The Thai Journal of Veterinary Medicine

Volume 41
Issue 2 *July, 2011*

Article 10

7-1-2011

Seminal Plasma MDA Concentrations Correlating Negatively with Semen Quality in Asian elephants

Sitthawee Thongtipsiridech

Podjana Imrat

Thanida Srihawong

Sittidet Mahasawangkul

Chanin Tirawattanawanich

See next page for additional authors

Follow this and additional works at: https://digital.car.chula.ac.th/tjvm

 Part of the Veterinary Medicine Commons

Recommended Citation

Thongtipsiridech, Sitthawee; Imrat, Podjana; Srihawong, Thanida; Mahasawangkul, Sittidet; Tirawattanawanich, Chanin; and Saikhun, Kulnasan (2011) "Seminal Plasma MDA Concentrations Correlating Negatively with Semen Quality in Asian elephants," *The Thai Journal of Veterinary Medicine*: Vol. 41: Iss. 2, Article 10.
Available at: https://digital.car.chula.ac.th/tjvm/vol41/iss2/10

This Article is brought to you for free and open access by the Chulalongkorn Journal Online (CUJO) at Chula Digital Collections. It has been accepted for inclusion in The Thai Journal of Veterinary Medicine by an authorized editor of Chula Digital Collections. For more information, please contact ChulaDC@car.chula.ac.th.

Seminal Plasma MDA Concentrations Correlating Negatively with Semen Quality in Asian elephants

Authors

Sitthawee Thongtipsiridech, Podjana Imrat, Thanida Srihawong, Sittidet Mahasawangkul, Chanin Tirawattanawanich, and Kulnasan Saikhun

This article is available in The Thai Journal of Veterinary Medicine: https://digital.car.chula.ac.th/tjvm/vol41/iss2/10

Original Article

Seminal Plasma MDA Concentrations Correlating Negatively with Semen Quality in Asian elephants

Sitthawee Thongtipsiridech[1,4,5*] Podjana Imrat[2] Thanida Srihawong[4,5]
Sittidet Mahasawangkul[3] Chanin Tirawattanawanich[1] Kulnasan Saikhun[6]

Abstract

The aim of this study was to determine whether lipid peroxidation is correlated with semen quality in Asian elephant bulls. Malondialdehyde (MDA) in seminal plasma from ejaculates with varying percentages of progressive motility was measured using Thiobarbituric Acid method. Correlation between the MDA levels and percentages of progressive motility and normal morphology were performed. Results revealed that the MDA levels were significantly negative, which correlated (p<0.05) with the percentages of progressive motility and normal morphology (R=0.2131 and 0.1685, respectively). The results also showed that the MDA levels were significantly difference between each bull (p<0.01). Furthermore, when the ejaculates were grouped according to motility scores into two groups; low- (<40%) and high-percentage of progressive motility (>40%), a significant difference was detected between the MDA means (±SD) of the low- (20.7±11.4 nmol/ml) and high-percentages of progressive motility (14.4±7.8 nmol/ml) groups. The results obtained from this study suggested that MDA could be a potential parameter applicable for the assessment of elephant semen quality. It could as well be deduced from this study that oxidative stress might play a key role in low fertility due to poor semen quality in captive male elephants. This data provides beneficial information to better understanding of elephant reproduction in captivity.

Keywords: elephant, malondialdehyde, oxidative stress, semen

[1] *Faculty of Veterinary Medicine, Kasetsart University, Nakhonpathom, Thailand*
[2] *Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.*
[3] *National Elephant Institute, Forest Industry Organization, Lampang, Thailand*
[4] *Center for Agricultural Biotechnology, Kasetsart University, Nakhonpathom, Thailand.*
[5] *Center of excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok, Thailand*
[6] *Institue of Molecular Biosciences, Mahidol University, Nakhonpathom, Thailand*
***Corresponding author E-mail:** fvetnit@ku.ac.th, nthongtip@yahoo.com*

บทคัดย่อ

ความสัมพันธ์แบบแปรผกผันระหว่างระดับของ MDA ในน้ำเลี้ยงเซลล์อสุจิกับคุณภาพน้ำเชื้อช้างเอเชีย

สิทธวีร์ ทองทิพย์ศิริเดช [1,4,5] พจนา อิ่มรัตน์ [2] ฐานิดา สีหวงษ์ [4,5] สิทธิเดช มหาสาวังกุล [3] ชนินทร์ ติรวัฒนาวานิช [1] กุลณสรรค์ สายขุน [6]*

