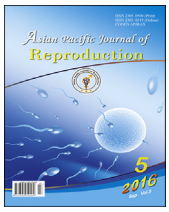
Contents lists available at ScienceDirect

Asian Pacific Journal of Reproduction

journal homepage: www.apjr.net



Review http://dx.doi.org/10.1016/j.apjr.2016.07.013

The important role of protamine in spermatogenesis and quality of sperm: A mini review

Muslim Akmal[1*], Aulanni'am Aulanni'am[2], M. Aris Widodo[3], Sutiman B. Sumitro[4], Basuki B. Purnomo[3], Widodo[4*]

[1]*Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh, Aceh, Indonesia*

[2]*Faculty of Veterinary Medicine, Brawijaya University, Malang, East Java, Indonesia*

[3]*Medicine Faculty, Brawijaya University, Malang, East Java, Indonesia*

[4]*Biology Department, Faculty of Mathematics and Natural Science, Brawijaya University, Malang, East Java, Indonesia*

ARTICLE INFO

Article history:
Received 6 May 2016
Received in revised form 14 Jul 2016
Accepted 14 Jul 2016
Available online 6 Aug 2016

Keywords:
Sperm
Inhibin B
Protamine
Spermatogenesis
Male fertility

ABSTRACT

Objective: To prove that the decrease in the expression of protamine 2 affects the fertilization ability and the number of child production.
Methods: During the development stage of elongating spermatids, human spermatozoa chromatin undergoes a complex transition where histone is extensively replaced by protamine. Histone substitution by protamine 1 and 2 plays a significant role in condensing chromatin required to induce the quality of potent spermatozoa. The substitution also has a key role in protecting spermatozoa from effects of free radicals which can degrade the spermatozoa quality.
Results: The results revealed that protamine deficiency instigated a severe disruption of spermatogenesis affecting male infertility. In addition, the protamine expression disorder caused a decrease in number, motility, and morphology of spermatozoa.
Conclusion: Our results of a study in mice confirmed that inhibin B injection caused a reduction in the expression of protamine 2 in cauda epididymis. It has implications in the decrease of motility, concentration, and spermatozoa viability so that it affects the fertilization ability and the number of child production.

1. Introduction

Spermatogenesis in mammals is a complicated process involving the division and differentiation of spermatogonial stem cells into mature spermatozoa. The spermatogenesis process consists of several phases, namely the mitosis proliferation of spermatogonial stem cells to produce spermatocytes, the division of spermatocyte meiosis to produce haploid round-spermatids, and the spermiogenesis or final stage involving the early stages of round spermatids to be mature elongated-spermatids [1].

During spermiogenesis, haploid spermatids undergo a series of changes in the composition and compactness of chromatin [2]. Meanwhile, in the round spermatid, the bond between deoxynucleic acid (DNA)-histone will be replaced by transition proteins, whereas in elongated spermatids, the transition protein will be replaced by protamine. Alterations from histone to protamine instigate spermatozoa chromatin condensation [3]. The process begins with changes of histone triggered by transition protein 1 and 2 and eventually replaced by protamine [4]. Alterations in transition proteins caused by protamine occur in the stage of elongating spermatid, and in humans, where 85% histone is replaced by protamine [2]. The content of protamine is indispensable for the final phase maturation of spermatozoa nucleus [5].

Facts reveal that a number of DNA in humans' mature spermatozoa binds to protamine [6]. In mammals, substituting histone by protamine is crucial in condensing and solidifying DNA into the spermatozoa head during spermatogenesis [7]. Somatic histone alterations by protamine result in a very compact DNA. It triggers the DNA in order to be protected from free radicals, such as free water and other compounds which are dissolved in water and causing the DNA damage [8]. Besides, alterations in a number of histones by protamine 1 (P1) and protamine 2 (P2) play a significant role in facilitating the compactness of chromatin package required for normal function of spermatozoa [9].

*Corresponding authors. Muslim Akmal, Faculty of Veterinary Medicine, Syiah Kuala University, Indonesia.
E-mail: muslim_akmal70@yahoo.com
Widodo, Biology Department, Brawijaya University, Indonesia.
E-mail: widodo@ub.ac.id
Peer review under responsibility of Hainan Medical College.

