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Judul Artikel Ilmiah : **The Different Effects of Zinc Supplement and Velvet Bean Mucuna Pruriens Extracts Toward Fertility of Balbc/Mice**  
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Prof. Dr. Sri Sumarmi, S.KM., M.Si  
 NIP 196806251992932002  
 Unit kerja: FKM Universitas Airlangga

Reviewer 2



Prof. Dr. Merryana Adriani, S.KM., M.Kes  
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Reviewer 1

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Unit kerja: Fakultas Kesehatan Masyarakat Universitas Airlangga

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Surabaya 8 Januari 2020

Reviewer 2

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Prof. Dr. Merryana Adriani, S.KM., M.Kes

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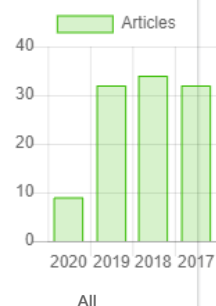
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Lecturer of Medical Faculty UNUSA

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<a href="#">Glycemic control with ipragliflozin, a novel selective SGLT2 inhibitor, ameliorated endothelial dysfunction in streptozotocin-induced diabetic mouse</a> HM Salim, D Fukuda, S Yagi, T Soeki, M Shimabukuro, M Sata Frontiers in cardiovascular medicine 3, 43	59	2016
<a href="#">Dipeptidyl peptidase-4 inhibitor, linagliptin, ameliorates endothelial dysfunction and atherogenesis in normoglycemic apolipoprotein-E deficient mice</a> HM Salim, D Fukuda, Y Higashikuni, K Tanaka, Y Hirata, S Yagi, T Soeki, ... Vascular Pharmacology 79, 16-23	35	2016
<a href="#">Teneligliptin, a dipeptidyl peptidase-4 inhibitor, attenuated pro-inflammatory phenotype of perivascular adipose tissue and inhibited atherogenesis in normoglycemic ...</a> HM Salim, D Fukuda, Y Higashikuni, K Tanaka, Y Hirata, S Yagi, T Soeki, ... Vascular pharmacology 96, 19-25	34	2017
<a href="#">Predictive factors for efficacy of dipeptidyl peptidase-4 inhibitors in patients with type 2 diabetes mellitus</a> S Yagi, K Aihara, M Akaike, D Fukuda, HM Salim, M Ishida, T Matsuura, ... Diabetes & metabolism journal 39 (4), 342	23	2015
<a href="#">Ticagrelor, a P2Y12 antagonist, attenuates vascular dysfunction and inhibits atherogenesis in apolipoprotein-E-deficient mice</a> B Ganbaatar, D Fukuda, HM Salim, S Nishimoto, K Tanaka, Y Higashikuni, ... Atherosclerosis 275, 124-132	20	2018
<a href="#">Predictors for the treatment effect of sodium glucose co-transporter 2 inhibitors in patients with type 2 diabetes mellitus</a> S Yagi, K Aihara, T Kondo, K Kurahashi, S Yoshida, I Endo, D Fukuda, ... Advances in therapy 35 (1), 124-134	10	2018
<a href="#">Canagliflozin prevents diabetes-induced vascular dysfunction in apoE-deficient mice</a> A Rahadian, D Fukuda, HM Salim, S Yagi, K Kusunose, H Yamada, ... Journal of atherosclerosis and thrombosis, 52100	9	2020



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# THE DIFFERENT EFFECTS OF ZINC SUPPLEMENT AND VELVET BEAN MUCUNA PRURIENS EXTRACTS TOWARD FERTILITY OF BALBC/ MICE

Pardipta Pradipta Kurniasanti<sup>1</sup>, M. Zen Rahfiludin<sup>2</sup>, Sri Winarni<sup>3</sup>

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<sup>3</sup>Lecturer on Biostatistics of Public Health Faculty, Diponegoro University

