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PROCEEDING

International Seminar

THE ROLE OF VETERINARY SCIENCE
TO SUPPORT MILLENNIUM DEVELOPMENT GOALS

and

THE 12th ASIAN ASSOCIATION OF VETERINARY SCHOOLS CONGRESS



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List of Content

	Page
Arts in Pig, Sheep and Goat: Experiences in Thailand M Techakumphu, C. Tretipskul, N, Anakkul, S. Panyaboriban ¹ , J. Suwimon- teerabutr and T. Tharasanit	1
Food Security - The Role of Veterinarians in an Emerging World Problem ID Robertson	6
Past, present and Future of Asian Society off Zoo and Wildlife Medicine (aszwm) Junpei Kimura	11
Standardized Veterinary Competence to Support Global Food Security and Infectious Animal Disease Control Wiwiek Bagja	12
How to Implement Policies and Regulations to Control Antimicrobial Resistance in Animal Husbandry? Yong Ho Park	13
Warning for Animal Parasites on HIV/AIDS Patients Nasronudin	16
Vector Vaccines: a New Generation of Veterinary Vaccines M. Hair-Bejo	21
One Health System for Controlling Zoonotic Diseases Srihadi.Agungpriyono, Denny Widaya Lukman	25
Development of a Novel Oral Contraceptive for the Population Control of Wild Mammals Based on the Neuroendocrine Mechanism Generating Gonadotropin Releasing Hormone (GNRH) Pulse Generation Kei-ichiro Maeda, TanuPinyopummintr, Hiroko Tsukamura	27
Reflection of Animal Welfare Principles as a Part of Professionalism Romziah, S.	31
Non Typhoidal Salmonellosis as Food Borne Disease Dadik Raharjo	37
Current Issue on Feed Additives Utilization in Indonesia Budi Tangendjaja	42
Mitochondrial Genetic Defect And Disease in Human Agung Pranoto	51

Semen Characteristics of Captive Sumatran Tiger (<i>Panthera tigris Sumatrae</i>) Ni Wayan Kurniani Karja, Mokhammad Fahrudin, Mohamad Agus Setiadi, Ligaya ITA Tumbelaka, Retno Sudarwati, Yohana Tri Hastuti, Bongot Huaso Mulia Ardyta Widianti, Keni Sultan, Kazuhiro Kikuchi, Takeshige Otoi	52
Rabies In Animals In Bali Province from 2008-2012 I Ketut Eli Supartika, I Ketut Wirata, I Gede Joni Uliantara, I Wayan Masa Tenaya, I Ketut Diarmita	56
Effectiveness of Red Algae (<i>Eucheuma Spinosum</i>) as Pathogenic Antibacterial in Coastal Organisms and Human Fattah, Afhariman, Muslimin, L, R, W Andy Omar, S. Bin	63
Anti-<i>Coxiella Burnetii</i> Antibody Specific for Q Fever Diagnosis Immunohistochemically in Ruminant Agus Setiyono, Mawar Subangkit, William Marea, Vivi Dwi Santi, Lia Elvira, Mutya Fadhilah and Sulphi Aufo	69
Identification of Patogenic Bacteria <i>Escherichia Coli</i> O157:H7 and <i>Staphylococcus Aureus</i> from Pasteurised and Non Pasteurised Bovine Fresh Milk Lucia R.Winata Muslimin, Dwi Kesumasari, M. Aqshar Marsani, Nurul Inayah, Ainin arsylini, and A.Aswan Salam	73
Clinical Sign Pattern of Infection <i>Microsporium canis</i> on Dogs Gerson Yohanes I Sakan, Puspa Wikan Sari, Yanuartono and Soedarmanto Indarjulianto	77
Detection of Autoimmune Thyroiditis Diseases (Aitd) : Based on Thyroid Peroxidase (TPO) Autoantibody by Immonochromatography Rapid Test Aulanni'am, Agung Pramana. W.Marhendra and Dyah Kinasih Wuragil	81
The Effect of Probiotic on Autoimmune Thyroiditis Model (AITD) Rat (<i>Rattus norvegicus</i>) Induced Sodium Iodide (NaI) Supplementation Hendra Legatawa, Wakhidatus Inrya, Adib Musta'in, Rizki Rosmallasari, Bayu Noviaji, Dyah Kinasih Wuragil and Agung Pramana W. Marhendra	85
Analysis of <i>Salmonella spp.</i> from Poultry Carcasses Industries in Malang, Indonesia Dyah Kinasih Wuragil, Masdiana C. Padaga	90
Molecular Genetic Analysis of Indigenous Bima Horse (<i>Equus Caballus</i>) Based on Cytochrome B Sequences Yuriadi, Rini Widayanti, Wayan Tunas Artama, Charles Rangga Tabbu.....	94
The Study of Binahong Leaves Extract (<i>Anredera cordifolia</i>) Ointment Ethanol Fraction on Skin Incision Wound Healing Process in Dog (<i>Canis familiaris</i>) Slamet Raharjo, Sri Hartati, Agus Budi Santosa, Fajar Kurnniawan	102

