

# PROVISION OF ARAK BALI REDUCES SPERMATOZOA QUALITY OF WHITE RATS (*Rattus norvegicus*)

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## PROVISION OF ARAK BALI REDUCES SPERMATOZOA QUALITY OF WHITE RATS (*Rattus norvegicus*)

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### ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh pemberian arak bali terhadap kualitas spermatozoa meliputi morfologi, motilitas, viabilitas, integritas membran spermatozoa tikus (*Rattus norvegicus*). Penelitian dilakukan dalam 2 tahap yaitu: tahap I penyebaran kuisioner yang dilakukan di 5 kabupaten di Bali untuk mengetahui jenis dan frekuensi konsumsi arak bali dan tahap II dilakukan pemberian perlakuan arak bali pada hewan coba. Penelitian ini menggunakan 24 ekor tikus (170-200 gram), dibagi menjadi 4 kelompok yaitu: satu control dan tiga perlakuan (diberi arak bali yang mengandung 40% alcohol sebanyak 0,1 dan 0,5 mL serta alcohol sintesis sebanyak 0,1 mL, selama 45 hari. Hasil penelitian menunjukkan bahwa dari lima kabupaten di Bali, sebagian besar masyarakatnya mengkonsumsi arak bali komersial dan sebagian besar frekuensi konsumsinya satu minggu lebih dari satu botol (350 mL). Pemberian arak bali pada hewan coba, menurunkan kualitas (morfologi, motilitas, viabilitas, integritas membran), semakin besar volume yang diberikan semakin menurun kualitas spermatozoanya. (FMI 2016;52:235-240)

**Kata kunci:** arak bali, kualitas spermatozoa (morfologi, motilitas, viabilitas, integritas membran), tikus (*Rattus norvegicus*)

### 1 ABSTRACT

This study aimed to determine the effect of arak bali on the quality of spermatozoa include morphology, motility, viability, membrane integrity of spermatozoa rat (*Rattus norvegicus*). The study was conducted in two phases: the first phase of the deployment of questionnaires conducted in five districts in Bali to determine the type and frequency of arak bali consumption and phase II made arak bali giving treatment in experimental animals. This study used 24 male rats (170-200 grams), divided into four groups: one control and three treatments (by arak bali containing 40% alcohol as much as 0.1 and 0.5 mL and 0.1 mL much alcohol synthesis, for 45 days. the results showed that of the five districts in Bali, most people consume arak bali commercial and most of the frequency of consumption of the week more than one bottle (350 mL). the provision of arak bali in experimental animals, degrade the quality (morphology, motility, viability, membrane integrity), the greater the volume given declining spermatozoa quality. (FMI 2016;52:235-240)

**Keywords:** arak bali, the quality of sperm (morphology, motility, viability, membrane integrity), rat (*Rattus norvegicus*)

### INTRODUCTION

Arak Bali is a traditional Balinese drinks that contain alcohol is high at 30-50%. Arak Bali is made from the fermentation of coconut water, fruit juice (palm), and water the rice, the most consumed and traded arak bali usually made from rice water. Processing of rice water into arak (wine) is commonly done by people in Bali, usually rice water used is water white glutinous rice is added to the starter or yeast. Fermented and then distilled by simple distillation apparatus, and will produce bioethanol (wine) (Iempang 2006).

Arak Bali containing alcohol enters the body will undergo a series of biochemical processes. Alcohol is consumed will be metabolized by the liver enzyme alcohol-assisted dehydrogenase (ADH) and coenzyme nicotinamide-adenine-dinokleotida (NAD) into acetaldehyde, and then by the enzyme aldehyde dehydrogenase (ALDH) is converted into acetic acid. Besides fat-soluble alcohol can easily cross the cell membrane,

through the mechanism of protein oxidation and lipid cell membranes, causing increased respiration activity of cells that contributes to the high levels of ROS can damage the structure and function of cells. (Zakhari 2006). Excessive alcohol consumption in men can cause fertility problems through low number and motility of spermatozoa (Maneesh et al 2006). Number and motility of spermatozoa indicate decreasing quality of spermatozoa, which in turn leads to infertility.

Thus it is necessary to do research on sperm fertility test to see quality include motility, morphology, viability and membrane integrity of spermatozoa to the experimental animals (rats) with the provision of the volume variation arak bali drinks containing alcohol.