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**Abstract:** Good quality velvet beans and zinc supplement are well studied and known as rich source of aphrodisiac. The following reseach was carried out to study the difference effect of zinc supplement (ZS) and velvet bean extracts (VBE) towards fertility of BALB/c mice i.e the number of sperm, the motility of sperm, the viability of sperm, the morphology of sperm, the existence of vagina plug, and the birth condition of mice. This study was an experiment using Posttest Only Controlled Group Design with three groups. The first group (A1) as control, the second group (A2) receives subcutaneous injection of 2-methoxyethanol fraction as much as 200 mg/kg weight/day for 5 days, followed with ZS at a dosage of 0,026 mg/day/head, and the third group (A3) receives subcutaneous injection of 2-methoxyethanol fraction as much as 200 mg/kg weight/day for 5 days, followed with VBE at a dosage of 56 mg/kg weight /day for 30 days. The quality of spermatozoa of A1, A2, and A3 was significant difference ( $p < 0.05$ ). Both of A2 and A3 were no significant difference of mean number of sperm ( $p = 0.274$ ), mean percentage of sperm motility ( $p = 0.739$ ), mean percentage of sperm viability ( $p = 0.141$ ) and mean value of morphology of sperm ( $p = 0.394$ ). The quality of spermatozoa and the total number of baby mice lived and died of A1, A2 and A3 were significant difference. In addition, there was no significant difference in the existence of female mice's vaginal plug of A1, A2 and A3. This study showed that ZS and VBE can increase the quality of spermatozoa of BALB/C mice.

**Keywords:** zinc supplement, velvet bean extracts, fertility, spermatozoa of mice.

## INTRODUCTION

Infertility case has been a world issue (Sardjono et al., 2016; Martinez et al., 2012; Mathur, 2009). A review study showed a significant decrease in mean sperm count from 113 x 10<sup>6</sup> per ml in 1940 to 66 x 10<sup>6</sup> per ml in 1990 (Carlsen et al., 1992). In Australia, 1 out of 20 males has infertility problem and 50% of all infertility problem are associated with male (McLahlan et al., 2001). Infertility increases 15-20% from approximately 50 million couples in Indonesia. This infertility is consecutively caused by male (40%) and female (40%), both of female and male (10%), and another 10% of unidentified reasons. It means that the number of infertility increases in the last 50 years (Sardjono et al., 2016).

Various types of modern medicine have been applied to solve this case but many of which yield negative effects (Hart, 2005).

Zinc supplement is one of the essential micronutrients to increase the number, quality and motility of sperm for low fertility in men. Zinc within normal amount in male body will support reproduction system. Zinc supplementation at a dose of 0,026 mg/day/head turns out to be optimal and shows a significant increase on the number and motility of sperm in mice (Widya, 2012). Zinc supplement can reduce reproduction potential of mice dosage dependently by affecting proliferation of spermatogonia (Sedigh et al., 2016). In addition to zinc supplement, 96% ethanol fractions and isolated velvet bean on quality of male mice spermatozoa are exposed to 2- methoxyethanol with a dose of 56 mg/kg/day.

# EPILEPSI POST TRAUMA DENGAN GEJALA PSIKOTIK

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Abstrak: Data WHO menunjukkan epilepsi menyerang 1% penduduk dunia. Angka kejadian epilepsi masih tinggi terutama di negara berkembang yaitu mencapai 114 per 100.000 penduduk per tahun.<sup>2</sup> Pada kelompok dewasa muda, epilepsi dapat terjadi salah satunya disebabkan oleh trauma kepala.<sup>3</sup> Kasus ini melaporkan penderita laki-laki usia 24 tahun dengan keluhan kejang yang disertai dengan gejala psikotik. Didapatkan riwayat pasien kejang pertama kali dua bulan setelah mengalami trauma kepala yaitu pada tahun 2003. Saat itu pasien mengalami cedera otak berat dan dari CT scan kepala didapatkan perdarahan di otak. Dua bulan setelah trauma kepala itu pasien mengalami kejang berulang disertai keluhan yang dominan adalah marah-marah hingga pernah sampai mau membunuh orang. Dari analisis EEG menunjukkan hasil epilepsi lobus frontotemporal. Setelah pasien mendapatkan pengobatan phenytoin dan risperidone, kejang mulai terkontrol. Namun bila tidak minum obat pasien kejang lagi. Hal ini dialami oleh pasien hingga saat kasus ini dilaporkan, yaitu sepuluh tahun post trauma.