Improving Milk Quality and Udder Health of Etawah Crossedbred Goat by Good Milking Procedure Yuni Suranindyah, Sari Retno Diwanti, Ditto Aji Diantha, Nurliyani	107
Blood Chemistry Parameters of Adult Female Turi Ducks Irkham Widiyono, Sri Hartati, Hary Purnamaningsih	112
The Influence of <i>Temu Hitam (Curcuma aeruginosa roxb.)</i> Rhizomes Ethanolic Extract Against Total Intraepithelial Lymphocyte Small Intestine on Layer Chicken Which Infect by <i>Ascaridia galli</i> Handayu Untari, Eka Pramyrtha Hestianah	117
Potential of Beluntas (<i>Plucea indica</i> L.) in Animal Feed to Decrease the Ammonia, Hydrogen Sulfide and Water Levels on Broiler Excreta Taufik Hidayatulloh, Anggun Rahmawati, Zakia Sheila Faradilla	121
The Xenobiotic Metabolism in Lead Intoxication Mice with Vitamin C Supplementation Juliana Christyaningsih	127
The Analysis of Distribution of <i>Mycobacterium bovis</i> Infection with Conventional Techniques, Polymerase Chain Reaction (PCR) and Geographical Information System (GIS) in Dairy Cow Cattle in Enrekang Regency Sartika juwita, Moch. Hatta, Lucia Muslimin, Ahmad Nadif	135
The Effect of Cigarette Smoke Exposure due to Placental Apoptosis and Gestation Outcomes at Gestation Disorders Mechanism in White Rat (<i>Rattus Norvegicus</i>) Portia Sumarsono, Sruti Listra Adrenalin, Ika Wahyuni, Bayu Digka, Christian Marco, and Widjiati	143
Some Factors that May Increase the Potency of <i>Trypanosomiasis</i> that was Caused by <i>Trypanosoma Evansi</i> to Become Zoonosis: A Review Herlina Susijanti, Fx. Satria Pinanditya, Rian Hari Suharto	148
Antibiotic Resistance in <i>Staphylococcus intermedius</i> Strain Isolated from Dogs with Dermatological Disorders Mustofa Helmi Effendi, Ngakan Made Rai Widjaja and Ristin Riwayanti	152
Combination of <i>Spirullna</i> and Fermented Rumen Content Meal As Substitution in Feed Toward Feed Efficiency of Male Broiler Mirni Lamid	156
Potential of Vitamin E (α-Tocopherol) Against on Spermatogenic Cells and Seminiferous Tubule Diameter Testes of Mice (<i>Mus Muscular</i>) Induced with 2, 3, 7, 8-Tetrachlorodibenzo-P-Dioxin (TCDD) Rosida Achlis, Ismudiono, Hani Plumeriastuti	160