The results showed that the increase in protamine expression leads to the deterioration in the spermatozoa quality and reduces the embryogenesis quality in couples which undergo in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) [10]; on the other hand, defects of protamine gene cause the disability of spermatozoa DNA and male infertility [7]. Torregrosa N *et al.* [11] found that the change of P1:P2 ratio within the spermatozoa leads to infertility. Moreover, [12] revealed the decrease in P2 expression in infertile male patients. It shows that P2 has a significant role in maintaining male's fertility.

2. Spermatozoa protamine

Protamine has a key role in spermatozoa chromatin condensation. Its deficiency causes negative effects on morphology and male fertility. It shows that protamine is required in the design and function of spermatozoa [13]. Protamine is a basic core protein contained in the head of spermatozoa [14] with a molecular weight about 5 kDa–8 kDa [15]. The content of protamine in the core of spermatozoa's head is vital to induce a compact spermatozoa chromatin condensation which is influential for male fertility [16].

In the core of spermatozoa's head, there are P1 and P2 [17]. P1 is synthesized as a mature protein, while P2 as a precursor [18], and they are located in the core of human's spermatozoa head [19]. P1 is found in spermatozoa of all mammals, while P2 spermatozoa is in mice, hamsters, stallions, some primates and humans [2]. Data presented that P1 and P2 are the abundant core proteins contained in the spermatozoa's head, and they function in packaging or protecting the male genome [20]. The results showed that the insufficiency of P1 or P2 causes infertility in mice, whereas P2 deficiency causes damage in spermatozoa's DNA and embryo mortality [21]. In general, these proteins are involved in various spermatogenesis mechanisms and sperm motility (Figure 1).

3. Role of protamine in spermatozoa's DNA abnormalities and disability

Fertilization involves a direct interaction amongst spermatozoa and oocytes, a merger of the cell membrane, and a union of male and female gamete genome [22]. The process can thoroughly take place when supported by the compact spermatozoa DNA integrity [23]. Spermatozoa's DNA integrity plays a significant role in delivering accurate genetic information [24].

Spermatozoa's DNA must be well protected, such as by somatic cell's chromatin in order to be resistant toward nucleases [25]. Normally, spermatozoa's chromatin is well structured, the structure is compact with DNA content, and the nucleoprotein is heterogeneous [26]. The compactness of spermatozoa chromatin is due to the bonding between DNA and proteins of core spermatozoa, particularly the protamine [27]. Various abnormal forms of spermatozoa's chromatin or DNA damage result in male infertility [24]. Damages in spermatozoa's DNA are supposed to play a role as an infertility biomarker [28]. A number of causes why spermatozoa DNA damages are the protamine deficiency, DNA fragmentation, and abnormal chromatin composition [29].

Protamine plays an important role in male's normal fertility. The deficiency in P1 and P2 causes subfertile or severe infertile condition [10]. The results indicated that DNA damage in human spermatozoa produces the disruption of reproductive outcomes [30]. Damages in germ cells' DNA can increase mutations which ultimately lead to birth defects, genetic diseases, and cancer [31]. Damages in spermatozoa's DNA are considered to be closely related to the occurrence of male infertility and abnormal spermatogenesis [29]. An abnormal expression of protamine results in pathology connected with the spermatogenesis disruption [9].

4. Correlation between inhibin B and protamine

Inhibin is a glycoprotein hormone secreted by testis' Sertoli cells [32]. Inhibin B is significantly secreted by the Sertoli cells of testes [33], and the most important form of inhibin in male [34]. Inhibin B secretion by Sertoli cells is stimulated by follicle stimulating hormone (FSH) [35], whereas inhibin B regulates the FSH secretion through negative feedback regulatory [36]. A feedback control system of FSH is significantly regulated by inhibin B [37].