**Kata kunci:** Epilepsi Post Trauma, *psychotic epilepsy*

## PENDAHULUAN

Epilepsi merupakan salah satu penyakit neurologis yang sering ditemukan. Data WHO menunjukkan epilepsi menyerang 1% penduduk dunia. Epilepsi dapat terjadi pada siapa saja di seluruh dunia tanpa batasan ras dan sosial ekonomi. Angka kejadian epilepsi masih tinggi terutama di negara berkembang yaitu mencapai 114 per 100.000 penduduk per tahun. Prevalensi epilepsi pada bayi dan anak-anak cukup tinggi, menurun pada dewasa muda dan pertengahan, kemudian meningkat lagi pada kelompok usia lanjut yang disebabkan oleh penyakit serebrovaskuler.<sup>2</sup> Pada kelompok dewasa muda, epilepsi dapat terjadi akibat trauma, neoplasma, gangguan vaskuler, maupun penggunaan alkohol dan obat-obat sedatif lain.<sup>3</sup>

Epilepsi secara garis besar dapat digolongkan menjadi epilepsi idiopatik, kriptogenik, dan simtomatik. Epilepsi pasca trauma termasuk dalam epilepsi simtomatik. Merupakan sekuel dari trauma kepala yang paling sering terjadi, dengan insiden 5% pada penderita

dengan trauma kepala tertutup dan 50% pada penderita dengan fraktur tulang tengkorak dan jejas pada otak.<sup>4</sup> Tipe kejang pada epilepsi pasca trauma dapat berbeda-beda bergantung pada lesi yang diakibatkan oleh trauma tersebut.

## TINJAUAN PUSTAKA

### Definisi Epilepsi

Epilepsi adalah suatu keadaan yang ditandai oleh bangkitan epilepsi berulang berselang lebih dari 24 jam yang timbul tanpa provokasi. Yang dimaksud dengan bangkitan epilepsi adalah manifestasi klinik yang disebabkan oleh aktivitas listrik otak yang abnormal dan berlebihan dari sekelompok neuron. Manifestasi klinik ini terjadi secara tiba-tiba dan sementara berupa perubahan perilaku yang stereotipik, dapat menimbulkan gangguan kesadaran, gangguan motorik, sensorik, otonom, ataupun psikik.<sup>1</sup>

### Etiologi Epilepsi

Etiologi epilepsi dapat dibagi ke dalam 3 kategori, yaitu:

# PERBAIKAN FUNGSI TROFOBLAST PADA KADAR $\beta$ hCG TIKUS BUNTING PREEKLAMPSIA PADA BAHAN BIOLOGIS TERSIMPAN PASCA PERLUKUAN DENGAN SPIRULINA

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**Abstract:** Preeclampsia is multisystem specific disorder in pregnancy. Preeclampsia has signed by increased cytokine Interleukin-6 and  $\beta$  hCG (human Chorionic Gonadotropin). Spirulina is green-blue alga has bioactive compound namely antioxidant, vitamin, mineral. And has benefit namely antiinflammation. The aim of this research was to know how spirulina influence to repair trophoblast in Hcg concentration on rat pregnancy (biology material collecting). The type of this research is an experimental laboratoric with post test only control group design. A total of 25 rats with pre-eclampsia models induced by Interleukin 6 are divided into 5 groups: the untreated control group, the positive control group with Interleukin 6 induction for three days, the treatment group with a dose of 10 mg / day, 20 mg / day and 40 mg of spirulina. / day for five day, then the blood serum produced was measured with  $\beta$  hCG levels using ELISA (Enzyme-Linked Immunosorbent Assay). The results of the Spirulina 10 mg / day are higher at  $85.11 \pm 25.70$  mIU / ml from a dose of spirulina 20 mg / day at  $79.65 \pm 10.65$  mIU / ml. In the level of  $\beta$  hCG, the dose group of spirulina 10 mg / day and the group there is no significant difference ( $0.730 > 0.05$ ), the  $\beta$  hCG level group of spirulina 40 mg / day was  $93.28 \pm 17, 12$  mIU / ml from the dose group of spirulina 10 mg / day was  $85.11 \pm 25.70$  mIU / ml. The administration of spirulina for five days was able to reduce  $\beta$  hCG levels at a dose of 10 mg / day, 20 mg / day and 40 mg / day and the dose that was most effective in reducing  $\beta$  hCG levels significantly ( $P < 0.05$ ) was a dose of 10 mg than dose of 20 mg / day and 40 mg / day.

