Acrosome Status of Ram Spermatozoa after Storage in Epididymis at 4 °C

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INTRODUCTION

The epididymis is an organ in which maturing spermatozoa are stored. The recovery of the epididymyal spermatozoa from dead animals and their cryopreservation are useful tool to rescue genetic material that otherwise would be lost, either from highly productive animals or from endangered species [1, 2, 3]. We have previously described that motile and viable spermatozoa could be recovered from ram epididymides up to 48 h after cold storage at 4°C, but the motility and fertilizing ability of spermatozoa gradually decreased as the storage period was prolonged [4], indicating that the spermatozoa loses their fertilizing potential after storage for several days at 4°C. Assessment of sperm quality conventionally relies on microscopic evaluation to estimate sperm viability and the percentage of motile spermatozoa, and percentage of normal sperm morphology are not truthfully predict male fertility because lack of objectivity and human bias [5]. Thus, functional status of sperm (acrosome, mitochondria, etc) analyses have been gain importance during last decades. Therefore, in this study, we evaluate the acrosomal integrity of spermatozoa after storage in epididymis at 4°C for several days. It is well known that acrosome membrane integrity is considered to be important as critical elements regarding fertilization.

MATERIALS AND METHODS

Testes with attached epididymides from adult rams were obtained at local slaughterhouse. They were transported to laboratory at room temperature. From one testes of each pairs, the epididymis was dissected free and spermatozoa were recovered from it in a culture dish containing m-PBS to serve as a control group. The remaining testes of the pair were put into plastic bag and stored in refrigerator of around 5 °C for 24 h group), 48 (48 h group), 72 (72 h group), and 96 h (96 h group), and afterwards the spermatozoa were recovered for evaluation of the acrosome status.

Acrosomal status was assessed with fluorescens stains. The fluorochrome combination for simultaneous evaluation of plasma membrane integrity and acrosome integrity were propidium iodine (PI) and fluorescent isothiocyanate-conjugated peanut (Arachis hypogaea) agglutinin (PNA-FITC, Sigma). The samples were spread on slides, air-dried at room temperature, and fixed with absolute ethanol for 10 min at room temperature. After drying, 30 μL of FITC-PNA (100 μg/mL) in PBS was spread over each slide and incubated in a dark, moist chamber for 30 min at 37°C. Then 5 μL (1 mg/mL) were spread over the slide and incubated for 5 min. The slides were then rinsed with PBS, air dried and overlaid with a coverslip. The samples were evaluated by using a fluorescence microscope at x400 (wavelength 488-nm). Spermatozoa were divided into two catagories (intact or reacted) according to their staining pattern. The acrosome integrity was expressed as the mean percentage of spermatozoa with intact acrosomes.

RESULTS AND DISCUSSION

The cauda epididymides of live animals an excellent environment for sperm storage in a quiescent state. Thus, spermatozoa stored within this structure retain their motility and fertilizing ability. On the other hand, it is generally assumed that gametes within the body animals generate quickly after death. The viability of germ cells must be affected by the duration and the temperature
at which the dead animal was held before the gametes are collected. In our study, we found that the percentage of spermatozoa with intact acrosome were similar between ejaculated and epidydymal spermatozoa obtained immediately after the death of the males. Then a slower decrease in sperm acrosome integrities were seen as storage time progressed. Kaabi et al. [6] reported that the percentage of intact acrosomes of spermatozoa collected from cauda epididymis after 48 h of storage were significantly lower to those of spermatozoa collected from epididymis without storage in refrigerator, indicating that acrosomes of ram epididymal spermatozoa might sensitive to long storage periods. In boar epididymal Kikuchi et al. [7] also reported similar results. According to these authors, sperm motility and oocytes penetration ability (reflected by acrosome integrity) are affected by different mechanism during cold storage of epididymides. In conclusion, the results of this study show that acrosome for ram spermatozoa were damage when storage period of epididymides was prolonged.