The bond between FSH and its receptor (FSH-R) in Sertoli cells will induce the activation of five molecular pathways,

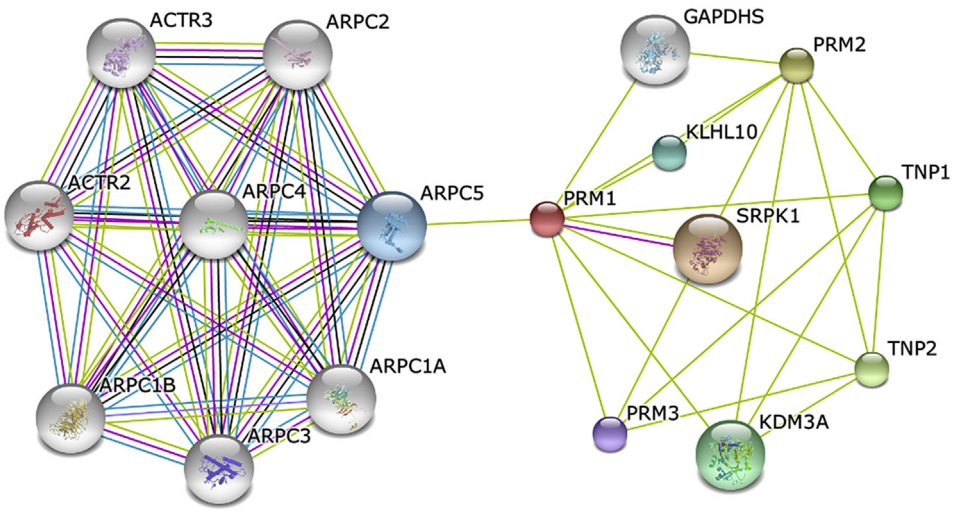


Figure 1. Protein network protamine (PRM1).
PRM1 protein has linkage to several proteins accumulated in two biological mechanisms: regulation of actin filament polymerization (left cluster) and spermatogenesis (right cluster). Data was retrieved from String-DB.

namely cyclic adenosine monophosphate-protein kinase A (cAMP-PKA) pathway, calcium pathway, mitogen-activated protein (MAP) kinase pathway, phospholipase A2 pathway, and the phosphatidylinositol 3-kinase pathway. Three of five pathways, namely cyclic adenosine monophosphate-protein kinase A (cAMP-PKA) pathway, calcium pathway, and mitogen-activated protein (MAP) kinase pathway will induce the activation of cAMP-response element binding protein (CREB) in the nucleus of Sertoli cells [38]. Moreover, the cAMP-PKA pathway also facilitated phosphorylation of cAMP-responsive element modulator (CREM) on the serine 117 [39].

CREM has a key role in spermatogenesis [40]. The results showed that it is crucial in the development of human's spermatids [41]. It is also required in regulating the gene expression in the haploid of spermatids [42]. Alterations in CREM expression interfere the spermatid maturation within a number of cases in idiopathic male infertility [41]. In addition, the results showed that there is a close link between the spermatid maturation disorder and the incidence of infertility in men [39,43]. The study also showed that mice with deficient CREM will affect the protamine expression, thus it causes infertility due to the disorder of round spermatid maturation [43].

Protamine, a major core protein of spermatozoa, also functions as DNA binder and compaction into the nucleus of spermatozoa head [44]. It is vital for the chromatin formation required in the normal function of spermatozoa [45]. An abnormal expression of protamine causes a decrease in the number of spermatozoa, in motility and morphology of spermatozoa, an increase of damage in spermatozoa chromatin [46], a decrease in the spermatozoa viability, in the damage of spermatozoa DNA [47] and male infertility [48]. The results showed that P2 is crucial in maintaining the integrity of human's spermatozoa chromatin [21] whereas the deficiency of P2 is thought to be a contributing factor in producing immotile spermatozoa [49].

Our results in rats (*Rattus novergicus*) showed that the injection of inhibin B incites a decrease in the concentration of FSH [50], which in turn has implications for the decreased expression of CREM in testis tissue [51]. The reduction of CREM triggers a decrease in P2 expression within the spermatozoa's head in the cauda epididymis. Thus it causes the decline in spermatozoa motility [52] in concentration and viability of spermatozoa [51]. Low motility of spermatozoa will reduce the spermatozoa ability to fertilize an egg cell [53,54]. Thus it decreases the number of children generated by *in vivo* fertilization [55].

