

## The Effect of Monosodium L-Glutamate (MSG) Treatment for Short and Long Terms to The Semen Quality of Adult Male Rats

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### Abstract

This study was conducted to investigate the effect of MSG treatment for short and long term on the semen quality of adult male rats. Twelve male adult Wistar rats with 200-300g of body weight (BW) and 3-4 month of age were used in this study. The animals were divided randomly into 3 groups. M0 was used as a control, M1 and M2 were given with MSG 4mg/gBW for 15 and 45 days respectively. The experimental animals were sacrificed on the days 16<sup>th</sup> (to M1 group) and 46<sup>th</sup> (to M0 and M2 groups). The epididymis was isolated and semen quality (motility, viability, concentration, and abnormality of sperm) was evaluated. The results showed motility and concentration of M1 and M2 were not significantly decreased compared to M0. MSG treatment also significantly reduced viability and increased abnormality of sperm. Analysis of sperm abnormality character shows that the use of long-term MSG caused a formation of the primary abnormality (round and double head sperm) and increased the secondary abnormality (bent neck, curve tail, coiled tail, headless, and tailless) compared to control. Conclusion, semen quality decreases with consumed MSG for the long term. For this reason, reconsidering the use of MSG as an enhancer for the taste of food is very important.

**Keywords:** epididymis, Monosodium L-Glutamate, semen quality.

### INTRODUCTION

Monosodium L-Glutamate (MSG) is a white crystal-like substance that contains 78% of glutamic acid, 22% of sodium and water [1]. The Majority, MSG used as a food additive in everyday life. MSG can increase the taste of *umami* in food and stimulated of appetite [2]. The production of MSG with the conventional method was made by the molasses fermentation process of *Micrococcus glutamicus* bacteria [3].

Glutamate given orally into the body will be absorbed by the stomach and small intestine. Then, glutamate entered the bloodstream and dissociated into sodium and L-glutamate [4]. L-glutamate then binds to the NMDA (N-Methyl-D-Aspartate) receptor in the testes. The bond between L-glutamate and NMDA receptor can open Ca<sup>2+</sup> ion channels which cause Ca<sup>2+</sup> influx into the intracellular and activate enzymes such as phospholipase and protein kinase. These enzymes lead to the degradation of proteins and membranes [5]. The degradation process of proteins and membranes is accompanied by the release of free radicals in the form of superoxide radical (O<sub>2</sub><sup>-</sup>). Superoxide radical (O<sub>2</sub><sup>-</sup>) converted to Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) by the enzyme

Superoxide Dismutase (SOD). H<sub>2</sub>O<sub>2</sub> will react with Fe<sup>2+</sup> through an Fenton reaction to form Hydroxy Radical (OH<sup>-</sup>) and lipid peroxidation [6]. The existence of Hydroxy Radical (OH<sup>-</sup>) and lipid peroxidation leads transformation and fragmentation of sperm DNA, necrosis in cells, increasing abnormal of sperm, decreased sperm count [7].

Treatment of MSG at 4mg.g BW<sup>-1</sup> in rats for 14 and 28 days induced alteration of sperm function in testes include significantly decrease of conversion of spermatogonia to primary spermatocytes and increase the number of inactive spermatogonia compared to controls [8]. Moreover MSG treatment in longterm can cause formation of Reactive Oxygen Species (ROS) [9]. ROS significantly promote in the level of sperm abnormality including abnormal head, normal sperm without the head, normal sperm without the tail (headless), and head without tail (tailless) [10].

In this research, we analyzed the toxic effects of treatment MSG 4mg.gBW<sup>-1</sup> for 15 and 45 days on the semen quality in adult male rats. This study differs from previous studies because it uses two different duration of MSG treatment. The duration treatments are short term for 15 days and long term for 45 days. In addition, sperm abnormality parameters in this study are presented in more detail by counting the number of cells in each type of sperm abnormality.

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## MATERIAL AND METHODS

Twelve male *Wistar* rats (3-4 months old and 200-350g body weight (BW) were obtained from the Animal Unit Laboratory of Gajahmada University, Yogyakarta, Indonesia. Experimental protocols were accepted by The Research Ethics Committee of Brawijaya University Malang, in accordance to the principles of Animal Care (No: 1067-KEP-UB).

Experimental animals were randomly divided into 3 groups (n = 4 per group). Control group (M0): fed standard dies and receiving mineral water; M1 group: given MSG 4mg.gBW<sup>-1</sup> for 15 days, and M2 group: given MSG 4mg.gBW<sup>-1</sup> for 45 days. MSG was daily administered by oral gavages. The animals were housed in conventional plastic cages measuring 39 x 30 x 12 cm (4 animals per cage) with wood shavings as bedding and maintained under standard laboratory conditions (in room temperature with a 12h/12-h light/dark cycle). Standard Food (Nova Rabbit Food, Perfect Companion Indonesia Ltd., Indonesia) and water (AQUA Golden Mississippi Indonesia Ltd.) were available ad libitum. Experimental animals were sacrificed using cervical dislocation on days 16<sup>th</sup> (M1) and 46<sup>th</sup> (M0 and M2) and the epididymis were removed and stored in buffer.