### MATERIALS AND METHODS

This research was conducted in two stages: the first step of distributing questionnaires in five districts in Bali,

Badung, Gianyar, Tabanan, Karangasem, Denpasar. Questionnaire aims to find out the type of arak bali which is often consumed and the frequency of arak bali consumption. The second phase is done giving treatment in experimental animals using white rats (*Rattus norvegicus*) weighing 170-250 grams and 3 months of age. A total of 24 male rats were divided four kolompok, each group there are six replication (6 rats). One control group was given 0.1 ml of aqua and three treatment groups with a variation of 40% volume of alcohol contained in the arak bali (0.1 and 0.5 mL) and 40% alcohol synthesis as much as 0.5 mL. Each administration in rats conducted orally every day for 45 days.

### Collection of epididymal spermatozoa

On day 46 mice were sacrificed and subsequently dissected testis cleared from other networks and taken part cauda epididymis. Spermatozoa collection is done by making a suspension of spermatozoa with epididymal small cut in the 2 ml 0.9% NaCl solution with a pH of 7.2 to 7.4. Suspension spermatozoa are ready to test the quality.

### Counting speed motility

Suspension of epididymal spermatozoa taken one drop using a pipette and then dripped on glass objects sunken and covered with a glass lid and then viewed under the digital inverted microscope (Olympus). Spermatozoa were observed spermatozoa moving swiftly forward and hyperactive with a magnification of 400x. Do as much as 6x repeated observation of each rat's tail. Each repetition of the observations made counting speed of 100 cell motility.

### Observation of morphology of spermatozoa

Sperm morphology was observed using eosin staining method Nigrosin 1% and 10%, by making a smear on a glass spermatozoa object, then given 1 drop eosin 1% and 1 drop Nigrosin 10%, then homogenized and made smears, after 3-5 minutes washable with running water, then observed under a light microscope (Olympus) with 400x magnification.

## RESULTS

### Results Phase I

Of the 100 respondents in 5 districts of the results obtained 70 respondents consumed arak bali with commercial species and 30 respondents consumed arak bali types of non-commercial. Commercial species is a

type of free arak bali traded and already has a letter from the Ministry of Health BPOM and Bali area. Meanwhile, non-commercial arak bali is a traditionally produced wine and does not have permission to be traded (Fig. 1).



Fig. 1. The packaging of several types of arak bali. (A) arak bali non-commercial, (B) arak bali commercial

Based on the results of the questionnaire in five districts showed that most people choose arak bali commercial species (70%), while 30% choose the type of non-commercial. Frequency of consumption, 6% consumed once a week, 70% consumed more than once a week, and 26% consumed arak bali every day of the week. Additionally result arak bali consume 69% as much as 1 bottle per day and 31% more than one bottle per day. It indicates the number of people who consume arak Bali bali.

### Phase II study

Arak bali influence on the rate of sperm motility

After an examination of the animal, the obtained results of sperm motility speed calculation in the control group and the treatment group. The results of these calculations can be seen in Fig. 2.

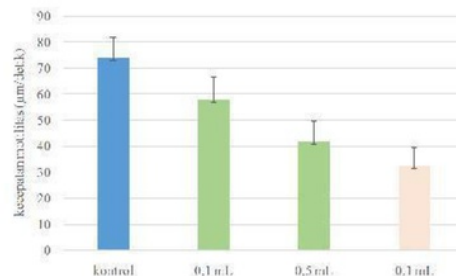


Fig. 2. Effect of arak bali against rat sperm motility rate ( $\mu\text{m}/\text{sec}$ ). The control group (blue), the group

treated with alcohol contained in Arak Bali 0.1 and 0.5 mL (green), and 0.1 mL of alcohol synthesis (in orange)

From the figure above it appears that giving alcohol terkandung in Arak Bali decrease sperm motility rate of mice. Having tested statistically using ANOVA seemed there were significant differences in all study groups. The greater the volume is given, the lower speed motility. However, a decrease in motility speed when compared with the provision of alcohol synthesis, then the speed drops motility over again.