We concluded that fertilization requires good quality spermatozoa. Disturbances in spermatogenesis result in the low-quality production of spermatozoa. Protamine is the molecule responsible for the spermatozoa quality. Inhibin B injection causes a decrease in motility, concentration, viability of spermatozoa, and the decrease in the number of children generated by *in vivo* fertilization. It is due to the reduction of CREM expression in testis tissue, which in turn interferes in the P2 expression within the network of cauda epididymis. It confirms the importance of protamine role particularly P2 in maintaining spermatogenesis and spermatozoa quality as well as indicates the possible development of inhibin B as the candidate of male's peptide-based hormonal contraception.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] He Z, Kokkinaki M, Pant D, Gallicano GI, Dym M. Small RNA molecules in the regulation of spermatogenesis. *Reproduction* 2009; **137**: 901-911.

[2] Steger K. Transcriptional and translational regulation of gene expressions in haploid spermatids. *Anat Embryol* 1999; **199**: 471-478.

[3] Steger K, Failing K, Klonisc T, Behre HM, Manning M, Weidner W, et al. Round spermatid from infertile men exhibit decreased protamine-1 and -2 mRNA. *Hum Reprod* 2001; **16**(4): 709-716.

[4] Ward WS, Kimura Y, Yanagimachi R. An intact sperm nuclear matrix may be necessary for the mouse paternal genome to participate in embryonic development. *Biol Reprod* 1991; **60**: 702-706.

[5] Loir M, Lanneau M. Structural function of the basic nuclear proteins in ram spermatids. *J Ultrastruct Res* 1984; **86**: 262-272.

[6] Conwell CC, Vilfan ID, Hud NV. Controlling the size of nanoscale toroidal DNA condensates with static curvature and ionic strength. *Proc Natl Acad Sci USA* 2003; **100**: 9296-9301.

[7] Siasi E, Aleyasin A, Mowla J, Sahebkashaf H. Association study of six SNPs in PRM1, PRM2 and TNP2 genes in iranian infertile men with idiopathic azoospermia. *Iran J Reprod Med* 2012; **10**(4): 329-336.

[8] Björndahl L, Kvist U. Human sperm chromatin stabilization: a proposed model including zinc bridges. *Mole Hum Reprod* 2010; **16**(1): 23-29.

[9] Carrell DT, Emery BR, Hammoud S. Altered protamine expression and diminished spermatogenesis: what is the link? *Hum Reprod Update* 2007; **13**(3): 313-327.

[10] Oliva R. Protamines and male infertility. *Hum Reprod Update* 2006; **12**(4): 417-435.

[11] Torregrosa N, Domínguez-Fandos D, Camejo MS, Shirley CR, Meistrich ML, Ballescà JL, et al. Protamine 2 precursors, protamine 1/protamine 2 ratio, DNA integrity and other sperm parameters in infertile patients. *Hum Reprod* 2006; **21**(8): 2084-2089.

[12] Aoki VW, Liu L, Carrell DT. Identification and evaluation of a novel sperm protamine abnormality in a population of infertile males. *Hum Reprod* 2005; **20**: 1298-1306.

[13] Lŭke L, Vicens A, Tourmente M, Roldan ERS. Evolution of protamine genes and changes in sperm head phenotype in rodents. *Biol Reprod* 2014; **90**(3): 67.

[14] McKay DJ, Renaux BS, Dixon GH. Human sperm protamines. Amino-acid sequences of two forms of protamine P2. *Eur J Biochem* 1986; **156**: 5-8.

[15] Rooney AP, Zhang J. Rapid evolution of a primate sperm protein, relaxation of functional constraint or positive Darwinian selection? *Mol Biol Evol* 1999; **16**: 706-710.

[16] Hammadeh ME, Hamad MF, Montenarh M, Fischer-Hammadeh C. Protamine contents and P1/P2 ratio in human spermatozoa from smokers and non-smokers. *Hum Reprod* 2010; **25**(11): 2708-2720.

[17] Corzett M, Mazrimas J, Balhorn R. Protamine 1: protamine 2 stoichiometry in the sperm of eutherian mammals. *Mol Reprod Dev* 2002; **61**: 519-527.

[18] Gusse M, Sautière P, Bélaïche D, Martinage A, Roux C, Dadoune JP, et al. Purification and characterization of nuclear basic proteins of human sperm. *Biochim Biophys Acta* 1986; **884**: 124-134.

[19] Aoki VW, Carrell DT. Human protamines and the developing spermatid: their structure, function, expression and relationship with male infertility. *Asian J Androl* 2003; **5**: 315-324.

