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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

ACADEMIC EXCELLENCE
DIES NATALIS
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# 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, electolyte 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 phosphorrylation 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.

## REFERENCES


Darnell J, Lodish H, Baltimora D., 1990. Molecular cel biology. 2nd Edition, New York : Scientific America Books, p 715

Foster DN, Froudman JA, Harmon SA, Foster LK., 1998. Baculovirus-mediated expression of chicken GH. Tektran p 14

Ihle JN., 1996. Cell 84 : 331-334

Kaiya H, Van Der Geyten S, Kojima M, Hosoda H, Kitajima Y, Matsumoto M, Geeliissen S, Darras VM and Kanagawa K. 2001. Chicken Ghrelin : purification cDNA cloning, and biological activity. Endocrinology 143 : 3463

Killefer K, Kenny PB., 2000. Anim Vet Sci 1877.

Ram PA and Waxman DJ., 1999. J. Biol. Chem. 274:35553-35561

Ram PA, Park SH, Choi HK and Waxman DJ., 1996. J. Biol. Chem. 271:5929-5940

Smit LS, Meyer DJ, Argetsinger LS *et al.*, 1999 Handbooks of Physiology. Oxford University Press : New York, pp445-480.

Younken RV, Zaou Y, Wang X et al., 2000. J Endocrinol 166:620-690

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 peroxyl (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 37ºC. 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 magnifycation 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/$10^8$ 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 signify-cantly different experience, but after centrifugation was not significantly different experience this can be caused by the influence of centrifugation. Post-thawing Spermatozoa before centrifu-gation 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 move-ment 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 pro-gressive 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 spermato-zoa 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.

## REFRENCES


Afiati, F., E.M. Kaiin, M. Gunawan, S.Said dan B. Tappa. 2004. Perbaikan Teknik Pembekuan Sperma: Pengaruh Suhu Gliserolisasi dan Penggunaan Kaset Straw. Seminar Nasional Teknologi Peternakan dan Veteriner: 67-71.

Arifriantini. RI. dan TL. Yusuf. 2012. Keberhasilan Penggunaan Tiga Pengencer dalam Dua Jenis Kemasan pada Proses Pembekuan Semen Sapi Frisien Holstein. Institute Pertanian Bogor. Bogor.

Benaroudj, N., D.H. Lee and A.L. Goldberg 2001. *Trehalose accumulation during cellular stress protects cells and cellular proteins from damage by oxygen radicals*. J. Biol. Chem., 276: 24261-24267.

Darmawan, H. 2007. Production of ROS and its effect on mitochondrial and nuclear DNA, human spermatozoa, and sperm function. Media Jurnal Indonesia.16 (2):127-133.

Dwiyanto, K., dan Herliantien. 2006. Aplikasi Teknologi Inovatif *Sexing* dalam Program Inseminasi Buatan dan Usaha *Cow-Calf Operation*. Wartazoa. 16 (4): 171-180.

Fauzi, T.M. 2008. Pengaruh Pemberian Timbale Asetat dan Vitamin C Terhadap Kadar Malondialdehid dan Kualitas Spermatozoa Di dalam Sekresi Epididimis Mencit Albino (Mus musculus m) Strain balb/c [Tesis]. Universitas Sumatra Utara. Medan.

Hardijanto., T. Sardjito., T. Hernawati., S. Susilowati dan T.W.Suprayogi. 2010. *Buku Ajar Inseminasi Buatan*. Airlangga University Press, Universitas Airlangga. Surabaya.

Kusriningrum. 2008. *Perancangan Percobaan*. Airlangga University Press. Surabaya. Setyawati, A.N. 2010. Handout Ajar Siklus Krebs. Biokimia Fakultas Kedokteran Universitas Diponegoro. Setyawati, A.N. 2010. Handout Ajar Siklus Krebs. Biokimia Fakultas Kedokteran Universitas Diponegoro.

Sujoko, H., A.S. Mohamad, dan B. Arief. 2009. Seleksi Spermatozoa Domba Garut dengan Metode Sentrifugasi *Gradien Densitas Percoll*. Jurnal Veteriner 10 (3) : 125-132.

Surachman, M., Herdis, M. A. Setiadi, dan M. Rizal. 2006. Cryopreservation of Ram Epididymal Spermatozoa Using Lecithin-Based Extender. Jurnal Indonesia Trop.Anim.Agric. 31 (2) : 83-89.