with PBS to a volume of 0.05 ml and Freund's complete adjuvant (FCA) 0.05 ml (ratio 1:1). Later in the vortex for 1 hour. The mixture was injected intraperitoneal. The control was immunized without protein. Immunization second, after the next 14 days with a mixture of antigens in 0.05 ml PBS and Freund's incomplete adjuvant (FICA) 0.05 ml. Immunization third, after 14 days and the next by way of the same material as the second immunization. Immunization fourth, after 7 the next day with protein in PBS without adjuvant.

One week of the last immunization, blood was taken via cardiac mice. Blood was collected in Eppendorf tubes. Blood was left at room temperature for 2 hours. Further blood in a centrifuge at 3000 rpm for 10 min temperature of 4°C. Serum was separated from blood and was collected for the agglutination test.

2.2 Agglutination test

Incubation of mice spermatozoa of with antibody against protein subunits (46, 66, and 73 kDa) rabbit sperm membrane. Sperm agglutination was motile sperm attached

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

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

2.3 Data Analysis

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

3. RESULTS & DISCUSSION

3.1 Number of agglutination

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

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

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

against the 66 kDa subunit protein, the amount of agglutination 14.13 ± 2.899 significantly different from the group treated with the antibody against the 73 kDa subunit protein

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

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

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

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

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

3.2 Type of agglutination

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

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

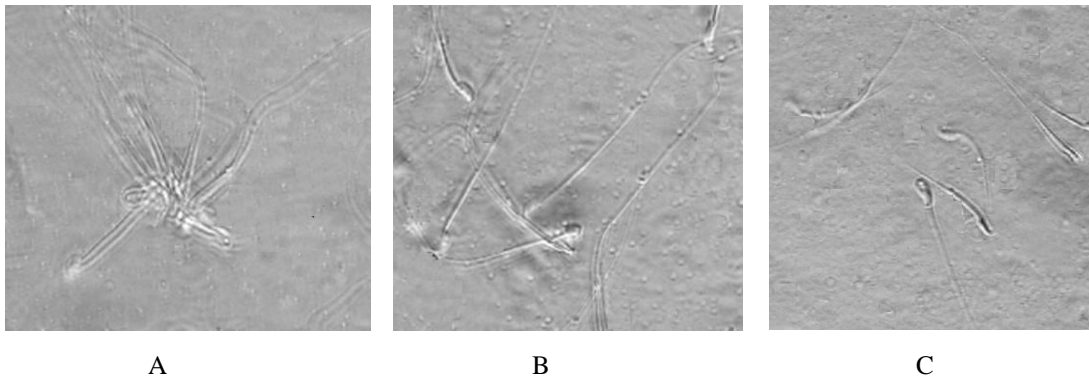
| Replication | The number aglutination of mice sperm after antibody incubation | | | |
|-------------|---|----------------------------------|----------------------------------|----------------------------------|
| | 0 kDa | 46 kDa | 66 kDa | 73 kDa |
| 1 | 7,56 | 18,33 | 18,11 | 23,22 |
| 2 | 5,22 |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

Description: The same Superscript showed no significant difference ($\alpha = 0.05$).

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

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

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

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

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

Type of agglutination between the heads after incubation in antibodies against the 73 kDa subunit protein

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

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

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

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

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

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

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

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

Effect of agglutination is a decreased ability of sperm motility. Naturally, spermatozoa are allowed in certain media will undergo agglutination or clumping. Indirectly, agglutination will reduce the level of efficiency of spermatozoa in an egg (Haviz *et al.*, 2008). Reduction or

disappearance of the ability of spermatozoa to fertilize eggs affect the failure of fusion of sperm and ovum at fertilization. This situation will result in the individual male infertility. Representative schemes antibody that blocks the receptor spermatozoa can be seen in Figure 2.

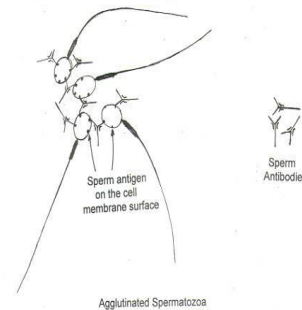


Fig. 2 Antibodies that block receptor spermatozoa (Calamera *et al.*, 2002).

4. CONCLUSION

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

ACKNOWLEDGEMENT

The authors thank the Penelitian Hibah Bersaing Dikti 2008-2009 for funding the project.

