

PROCEEDING



THE THIRD INTERNATIONAL SEMINAR ON ANIMAL INDUSTRY

**“Sustainable Animal Production
for Better Human Welfare and Environment”**

**September, 17-18 2015
IPB International Convention Center
Bogor-Indonesia**



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FOREWORD FROM CHAIRPERSON OF ORGANIZING COMMITTEE

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Director General of Livestock Services and Animal Health, Ministry of Agriculture, Republic of Indonesia,
Prof. Dr. Ir. Muladno, MSA.

Dean of Animal Science Faculty, Bogor Agricultural University, Prof Dr Luki Abdullah M.Agr.Sc.

All participants of the International Seminar on Animal Industry 2015

Good morning ladies and gentlemen,

It is my great pleasure to welcome you all, distinguished guests, speakers and participants, to the Third International Seminar on Animal Industry (ISAI 3rd, 2015) held at the IPB International Convention Center, Bogor Indonesia. This seminar with the theme **"Sustainable Animal Production for Better Human Welfare and Environment"** is organized by Faculty of Animal Science, Bogor Agricultural University in collaboration with Association of Indonesia Animal Scientists.

Following the recommendations from Isai 1 and Isai 2, which were held in Indonesia in 2009 and 2012, the strategic issues of Isai 3rd is emphasized on animal production systems and technology and the use of natural resources in relation with environmental aspects, toward a sustainable animal production. There will be 97 papers presented during the two days seminar; 9 by invited speakers, 69 for oral and 28 for posters presentations. The speakers came from different countries including Australia, Egypt, France, Korea, German, Netherland, Indonesia, Malaysia, Nigeria, Pakistan, Thailand, USA.

This is a great opportunity for scientists, researchers, private sectors and policy makers to discuss, share information and experiences on interesting topics in animal production in a broad sense, including good farming practices, recent technologies and save animal products. I believe, there is an open window for initiating and strengthening collaboration among scientists and institutions during and after the seminar.

On behalf of the Organizing Committee, I would like to express my sincere appreciation and thanks to IPB, and some units within, including Institute of Research and Community Empowerment, Faculty of Animal Science, Department of Animal Production and Technology, Department of Nutrition and Feed Technology, Diploma Program, Management and Business Program for all advice and funding support.

The success of this seminar could only be achieved with all the valuable supports and sponsorship from some recognized institutions in this country. In this regards, I would like to address my grateful thanks to Directorate General of Livestock Services and Animal Health-Indonesia Ministry of Agriculture for funding support, and Infovet and Trobos, Green TV as promotion agency. To: Sierad Produce, Kaltim Prima Coal, BRIngin Life, Adaro Indonesia, Trouw Nutrition Indonesia, Nutricell Pasific, Sweni Transfer Indonesia, Charoen Phokphand, Wide & Pin, Pupuk Kujang, and ANTAM thank you so much with big appreciation, for having being part of this important event and such enormous contributions.

My recognition and gratitude are also forwarded to the Steering Committee for advice and assistanship, to international and national reviewers and the Scientific Committee for hard working and such great contribution. Last but not least, to all my dear colleagues of the Organizing Committee members, who have been working smartly and full of dedication and passion, to make this seminar a great successful event.

To all participants, hopefully, the two days seminar may bring fresh ideas, and enhancing collaborations for future success toward sustainable animal production. Big apologies for any inconveniences during the seminar, wish you all having good times, and fruitful discussions.

During your short stay, please enjoy the surrounding of Bogor city, the Museum of Presidential Palace and Historical Botanical Garden of Bogor.

Bogor, September 17th, 2015

The Isai 3rd 2015,

Chairperson of Oraganizing Committee

Asnath M. Fuah

REMARKS FROM DEAN OF ANIMAL SCIENCE FACULTY

Prof. Dr. Muladno, MSA
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Prof. Dr. Ir. Herry Suhardiyanto, M.Sc.
Rector of IPB

Dr. Ir. Asnath Maria Fuah
Chairperson, The 3rd International Seminar on Animal Industry

Our Colleagues from Indonesian universities and research institutes,
Distinguished foreign participants and speakers,

Representative of livestock services officers of local government from all over Indonesia,

Distinguished guests, ladies and gentlemen.

Assalamu'alaikum warahmatullaahi wabarakatuh,

I am pleased to welcome you all to Bogor city for attending "The 3rd International Seminar on Animal Industry 2015" held at Faculty of Animal Science, Bogor Agricultural University (IPB). As the Dean of Faculty, I am also really honored to host this conference.