**Keywords:** Preeclampsia, Interleukin 6,  $\beta$  hCG, Spirulina

## PENDAHULUAN

Salah satu faktor yang merupakan penyulit atau gangguan atau komplikasi yang menyertai ibu saat hamil adalah patologi kehamilan (Sujiyatini, 2009). Sekitar lebih dari 63.000 ibu meninggal setiap tahunnya didunia disebabkan preeklampsia. Angka kematian ibu tertinggi terdapat pada negara negara yang berpenghasilan rendah dan menengah, namun demikian preeklampsia juga berpotensi pada negara negara maju (Marie Bolin, 2012). Preeklampsia merupakan gangguan multisistem spesifik pada kehamilan yang ditandai dengan perkembangan hipertensi dan proteinuria (Lana K, 2004). Hingga saat ini preeklampsia masih merupakan "the disease of theories", dimana patofisiologinya masih belum jelas diketahui. Sebagai penanda terjadinya preeklampsia. Pada kasus preeklampsia terjadi

peningkatan kadar  $\beta$  hCG serum (Priyatini T, 2004).

Human chorionic gonadotropin (hCG) merupakan hormon glikoprotein terdiri dari dua subunit kovalen non ,  $\alpha$  dan  $\beta$  , dan diproduksi oleh sel sinsitiotrofoblas plasenta. Puncak dari kadar  $\beta$  hCG adalah minggu ke 8 – 10 kehamilan dan kadar paling rendah pada minggu 18 – 20 kehamilan. (Kanika Mandi Chaudhury *et al*, 2012). Fungsi  $\beta$  hCG pada kehamilan adalah sebagai penghasil progesteron, implantasi dan sebagai regulasi sistem kekebalan (W. Norris, 2011).

Spirulina platensis merupakan salah satu varian dari mikroalga Spirulina sp. yang banyak dimanfaatkan oleh masyarakat karena kandungan nutrisinya yang lengkap. (Oliveira *et al* 2008). Fikosianin merupakan salah satu dari tiga pigmen

# THE DIFFERENT EFFECTS OF ZINC SUPPLEMENT AND VELVET BEAN MUCUNA PRURIENS EXTRACTS TOWARD FERTILITY OF BALBC/ MICE

*by* M. Zen Rahfiludin

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## THE DIFFERENT EFFECTS OF ZINC SUPPLEMENT AND VELVET BEAN MUCUNA PRURIENS EXTRACTS TOWARD FERTILITY OF BALBC/ MICE

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**Abstract:** Good quality velvet bean and zinc supplement are well studied and known as rich source of aphrodisiac. The following research was carried out to study the difference effect of zinc supplement (ZS) and velvet bean extracts (VBE) towards fertility of BALB/c mice i.e the number of sperm, the motility of sperm, the viability of sperm, the morphology of sperm, the existence of vagina plug, and the birth condition of mice. This study was an experiment using Posttest Only Controlled Group Design with three groups. The first group (A1) as control, the second group (A2) receives subcutaneous injection of 2-methoxyethanol fraction as much as 200 mg/kg weight/day for 5 days, followed with ZS at a dosage of 0,026 mg/day/head and the third group (A3) receives subcutaneous injection of 2-methoxyethanol fraction as much as 200 mg/kg weight/day for 5 days, followed with VBE at a dosage of 56 mg/kg weight/day for 16 days. The quality of spermatozoa of A1, A2, and A3 was significant difference ( $p < 0.05$ ). Both of A2 and A3 were no significant difference of mean number of sperm ( $p = 0.274$ ), mean percentage of sperm motility ( $p = 0.739$ ), mean percentage of sperm viability ( $p = 0.141$ ) and mean value of morphology of sperm ( $p = 0.394$ ). The quality of spermatozoa and the total number of baby mice lived and died of A1, A2 and A3 were significant difference. In addition, there was no significant difference in the existence of female mice's vaginal plug of A1, A2 and A3. This study showed that ZS and VBE can increase the quality of spermatozoa of BALB/C mice.