REFERENCES

- [1] Bellanti, J. A. 1993. *Imunologi III*, Edisi Bahasa Indonesia. Gadjah Mada University Press, Yogyakarta.
- [2] Bohring C., E. Krause, B. Habermann, and W. Krause. 2001. Testis and Spermatogenesis. Isolation and Identification of Sperm Membrane Antigens Recognized by Antisperm Antibodies, and Their Possible Role in Immunological Infertility Disease. *Molecular Human Reproduction*, 7(2): 113 - 118.
- [3] Calamera, J.C., G.F. Doncel, S. Brugo-Olmedo, A. Sayago, and A.A. Acosta. 2002.. Male antisperm antibodies: association with a modified sperm stress test and lipid peroxidation. *Andrologia*, 34(2): 63-68.
- [4] Campbell, N.A., B.R. Jane, and G.M. Lawrence. 2004. *Biology*, 5th edition. Benjamin Cummings.
- [5] Cesari, A., M R Katunar, M A Monclus, A Vincenti, J C de Rosas and M W Fornés. 2005. Serine Protease Activity, Bovine Sperm Protease, 66 kDa (BSp66), is Present in Hamster Sperm and is Involved in Sperm-Zona Interaction. *Reproduction*, 129: 291-298.
- [6] Domagala, A. and Kurpisz, M. 2004. Identification of Sperm Immunoreactive Antigens for Immun contraceptive Purposes: A Review. *Reproductive Biology and Endocrinology*, 2: 11-18.
- [7] Evans, J.P. 2002. The Molecular Basis of Sperm-Oocyte Membrane Interactions during Mammalian Fertilization. *Human Reproduction Update*, 8(4), 297-311.
- [8] Gahmberg, C.G. and Tolvanen, M. 1996. Why Mammalian Cell Surface Proteins are Glycoproteins. *Trends Biochemistry Sciences*. 21:308-311.
- [9] Hafez, E. S. E. and Hafez, S. D. 2005. *Atlas of Clinical Andrology*. Taylor and Francis Group. United States.

- [10] Haila, A.A. and Daulat, R.P.T. 2001. Acid Glycohydrolases in Rat Spermocyte, Spermatids, and Spermatozoa: Enzyme Activities, Biosynthesis and Immunolocalization. *Biological Procedures Online*, 3: 35-42.
- [11] Haviz, M., A. Boediono, M.A. .Setiadi, S. Agungpryono, dan M. Fahrudin. 2008. Kajian Aglutinasi Spermatozoa Melalui Karakteristik Plasma Yang Dikoleksi Dari Epididimis Dan Seminalis Domba. *Jurnal Veteriner*, Institute Pertanian Bogor, Bogor.
- [12] Manyonda, I.T., 2006. *The Immunology of Human Reproduction*. Taylor & Francis Group, London.
- [13] Naz, R.K., 1996. Application of Sperm Antigen in Immunocontraception. *Frontier in Bioscience* 1, e87-95, September 1, 1996. <http://www.bioscience.org/1996/v1/e/naz1/htmls/2/htm>.
- [14] Naz, R.K. and C.M. Vanek, 1998. Spermatozoa-Specific Protein and Their Role in Contraceptive Development. *Bioscience* 1, e39-48, 30 April 1998. <http://www.bioscience.org/1998/v1/e/naz1/htmls/2/htm>.
- [15] Naz, R.K. and X. Zhu, 1998. Recombinant Fertilization Antigen-1 Causes a Contraceptive Effect in Actively Immunized Mice. *Biology of Reproduction* 59: 1095-1100
- [16] Naz, R.K., S.C. Chauhan, and R.N. Trivedi. 2002. Monoclonal Antibody Against Human Sperm-Specific YLP 12 Peptide Sequence Involved in Oocyte Binding. *Archives of Andrology*, 48: 169-175.
- [17] Naz, R.K. 2004. Human Synthetic Peptide Vaccine for Targeting Sperm. *Archives of Andrology*, 50:113–119.
- [18] Patrat, C., C. Serres, and P. Jouanne. 2000. The Acrosome Reaction in Human Spermatozoa. *Biology of the Cell*, 92: 255–266.
- [19] Suri, A., 2004. Sperm Specific Protein-Potential Candidat Molecules for Fertility Control. *Reproduction Biology and Endocrinology*, 2(10): 1-6.
- [20] Suri, A. 2005. Sperm-Based Contraceptive Vaccines: Current Status, Merits and Development. *Expert Reviews in Molecular Medicine*, 7: 1-16.
- [21] Wahyuningsih, S.P.A., A. Hayati, dan I. Mustofa,. 2008. *Pembakuan epitop membran protein membran spermatozoa kelinci sebagai dasar pengembangan imunokontrasepsi pada pria*. Lembaga penelitian Universitas Airlangga.