วัตถุประสงค์ของการทดลองนี้คือเพื่อประเมินความสัมพันธ์ของการเกิดภาวะ lipid peroxidation กับคุณภาพน้ำเชื้อของช้างเอเชีย โดยทำการตรวจวัดปริมาณของมาลอนไดอัลดีไฮด์ หรือ MDA ในน้ำเลี้ยงเซลล์อสุจิที่แยกได้จากน้ำเชื้อของช้างที่มีร้อยละของการเคลื่อนที่ไปข้างหน้าของเซลล์อสุจิที่แตกต่างกันด้วยวิธี thiobarbituric acid method จากนั้นทำการวิเคราะห์ความสัมพันธ์ของระดับ MDA กับค่าคุณภาพน้ำเชื้อสองค่าคือ ร้อยละของการเคลื่อนที่ไปข้างหน้าของเซลล์อสุจิและร้อยละของเซลล์อสุจิที่มีรูปร่างปกติ ผลการวิเคราะห์พบว่าระดับของ MDA มีความสัมพันธ์แบบแปรผกผันกับค่าทั้งสองอย่างมีนัยสำคัญ (R=0.2131 และ 0.1685 ตามลำดับ) และพบว่าระดับของ MDA นั้นแตกต่างกันไปในช้างแต่ละเชือกอย่างมีนัยสำคัญ (p<0.01) นอกจากนี้เมื่อทำการแบ่งกลุ่มน้ำเชื้อออกเป็นกลุ่มที่มีร้อยละของการเคลื่อนที่ไปข้างหน้าของเซลล์อสุจิที่ต่ำ (<40%) กับกลุ่มที่มีร้อยละของการเคลื่อนที่ไปข้างหน้าของเซลล์อสุจิที่สูง (>40%) พบว่ากลุ่มที่มีร้อยละของการเคลื่อนที่ไปข้างหน้าของเซลล์อสุจิที่สูงมีระดับของ MDA ต่ำกว่าอย่างมีนัยสำคัญ ผลที่ได้รับแสดงให้เห็นว่า MDA สามารถใช้ในการช่วยตรวจสอบคุณภาพของน้ำเชื้อช้างที่มีคุณภาพต่ำที่มีสาเหตุมาจากการเกิด lipid peroxidation ได้ ข้อมูลที่ได้รับจากการศึกษานี้ทำให้เราเข้าใจสรีรวิทยาระบบสืบพันธ์ของช้างมากขึ้น

คำสำคัญ: ช้าง มาลอนไดอัลดีไฮด์ oxidative stress น้ำเชื้อ

[1] *คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์* [2] *คณะสัตวแพทยศาสตร์ มหาวิทยาลัยอูเทรค ประเทศเนเธอร์แลนด์*

[3] *ศูนย์อนุรักษ์ช้างไทย สถาบันคชบาลแห่งชาติ องค์การอุตสาหกรรรมป่าไม้ จ. ลำปาง*

[4] *ศูนย์เทคโนโลยีชีวภาพเกษตร มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จ. นครปฐม 73140*

[5] *ศูนย์ความเป็นเลิศด้านเทคโนโลยีชีวภาพเกษตร สำนักพัฒนาบัณฑิตศึกษาและวิจัยด้านวิทยาศาสตร์และเทคโนโลยี (สบว.) สำนักงานคณะกรรมการอุดมศึกษา* [6] *สถาบันชีววิทยาศาสตร์โมเลกุล มหาวิทยาลัยมหิดล นครปฐม*