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TABLE OF CONTENTS

Welcome from President RNAS+ iii
Welcome from President SEAVSA & Head of AFKHI iv
Schedule at Glance v
Table of Contents vii

Meeting Report

R-01 Summary Report of the 14th RNAS+ Meeting 1
Lydia R. Leonardo

Oral Presentation

O-01 Detection of Acrosomal Damage of Ram Spermatozoa using Lectin Histochemical Technique during Freezing Process 11
Lisa Dwi Fannessia, Ni Wayan Kurniani Karja, I Ketut Mudite Adnyane, Mohamad Agus Setiadi

O-02 Piper and Zingiberaceae are Potencial as Antibacterial Agent of Chronic Respiratory Disease in Poultry 13
Min Rahminiwati, Yulin Lestari, Aulia A Mustika, Agung Zaim

O-03 Renal Adenocarcinoma with Marked Desmoplasia in a Lion 16
(Panthera leo): Pathomorphological Study
Ekowati Handharyani, Syafri Edwar, Endah Rumiati, Yuli Purwandari Kristioningrum, Adi Winarto

O-04 Maturation and Fertilisation of Sheep Oocytes Matured in Sericin Supplemented Media in Vitro 18
Cut Yasmin, Mohamad Agus Setiadi, Ni Wayan Kurniani Karja

O-05 The Exploration of Eimeria tenella Sporocysts Inoculation on Featuring Cecum and Oocysts Production in Chicken, an Initial Exploration of Sporocysts Potency as Vaccine Material Candidate 20
Muchammad Yunus

O-06 The Prospect of Medical Devices for Early Detection of Autoimmune Diseases based on Reverse Flow Immunochromatography Technique 23
Aulanni'am

O-07 Diagnose and Treatment Evaluation of Microsporum canis Infection in Dogs 25
Soedarmanto Indarjulianto, Yanuwarto, Sitarina Widyarini, Putu Ayu Sisyawati Putriningsih

O-08 Distribution of Ghrelin and It's Receptor in the Stomach: Immunohistochemical Study on Obese Rats (Rattus norvegicus) 27
Teguh Budipitojo, Hevi Wihadmadyatami, Gianies Riza Aristya, Yuda Heru Fibrianto, Dela Ria Nesti

O-09 Fertilizing Ability of Post-Thaw of Epididymal Spermatozoa Stored for 48 H at 4°C Prior Cryopreservation in Domestic Cat 29
Sri Gustari, Hermawan Andri Wibowo, Hardi Purwo S, Ervina Yulianti, Setyo Budhi, Ni Wayan Kurniani Karja

O-10 Histology of Cerebellum of Kalong Kapauk (Pteropus vampyrus) using Cresyl Violet Staining 31
Tri Wahyu Pangestiningsih, Pipin Dwi Kartikasari, Atta Hida Sarassanti, Syahida Eviliana Zulaikha

O-11 Identification of Meatball Adulteration by Porcine Detection Kit and Polymerase Chain Reaction (PCR) 34
Dyah Ayu Widiasih, Mutiara Ulfa, Christina Yuni Admantin, Zuli Amanah, Aris Haryanto

O-12 Prevalence of Leptospirosis in Cattle in Sub-District Pengasih Kulon Progo 35
Estu Widodo, Widagdo Sri Nugroho, Bambang Sumiarto
O-13 Potency of Testosterone Hormone Therapy in the Guinea Pig (Cavia porcellus) as an Alzheimer's Disease Model  
Yuli Purwandari Kristianingrum, Ekowati Handharyani, Dondin Sajuthi, Erni Sulistiawati

O-14 Studies on Turkey’s (Meleagris gallopavo) Semen Collection Method as an Animal Model for Collections of Merak Jawa’s (Pavo muticus) Semen in Vivo  
Budianto Agung, Sri Gustari, Surya Agus Prihatmo, MMP Sitrat

O-15 The Correlation between Femur and Humerus Length, Carpal Tarsal, and Sole Circumferences with the Main Body Size of Sumatran Elephants (Elephas maximus sumatranus)  
Hery Wijayanto, Tri Wahyu Pangestiningsih, Woro Danur Wendo

O-16 Clinical Laboratory Study of Blood Parasites Infected Dairy Cattle at Tandangsari, Sumedang Region  
Agus Wijaya, Bayu Febrom Prasetyo, Leni Maylina

O-17 Enrichment of Black Seed (Nigella sativa) Extract in In Vitro Culture of Rat (Rattus norvegicus) Bone Cells  
Fitri Susana, Wahono Esthi Prasetyaningtyas, Arief Boediono, Kusdiwantoro Mohamad