First, let me introduce briefly about Bogor city. Bogor is one of the major scientific and educational centers in Indonesia. A significant part of academic and research base was laid in the period of Dutch colonization. In particular, since the beginning of the 19th century there were established laboratories and professional schools focused primarily on improving the efficiency of the colonial agriculture. Similar to the prevailing profile of research and academic activity was retained in Bogor after gaining independence. As in the second half of 20th century, and in the 2000s strongest areas were Agricultural sciences, Biology, Animal and Veterinary Sciences. The main educational and scientific center with the utmost national importance is the Bogor Agricultural University (IPB). It is therefore the city regularly hosted various international events, such as international seminars and conferences.

I would like to express my gratitude to IPB for supporting us to hold this conference, and also to the organizing committee of the present conference for their hard work and persistence. I convey my sincere gratitude to all the parties which is supporting this event, such as Directorate General of Livestock and Animal Health-Ministry of Agriculture Republic of Indonesia, Infovet Trobos, Agrina, Green TV as promotion agency and Sierad Produce, Kaltim Prima Coal, BRIngin Life, Adaro Indonesia, Trouw Nutrition Indonesia, Nutricell Pasific, Sweni Transfer Indonesia, Charoen Phokphand, Wide & Pin, Pupuk Kujang, and ANTAM thank you so much with big appreciation, for having being part of this important event and such enormous contributions. I am very pleased to see here the delegates from various foreign countries as well as representatives from many domestic institutions.

I hope you find this conference and the city, both interesting and stimulating and that you enjoy meeting up with your professional colleagues as well as having pleasure time during your stay in Bogor.

Thank you very much and
Wassalamu'alaikum warahmatullaahi wabarakaatuhu.

Bogor, 17 September 2015
Prof. Dr. Ir. Luki Abdullah, MSc.Agr
DEAN

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Effect of Caffeine on Morphology of Epididymis Spermatozoa of Bali Bull

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Abstract

There is an unexpected loss of breeding animals of high genetic value for any reason, such as loss of libido, reproductive tract injury, or death. This loss can be reduced by harvesting spermatozoa from the epididymis with cryopreservation. However, after cryopreservation, there is still a decline of 50% in sperm post-thaw viability after thawing process. This research was conducted to evaluate the use of caffeine to improve cryopreservation quality of Bali bull's spermatozoa. Selection for sperm quality was based on sperm morphology. Cauda epididymis was obtained from slaughterhouse and the semen was collected and examined with caffeine after cryopreservation. The spermatozoa were stained with Acredine Orange by microscopy laser-scanning confocal microscope (BioRad MRC-1024). The result demonstrated that control have high value of normal sperm cell than those treated with caffeine.

Key words: cauda epididymis, abnormality, caffeine

Introduction

Bali cattle are widely spread in Eastern Indonesia because they have good adaptability and have a fairly high carcass that reaches 57%. Bali cattle were originated from a wild ox of java that had been domesticated in Indonesia. Well-maintained Bali cattle will show a good performance, so the male Bali cattle that are genetically well will show a color change from brown brick to a black color. In order not to lose these genetic resources sperm cryopreservation of Bali cattle should be conducted.

Cryopreservation is one way of handling the sperm that is long lasting and can be used at any time as needed. However, cryopreservation of sperm is often experienced problems such as loss of viability of sperm that can occur because of freezing process. Loss of post thawing motility can reach 50% (Lemma, 2011), and therefore it is needed for the addition of additives before artificial insemination (AI). Caffeine is one of the ingredients that can be added in post-thawing sperm.

Caffeine is an alkaloid found in plants formed of white powder with the mechanism of action to inhibit the activity of nucleotide phosphodiesterase. Nucleotide phosphodiesterase inhibited the production of cAMP and cAMP levels are relatively low due to the activity of nucleotide phosphodiesterase. The addition of caffeine can suppress the activity of nucleotide phosphodiesterase that eventually increases cAMP levels (Hasbi et al, 2011). Increased nucleotide phosphodiesterase will increase motility, thus further improving sperm metabolism when in certain circumstances it is possible to cause damage or abnormal spermatozoa.