***ผู้รับผิดชอบบทความ E-mail:** fvetnit@ku.ac.th, nthongtip@yahoo.com*

Introduction

The risk of extinction of Asian elephant is critical and has increasingly gained a social concern. Their populations in the wild are threatened by habitat loss and fragmentation, decreased food sources and poaching. Self-sustained genetic diversity in avoidance of the inbreeding opportunity has become limited. Although a reasonable number of elephants has been maintained in captivity, the breeding programs are currently poorly developed. The small number of bulls used in the breeding program raises the awareness of inbreeding risk in the captive population. To establish a countermeasure to such problem, assisted reproductive techniques, for example artificial insemination (AI), have been pursued to allow breeding between geographically distant animals. This is considered an indispensable element of a successful captive breeding program for the conservation of genetic diversity in this species (Schmitt et al., 2001). However, poor sperm motility is a common finding in domesticated Asian elephant bulls, which may restrict their suitability for AI and semen preservation. While the causes of poor sperm motility in Asian elephant remain un-clear, in other species lipid peroxidation has been proposed to play an important role in compromising sperm quality, primarily because the plasma membrane of mammalian sperm contains abundant polyunsaturated fatty acids susceptible to the attack by free radicals (Alvarez et al., 1987; Aitken et al., 1993; Sikka, 1996). On the other hand, sperm cells can be protected from oxidative damage by antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, ascorbate, urate, α-tocopherol, pyruvate, glutathione, taurine and hypotaurine, which may be present in the seminal plasma (Saleh and Agarwal, 2002). Malondialdehyde (MDA) is a stable lipid peroxidation product of which elevated

concentrations in the seminal plasma have been associated with reduced semen quality in other species such as human (Aitken et al., 1989; Das et al., 2009), dog (Cassani et al., 2005) and ram (Andreea and Stela, 2010). The relationships between Asian elephant semen quality and MDA, however, have not yet been reported. The aim of this study was to determine whether lipid peroxidation is likely to contribute to poor semen quality in Asian elephant bulls by measuring MDA in seminal plasma from ejaculates with varying percentages of progressive motility.

Materials and Methods

Chemicals: All chemicals in the present study were purchased from Sigma Chemical Company (Sigma, St. Louis, MO, USA) unless stated otherwise.

Animals: Six elephant bulls, age ranging from 19 to 46 years old and housed at the National Elephant Institute (NEI), Forest Industry Organization (FIO), Lampang, Thailand were recruited in this study. The elephants were fed with grass, banana and sugar cane and were allowed a free approach to water. The selection of the bulls used in this study was based on their previous history of semen quality. Bulls that gave more than eighty percent of collection success with the percentage of progressive motility of higher than 40% were selected.

Semen collection and evaluation: Semen samples were monthly collected from April- December, 2010, except in November, by manual collection using the protocol previously described by Schmitt and Hildebrandt (1998). Ejaculates were immediately analyzed for volume, sperm concentration, progressive motility, sperm viability and pH (Kidd et al., 2001). The sperm concentration was determined using a hemocytometer. The progressive motility was visually estimated under a phase-contrast microscopy by two independent investigators. Sperm morphology was examined under a phase contrast microscope. The sperm viability (live/dead ratio) was counted by using an eosin-nigrosin staining. Two hundred spermatozoa were counted per slide (BjoÃ rndahl et al., 2003). Due to the two day series of semen collection, the semen quality data from date that had a higher progressive motility were chosen in this study.

Seminal plasma and serum collection: Seminal plasma was collected from all ejaculates by centrifugation at 1,000 g for 10 min, then the supernatant was collected. The obtained seminal plasma samples were frozen in Dry Shipper (CX 100 Cryo Express Taylor-Wharton, HCI Cryogenics B.V., Postbus, AC, HEDEL) and transported to the laboratory, where they were stored at -20°C until analysis.

Seminal plasma MDA: MDA in the seminal plasma or seminal MDA was determined by Thiobarbituric Acid (TBA) assay described by Yagi (1984). The reaction of MDA with TBA has been used to estimate the lipid peroxidation in biological material. The reaction reveals a red MDA-TBA adduct, the product of 2 mol of TBA plus 1 mol of MDA. The colored complex can be evaluated spectrophotometrically (Spectronic Hελios γ, Thermo Electron Corporation, Cambridge, GB) from its visible absorbance at 532 nm. The values of MDA reactive material were shown as MDA quantities for plasma volume (nmol/ml).

Statistical analysis: Statistical analysis was performed by using SPSS 13.0 software (SPSS Inc, Chicago, IL, USA). The correlation between seminal MDA concentrations and percentage of progressive motility and normal morphology were analyzed by Linear Regression. The effect of bulls on MDA levels was tested between each bull by ANOVA. The comparison of mean±SD of seminal MDA between low- and high- percentage of progressive motility groups was done by *t*-test. Differences were considered significant at $p<0.05$.

