Comparison of Two Different Method for Sperm Concentration Measurement of Ram and Buck Semen

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ABSTRACT

Sperm concentration is the number of sperm cells contained in one milliliter semen. Careful evaluation and validation of sperm concentration is very important in artificial insemination technology, because an accurate estimation of concentration will determine extension rates that optimize farm animal's utilization and fertility of extended semen. Ram and buck had a high sperm concentration and low in sperm volume. The concentration of the sperm can be calculated in various ways, from simple technique to see the distance between the head and to use computerize assisted sperm analysis. This research aimed to compare the concentration of buck and ram sperm using a counting chamber and photometer SDM6. Ten animals consist of 5 sexually mature rams and 5 bucks used as sperm source. Semen was collected using artificial vagina. Each individual semen were evaluated for sperm concentration using counting chamber (Neubauer improvement) and photometer SDM6 (Minitub, Germany). Result demonstrated that sperm concentration of buck and ram semen was not differ between photometer SDM6 and counting chamber ($p<0.05$), the sperm concentration of ram and buck semen were 4864.17 ± 2044.9210^6 and $5432\pm1350.44\ 10^6$ per mL respectively. In conclusion photometer SDM6 can be used as a new method for sperm concentration assessment with a faster time and accurate as counting chamber

Key Words: Counting chamber, Photometer SDM6, Ram and buck, Sperm concentration

INTRODUCTION

Sperm concentration is the number of sperm cells contained in one milliliter semen.The success of an artificial insemination (AI) program depends to a large degree on the accurate determination of sperm concentration. Careful evaluation and validation of sperm concentration is very important in artificial insemination technology, because an accurate estimation of concentration will determine extension rates that optimize farm animal's utilization and fertility of extended semen (Knox et al., 2002; Kirkman-Brown et al., 2009). The concentration of the sperm can be calculated in various ways, from simple technique to see the distance between the head and to use computerize assisted sperm analysis.

Photometric devices are most common methods of estimating sperm concentration. In these devices, a beam of light is passed through the sample and the amount of light transmitted is measured by phototube, which is then inversely correlated with sperm concentration in the sample. Photometers is the instrument itself converts it to sperm/ml (Atiq et al., 2011). Among other methods of sperm concentration measurement, improved Neubauer hemocytometer chamber is the standard for sperm counting (Lu et al., 2007).

Photometer SDM5 and SDM6 (Minitüb GmHb) is used in all Artificial Insemination Centre, at Indonesia. This device used for the evaluation of bovine and ram or buck spermatozoa concentration. Ram and buck had a high number of spermatozoa concentration and low in sperm volume (Garner and Hafez, 2000). This study was designed to validate the photometer measurements of spermatozoa concentration with the help of manual methods namely,

improved Neubauer hemocytometer chamber. By comparing the measurements of two instruments with same semen samples, the reliability of photometer measurements was assessed.

MATERIALS AND METHODS

Ten animals consist of 5 sexually mature rams and 5 bucks maintained as regular at Division of Reproduction and Obstetric, Department of Veterinary Clinic, Reproduction and Pathology Faculty of Veterinary Medicine, Bogor, Indonesiawere used as semen source. Semen was collected using artificial vagina. After recording the semen physical characteristics such as volume and motility, the semen was evaluated for spermatozoa concentration. Semen samples with watery appearance were discarded because photometer was programmed to read sperm concentration of more than $40x10^6$ sperms/ml.

Two different sets of procedures i.e. methods using photometer (SDM6, Minitüb Gm Hb), and improved Neubauer hemocytometer were used for spermatozoa concentration measurement. Each individual semen were evaluated for sperm concentration using counting chamber (Neubauer improvement) by diluted the semen 1:500 ratio with formal-saline on test tube and gently mixwell the solution. After mixing, one drop of solution were placed in a clean and dry hemacytometer covered by cover slip and count the sperm dispersed in the middle square and the four corner squares of the 25 squares in the grid. Number of spermatozoa was counted in 100 squares with the help of manual counter. The number of spermatozoa from 5 square were multiplied by 25 x 10^6 (Arifiantini, 2012).