The Activity of Vitamin E (α-Tocopherol) as an Antioxidant on Histopathology of Balb/C Mice's Liver Exposed by 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD) Ajeng Erika Prihastuti Haskito, Dewa Ketut Meles, Hani Plumeriastuti	166
α Tokerol in Sperm Muscovy Retailed During Storage to Temperature 27°C. Fitrizni	174
Prevalence and Infection Rate of Gastrointestinal Nematodosis of Limousine and Simmental Crossbreed in the Loceret District Nganjuk Hasutji Endah Narumi, Mamluatus Sa'diyah, Setiawan Koesdarto	177
The Use of <i>Spirulina</i> in Substitution of Rumen Content Meal Wich is Fermented in Feed on Carcass Percentage of Male Broiler Mia Anjar Sari, Wurlina, Mirni Lamid	181
Crude Fiber Digestibility Value of Complete Feed with Omega 9 in the Javanese Fat Tailed Sheep Ninik Rahayuningsih, Tri Nurhajati, Romziah, S, Mirni, L, Retno, S.P	183
Understanding the Biological Products and Development of Biosimilars Nurina Hasanatuludhhiyah, Abdul Khairul Rizki Purba	185
Protein Digestibility Value of Complete Feed With Omega 9 on Javanese Fat Tailed Sheep Virdhanur Chorina, Tri Nurhajati, Romziah Sidik, Mirni Lamid	191
Embryo Collection toward Different Doses of PMSG in Rats (<i>Ratus norvegicus</i>) Bambang Poernomo S	194 ✓
Identical Twins Production of Rat (<i>Ratus norvegicus</i>) Through a Metal Razor Blade Bambang Poernomo S	197 ✓
Clinical Case and Incidence Rate of Mite Infestation on Dog by Scraping Examination at Veterinary Teaching Hospital of Faculty of Veterinary Medicine, Bogor Agricultural University Agus Wijaya	201
Effect of Oviduct Flushing Fluid Addition on Polyspermy Rate of Goat Oocyte in <i>In Vitro</i> Fertilization Yayuk Kholifah, Sri Pantja Madyawati, Wurlina	205
Therapeutic Effectiveness of Rat Bone Marrow Stem Cells in Rats (<i>Rattus Novergicus</i>) Model Exposed to Particulate Matter on Congenital Defects Sri Pantja Madyawati, Widjiati, Rimayanti, Agung Budianto	209

Expression of Cytochrome C as Apoptotic Indicator and it Relation With Sperm Viability and Motility of Domba Ekor Gemuk Frozen Semen In Different Thawing Duration Rahmalia Dwi Suindarti, Imam Mustofa, Suherni Susilowati	213
Effects of the Timing of Insemination by The Use of a Heat Detector on the Incidence of Metestrous Bleeding and Non-Return Rate at Day 21 Ismudiono, Pudji Srianto and Trilas Sardjito	217
Rapid Detection and Phylogenetic Analysis of West Nile Virus as Zoonosis New Emerging Disease in Patients with Fever of Unknown Origin (FUO) in Surabaya E. Bimo Aksono H, Nasronudin, Maria Inge Lusida, Aldise Marieta N, M. Qushai, N. Fajar, Lilis Mundri Jannah, Brian Eka Rachman, Musofa Rusli	221
Comparative Study of Pathogenicity Of H5n1 Virus Between in Tree Sparrow, Scaly Breasted Munia, And Backyard Chickens As Natural Source of Infection in East Java – Indonesia Emmanuel Djoko Poetranto, Djoko Legowo, Suwarno, Fedik A Rantam.....	226
Toxoplasmosis :Changes in Trophoblast Apoptosis index Mice (<i>Mus musculus</i>) Given Anti-<i>Toxoplasma gondii</i> ESA (Excretory Secretory Antigen) Immunoglobulin Y Lucia Tri Suwanti, Hani Plumeriastuti, Dessy Fajarwati	233
Egg Yolk Derived Anti-Rabies Antibody Production as Immunotherapy Agents Suryo Kuncorojakti	240
Relations of Weight And Age to The Front Feet Sole Area of Merino Ram Benjamin Christoffel Tehupuring, Dady Soegianto Nazar, Sarmanu.....	244
Molecular Characterization of Nucleoprotein <i>Antigenic Sites</i> of Indonesian Isolate Rabies Virus Jola Rahmahani and Suwarno	248
Homology Analysis of G Protein Coding Genes of Rabies Virus Sulawesi Isolate Against Pasteur Strain Riski Arya Pradikta and Suwarno	254
The Production of Plastic Progesterone Implants for Estrus Synchronization in Big Tail Sheep From Sapudi Island Sunaryo Hadi Warsito, Setyawati Sigit, Herry Agoes Hermadi	258
Production of Frozen Dry Equine Chorionic Gonadotrophin (eCG) from Pregnant Mare Sera Herry Agoes Hermadi, Laba Mahaputra	262
Acrosin Half-Breed Etawa Goat (PE) Sperm Characteristic to Increase Spermatozoa Quality Budi Utomo	270