Materials and Methods

Cryopreservation sperm

Collection of spermatozoa from cauda epididymis by making an incision in the cauda epididymis then put in a test tube medium Tris until 3 minutes, and then continued the process of cryopreservation. Add egg yolks 20% in medium tris, ekulibrasi at a temperature of 4 degrees celsius for 2 hours and then to add to the straw-sized 0,25ml. Take place it in a styrofoam plate in liquid nitrogen vapor for 20 minutes and then put in a storage container for the liquid nitrogen.

Morphological examination of spermatozoa

The frozen semen was thawed in a water bath at 38°C for 30 seconds, centrifuged at 1800 rpm for 5 second before being used in each experiment. Sperm head morphology was studied in thin smears prepared, fixation 2 h in acetic acid: methanol = 1:3) and stained with Acredine Orange (AO). Two hundred spermatozoa were counted in each smear at a magnification of 1200x in a light microscope laser-scanning

confocal microscope (BioRad MRC-1024). Two hundred spermatozoa were counted in each preparation and the abnormalities (acrosomes, nuclear pouches, proximal and distal cytoplasmic droplets, midpiece and abnormal tails) were classified according to a system developed by Bane (1961). The number of spermatozoa showing each class of abnormality was expressed as a percentage of the counted spermatozoa.

Statistical analysis

The differences between concentrations were compared and results were expressed as mean $\pm$ Sd. Analysis of variance (ANOVA) using the SPSS software version 18 with Tukey test was performed to verify statistical significance. The p-values of <0.05 were considered as statistically significant.

Result and Discussion

Morphological evaluation of spermatozoa is very important because it indicates the spermatogenesis process running normally during sperm maturation in the epididymis that can be seen from the abnormality. Morphological parameters with low value would be detrimental because it can decrease the fertility of sperm after insemination.

Epididymis is a place for maturation of spermatozoa. Spermatozoa were left testes still have a morphology that is not perfect and sperm membrane integrity is still weak, so that possible damage to spermatozoa is possible higher than the ejaculation of sperm membrane integrity. Cryopreservation process on epididymal spermatozoa is also very possible spermatozoa is damaged or abnormality, due to changes in temperature (cold shock) when the cryopreservation. Table 1 will be obvious epididymal spermatozoa damage as a result of incomplete morphology of spermatozoa leave the testes and cryopreservation process.

Abnormality	Level of Caffeine (mg/ml in %)			
	0	2	4	6
Normal	45.30 (1.97 $\pm$ 0.42) ^a	44.32 (1.70 $\pm$ 0.77) ^{ab}	43.73 (1.37 $\pm$ 0.63) ^b	43.20 (1.44 $\pm$ 0.68) ^b
Pear Shape	1.68 (0.21 $\pm$ 0.00) ^a	1.41 (0.47 $\pm$ 0.21) ^a	1.09 (0.55 $\pm$ 0.26) ^a	2.68 (0.45 $\pm$ 0.36) ^a
Narrow	2.74 (0.74 $\pm$ 0.20) ^a	2.11 (0.53 $\pm$ 0.36) ^a	1.8 (0.36 $\pm$ 0.00) ^a	0.90 (0.30 $\pm$ 0.00) ^a
Narrow at the base	1.47 (0.25 $\pm$ 0.09) ^a	0.7 (0.35 $\pm$ 0.00) ^a	1.44 (0.36 $\pm$ 0.00) ^a	0.90 (0.30 $\pm$ 0.00) ^a
Abnormal contour	0.42 (0.21 $\pm$ 0.00)	-	-	0.60 (0.30 $\pm$ 0.00)
Round underdeveloped	0.84 (0.21 $\pm$ 0.00)	-	1.08 (0.36 $\pm$ 0.00)	2.08 (0.42 $\pm$ 0.16)
Underdeveloped	7.63 (0.51 $\pm$ 0.28) ^a	9.17 (0.57 $\pm$ 0.34) ^{ab}	11.22 (0.51 $\pm$ 0.27) ^{ab}	10.37 (0.69 $\pm$ 0.38) ^b
Macrocephalic	1.05 (0.35 $\pm$ 0.12) ^a	2.45 (0.35 $\pm$ 0.00) ^a	3.96 (0.36 $\pm$ 0.00) ^a	1.79 (0.36 $\pm$ 0.13) ^a
Microcephalic	4.63 (0.31 $\pm$ 0.14) ^a	5.29 (0.59 $\pm$ 0.47) ^a	5.08 (0.64 $\pm$ 0.43) ^a	3.84 (0.64 $\pm$ 0.29) ^a
Decapitated Head	-	-	-	1.49 (0.37 $\pm$ 0.15)
Diadem	-	2.11 (0.42 $\pm$ 0.16)	1.44 (0.36 $\pm$ 0.00)	-
Simple bend tail	24.81 (1.18 $\pm$ 0.61) ^a	27.29 (1.24 $\pm$ 0.51) ^a	26.93 (0.93 $\pm$ 0.57) ^a	29.62 (1.02 $\pm$ 0.53) ^a
Double tail	1.48 (0.30 $\pm$ 0.19)	-	-	-
Coiled tail	1.27 (0.42 $\pm$ 0.22)	-	-	-
Droplet Cytoplasmic distal	1.90 (0.38 $\pm$ 0.18)	2.46 (0.41 $\pm$ 0.15)	-	-
Head sperm	2.73 (0.23 $\pm$ 0.06) ^a	1.41 (0.47 $\pm$ 0.21) ^b	0.72 (0.36 $\pm$ 0.00) ^{ab}	0.90 (0.30 $\pm$ 0.00) ^{ab}
Tail sperm	2.10 (0.26 $\pm$ 0.10) ^a	1.06 (0.53 $\pm$ 0.25) ^a	1.08 (0.36 $\pm$ 0.00) ^a	1.49 (0.37 $\pm$ 0.15) ^a
Total abnormality	54.70 (0.48 $\pm$ 0.47) ^a	55.68 (0.69 $\pm$ 0.49) ^b	56.27 (0.60 $\pm$ 0.43) ^{ab}	56.80 (0.67 $\pm$ 0.47) ^b