Dilution factors for photometer SDM6 (Minitub, Germany) conducted according to the manual operation procedure.Photometerically, spermatozoa concentration was determined at 546 nm wave length with the help of a prewarmed and calibrated photometer.

To exclude person to person variation, allthe samples were analyzed by one person.Data were statistically analysed using single factor analysis of variance, to compare the sperm concentration between two devices using statistical T analysis (SPSS 17 program).

RESULTS AND DISCUSSION

There were an individual variation among rams and bucks spermatozoa concentration. The spermatozoa concentration in buck semen were 3612±179.36to 7981±649.03$x10^6$/mL (Table 1). The higest spermatozoa concentration demonstrated by buck number 1, with the spermatozoa concentration 7602.67±91.82 and 7981±649.03 $x10^6$/mLand lowest was demonstrated by buck number 4 with only 3.612±179.36 and 4364.33±573.93 $x10^6$/mL measured by photometer SDM6 and counting chamber respectively.

The higest spermatozoa concentration in ram semen demonstrated by ram number 2 and 4, with the spermatozoa concentration were 6217.33±616.68 and 6630.00±300.51 $x10^6$/mL, the lowest concentration was demonstrated by ram number 1 and 5 with only 2174.67±46.49 and 2284.67±84.01$x10^6$/mL (Table 2) measured by counting chamber.

Table 1. Average of individual spermatozoa concentration (10^6/mL) in buck measured by two methods

Bucks	Photometer SDM6	Counting Chamber
1	7602,67±91,82[a]	7.981,00±649,03[a]
2	4588,33±199,25[c]	5.268,67±567,60[b]
3	4621,00±315,25[c]	5.056,00±451,68[b]
4	3612,00±179,36[d]	4.364,33±573,93[c]
5	4972,00±155,35[c]	6.254,00±341,95[bc]

Values within each column with different superscripts differ significantly at $p<0.05$

Table 2. Average of individual spermatozoa concentration (10^6/mL) in ram measured by two methods

Rams	Photometer SDM6	Counting Chamber
1	2284.67±84.01[d]	4305.33±680.28[b]
2	6630.00±300.51[a]	7368.33±1490.59[a]
3	4341.00±50.69[c]	4733.33±454.28[c]
4	6217.33±616.68[a]	7870.67±2264.47[a]
5	2174.67±46.49[d]	2716.33±103.18[d]

Values within each column with different superscripts differ significantly at $p<0.05$

Non significant difference in the sperm concentration determined by the two methods tested was detected (Table 3).

Table 3. Average spermatozoa concentration (10^6/mL) in Ram and buck measured by two methods

Method	Ram	Buck
Photometer SDM 6	4330±1964	5079±1398
Neubauer chamber	5399±2276	5785±1372
Average (±SD)	4864.17±2044.92	5432±1350.44

The spermatozoa concentration in buck was higher than reported by El-Kon et al., (2010) in Damascus buck the spermatozoa concentration was only 2718±32.60x10^6 cells/mL and Arifiantiniet al.,(2014) in Etawah grade with the spermatozoa concentration only 2020.00±107.24 and 2375.00±61x10^6 cells/mL. These results are consistent with those reported by Wildeus (2000) that buck spermatozoa ranging from 1500 to 5000 x10^6cells /mL.

Ram spermatozoa concentration in this research was consistent with Herdis et al., (2005) in Garut ram and Guedes dan Soto-Blanco, (2010)in Santa Inêsram with the average of spermatozoa concentration were 4.368±303 x$10^6$and 4.110±0.53x10^6cells /mL.

Improved Neubauer hemocytometer is a "gold standard" technique for spermatozoa concentration measurement in the world (World Health Organization 1999; Lu et al., 2007). The measurment of spermatozoa by Neubauer hemocytometer chamber was time consuming and cannot be used in routine evaluation of semen samples in an AI laboratory. Average time to prepare and evaluate semen samples by hemocytometer took longer (8-10 min) as compared to the optical density method (2 min). Improved Neubauer hemocytometer require a skillful and experienced laboratory technician because human error cannot be excluded from these methods.