[20] Lewis JD, Song Y, de Jong ME, Bagha SM, Ausio J. A walk though vertebrate and invertebrate protamines. *Chromosoma* 2003; **111**: 473-482.

[21] Cho C, Jung-Ha H, Willis WD, Goulding EH, Stein P, Xu Z, et al. Protamine 2 deficiency leads to sperm DNA damage and embryo death in mice. *Biol Reprod* 2003; **69**: 211-217.

[22] Primakoff P, Myles DG. Penetration: adhesion, and fusion in mammalian sperm–egg interaction. *Science* 2002; **296**: 2183-2185.

[23] Ahmadi A, Ng SC. Fertilizing ability of DNA-damaged spermatozoa. *J Exp Zool* 1999; **284**: 696-704.

[24] Agarwal A, Said TM. Role of sperm chromatin abnormalities and DNA damage in male infertility. *Hum Reprod Update* 2003; **9**: 331-345.
[25] Sotolongo B, Huang TT, Isenberger E, Ward WS. An endogenous nuclease in hamster, mouse, and human spermatozoa cleaves DNA into loop-sized fragments. *J Androl* 2005; **26**: 272-280.
[26] Manicardi GC, Bianchi PG, Pantano S, Azzoni P, Bizzaro D, Bianchi U, et al. Presence of endogenous nicks in DNA of ejaculated human spermatozoa and its relationship to chromomycin A3 accessibility. *Biol Reprod* 1995; **52**: 864-867.
[27] Ward WS, Coffey DS. DNA packaging and organization in mammalian spermatozoa: comparison with somatic cells. *Biol Reprod* 1991; **44**: 569-574.
[28] Simon L, Brunborg G, Stevenson M, Lutton D, McManus J, Lewis SEM. Clinical significance of sperm DNA damage in assisted reproduction outcome. *Hum Reprod* 2010; **25**(7): 1594-1608.
[29] Zini A, Libman J. Sperm DNA damage: clinical significance in the era of assisted reproduction. *CMA J* 2006; **175**(5): 495-500.
[30] Kodama H, Yamaguchi R, Fukuda J, Kasai H, Tanaka T. Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. *Fertil Steril* 1997; **68**: 519-524.
[31] Fraga CG, Motchnik PA, Wyrobek AJ, Rempel DM, Ames BN. Smoking and low antioxidant levels increase oxidative damage to sperm DNA. *Mutat Res* 1996; **351**: 199-203.
[32] Ying SY. Inhibins, activins and follistatin: gonadal proteins modulating the secretion of follicle-stimulating hormone. *Endocr Rev* 1988; **9**: 267-293.
[33] Marchetti C, Hamdane M, Mitchell V, Mayo K, Devisme L, Rigot JM, et al. Marked differences in protamine content and P1/P2 ratios in sperm cells from percoll fractions between patients and controls. *J Androl* 2003; **24**: 438-447.
[34] Anawalt BD, Bebb RA, Matsumoto AM, Groome NP, Illingworth PJ, McNeilly AS, et al. Serum inhibin B levels reflect Sertoli cell function in normal men and men with testicular dysfunction. *J Clin Endocrinol Metab* 1996; **81**: 3341-3345.
[35] Crofton PM, Evans AEM, Groome NP, Taylor MRH, Holland CV, Kelnar CJH. Inhibin B in boys from birth to adulthood: relationship with age, pubertal stage, FSH and testosterone. *Clin Endocrinol* 2002; **56**: 215-221.
[36] De Kretser DM, Meinhardt A, Meehan T, Phillips DJ, O'Bryan MK, Loveland KA. The roles of inhibin and related peptides in gonadal function. *Mol Cell Endocrinol* 2000; **161**: 43-46.
[37] Anderson RA, Sharpe RM. Regulation of inhibin production in the human male and its clinical applications. *Inter J Androl* 2000; **23**: 136-144.
[38] Walker WH, Cheng J. FSH and testosterone signaling in Sertoli cells. *Reprod* 2005; **130**: 15-28.
[39] Groussin L, Bertherat J. Transcriptional regulation by cyclic AMP is essential for development, reproduction and survival: lessons from the transgenic mice. *Eur J Endocrinol* 1998; **139**: 571-572.