The Prevalence of Intestinal Tract Worm Disease of Beef Cattle Brahman and Peranakan Ongole (Po) in the Subdistrict Sugio, Lamongan Faris Amsyari Khozin, Sri Mumpuni Sosiawati, Husni Anwar Kusnoto	282
Osteopontin Maintain Post-Thawed Sperm Mitochondrial Potential Membrane of Friesian Holstein Bull Tatik Hernawati, Yudit Oktanella, Abdul Samik, Ngakan Made Rai Widjaja	287
Protein Utilization of Spirulina in Response to Protein Efficiency Ratio in Laying Hens Widya Paramita Lokapirnasari	293
28-DAY NON RETURN RATES OF DAIRY COWS AS BOTH ACCEPTORS AND RECIPIENTS Trilas Sardjito, Pudji Srianto and Ismudiono	296
Hymenolepiasis Nana, is a Scarce Case in Zoonosis R. Heru Prasetyo	299
Financial Analysis of Layer Chicken Farms in Sub-District Kedungpring of Lamongan Sunaryo Hadi Warsito	302
The Potency of Protein Ghrelin and Neuropeptide Y as Materials for Energy Balance to Set Feed Efficiency of Broiler Chicken Nove Hidajati Romziah Sidik, Ratna Damayanti	306
Proteins Signal Transducers and Activators Transcription (STAT) 5a and 5b as a Candidate Growth Promoter on Broiler Chicken Anwar Ma'ruf, Romziah Sidik and Kuncoro Puguh S	310
Comparison of The Spermatozoa Quality of Post Thawing Simental Cow That Centrifugated Use Yolk Skim Diluter and Soya Lecithin With Malondialdehyde (MDA) Level Measurement Novia Candrawati, Suherni Susilowati, Bambang Purnomo	314 1
Incidence Rate and Small Animal Geriatric Diseases in Veterinary Teaching Hospital Airlangga University Surabaya on 2010-2011 Nusdianto Triakoso	318
(In Vitro) Antibacterial Activity of the Supernatant of Shrimp Pond Isolate Bacillus subtilis Against Aeromonas hydrophila and Staphylococcus aureus Erni Rosilawati Sabar Iman, Elyza Noor Fitria, Suzanita Utama	323
The Effect of Anti- Toxoplasma gondii Esa Immunoglobulin Y (IgY) Againsts Liver Damage in Mice Gestation were Infected Tachyzoite Toxoplasma gondii Lucia Tri Suwanti, Hani Plumeriastuti, Basuki Suryo Jatmiko	328
Effect Various Height of Equilibration Nitrogen Vapour on Post Thawing Semen Quality at Madura Bull Cattle Hermin Ratnani and Suyadi	335