Arak bali influence of the morphology of spermatozoa

Giving Arak Bali in male mice may affect the morphology of spermatozoa. A decline in the percentage of morphologically normal spermatozoa significant rats after administration of arak bali for 45 days with a variation of volume. Morphological changes in spermatozoa is shown in Fig. 3.

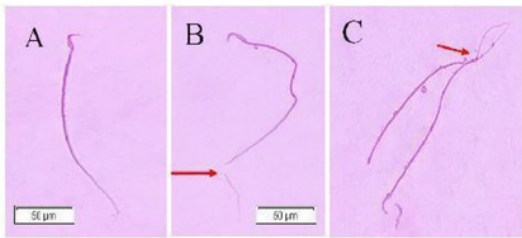


Fig. 3. Morphology of rat spermatozoa. A. morphologically normal and B-C abnormal morphology in the tail

The mean decrease in the percentage of morphologically normal spermatozoa mice is shown in Fig. 4. In normal circumstances (control) approximately 80% of normal sperm morphology, but by giving a good alcohol contained in arak bali or alcohol synthesis for 45 days resulted in lowering the percentage of morphologically normal.

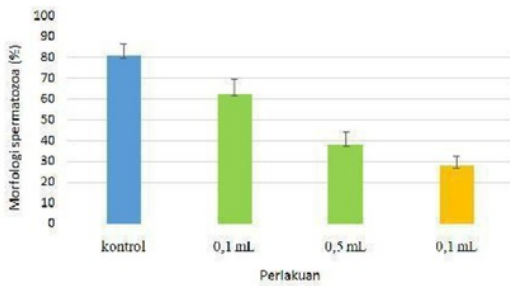


Fig. 4 Effect of arak bali of the morphology of spermatozoa mice. The control group (blue), the group treated with alcohol contained in Arak Bali 0.1 and 0.5 mL (green), and 0.1 mL of alcohol synthesis (in orange)

Effect of arak bali against spermatozoa Viability

Spermatozoa surviving marked by not absorb dye eosin-Nigrosin, so it looks pale. While dead spermatozoa appear red (Fig. 5).

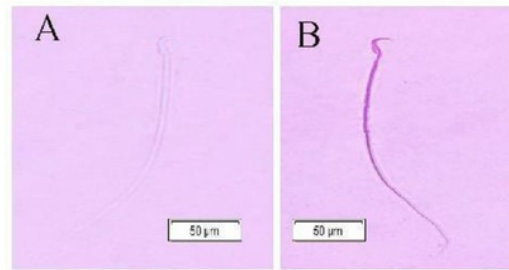


Fig. 5. The viability of spermatozoa of mice after the administration of arak bali. A. spermatozoa alive and dead spermatozoa B.

The mean percentage of live sperm viability in the control group and the group treated with arak bali volume variation can be seen in Fig. 6.

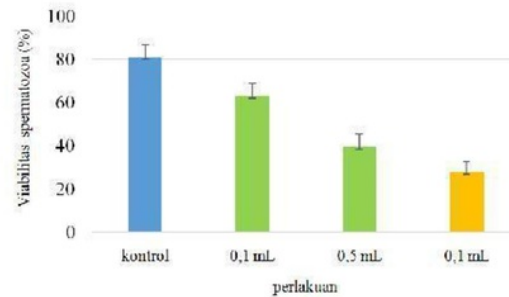


Fig. 6. Effect of arak bali on the viability of rat spermatozoa. The control group (blue), the group treated with alcohol contained in Arak Bali 0.1 and 0.5 mL (green), and 0.1 mL of alcohol synthesis (in orange)

Arak bali influence on sperm membrane integrity

The results of data analysis showed no effect of arak bali against sperm membrane integrity. These results are presented in Fig. 7. From Fig. 7 it appears that administration of arak bali also affect sperm membrane integrity mice. The more volume given declining per-



centage of sperm membrane integrity was good. Results of statistical analysis showed a significant difference in each treatment group.