**Keywords:** zinc supplement, velvet bean extracts, fertility, spermatozoa of mice.

### INTRODUCTION

Infertility case has been a world issue (Sardjono et al., 2016; Marti et al., 2012; Mathur, 2009). A review study showed a significant decrease in mean sperm count from  $113 \times 10^6$  per ml in 1940 to  $66 \times 10^6$  per ml in 1990 (Carlsen et al., 1992). In Australia, 1 out of 20 males has infertility problem and 50% of all infertility problem are associated with male (McLahlan et al., 2001). Infertility increases 15-20% from approximately 50 million couples in Indonesia. This infertility is consecutively caused by male (40%) and female (40%), both of female and male (10%), and another 10% of unidentified reasons. It means that the number of infertility increases in the last 50 years (Sardjono et al., 2016).

Various types of modern medicine have been applied to solve this case but many of which yield negative effects (Hart, 2005).

Zinc supplement is one of the essential micronutrients to increase the number, quality and motility of sperm for low fertility in men. Zinc within normal amount in male body will support reproduction system. Zinc supplementation at a dose of 0,026 mg/day/head turns out to be optimal and shows a significant increase on the number and motility of sperm in mice (Widya, 2012). Zinc supplement can reduce reproduction potential of mice dosage dependently by affecting proliferation of spermatogonia (Sedigh et al., 2016). In addition to zinc supplement, 96% ethanol fractions and isolated velvet bean on quality of male mice spermatozoa are exposed to 2-methoxyethanol with a dose of 56 mg/kg/day.



It improves the number, motility rate, and the percentage of spermatozoa viability and the percentage of normal sperm morphology (Winarni, 2010). Various study had be done to investigate the aphrodisiac activity of velvet bean extracts (Gupta et al., 2011; Sukhla et al., 2010; Sekar et al., 2009; Ahmad et al., 2008). Therefore the following research was carried out to compare the difference effect of zinc supplement and velvet bean extracts toward fertility of BALB/c mice.

**Materials and Methods** Zinc supplement (ZS) Zinc supplement was a syrup (Zinkid) purchased from the clinic of pharmacy, then the dosage was diluted up to 0.026 mg/day/head. Source of 2-methoxyethanol The toxic of 2-methoxyethanol fraction as much as 200 mg/kg weight/day were purchased from (collection of Mrs. Hayati) Biological Laboratory of Unair, Surabaya, Indonesia.

Velvet bean extracts (VBE) preparation Velvet beans were sun dried to reduce water content and shelled to obtain the seed (nib). Nib was defatted by maceration with 1500 ml acetone and shaken for 48 h at room temperature. It was filtered, then filtrate of velvet powder was extracted by maceration with water and 96% ethanol (1:1) (sample to solvent ratio of 1:2 w/v ). The extraction process was carried out for 24 h at room temperature (25°C). Once the extraction was complete, it was filtered by vacuum (Winarni, 2010). The resulting velvet bean extracts was condensed by a rotary evaporator at 40°C, 50 rpm and dried by vacuum oven and designed as velvet bean extracts (VBE).

## MATERIAL AND METHODS

### *Animal*

The kind of rat was the BALB/c mice strain Mus Muculus obtained from the Biological Laboratory of Universitas Negeri Semarang (UNNES), Indonesia.

### *Preparation of laboratory experimental and control research*

The number of mice used for all three groups were 45 BALB/c male mice (8 w old, weighing about 20-30 g were used for the investigation). The ratio of BALB/c female mice and BALB/c male mice was 1:2 each group. It means that a male mice was mated with 2 female mices, and 15 mices of each group (9 mices were examined its sperm quality, 6 mices were mated).

### *Monitoring of the existence of vaginal plug*

Male and female mice were mated in a week. Every morning for a week, the female mice examined whether there was vagina plug with the use of apusan vagina. When vagina plug was found in red colour, it indicates that intercourse was done. It was defined as the first day of female mice pregnancy (Adnan, 2006).

### *The number of sperm*

Neck-dislocated died mice were located on tray for surgery. Their cauda epididymis was isolated using NaCl. Sperm liquid was emptied from cauda epididymis by a syringe before it was dissolved with 2 ml NaCl until it became homogenous. The calculation of the sperm concentration was using haemocytometer improved Neubauer before it was observed by a microscope of 400x magnifications. The calculation was conducted for four boxes of counting chamber, prior to average calculation. The result of the calculation was the sperm concentration in 10<sup>-4</sup> ml sperm suspension (Hayati, 2007):

$$\text{Number of cell/ml} = \text{number of spermatozoa (n)} \times 104 \times \text{dilution factor}$$

Sperm motility was observable from sperm suspension dropped on Neubauer counting chamber observed by a microscope of 400x magnifications. Sperm motility was valued on the basis of percentage of good sperm motility, that was sperm

which moves fast, straight forward and active (Aleissa, 2014).