The Potential of Various feed Pellet to Weight Gain and Feed Conversion of Rex Rabbit (<i>Oryctolagus cuniculus</i>) Nimas Ayu Pertiwi, Romziah Sidik, Dady Soegianto Nazar	345
Ideal Manajemen System for the Feasibility Ranch Purebred Cats (Cattery) In Surabaya, Sidoarjo and Gresik Ratna Widyawati, Koesnoto Supranianondo, Bambang Sektiari L	348
Seroprevalence of Influenza Virus H5 Isolated from Mojosari Broiler Ducks (<i>Anas Javanicus</i>) Originated From Two Subdistricts in Jombang District East Java Province A. P. Rahardjo, A. T. S. Estoe pangestie, N. H. Risanti.....	355
Effect of Epigallocatechin-3-Gallate (Egcg) Content in The Green Tea as a Diet for Expression of Transforming Growth Factor B (Tgf-B), Impaired Folliculogenesis and Reproductive Status of Rats (<i>Rattus Norvegicus</i>) Widjiati, Ika Wahyuni, Portia Sumarsono, Sruti Listra adrenalin, Christian Marco Hadi	362
Increased Neural and Glial Cells Death of Embryonic Cerebral Cortex Exposed to Carbofuran Insecticide in Prenatal Period Epy Muhammad Luqman, Ari Gunawan, Harjanto, I Ketut Sudiana dan Widjiati	366
The Events of Helminthiasis in Digestive Tract of Pre and Post Weaning on Cattle in Lumajang Plateau Region Ferri Andrianto, Setiawan Koesdarto, Nanik Sianita Widjaja	376
Comparisons of Nutritive Value Between Dairy Cow Milk and Yoghurt Romziah S., Tri Bhawono D., Mirni L., Nenny H	382
The Blood Urea Nitrogen (BUN) And Creatininconcentration in Local Male Cats after Feeding by Dry Commercial Food Lita Rakhma Yustinasari, Suryo Kuncorojakti	390
Potencial Test of Local Product PMSG (<i>Pregnant Mare Serum Gonadotropin</i>) Polyclonal Antibody (Abpo PMSH) Originated from Male Rabbit (<i>Orictolagus Cuniculus</i>) on Mice (<i>Mus Musculus</i>) Foetus Number Indra Rahmawati	394
The Biopotency of PMSG (Pregnant Mare Serum Gonadotrophin) from Local Horse Toward Pregnancy Totally in Madura Cattle Muharti Rahaju, Herry Agoes Hermadi, Fedik Abdul Rantam.....	401
The Use of Recycle Soybean Fermented Cake (Tempe) with Cellulolytic Bacteria From <i>Spodoptera litura</i> (Ulat Grayak) as Corn Substitution to Carcass and Abdominal Fat Percentage of Duck Sri Hidanah and Dady Soegianto Nazar	406
Effect Of Non-Competitive Antagonist NMDA Receptors (N-methyl-D-aspartate), Ketamine, on NR2B Subunit Expression of NMDA Receptors In Neuropathic Pain Management Indiastuti D.N, Setiawati Y., Khotib J	411

The Potency of Kelor Leaves (<i>Moringa oleifera</i>) for Treatment of Hypercholesterolemia in Mice (<i>Mus musculus</i>) M. Gandul Atik Yuliani, M. Chalid Ardiansyah, Yuanistia Shally, Haydy Layli Oriliza, Qurrota A'yuni, Nur Faidah	418
Bacteriocin Produced From Lactic Acid Bacteria as an Antibacteria and The Effect as Therapeutic of Dairy Cattle Sub Clinic Mastitis Nenny Harijani	423
Exploration of Antibacteria From Lactic Acid Bacteria Against to Escherichia Coli and The Effect to Therapeutical of Dairy Cattle Sub Clinic Mastitis Hani Plumeriastuti	426
The Role of External Heat Shock Protein 70 (HSP70) Supplementation On Expression of Caspase 3 in Oocyte During Vitrification Rimayanti	429
The Potency of Repetitive DNA Fragment for Molecular Diagnosis of Toxoplasmosis Dyah Ayu OktavianieArdhiana Pratama, Sumartono and Wayan T. Artama	434
Molecular Analysis of a Variable Region on Protective Antigen Gene of Selected <i>Bacillus Anthracis</i> Isolates from Central Java And Yogyakarta Special Region Maxs Urias Ebenhaizar Sanam, Widya Asmara, Agnesia Endang Tri Hastuti Wahyuni, Michael Haryadi Wibowo	441
Antibacterials Effect Of <i>Streptomyces</i> Sp-Mws1, <i>Streptomyces</i> Sp-Mws3, And <i>Streptomyces</i> Sp-Mws6 On Non Extended-Spectrum B-Lactamase (Esbl)-Producing <i>Klebsiella Pneumoniae</i> Yuanl Setlawati, Danti Nur Indiasuti, Wiwin Retnowati.....	449
Relationship Management System Animal Hospital With Pattern Service Disease In Animal Hospital Surabaya Miyayu Soneta Sofyan	457
Neutrophilia As a Spesific Clinical Sign to Differentiate Acute Cholangiohepatitis With Others Liver Inflammatory Diseases in Cat Wiwik Misaco Yuniarti, Bambang Sektiari Lukiswanto	465
Effect of Transport on Glucose in Sheep Sarmin, Amelia Hana, P udji Astuti, Yuda.Heru Fibrianto, Claude Mona Airin	471