O-18 Kapok (Ceiba pentandra) Fiber and Used Oil Fueled Portable Incinerator as Biosecurity Enforcement Tool in Indonesia  
Esdinawan Carakantara Satria, Fadjar Satria, Irzaman, Sri Murtini, I Wayan Teguh Wirawan

O-19 Histopathology Study the Benefits of Black Cumin (Nigella sativa) Extract for Respiratory Organ of Mice (Mus musculus) as Animal Model  
Sri Estuningsih, Agung Sudomo, Dewi Ratih Agungpriyono

O-20 Hypoglycemic Effect of Ethanol Swietenia mahagoni Seed Extract on Experimental Diabetic Rats  
Tutik Wresdiyati, Siti Sa’diah, Adi Winarto

O-21 Naturally Tetrahymena spp Protozoan Infection in Guppies (Poecilia reticulata)  
Dewi Ratih Agungpriyono, Fatma Dewi Pravita Putri, Sri Estuningsih

O-22 Liver and Gall Bladder Ultrasound Morphometry of Indonesian Domestic House Cat (Felis catus)  
Rr. Soesatyoratith, Kurniawan Prasetya, Deni Noviana

O-23 Detection of Methicillin-Resistant Staphylococcus aureus (MRSA) Isolated from Dairy Cattle Milk  
Agnesia Endang Tri Hastuti Wahyuni, Agustina Dwi Wijayanti, Fx. Satria Pinanditya, Supriyanto

O-24 Scriptaid and Trichostatin Improve in Vitro Developmental Competence in Mice Cloned Embryos  
Harry Murti, Mokhamad Fahrudin, Mohamad Agus Setiadi, Boenjamin Setiawan, Arief Boediono

O-25 Effects of Crude Extracts Lecaena leucocephala on the in Vitro Migration of Sheep Gastrointestinal Nematode Larvae and the Mortality of C. elegans  
Yusuf Ridiwan, Fadjar Satria, Stig Milan Thamsborg

O-26 The Use of Recombinant DNA Vaccine to Schistosomiasis  
Kurniastih

Poster Presentation

P-01 Acrosome Status of Ram Spermatozoa after Storage in Epididymis at 4 °C  
Ni Wayan Kurniani Karja, Mokhamad Fahrudin, Kusdiwantoro Mohamad, Mohamad Agus Setiadi

P-02 Anatomy of the Male Reproductive Organ of Water Monitor Lizard, Varanus salvator bivittatus (Reptil: Varanidae)  
Mahfudz, Chairun Nisa, Adi Winarto

P-03 Anatomy of the Male Reproductive Organs of Javan Pangolin (Manis javanica)  
Yusrizal Akmal, Chairun Nisa, Savitri Novelina
P-04 Morphological Characteristic of Appendicular Skeleton of Water Monitor Lizard (Varanus salvator)  
Eling Purwanto, Nurhidayat, Savitri Novelina

P-05 Characterization of Staphylococcus aureus Isolated from Dairy Cattle Milk  
Agnesia Endang Tri Hastuti Wahyuni, Michael Haryadi Wibowo

P-06 The Use of Contrast Media (Iohexol) with Angiography Technique to Measure the Density of Feline Urinary Tract  
R Harry Soehartono, Awit Diah A Naomi

P-07 The Development of Luteinizing Hormone (LH) Cells of Long-Tailed Monkey (Macaca fascicularis) during Prenatal Period  
Nurhidayat, R. Anny Karyani, Supratikno

P-08 Echocardiography Evaluation in Piglet (Sus scrofa) during Recruitment Maneuver on Pediatric Acute Lung Injury Model  
Gunanti, Siti Khaerotun Nufus, Riki Siswandi, Ririe Fachrina Malisie, Antonius Pudjiadi

P-09 Histo-dynamical Study of Posterior Pituitary of Long-Tailed Macaque (Macaca fascicularis) during Prenatal Period  
Supratikno, Iga Ismaya, Nurhidayat

P-10 In vitro Embryo Production Using Simmental Cattle (Bos taurus) and Brahman Cattle (Bos indicus) Frozen Semen  
Alif Iman Fitrianto, Anny Rosmayanti, Arief Boediono