CONCLUSION

It can be concluded from the present study that the use of photometer in semen evaluation for sperm concentration reduces chances of human error and time consumption effectively

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Cataloguing-in-Publication Data

The 16th Asian-Australasian Associations of Animal Production Socities

Proceedings Full Papers

Sustainable Livestock Production in the Perspective of

Food Security, Policy, Genetic Resources, and Climate Change

10-14 November 2014, Yogyakarta, Indonesia / editors Subandriyo *et al*;

2825 p: ill.; 21 x 29,7 cm

Organized by Indonesian Society of Animal Sciences

In Collaboration with Ministry of Agriculture

Faculty of Animal Sciences Universitas Gadjah Mada

ISBN 978-602-8475-87-7

1. Livestock 2. Food Security 3. Policy

4. Genetic Resources 5. Climate Change

I. Title II. Subandriyo

AAAP

Asian-Australasian Association of Animal Production Societies

✧ **Scope of AAAP**: AAAP is established to devote for the efficient animal production in the Asian-Australasian region through national, regional, international cooperation and academic conferences.

✧ **Brief History of AAAP**: AAAP was founded in 1980 with 8 charter members representing 8 countries-those are Australia, Indonesia, Japan, Korea, Malaysia, New Zealand, Philippines and Thailand. Then, the society representing Taiwan joined AAAP in 1982 followed by Bangladesh in 1987, Papua New Guinea in 1990, India and Vietnam in 1992, Mongolia, Nepal and Pakistan in 1994, Iran in 2002, Sri Lanka and China in 2006 , thereafter currently 19 members.

✧ **Major Activities of AAAP**: Biennial AAAP Animal Science Congress, Publications of the Asian-Australasian Journal of Animal Sciences and proceedings of the AAAP congress and symposia and Acknowledgement awards for the contribution of AAAP scientists.

✧ **Organization of AAAP**:

· President: Recommended by the national society hosting the next biennial AAAP Animal Science Congress and approved by Council meeting and serve 2 years.

· Two Vice Presidents: One represents the present host society and the other represents next host society of the very next AAAP Animal Science Congress.

· Secretary General: All managerial works for AAAP with 6 years term by approval by the council

· Council Members: AAAP president, vice presidents, secretary general and each presidents or representative of each member society are members of the council. The council decides congress venue and many important agenda of AAAP

✧ **Office of AAAP:** Decided by the council to have the permanent office of AAAP in Korea. Currently # 909 Korea Sci &Tech Center Seoul 135-703, Korea

✧ **Official Journal of AAAP**: Asian-Australasian Journal of Animal Sciences (Asian-Aust. J. Anim. Sci. ISSN 1011-2367. http://www.ajas.info) is published monthly with its main office in Korea

✧ **Current 19 Member Societies of AAAP:**

ASAP(Australia)**, BAHA**(Bangladesh), **CAASVM**(China), **IAAP**(India), **ISAS**(Indonesia), **IAAS**(Iran), **JSAS**(Japan), **KSAST**(Korea), **MSAP**(Malaysia), **MLSBA**(Mongolia), **NASA**(Nepal), **NZSAP**(New Zealand), **PAHA**(Pakistan), **PNGSA**(Papua New Guinea), **PSAS**(Philippines), **SLAAP**(Sri Lanka), **CSAS**(Taiwan), **AHAT**(Thailand), **AHAV**(Vietnam).