Results

A total of forty eight attempts of semen collection were done in six bulls during eight months by which 40 ejaculates (90.91%) without urine contamination were obtained. The rest four attempts (9.01%) were un-ejaculates and ejaculates with urine contamination and, therefore, were excluded from this study. Mean±SD of semen characteristics are showed in Table 1. The average of percentages of progressive motility, sperm concentrations, volumes, pH, percentages of live sperm, percentages of normal morphology and seminal MDA levels were 33.4±27.1%, 1201.1±619.9x10^6 sperms/ml, 24.2±17.6 ml, 7.2±0.7, 52.6±29.7%, 81.4±15.9% and 18.0±10.3 nmol/ml, respectively. The correlation between seminal MDA level and percentage of progressive motility and normal morphology showed the significant negative correlation between MDA

Table 1 Means±SD of semen characteristics and seminal MDA levels of Asian elephant semen.

Semen parameters	Mean±SD (n=40)
Progressive motility (%)	33.4±27.1
Sperm concentration (x 10^6 sperms/ml)	1201.1±619.9
Volume (ml)	24.2±17.6
Semen pH	7.2±0.7
Live sperm (%)	52.6±29.7
Normal morphology (%)	81.4±15.9
Seminal MDA (nmol/ml)	18.0±10.3

Table 2 Correlation between seminal MDA level and percentage of progressive motility and normal morphology of Asian elephant semen.

Semen parameters	*R*-squared	Correlation	*P* value
Progressive motility (%) (n = 39)	0.2131	-0.4616	0.003
Normal morphology (%) (n = 29)	0.1685	-0.4105	0.027

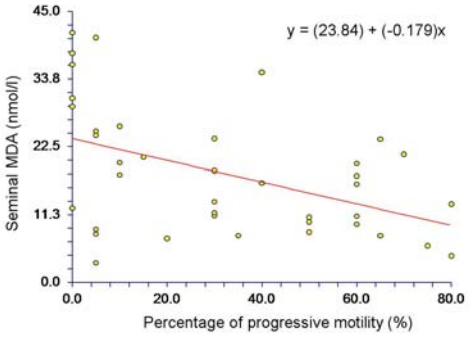

Figure 1 Correlation between seminal MDA and percentage of progressive motility of Asian elephant semen.

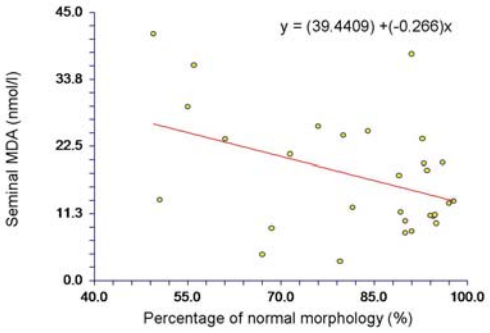

Figure 2 Correlation between seminal MDA level and percentage of normal morphology of Asian elephant semen.

concentrations and percentage of progressive motility and normal morphology (R=0.2131 and 0.1685, p<0.05) (Table 2, Fig 1 and 2). The result also showed the significantly difference of MDA levels between each bull (p<0.01). Mean±SD of MDA levels in the oldest elephant (46 years old) was not significantly higher than the youngest elephant (19 years) (Table 3). Furthermore, when the ejaculates were grouped according to motility scores into two groups, low- (<40%, n=23) and high-percentages of progressive motility (>40%, n=16), the mean±SD of MDA concentrations were significantly different between the groups having low- (20.7±11.4 nmol/ml) and high-percentages of progressive motility (14.4±7.8 nmol/ml) (Table 4).

Discussion

In this study, although we focused on the bulls anticipated to have a better semen qualities than others according to their previous history of semen quality, the same scenario including sub-fertility and male to male variability remains as compared to our previous reports, only slight improvement in some semen parameters was detected (Thongtip et al., 2008; Sivilaikul et al., 2010). Regarding the semen collection in the present study, 40 ejaculates (90.91%) without urine contamination were obtained and only 4 attempts (9.01%) were of un-ejaculates and ejaculates

Table 3 Comparison of means±SD of seminal MDA levels in each elephant.

Elephants	Age (Year)	Seminal MDA (Mean±SD)
EM 1 (N=6)	36	29.5±9.6[a]
EM 2 (N=6)	19	16.8±6.6[ab]
EM 3 (N=7)	46	13.3±4.3[ab]
EM 4 (N=7)	36	17.5±8.1[ab]
EM 5(N=6)	36	26.6±12.2[a]
EM 6 (N=8)	36	8.4±3.6[b]

Table 4 Comparison of mean±SD of seminal MDA levels in low- and high-percentages of progressive motility group.