COMPARISON OF THE SPERMATOZOA QUALITY OF POST THAWING SIMENTAL COW THAT CENTRIFUGATED USE YOLK SKIM DILUTER AND SOYA LECITHIN WITH MALONDIALDEHYDE (MDA) LEVEL MEASUREMENT

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ABSTRACT

This study purposes to compare the quality of spermatozoa by using two diluter that are yolk skimmed and soya lecithin, in frozen bovine spermatozoa simental before and after centrifugation. Then Malondialdehyde levels checked only on spermatozoa frozen after centrifugation. Examination of sperm quality includes sperm motility, sperm viability checks by using negrosin eosin staining method, and examination of intact plasma membrane of spermatozoa using Hypo-osmotic Swelling Test. The level of MDA using the TBA (Thyobarbiturat Acid) method. Test results on the quality of spermatozoa showed no significant difference ($p > 0.05$) in the time before and after centrifugation, but the motility examination showed significantly difference ($p < 0.05$) at the time prior to centrifugation. Examination showed MDA levels were not significantly different ($p > 0,05$).

Keywords: diluter, semen freezing, centrifugation, MDA levels.

INTRODUCTION

Freezing will induce permanent damage to spermatozoa, but the damage can be reduced by adding a protective substance called cryoprotectants. Cryoprotectants a protective agent is added to the diluent in the process of freezing, which is divided into intracellular and extracellular (Afiati, *et al.*, 2004). Speed reduction temperature affects the metabolism and viability of sperm cells. Drop in temperature too quickly can lead to cold shock to the spermatozoa in the semen, at temperatures slightly above freezing metabolism usually is perpetuated by a low degree of speed. Storage at temperatures below the freezing point of glycerol should be added into the liquid diluent in order to avoid the formation of ice crystals, electrolyte intracellular accumulation and intracellular destruction of a vital relationship (Hardijanto, *et al.*, 2010).

According Dwiyanto and Herliantien (2006) prior to distribution of frozen semen, frozen semen tested first whether they meet the minimum standards of quality frozen semen according SNI Frozen Semen 01-4869,1-1998 that have minimal motility after thawing (PTM) 40% and

the number of spermatozoa per ministraw dose of at least 25 million cells. Frozen semen diluent should contain a source of nutrients, buffers, anti cold shock material, antibiotics, and cryoprotectants can protect spermatozoa during freezing and thawing. This time has been widely used and diluents containing buffers such as tris (*hydroxymethyl*) amino-methan and recipes that can be made based on such Skim Yolk (SKT) which is universally used for frozen bovine semen (Arifiantini and Joseph, 2012).

Skim Yolk Diluent (SKT) using yolk as one of the constituent components, which are known to contain various types of microorganisms and the media may be spreading some infectious diseases. Supported by (Froning, in Surachman, *et al.* 2006) reported that eggs containing *Salmonella typhimurium* by a mean of 67,09 CFU/ml. One cement diluent made from Soybean Lecithin (EHS) and does not contain yolk is Andromed® (Minitube - Germany). This commercial cement diluent besides not contaminated microorganisms derived from yolk is also easy to use because it is available in a ready-made package.

muscle of normal mice (Smit et al , 1999)

STAT1 is also called P91 , was identified as a member of a gene factor 3 complex is stimulated by IFN (FU, 1992). Analysis of GH on cell signaling and cell deficiency JAK2 mutation in the GH receptor expression suggests that activation of Stats 1, 3, 5a and 5b requires GH -dependent JAK2 activation (Smit et al., 1997). This is consistent with the finding that the activation of Jaks required for STAT activation (Muller et al., 1993) . JAK1 or JAK2 overexpression actively expressed in COS cells will stimulate STAT1 bound to DNA (Silvennoinen, 1993) .