The percentage of sperm motility was measured by the following equation (Goyal et al., 2001; Hayati, 2007; Canyurt and Akhan, 2008): Spermatozoa motility (%) =  $\frac{\text{category A} + \text{B} \times 100\%}{100}$  : spermatozoa.

#### ***The viability of sperm***

Sperm viability was observable from sperm suspension dropped on colouring eosin Y 1% observed by a microscope of 400x magnifications. Sperm viability was valued on the basis of percentage of good sperm viability. That was sperm which indicates transparant color as live and red color as die (Aleissa, 2014).

The percentage of sperm viability was measured by the following equation (Goyal et al., 2001; Hayati, 2007; Canyurt and Akhan, 2008):  $\text{Spermatozoa viability (\%)} = \frac{\_}{\_}$  spermatozoa

#### ***The morphology of sperm***

Sperm morphology testing was conducted by differentiating the shape of normal and abnormal sperm of 100 sperms observed before it was made into percentage (Aleissa, 2014). Abnormal sperm includes abnormality such as broken, detached and thin head; broken, crooked and droplet cytoplasm middle part or broke, crooked and coil tail. The observation used a microscope of 400x magnifications (Henderson and Robaire, 2005; Sardjono et al., 2016).

#### ***Analysis of the data***

Results were expressed as mean  $\pm$  SD. The normality of the data was tested using Shapiro Wilks. Statistical tests uses Kruskal-Wallis Test, Mann-Whitney test, One-way ANOVA test, and followed by Post Hoc LSD test, with the significant level of 0.05.

## **RESULTS AND DISCUSSIONS**

### ***The number of sperm***

The mean number of sperm of A2 ( $3.29 \pm 0.55$  million/ml) and A3 ( $3.66 \pm 0.99$  million/ml) were greater than A1 ( $2.67 \pm 0.37$  million/ml) (Figure 1a). It indicated that higher number of sperm in A2 and A3 also gave a stronger spermatogenesis activity, whereas ZS and VBE have aphrodisiac content to increase the number of sperm. This is in line with the general knowledge that ZS can increase the number of sperm stem cells (Sedigh et al., 2016) and VBE can increase the number of sperm with stimulating the hormones of mice (Sardjono et al., 2016).

There was a significant difference of the number of sperm of A1, A2 and A3 by ANOVA Test ( $p = 0.019$ ), whereas LSD test showed the significant difference of A1 versus A3 ( $p = 0.006$ ) and A1 versus A2 ( $p = 0.069$ ) respectively. However, there was no significant difference of A2 versus A3 ( $p = 0.274$ ). This fact proved that the ZS and VBE were the same effect that affected the number of sperm of the mice. Thus, ZS and VBE are a powerful source of aphrodisiac food which is potential to increase the number of sperm.

### ***The motility of sperm***

The mean percentage of sperm motility in A2 ( $77.7 \pm 12.0\%$ ) and A3 ( $79.4\% \pm 7.7\%$ ) were greater than A1 ( $54.4 \pm 11.3\%$ ) (Figure 1b). It indicated that higher motility of sperm affected by the number of sperm and morphology of sperm. A living spermatozoa correlates highly to the motility of the sperm as being alive is an absolute requirement for a spermatozoa to be able to produce energy and move. Semen of mammal that has high fertility is characterized with a high level of living spermatozoa with normal morphology. Good motility depends on many things, including the morphology of sperm (Sardjono et al., 2016).

There was a significant difference of the percentage of sperm motility of A1, A2 and A3 by ANOVA Test ( $p = 0.001$ ), whereas LSD test showed the significant difference of the percentage of sperm motility of A1 versus A2 ( $p = 0.001$ ) and A1 versus A3 ( $p = 0.001$ ) respectively. However, there was no significant difference of A2 versus A3 ( $p = 0.739$ ). This fact proved that the ZS and VBE were the same effect that affected the motility of sperm of the mice too. It given correlates positively with the previous result and discussion (the number of sperm). A large amount of sperm with good sperm motility is sufficient for the insemination to take place successfully.