Different superscripts in the same row means significantly different ($P < 0.05$)

The results showed that a large degree of abnormality in the simple bend tail (24.81%, 27.29%, 26.93%, and 29.62%) respectively and underdeveloped (7.63%, 9.17%, 11.22%, and 10.37%), respectively with the 0, 2, 4, and 6 mg% caffeine. Abnormality in the tail is due to the post-collection process, whereas underdeveloped abnormalities caused by genetic factors, or during the process of spermatogenesis. Post-collection handling and sperm freezing process causes many spermatozoa abnormalities in the tail. Epididymis spermatozoa have membrane stability are more susceptible to cold shock and osmotic pressure (Hewitt et al. 2001). Underdeveloped abnormality or often called teratoid occurs because of degeneration of primordial cell in the seminiferous tubules (Barth and Oko 1989).

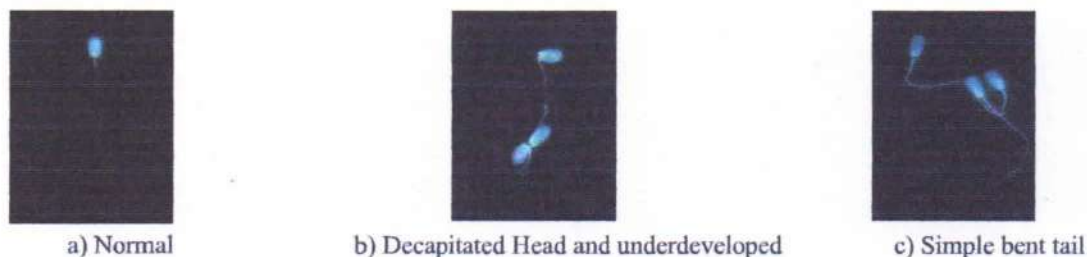


Figure 1. Morphology of epididymis spermatozoa

Total abnormalities of epididymis spermatozoa after the addition of caffeine increased from 54.70% in control to 55.68%, 56.27%, and 56.80%, in epididymis spermatozoa supplemented with 2, 4, and 6% caffeine, respectively. Increased abnormality shows the effect of caffeine can improve epididymis spermatozoa abnormalities. The abnormality occurs because caffeine can stimulate sperm intracellular cATP production thus increasing phosphorylase activity. Increased activity of glycogen phosphorylase will increase the production of glucose from glycogen, so the metabolism and motility of spermatozoa will increase (Soedarsono *et al.*, 1995 and Lieberman, 1988 in Soeparna *et al.*, 2010). Increased metabolism will produce byproducts in the form of lactic acid. Lactic acid will result in excessive toxic to spermatozoa (Richie, 1987 in Schunack *et al.*, 1990).

Conclusion

Addition more than 4 mg caffeine increase spermatozoa abnormalities.

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