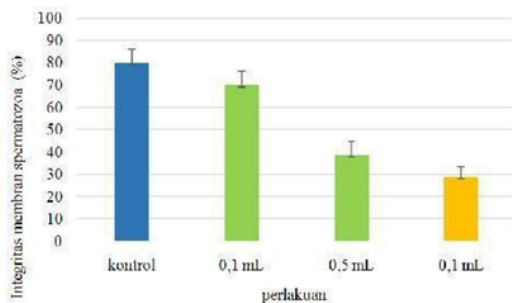


Fig. 7. Effect of arak bali against rat sperm membrane integrity. The control group (blue), the group treated with alcohol contained in Arak Bali 0.1 and 0.5 mL (green), and 0.1 mL of alcohol synthesis (in orange)

## DISCUSSION

### Arak bali influence on the rate of sperm motility

The results showed that the rats given arak bali with a volume of 0.5 mL motility speed smaller than the control. It also decreased motility rate in the group of rats given arak bali with a volume of 0.1 mL. Decrease speed of motility is caused by the alcohol contained in arak bali enter the body through the blood stream will go into the liver, in the liver alcohol is converted into the compound acetaldehyde by the enzyme alcohol-dehydrogenase (ADH) and the compound acetaldehyde is converted to acetic acid by enzyme aldehyde-Dehidrogenase (ALDH) (Koivisto 2007, Das et al 2008). Marchitti et al (2008) suggested that acetaldehyde is a molecule that is reactive by ALDH enzyme can be oxidized to acetate. In people who consume alcohol will be increased levels of acetaldehyde are toxic to various organs or tissues. Increased compound acetaldehyde in the body as a result of the fall of the ALDH enzyme function resulted in increased Reactive Oxygen Species (ROS). According Hoek et al (2002). Unstable antioxidants with high levels of ROS can reduce the rate of sperm motility. ROS can oxidize mitochondrial DNA located at the middle piece of sperm, which can impair the function of mitochondria to produce energy to move the flagella or tails of spermatozoa.

The movement of the sperm tail is controlled by a drive portion or dynein (dynein arm outer and inner dynein

arm) and radial spokes that make up microtubules. Movement of spermatozoa use the energy produced from the mitochondria, where the mitochondrial arranged in a spiral and are protected by the cell membrane. Mitochondria will produce energy through a process in which the movement of ATP hydrolysis occurs by converting chemical energy into kinetic energy through enzin ATPase (Conservation 2011). Arak Bali with a high alcohol content in spermatozoa resulting in high levels of ROS, ROS levels were higher resulting from the reduction in oxidant causing oxidative stress, thus resulting in cell membranes that melindungi mitochondria in the tail become damaged and interfere with the function of the mitochondria to produce ATP for movement (Hoek et al 2002).

ATPase enzyme serves to maintain internal homeostasis of sodium and potassium ions. If the enzyme ATPase activity is interrupted, then the sodium and potassium ion homeostasis would be disrupted so that increased intracellular concentrations of  $Na^+$ ,  $Na^+$  gradient across the cell membrane is decreased,  $Ca^{2+}$  ions expenses will also decrease. If the  $Ca^{2+}$  ions decreases the membrane will lose its ability to transport the materials dissolved into the cytoplasm. With the disruption of sperm membrane permeability will cause disruption of the transport of nutrients needed by the spermatozoa to movement.

### Arak bali influence of the morphology of spermatozoa

Morphology in groups of mice were given Arak Bali showed a decrease of normal morphology and an increase in abnormal morphology (disabled). In the group of mice given Arak Bali with volume of 0.5 mL are found defects in the tail, this shows that the influence of the morphology of spermatozoa arak bali.

The decline morphologically normal spermatozoa caused by exposure to alcohol in the arak bali which can increase ROS. The presence of ROS in the testes, may interfere with testicular function during the process of spermatogenesis. Disruption of the process of spermatogenesis damage spermatogenesis spermatogenic cells results, including the amount of cytoplasmic droplet and disruption during spermiogenesis. Spermiogenesis disorder resulting in the formation of sperm abnormalities, such as the head of spermatozoa into smaller or larger than normal, tail bent or broken, etc.