P-11 Microanatomical Study of Adrenal Gland of Newborn Long-Tailed Macaque (Macaca fascicularis)  
Danang Dwi Cahyadi, Supratikno, Nurhidayat

P-12 Policy Implementation Analysis for National Committee of Avian Influenza Control and Pandemic Preparedness (Komnas FBPI) in term of Avian Influenza Coordination Program in Indonesia  
Mira Fatmawati, Etih Sudarnika, Kedi Suradisastra

P-13 The Anatomy of Sumatran Rhino (Dicerorhinus sumatrensis) Body Muscles  
Andi Hiroiyuki, Nurhidayat, Chairun Nisa

P-14 The Morphology of the Female Reproductive Organs of Cave Swiftlet (Collocalia linchli)  
Savitri Novelina, RM Rizky Jayhari, Heru Setijanto

P-15 The Muscles Anatomy of Pelvic and Thigh Region of Javan Porcupine (Hystrix javanica)  
Supratikno, Oki Kurniawan Nur Cahyo, Srihadi Agungpriyono

P-16 The Successfulness of Embryo Production by in Vitro Fertilization using Frozen Semen of Bali Cattle (Bos javanicus) and Ongole (Bos indicus)  
G Andri Hermawan, Yanyan Setiawan, Arief Boediono

P-17 The Effect of Thoraco-Vagotomized Calves on Omasum by PGP 9.5 Immunohistochemistry  
R Harry Soehartono, Riona Desti

P-18 Morphological Characteristic of the Cranial Skeleton of Water Monitor Lizard (Varanus salvator)  
Wiwit Widiawati, Nurhidayat, Savitri Novelina

P-19 Electrocardiogram Analysis of Blood Autotransfusion on Local Indonesian Pig (Sus domestica) as Human Model  
Gunanti, Khansaa Mirajziana, Riki Siswandi, Peter Ian Limas, Basrul Hanafi

P-20 Effectiveness of Rat Bone Marrow Stem Cell Therapy to Rattus novergicus by Teratogenic Model of Particulate Matter on Expression of Kappa Beta (NFkβ) Nuclear Factor on Placenta  
Sri Pantja Madyawati, Widjati, Rimayanti
Proceeding of the 3 Joint International Meeting
Bogor INDONESIA, 13-15 October 2014

P-21 Identification of Avian Influenza Virus Subtype H5N1 Clade 2.3.2.1 from Duck as a Candidate Vaccine to Chicken
Suwarno, Nanik Siantita Widjaja, Jola Rahmahani

P-22 Profiles of Red Blood Cell and White Blood Cell of Rat Snake (Ptyas korros)
Aryani S Satyasinghitas, Ibra Maheshwari, Wahyu Aji Al Amin, Fajar S. Nur Hardiansyah

P-23 Distribution of Lysozyme Producing Cells in the Sheep Salivary Glands: Immuno-histochemical Study
I Ketut Madite Adnyane, Wahono Esthi Prasetyaningtyas, Adi Winarto

P-24 Antimicrobial Effectivity of Mikania micrantha Leaves Extract Against Penicillin Resistant Positive Gram Bacteria
RH Gumelar Yoga Tantra, Usamah Affiff, Siti Sadiyah

P-25 The Potency of Ghrelin and Neuropeptide Y Protein as Materials for Energy Balance Regulate Feed Efficiency of Broiler Chicken
Nove Hidajati, Romziah Sidik, Ratna Damayanti

P-26 Proteins Signal Transducers and Activators Transcription (STAT) 5a and 5b as a Candidate Growth Promoter on Broiler Chicken
Anwa Mar'uf, Kuncoro Pugh S.

P-27 Motion Mode Ultrasonography of Rabbit’s Heart during Long-Term Anesthesia
Septiana Eka Sari, Rr. Soesatyoratih, Devi Paramitha, Sitaria Siallogan, Deni Noviana

P-28 Effect of Zinc Supplementation on Serum Biochemistry in Dairy Calves
Sus Derthi Widhyari, Anita Esfandiari, Agus Wijaya, Retno Wulansari, Setyo Widodo, Leni Maylina

P-29 The Prevalence of Reproductive Disorder on Beef Cattle
Surya Agus Prihatno, Sri Gustari, Agung Budiyanto, Erfi Maha Nugrahadi S., Woro Danur Wendo, Dwi Cahyo Budi Setyawan