✧ **Previous Venues of AAAP Animal Science Congress and AAAP Presidents**

I	1980	Malaysia	S. Jalaludin	II	1982	Philippines	V. G. Arganosa
III	1985	Korea	In Kyu Han	IV	1987	New Zealand	A. R. Sykes
V	1990	Taiwan	T. P. Yeh	VI	1992	Thailand	C. Chantalakhana
VII	1994	Indonesia	E. Soetirto	VIII	1996	Japan	T. Morichi
IX	2000	Australia	J. Ternouth	X	2002	India	P. N. Bhat
XI	2004	Malaysia	Z. A. Jelan	XII	2006	Korea	I. K. Paik
XIII	2008	Vietnam	N.V. Thien	XIV	2010	Taiwan	L.C. Hsia
XV	2012	Thailand	C.Kittayachaweng	XVI	2014	Indonesia	Yudi.Guntara.Noor

Remark from Chairman of the 16th AAAP Congress

Dear all of the scientists, delegates, participants, ladies and gentlemen,

As the host of the 16th AAAP Animal Science Congress, we do impress, thankful, and present a high appreciation for your participation in joining the 16th AAAP Conference in Yogyakarta, Indonesia. We can see the very great enthusiasm of all the scientists to solve livestock problems as well as to share valuable information and knowledge for human prosperity all over the world.

A large numbers of representatives are participating in this conference, which indicates that the interest in the field of animal science is continuously increasing among member countries. We have invited some Plenary Speakers and Invited Papers who are qualified as scientists and bureaucrats in animal science field to share their valuable information and knowledge. Other participants can deliver their precious research through oral and poster presentations. This congress is also paralleled to symposium held by livestock organization and institution as well as some academic meetings.

The theme of the 16th AAAP Congress is "Sustainable Livestock Production in the perspective of Food security, Policy, Genetic Resources and Climate Change". We believe that animal production in Asia and Australasia has become important and strategic sector to provide high quality food, opening up job opportunities, as well as improving farmer's welfare. Animal science socities, therefore, have to support this growing interest by providing more appropriate and relevant technologies to improve efficiency of resources utilization to produce more animal protein food by member countries. Long term sustainable livestock production will, therefore, be significantly influenced by the national food policy, climate change issues, as well as conserved environments and genetic resources.

On behalf of 16th AAAP Committee and all associates, we wish all of the participants having a great achievement of success and fulfill the expectation as well as enjoying the interaction with all scientists participating the Congress.

High appreciation we may acknowledge to all of sectors, especially for His Majesty of Royal Palace of Yogyakarta, Sri Sultan Hamengku Buwono X, and Rector of Universitas Gadjah Mada, who have concerned to facilitate the Congress site host. Special thank to the Steering Committee, Scientific Committee, Reviewers and Editorial Boards for their great contribution to make the Congress successfully organized.

To you, your excellencies, invited guests and delegates, thank you for choosing to come to this conference and to Indonesia. We hope the arrangements we have put in place meet with your requirements. We wish you fruitful deliberations and an intellectually and socially rewarding stay in Yogyakarta.

We are looking forward to meeting you all in the future congress to continue.

Terimakasih (Thank you)

Budi Guntoro

Chairman of the 16th AAAP Congress

16th AAAP PRESIDENT'S REPORT

Selamat pagi!

Dear Ladies and Gentleman

Attendants of 16 AAAP congress:

It is my great pleasure and honor to welcome all of you at The 16th AAAP Congress on November 10 – 14, 2014 at Grha Sabha Pramana, Universitas Gadjah Mada, Yogyakarta Indonesia. This Congress is jointly organized by The Indonesian Society of Animal Science (ISAS), Indonesian Agency for Agricultural Research and Development, Indonesian Directorate General of Livestock and Animal Health Services-Ministry of Agriculture and Faculty of Animal Science Universitas Gadjah Mada. Universitas Gadjah Mada Campus is located in Yogyakarta, one of the Special Region in Indonesia where culture and tradition live in harmony with the modern nuance and educational spirit makes it a beautiful venue of this Congress.

The 16th AAAP Program consists of scientific and technical programs as well as social and cultural activities. The scientific and technical programs offer five plenary sessions, two satellite symposia, field trip, and many scientific sessions, both oral and poster presentations.