Increased levels of ROS will generate oxidative stress due to high levels of ROS and antioxidants the body is not able to reduce levels of oxidants, causing damage to cells, tissues and organs. In spermatozoa, the damage includes primary and secondary abnormalities. Primary

abnormality seen in the form of a small head, head amorphous, and the spiral tail, while the secondary abnormality is seen that spermatozoa with no head and no tail. This is caused by ROS affect the plasma membrane of spermatozoa containing phospholipids and unsaturated fatty acids in large amounts, where the unsaturated fatty acids are prone to ROS primarily hydroxyl radicals which is a derivative of the most reactive, this is because the hydroxyl radical will cause chain reaction called lipid peroxidation resulting in a disconnection of fatty acids into compounds that are toxic to sperm cells (Murray 2003)

Peroxidation process begins with the formation of carbon-centered radicals in phospholipid layer and then reacts with oxygen to form new free radicals that peroxy free radicals. Peroxyl radical reactive enough to attack the fatty acids in the vicinity that could form lipid hydroperoxide and carbon-centered radical new and called hydroxyl radicals. Hoarding on the membrane lipid hydroperoxide will cause disturbances in the function of cells (Murray 2003). This is a major agent of morphological changes from normal to abnormal spermatozoa

#### **Arak bali influence on the viability of spermatozoa**

Observations on viability of spermatozoa of the numbers of dead spermatozoa in the group given arak bali with a volume of 0.1 and 0.5 mL. Live spermatozoa characterized by spermatozoa do not absorb the dye or transparent, while the dead spermatozoa characterized by the head of the red after staining with eosin-Nigrosin.

Damage to the plasma membrane in dead spermatozoa causing sodium pump no longer function properly to regulate the circulation of substances into and out of the cell, so that the dye into the cells and spermatozoa into the red, especially on the head. Live spermatozoa have a plasma membrane intact so that pumps sodium to function properly. The enzyme Na<sup>+</sup> and K<sup>+</sup> ATPase found on the plasma membrane will be pumped back to the Na ions that bind to the dye eosin out of the cell.

Death spermatozoa can also be caused by damage to nuclear DNA resulting effect of free radicals or reactive oxygen species (ROS). DNA damage caused by oxidative stress that increases ROS formation, which can damage DNA fragmentation resulting in apoptosis. This is causing a lot of the discovery of dead spermatozoa (Moustafa et al 2004). Free radicals cause mutation of DNA and cytotoxicity. Cytotoxicity can cause death and a decrease in the number of cells in the network. The mechanism of cytotoxicity in cell one of which may be caused by oxidative stress (Agarwal et al 2003). Oxidative stress arising from excessive ROS

production and destruction of antioxidant defense mechanisms, causing many dead sperm cells.

#### **Arak bali influence on sperm membrane integrity**

Membrane integrity of spermatozoa in mice fed a arak bali showed significant declines. Good spermatozoa membrane integrity can be seen from the swelling of the tail section, while for membrane integrity of spermatozoa defective (bad) can be judged from swelling on the tail or straight.

Sperm cell membrane consists of a double lipid containing unsaturated fatty acids that are highly susceptible to ROS causing lipid peroxidation in the cell membranes of spermatozoa. Lipid peroxidation is an oxidation reaction which describes unsaturated fatty acids (fatty acids containing more than two carbon double bonds) into aldehyde (MDA) (Conservation 2011).

Lipid peroxidation in cells could affect the integrity of the cell membrane. In normal circumstances, the protection against superoxide radicals done scavenger system, ie by changing the superoxide ions (O<sub>2</sub><sup>-</sup>) into hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), assisted by the enzyme superoxide dismutase (SOD) with the addition of hydrogen ions. Hydrogen peroxide is then broken down into the water with the help of the enzyme catalase. The hydroxyl radical (OH) also reacts with unsaturated fatty acids in the cell membrane. This reaction led to formation of hydrogen peroxide. Unsaturated fatty acids undergo decomposition (decomposed) into many aldehydes with chain length varies. One among the aldehydes are the result peroxidation MDA (Conservation 2011)

MDA compound is very toxic to the cells thus causing damage to sperm membranes and decrease sperm membrane integrity resulting in decreased sperm quality (Sanocka et al 2004). This study is in line with research conducted by A.A. Oremosu and E.N. Akang (2014) regarding the influence of alcohol on reproductive hormones, oxidative stress and sperm parameters, which in this study stated that the high levels of MDA which is one factor of damage to the sperm membrane integrity.

#### **CONCLUSION**

The provision of arak bali containing alcohol of 40% and 40% alcohol synthesis levels for 45 days can reduce kualitas spermatozoa (motility, morphology, viability, membrane integrity of spermatozoa) mice.

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