#### **The viability of sperm**

The mean percentage of sperm viability in A2 ( $29.0 \pm 3.2\%$ ) and A3 ( $31.3 \pm 3.7\%$ ) were greater than A1 ( $17.4 \pm 2.8\%$ ) (Figure 1c). There was a significant difference of the percentage of sperm viability of A1, A2 and A3 by ANOVA Test ( $p = 0.001$ ). LSD test showed showed the significant difference of the percentage of sperm viability of A1 versus A2 ( $p = 0.001$ ) and A1 versus A3 ( $p = 0.001$ ) respectively. However, there was no significant difference of A2 versus A3 ( $p = 0.141$ ). The study shows that ZS and VBE can increase the viability of sperm as well as increase the number of sperm and the motility of sperm. This increase in viability sperm is inseparable from L-dopa component found in VBE. This compound not only increase sexual activity but also hormones regulating spermatogonia process such as FSH and LH (Sardjono et al., 2016). Whereas ZS concentration in seminal plasma is known to correlate with the viability of sperm (Akinloye et al., 2011; Colagar et al., 2009).

#### **The morphology of sperm**

The morphology of sperm of A2 ( $19.4 \pm 3.2\%$ ) and A3 ( $21.6 \pm 5.2\%$ ) was greater than A1 ( $13.3 \pm 3.5\%$ ). There was no significant difference

of the morphology of sperm of A1, A2, and A3 by Kruskal-Wallis test ( $p = 0.003$ ). When A1 was compared to A2 and A3, it showed the significant difference of the morphology of sperm by Mann-Whitney Test ( $p < 0.05$ ). It means the existence of ZS in A2 and VBE in A3 affected the normality of morphological sperm of BALB/c mice. This is in line with the general knowledge that VBE treatment succeeded in reducing morphological abnormal sperm in all dosage given (50, 100, 150, 200 and 250 mg/kg (Sardjono et al., 2016), which is our study use dosage of VBE as much as 56 mg/kg. However, there was no significant difference of A2 versus A3 ( $p > 0.05$ ) (Table 1). Increased number of sperm and motility of sperm as well as decreased abnormal sperm morphology of mice occur because of L-dopa and other components contained in VBE which influence the secretion of testosterone (Sardjono et al., 2016). Whereas ZS plays key role in immune system improvement and activity of hormones to affect spermatogonia process and the effects of ZS on prostate gland are very obvious (Sedigh et al., 2016).

#### **The existence of vagina plug**

Male mice in A1, A2, and A3 had the same capability to impregnate female mice. Overall our study showed that only one female mice in each group had no vagina plug. It means there was one male mice able to mate two female mices, but other male mice only one female mice.

#### **The birth condition of mice**

The ratio number of baby mice dead in A1 (0.22) was two among nine (18%; 7 alive out of 39 babies born), A2 (0.26) was five baby mices dead out of 19 (36%; 14 alive out of 39 babies born) and A3 (0.18) was four baby mices dead out of 22 (46%; 18 alive out of 39 babies born) (Figure 2). It showed that female mice given ZS and VBE produced more baby mice than control group. It

was likely due to mineral of zinc contained in VBE and also other vitamins and minerals. This result given correlation positively with the quality of spermatozoa. Our study showed that ZS and VBE increase the number, motility, viability, and morphology of sperm. Fertility amount was increased by ZS and VBE, which is due to intensive increasing in epididymis sperms. Since certain number of sperm in the semen was necessary for fertilization. This increasing will directly affect the number of fertilized ovules of female mice.

The mean weight of baby mice in A2 ( $0.55 \pm 0.13$  g) and A3 ( $0.53 \pm 0.14$  g) was greater than A1 ( $0.49 \pm 0.12$  g) (Figure 3). There was no significant difference of the weight of baby mice by ANOVA Test ( $p = 0.644$ ) and LSD Test ( $p > 0.05$ )

## CONCLUSIONS

Zinc supplement and velvet bean extracts increase the quality of spermatozoa (the number of sperm, the motility of sperm, the viability of sperm and the morphology of sperm) of BALB/c mice higher than control group and can be used as aphrodisiac food.

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