P-30 Effect of pH on the Stability of Anti Avian Influenza H5N1 IgG from Colostrum of Cows Vaccinated by H5N1
Anita Esfandiari, Fajar Kawitan, Sri Murtini, Sus Derthi Widhyari

P-31 The Effect of Pepsin and Trypsin Enzym on Anti H5N1 IgG Titer of Colostrum from Bovine Vaccinated with H5N1 Vaccine
Sri Murtini, Fitri Amalia, Anita Esfandiari, Sus Derthi Widhyari

P-32 Erythrocyte Profile of Three Breed Bulls at Balai Inseminasi Buatan, Lembang, West Java
Intan Pandini Restu Mukti, Chusnul Choliq, Leni Maylina

P-33 Ecosystems, Aquaculture and Potential Vulnerability to Schistosomes and Food-Borne Trematodes in Fresh Water Wetlands, Myanmar
Khin Thet Wai, Kay Thwe Han, Tin-Oo, Aung Ye Naung Win, Su Latt Tun Myint

P-34 Development and Optimization of Indirect ELISA for Detection of Human Antibody against Schistosoma japonicum
Fadjar Satrija, DG Noor Syamimi binti Daud, Samarang, Sri Murtini

P-35 Analysis of Community Knowledge and Behaviour to Cysticercosis/ Taeniosis in Kama Village at Jayawijaya Region, Papua
Olimince Asso, Irriyanti Asso

P-36 Observation on Temperature of Pork Cooked with Traditional Burning Stones (Bakar Batu) Cooking Technique of Jayawijaya Regency, Papua Province, Indonesia
Irriyanti Asso, Fadjar Satrija, Denny Widaya Lukman, Nyoman Sadra Dharmawan

P-37 Cysticercosis in Wild Boar and Domestic Pig in Way Kanan District, Lampung Province, Indonesia
Heri Yulianto, Fadjar Satrija, Denny Widaya Lukman, Mirnawati Sudarnasto

P-38 Trichinelllosis Prevalence in Pigs in Kopang City, East Nusa Tenggara Province
Andrijanto Hauferson Angi, Fadjar Satrija, Denny Widaya Lukman, Mirnawati Sudarnasto, Eth Sudarnika
P-39 Street Monkey Performance in Jakarta-Depok-Bogor and Zoonotic Disease Risk of Endogen Parasitic Infection
RP Agus Lelana, Diah Iskandriati, Uus Saepuloh, Entang Iskandar, Randall C. Kyes, Suryo Saputro, Lis Rosmanah, Elok Budi Retnani, Intan Ciptaning Putri, Silvia Arin Prabandari, Irma H. Suparto, Joko Pamungkas, Dondin Sajuthi

P-40 The Potential Zoonotic Soil Transmitted Helminths in Javan Slow Loris (Nycticebus javanicus)
Muhammad Mirzan Adi Wibowo, Elok Budi Retnani, R. P. Agus Lelana

P-41 Identifying Future Helmith Zoonotic of Indonesian Slow Loris (Nycticebus coucang)
Nafisatuil Ulfa, Elok Budi Retnani, RP Agus Lelana

P-42 Lice Infestation on Albino Buffalo (Bubalus bubalis) in Sukamaju Village, Ciampea, West Java
Susi Soviana, Firna Kristin Natalia Kolombo

P-43 Bilirubin Profile of Dog Infected Chronically by Babesia sp. and Haemobartonella sp.
Combination
Leni Maylina, Dondin Sajuthi, Anita Esfandiari, Agus Wijaya, Sus Derthi Widhyari

P-44 Optimization of ELISA Method for Detecting Schistosoma japonicum Excretory-Secretory Antigen in Human Schistosomiasis in Napu Valley, Central Sulawesi, Indonesia
Samarang, Fadjor Satrija, Sri Murtini, Made Agus Nurfana, Sitti Chadijah, Intan Tolistiawaty, Malonda Maksud, Andi Tenriangka

P-45 Biological Control of Snail Intermediate Hosts of Fish-Borne Zoonotic Trematodes, by the Black Carp, Mylopharyngodon piceus (Pisces : Cyprinidae)
Nguyen Mahn Hung, Henry Madsen

Index of Authors