During this event distinguished scientists from all over the world will present plenary papers ranging from livestock policy, food security, local genetic resources, climate change, animal welfare, international trade, as well as global research agenda. I believe that around 1,200 scientists as well as livestock producers, companies, graduate and postgraduate students from 40 countries are attending the Congress and more than 770 research papers will be presented. The Congress also provides not only opportunities to discuss and exchange information and experience with scientists from different regions of the world, but also a good environment to build up friendship between nations is our ultimate goals for the Congress outcome. Moreover, this congress also keeps its tradition to be a forum of communication among researchers, academician, industries and related stakeholders among Asian-Australasian countries.

The social and cultural programs are specially desgined to be very important for the congress participants since the promotion of friendship and future scientific cooperation are also central to this AAAP Congress. The Opening Ceremony will offer you the Congress Program at a glance. In addition, participants will also join at a warm Welcome Dinner gathering at Keraton Yogyakarta. Sri Sultan Hamengku Buwono X, His Majesty of The Royal Palace of Yogyakarta will give you the most memorable moment during this event.

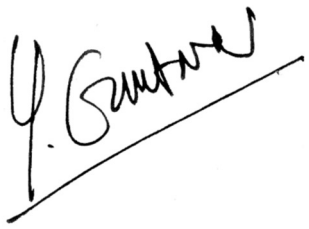
Moreover, cultural night offers us an opportunity to introduce significant culture from participants' countries and gives a spectacular performance to enjoy in order to strengthen our friendship and future cooperation. Field trip, on the other hand, provides a wonderful sightseeing to the most valuable ancient heritage around Yogyakarta, such as Borobudur and Prambanan Temples, and more other interesting places to visit. I do hope that you enjoy your stay in Yogyakarta and not miss all of these spectacular opportunities.

Closing Ceremony will be held on November 14, 2014 immediately after the last session of presentation. During this great moment we will welcome the next host of the 17th AAAP Congress to deliver a brief message. The AAAP Congress Award will provide and announce some participant who receive appreciation for their valuable research.

With all of our hospitability, we will try our best to make your brief visit to Yogyakarta and our beautiful country Indonesia, become a wonderful experience and memorable moments.

I wish you all a very pleasant and most enjoyable stay in Yogyakarta, Indonesia.

Terima kasih (Thank you).



Sincerely Yours
Mr. Yudi Guntara Noor
President
The 16th AAAP Congress

PREFACE

The proceedings of the 16th Congress of the Asian-Australasian Association of Animal Production Societies (AAAP) held on 10-14 November 2014 at Grha Sabha Pramana, Universitas Gadjah Mada, Yogyakarta, Indonesia, consist of two volumes. Those are Volume I of Plenary and Invited Papers and Volume II of Abstracts Contributed Papers. This is the second volume of the proceedings that contains a total of 754 abstracts, consist of 368 papers for oral presentation and 386 papers for poster. Papers were categorized into various disciplines, such as Nutrition and Feed Technology; Genetics and Reproduction; Physiology, Animal Welfare and Health Management; Product Technology and Food Safety; Waste and Environmental issues; Forage Agrostology; as well as Agribusiness, Marketing, Extension and Community Development. The scientific committee has initially received a total of 1,028 abstracts from 42 countries. After reviews have been made, 60 of them were rejected and 74 were cancelled by the authors. The reviewers consist of 4 international and 71 internal reviewers from 6 universities and 1 research institute in Indonesia. In the interest of time limitation for proceedings publication, we apologize for not including 140 submitted abstracts in the proceedings since they were not being followed up with full manuscripts until the extended due date we offered.

The scientific committee would like to thank all the reviewers and appreciate their effort to make significant contribution in reviewing the full manuscripts. Similarly, we would also like to thank supporting staffs at the secretariat office of the Faculty of Animal Science, Universitas Gadjah Mada as well as of the Indonesian Center for Animal Research and Development who have helped in the preparation of the proceedings. Finally, we would like to thank all the authors for their valuable contribution to the congress and make it useful for our societies.

Editorial Team

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