Motility groups	MDA (nmol/l)
Low-percentage of progressive motility (progressive motility <40%) (n=23)	20.7±11.4[a]
High-percentage of progressive motility (progressive motility >40%) (n=16)	14.4±7.8[b]

Values with difference superscripts (a and b) differ significantly within column.

with urine contamination, which was a considerably good result. This suggests that the bull elephants in this study were highly responsive to our semen collection stimulation. The average of percentages of progressive motility as reported herein was higher than those reported before (Schmitt and Hildebrandt, 1998; Thongtip et al, 2008; Sivilaikul et al., 2010). The ages of the elephants in this study (19-46 years of age) might be the factor in the better semen quality. We had previously reported the age effect on elephant semen quality that better semen quality were indicated in elephant bulls of 23-43 years of age (Thongtip et al., 2008). However, in this study, mean±SD of MDA levels in the oldest elephant (46 years old) was not significantly higher than the youngest elephant (19 years). It might be indicated that seminal MDA in elephant is not mainly related to elephant age. A significant negative correlation between seminal MDA concentrations and the percentages of progressive motility and normal morphology were revealed in this study. Furthermore, the seminal MDA comparison between the two groups, low- (<40%) and high-percentages of progressive motility (>40%), was significantly different. MDA, the end product of lipid peroxidation, may not be involved directly in the poor semen quality found in Asian elephants. Due to the lack of epididymis, elephant semen are stored at the ampulla gland. During the storage in ampulla gland, elephant sperm might be effected by free radicals which interact with polyunsaturated fatty acids in the phospholipids of the sperm plasma membrane. From our previous results, the long-term storage semen usually have a brownish colour. It also usually have a low pH and high proportion of dead sperm (personal datas). It has been reported that, in boar semen, chilled semen preservation induces lipid peroxidation, diminishes mitochondrial activity and increases plasma membrane permeability (Kumaresan et al., 2009). Furthermore, Reactive Oxygen Species (ROS) generated by spermatozoa has been indicated

as one of the mechanism that impaired sperm motility and plasma membrane integrity via lipid peroxidation (White, 1993; Cummings et al., 1994; Chatterjee and Gagnon, 2001). High level of MDA has been found in poor semen quality in some species such as human (Aitken et al., 1989; Nouri et al., 2008; Das et al., 2009) and ram (Andreea and Stela, 2010). MDA concentration in spermatozoa has been revealed to be significantly related to the number of immotile spermatozoa. The increase in incubation period in a condition without seminal plasma resulted in the decrease in the sperm motility meanwhile increased MDA concentration (Kobayashi et al, 1991).

The negative correlation between MDA concentrations and the percentages of progressive motility and normal sperm morphology in Asian elephant semen could help us to indicate the effect of oxidative stress on elephant semen quality. However, other parameters such as membrane integrity and mitochondrial activity which might also be affected by oxidative stress have not yet been tested and remain to be scrutinized. Other functions of the antioxidant system such as total antioxidant capacity (TAC), superoxide dismutase activity and glutathione system should be pursued. The data obtained in this study are useful for our better understanding about male elephant reproduction. Furthermore, seminal MDA may be an alternative parameter to evaluate Asian elephant semen quality before preservating as chilled or frozen semen.

Acknowledgement

The authors are grateful for the National Elephant Institute, Forest Industry Organization for their permission of sample collection and the Faculty of Veterinary Medicine at Kasetsart University for providing facilities. We thank Mr. Kornchai Kornkaewrat and Ms. Piyawan Suthanmapinanth for their technical asssistance. This research is supported by the Thailand Research Fund, the Center of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office and the Commission on Higher Education, Ministry of Education. (AG-BIO/PERDO-CHE).

References

Aitken, R.J., Clarkson, J.S. and Fishel, S. 1989. Generation of reactive oxygen species, lipid peroxidation and human sperm function. Biol Reprod. 40: 183-187.

Aitken, R.J., Harkiss, D. and Buckingham, D. 1993. Analysis of lipid peroxidation mechanisms in human spermatozoa. Mol Reprod Dev. 35: 302-315.