Indirect studies indicate that GH stimulates phosphorylation of Stats 1, 3 and 5 on serine or threonine in the liver. This phosphorylation increases the DNA binding of STAT1 , STAT3 DNA binding and DNA substantially alter STAT5 (Ram et al , 1996) . STAT 1 , 3 , and 5a contain the conserved consensus sequence for the phosphorylation of MAP kinase and preliminary studies indicate that MAP kinase is responsible for Seril phosphorylation of STAT1 , STAT3 and STAT 5a . While STAT 5b because it does not contain the conserved consensus sequence phosphorylation by kinases other than MAP kinase . STAT proteins 1 , 3 , 5a and 5b also contains protein kinase C and casein kinase for the phosphorylation process. This suggests that multiple signaling pathways may converge on STAT proteins for transcriptional activation by GH.

CONCLUSION

The molecular weight of STAT 5a protein in broiler during the growing period is 91 kDa and STAT 5b 90 kDa. It can be considered as scientific base for the production of bioactive materials in tissue cultivation and culture.

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Difference in speed centrifugation in preparation for IVF sperm washing will affect the motility, viability, and that sperm abnormalities. Excessive ROS production due to the availability of oxygen in large quantities in the spermatozoa. This can lead to sperm damage caused by lipid peroxidation. Lipid peroxidation caused by the free radicals, some examples of these free radicals are superoxide, hydroxyl and peroxy (Darmawan, 2007). Lipid peroxidation can also be decomposed by free radical compounds into compounds malondialdehyde (MDA) (Fauzi, 2008).

The general objective of this study was to compare the quality of spermatozoa using two diluter which are yolk skimmed and soya lecithin before and after centrifugation, was also checks levels of MDA after centrifugation.

MATERIAL AND METHODS

Semen samples obtained by using an artificial vagina shelter from Simental male cows 5-6 year old. Collecting done in the afternoon at 16:00 to 17:00 pm, one-time.

After the cement collected then immediately examined macroscopically and microscopically. The examination aims to determine whether the cement is still worth used to be frozen semen. Macroscopic examination include: volume, color, odor, consistency, and the degree of acidity or pH. Whereas for microscopic examination include: mass movements, individual movement, motility, and concentration of spermatozoa. Cement has a minimum percentage of 70% initial motility, sperm concentration of more than 600 million/ml, the percentage of live spermatozoa more than 80% is used as a sample.

Further making the yolk skim diluter and soy lecithin diluter, which is then followed by the process of freezing semen, motility after freezing semen examination and inspection of the plasma membrane integrity of spermatozoa as sperm quality inspection.

Motility examination after Semen Freezing

Frozen semen produced in maintaining the quality, the quality needs to be checked. *Test After Thawing* (after dilute again) aims to determine whether frozen semen is still fit for use. So in this test determined the percentage of live sperm minimum standard is 40% with 3 individual movements. How to perform the *test after thawing*, first prepare 2 ml diluent A in the water incubator temperature 37°C. Thawing 2 doses of frozen semen, cut both ends, drop cement into tubes containing diluent A. By using stick glass dropped cement over the object glass that have been prepared, covered with a cover glass. Look under the microscope with a magnification 10x10.

Examination of Plasma Membrane Intake of Spermatozoa

Examination of plasma membrane intake of spermatozoa using *Hypoosmotic Swelling Test* (HOST), inspection procedures using a medium Host as NaCl hypotonic (composition contained in the appendix. A total of 0.1 ml of cement was added with 0.9 ml of HOST medium, then incubated for one hour at 37°C. Incubated semen has been evaluated using 400 times magnification microscope. number of spermatozoa counted with a scale of 0% to 100%. Semen will show morphologic changes when incubated in hypotonic medium. Changes that occur include swelling at the tip of the tail, the tail is short and thick or swelling of all or part of the arch formed by the tail of spermatozoa.

Examination malondialdehyde (MDA) level of spermatozoa

Measurement of MDA levels were calculated using TBA (Thyobarbiturat Acid). MDA values expressed in nmol/10⁸ spermatozoa.