[40] Nantel F, Monaco L, Foulkes NS, Masquilier D, LeMeur M, Hendriksen K, et al. Spermiogenesis deficiency and germ-cell apoptosis CREM-mutant mice. *Nature (London)* 1996; **380**: 159-162.
[41] Weinbauer GF, Behr R, Bergman M, Nieschlag E. Testicular cAMP responsive element modulator (CREM) protein is expressed in round spermatids but is absent or reduced in men with round spermatid maturation arrest. *Mol Hum Reprod* 1998; **4**: 9-15.
[42] Steger K, Behr R, Kleiner I, Weinbauer GF, Bergmann M. Expression of activator of CREM in the testis (ACT) during normal and impaired spermatogenesis: correlation with CREM expression. *Mol Hum Reprod* 2004; **10**: 129-135.
[43] Blendy JA, Kaestner KH, Weinbauer GF, Nieschlag F, Schütz G. Severe impairment of spermatogenesis in mice lacking the CREM gene. *Nature* 1996; **380**: 162-165.
[44] Tanaka H, Miyagawa Y, Tsujimura A, Matsumiya K, Okuyama A, Nishimune Y. Single nucleotide polymorphisms in the protamine-1 and -2 genes of fertile human male populations. *Mol Hum Reprod* 2003; **9**(2): 69-73.
[45] Szczygie MA, Ward WS. Combination of dithiothreitol and detergent treatment of spermatozoa causes paternal chromosomal damage. *Biol Reprod* 2000; **67**: 1532-1537.
[46] Mengual L, Ballesca JL, Ascaso C, Oliva R. Marked differences in protamine content and P1/P2 ratios in sperm cells from percoll fractions between patients and controls. *J Androl* 2003; **24**: 438-447.
[47] Aoki VW, Emery BR, Liu L, Carrell DT. Protamine levels vary between individual sperm cells of infertile human males and correlate with viability and DNA integrity. *J Androl* 2006; **27**(6): 890-898.
[48] Clark AG, Civetta A. Protamine wars. *Nature* 2000; **403**: 261-263.
[49] Cho C, Willis WD, Gouling EH, Jung-Ha H, Choi YC, Hecth NB, et al. Haploinsufficiency of protamine-1 or -2 causes infertility in mice. *Nat Genet* 2001; **28**(1): 10-12.
[50] Akmal M, Aulanni'am, Widodo MA, Sumitro SB, Purnomo BB, Siregar TN, et al. Inhibin B menurunkan konsentrasi follicle stimulating hormone (FSH) pada tikus putih (Rattus norvegicus): upaya pengembangan kontrasepsi hormon pria berbasis peptida. *J Ked Hew* 2015; **9**(1): 42-46.
[51] Akmal M. *Inhibin B berpotensi menghambat proses spermatogenesis secara reversible melalui penurunan konsentrasi dan ekspresi Follicle-Stimulating Hormone (FSH), cAMP responsive element modulator (CREM), protamine P2, dan kualitas spermatozoa*. Disertasi. Fakultas Kedokteran Universitas Brawijaya Malang; 2011.
[52] Akmal M, Aulanni'am, Widodo MA, Sumitro SB, Purnomo BB. Inhibin B menurunkan motilitas spermatozoa (*Rattus norvegicus*): Upaya pengembangan kontrasepsi pria berbasis hormon peptida. *Media Kedokt Hewan* 2011a; **27**(2): 89-94.
[53] Baccetti B, Bruni E, Collodel G, Gambera L, Moretti E, Marzella R, et al. Reciprocal translocation in an infertile man: ultrastructural and fluorescence in-situ hybridization sperm study: case report. *Hum Reprod* 2001; **18**: 2302-2308.
[54] Chemes HE, Rawe PY. Sperm pathology: a step beyond descriptive morphology. Origin, characterization and fertility potential of abnormal sperm phenotypes in infertile men. *Hum Reprod Update* 2003; **5**: 405-428.
[55] Akmal M, Aulanniam, Widodo MA, dan Sumitro SB, Purnomo BB. Inhibin B menurunkan jumlah anak hasil fertilisasi *in vivo* pada tikus (*Rattus norvegicus*) secara *reversible*: upaya pengembangan kontrasepsi pria berbasis hormon peptida. *Media Kedokt Hewan* 2011b; **27**(1): 11-15.