Alvarez, J.G., Touchtone, J.C., Blasco, L. and Storey, B.T. 1987. Spontaneous lipid peroxidation and production of superoxide and hydrogen peroxide in human spermatozoa: superoxide dismutase as major protectant against oxygen toxicity. J Androl. 8: 338–348.

Andreea, A. and Stela, Z. 2010. Role of antioxidant additives in the protection of the cryopreserved semen against free radicals. Roman Biotechnol Letters. 15(3) (Supplement): 33-41.

BjoÃ rndahl, L., SoÃ derlund, I. and Kvist, U. 2003. Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. Hum Reprod. 18: 813-816.

Cassani, P., Beconi, M.T. and O'Flaherty, C. 2005. Relationship between total superoxide dismutase activity with lipid peroxidation, dynamics and morphological parameters in canine semen. Anim Reprod Sci. 86: 163-173.

Chatterjee, S. and Gagnon, C. 2001. Production of reactive oxygen species by spermatozoa undergoing cooling, freezing and thawing. Mol Reprod Dev. 59: 451-458.

Cummings, J.M., Jequier, A.M. and Kan, R. 1994. Molecular biology of the human male infertility: links with ageing, mitochondrial genetics and oxidative stress. Mol Reprod Dev. 37: 345–362.

Das, P., Choudhrari, A. R., Singh, A. K. and Singh, R. 2009. Correlation among routine semen semen parameters, sperm viability and malondialdehyde levels in human subjects with different fertility potential. Indian J Physiol Pharmacol. 53(3): 253-258

Kidd, S. A., Eskenazi, B. and Wyrobek, A. J. 2001. Effects of male age on semen quality and fertility: A review of the literature. Fertil Steril. 75: 237-248.

Kobayashi, T., Miyazaki, T., Natori, M. and Nozawa, S. 1991. Protective role of superoxide dismutase in human sperm motility: superoxide dismutase activity and lipid peroxide in human seminal plasma and spermatozoa. Hum Reprod. 6: 987-991.

Kumaresan, A., Kadirvel, G., Bujarbaruah, K.M., Bardoloi, R.K., Das, A., Kumar, S. and Naskar, S. 2009. Preservation of boar semen at 18 degrees C induces lipid peroxidation and apoptosis like changes in spermatozoa. Anim Reprod Sci. 110(1-2):162-171.

Nouri, M., Ghasemzadeh, A., Farzadi, L., Shahnazi, V. and Ghaffari Novin, M. 2008. Vitamins C, E and lipid peroxidation levels in sperm and seminal plasma of asthenoteratozoospermic and normozoospermic men. Iran J Reprod Med. 6: 1-5.

Saleh, R.A. and Agarwal, A. 2002. Oxidative stress and male infertility: From research bench to clinical practice. J Androl. 23: 737-752.

Schmitt, D.L., Hildebrant, T.B., Hermes, R.and Goritz, F. 2001. Assisted reproductive technology in elephants. Proc 1st Int Symp Assisted Reproductive Technology for Conservation Genetic Management of Wildlife, Omaha's Henry Doorly Zoo; 15-17.

Schmitt, D.L. and Hildebrandt, T.B. 1998.Manual collection and characterization of semen from Asian elephants (*Elephas maximus*). Anim Reprod Sci. 53: 309–314.

Sikka, S.C. 1996. Oxidative stress and role of antioxidants in normal and abnormal sperm function. Front Biosci. 1: 78-86.

Sivilaikul, S., Jitprom, A., Kularb, A., Kornkaewrut, K., Suthanmaphinuth, P., Mahasawangkul, S., Saikhun, K., Wajjwalku, W. and Thongtipsiridech, S. 2010. Relationship between seminal and serum calcium concentration with semen quality in the Asian elephant (*Elephas maximus*). Thai J Vet Med. 40(3): 251-255.

Thongtip, N., Saikhun, J., Mahasawangkul, S., Kornkaewrat, K., Pongsopavijitr, P., Songsasen, N. and Pinyopummin. A. 2008. Potential factors affecting semen quality in the Asian elephant (*Elephas maximus*). Reprod Biol Endocrinol. 6: 9.

White, I.G. 1993. Lipids and calcium uptake of sperm in relation to cold shock and preservation: A review. Reprod Fertil Dev. 5: 639-658.

Yagi, K. 1984. Assay for blood plasma or serum. Methods Enzymol. 105. 328-331.