First tested the homogeneity of the distribution of the data to determine the data normality, if data in the form of scores were tested using the Mann-Whitney test. Research data in the form of the ratio in the test for normality of distribution, so that the

normal data were analyzed by using Independent T test (student's t) (Kusriningrum, 2008).

RESULTS AND DISCUSSION

Examination results of Quality Simental Cow Sperm

The results of average and standard deviation of motility, viability, and plasma membrane intact spermatozoa after treatment was given two diluents: Yolk Skim (SKT) and Soybean Lecithin (EHS) which had been centrifuged and before centrifuged listed in Table 5.2 and Table 3.

Calculations on the viability and plasma membrane intact before and after centrifugation the yolk skim diluter and soy lecithin there is no difference ($p > 0,05$). Calculations on motility before centrifugation significantly different experience, but after centrifugation was not significantly different experience this can be caused by the influence of centrifugation. Post-thawing Spermatozoa before centrifugation still have full energy, whereas the post-thawing spermatozoa after centrifugation of spermatozoa energy is divided because some of the energy is divided nuntuk spermatozoa movement itself and also in part to balance

the movement of centrifuge. Accordance with the opinion Sujoko, *et al.*, (2009) the higher speed and centrifugation time resulted in friction among spermatozoa or spermatozoa with medium or bigger reaction tube wall. Instead centrifugation with speed and slow time, the presence of spermatozoa in the tube and spread away from the center of the reaction tube due to centrifugal force is greater than the centripetal, so not a lot of sperm to reach to the bottom of the test tube and cause the concentration of spermatozoa to settle to be low. The separation process may be due to differences in specific gravity and progressive motility of spermatozoa. Dead spermatozoa suspected of having a lower specific gravity than live spermatozoa, due to the release of the cell suspension for cell membrane has been damaged.

Examination results MDA spermatozoa Simental Cow

The results of average and standard deviation of MDA Simental cow spermatozoa after treatment was given two diluents: Skim Yolk (SKT) and Soybean Lecithin (EHS) which has been centrifuged listed in Table 4.

Table 2. Mean and Standard Deviation Percentage Examination Results Viability, Motility, Plasma Membrane Intact spermatozoa Simental Cow Before centrifugation.

Parameter	Before Centrifugation	
	SKT	LKK
Motility	35,00 ± 4,08 ^a	31,25 ± 2,50 ^b
Viability	30,07 ± 7,00 ^a	34,05 ± 7,22 ^a
Whole Plasma Membran	33,45 ± 8,00 ^a	43,77 ± 2,85 ^a

Superscripts in the same row with the same notation indicates not significantly different ($p > 0.05$)

Table 3. Mean and Standard Deviation Percentage Examination Results of Viability, Motility, Plasma Membrane Intact Spermatozoa Simental Cow After Centrifugation.

Parameter	After Centrifugation	
	SKT	LKK
Motility	11,44 ± 103,00 ^a	7,56 ± 68,00 ^a
Viability	29,90 ± 11,17 ^a	29,71 ± 6,90 ^a
Whole Plasma Membran	26,46 ± 7,23 ^a	35,14 ± 10,69 ^a

Superscripts in the same row with the same notation indicates not significantly different ($p > 0.05$)

Table 4. Mean and Standard Deviation Examination Results Percentage MDA and DNA Damage Sperm Nucleus of Simental Cow After centrifugation.

Parameter	SKT	LKK
MDA Level	8,80 ± 0,54 ^a	8,18 ± 0,67 ^a

Superscripts in the same row with the same notation indicates not significantly different ($p > 0.05$)

MDA level calculation of spermatozoa and sperm nuclear DNA damage Simental cows after post-thawing treated two diluents: Skim Yolk (SKT) and Soybean Lecithin (EHS) provides the results of the use of MDA spermatozoa unit nmol/ml). According Benaroudj et al., (2001) Metabolism in normal spermatozoa will generate free radicals in the day 2-5% of total oxidation products. Exposure to low temperatures will significantly increase free radicals and anti-oxidants in this condition naturally generated not able to offset the amount of free radicals that cause oxidative stress.

CONCLUSION

The conclusion of this study is diluter soybean lecithin as well as yolk diluter scheme is no different as evidenced by the results of MDA spermatozoa.

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