### Semen Collection and Analysis

Semen were collected from right cauda epididymis. The epididymis was placed and excised in a watch glass contain 1.5 mL of NaCl 0.9% at 37°C [11]. The motility, viability, abnormality, and concentration of sperm were examined by a light microscope at 100 and 400x magnifications.

### Sperm Motility (SM)

Total of 10µL semen were added to object glass. The movement of sperm at 5 microscope fields was observed by a light microscope at 100x magnification [12]. The percentage of sperm motility was recorded and scored by McMaster [13].

**Table 1.** Criteria of Sperm Motility Categorization by McMaster [13].

Score	Criteria
1	Very poor (0-20% progressive motile)
2	Poor (20-40% progressive motile)
3	Good (40-60% progressive motile)
4	Very good (60-80% progressive motile)
5	Excellent (80-100% progressive motile)

### Sperm Viability (SV) and Abnormality (SA)

Total of 10µL liquid semen was added to object glass and was stained with 1% eosin/5%

negrosin. Percentage of sperm viability and abnormality was examined by a light microscope at 400x magnification in 3 microscope fields (or up to 200 cells). This is the formulation [12,14]:

$$SV (\%) = \frac{\text{live sperm}}{\text{total sperm}} \times 100\%$$

$$SA (\%) = \frac{\text{abnormal sperm}}{\text{total sperm}} \times 100\%$$

#### Description:

SV = sperm viability

SA = sperm abnormality

### Sperm Concentration (SC)

The semen was diluted 50x (20µL of liquid semen was added to 980µL of fixative solution from (1:1) NaHCO<sub>3</sub> and formalin 10%). The spermatozoa were counted at 400x magnification using a Neubauer Hemocytometer. The number of sperm was calculated at 5 small chambers per sample using formulation (15):

$$SC = n \times k \times DF \times 10^4$$

#### Description:

SC = sperm concentration was expressed as 10<sup>6</sup>.mL<sup>-1</sup>,

n = number of sperm

k = number of small chamber calculated

DF = dilution factor

10<sup>4</sup> = room volume of hemocytometer

### Statistical Analysis

The data of control and experimental group were presented as mean values and Standard Error (SE). Significant differences among treatment groups was performed using one way ANOVA with SPSS 16.0 for windows program. Result of one way ANOVA analysis is significant difference at P<0.05 and the next analyzed with *Least Significant Difference* (LSD) test.

## RESULT AND DISCUSSION

The quality of semen was analyzed with the following results:

**Table 2.** The effects of MSG treatment on semen quality of the adult male rats

Semen Quality ± SE	Animal Group		
	M0	M1	M2
SM (%)	70.50 ± 6.85	55.00 ± 4.08	55.00 ± 10.61
SV (%)	76.57 ± 5.38	52.49 ± 8.59*	36.96 ± 6.75**
SA (%)	10.02 ± 1.62	32.41 ± 2.25**	37.94 ± 2.21**
SC (10 <sup>6</sup> .mL <sup>-1</sup> )	100.63 ± 5.53	79.38 ± 13.13	69.38 ± 11.79

**Notes:** SM = Sperm Motility, SV = Sperm Viability, SA = Sperm Abnormality, SC = Sperm Concentration, SE = Standard Error, M0 = Control, M1 = treatment MSG for 15 days, M2 = treatment MSG for 45 days, \*(p ≤ 0,05), \*\* (p ≤ 0,01)

### Sperm Motility

Treatment of MSG 4mg.gBW<sup>-1</sup> once daily for 15 days (M1) and 45 days (M2) no significantly reduced motility of sperm from 70.50 to 55% as compared to control group (Table 2). Although, there were no significant differences in the sperm motility between M1 and M2 but the use of MSG short and long-term induced a decrease in the percentage of sperm motility by 15.5% under the control value.

The result showed that treatment with MSG caused a decrease in sperm motility compared to control. In rats, the sperm motility value was acceptable as normal motility over 70% [16]. Reduction of sperm motility can be affected by disrupted of the spermatogenesis in testes and sperm maturation in epididymis [17,18]. The inhibition of the process of spermatogenesis was caused by a greater extent of oxidative damage in the testis. The long term (for 30 days) of MSG exposure to male rats enhance significantly of the lipid peroxidation level of the testes. The lipid peroxidation is the generation of Reactive Oxygen Species (ROS) [9]. High level of ROS in spermatozoa significantly decreased mitochondrial function and caused Adenosine Triphosphat (ATP) depletion [18]. The ATP was required by the sperm to mobility and capacitation process [20]. Moreover, ROS can cause the disruption of membrane fluidity. So, the ATP depletion and disruption of membrane fluidity can lead to loss of sperm motility [21]. ROS can reduce sperm motility and the ability of sperm capacitation through the pathways of membrane fluidity alteration, leading to an alteration in sperm motility parameters [22].

### Sperm Viability

The sperm viability on male rats of M0 group was reduced significantly after MSG treatment (M1 and M2 groups). The highest of sperm viability was found in M0 group and the lowest in the M2 group with a reduction by 39.61% (Table 1). The treatment of MSG at longer term for 45 days caused a very significantly reduced of the sperm viability from adult male rats.

The consumption of MSG-induced the destroyer of a lipid membrane by elevated ROS [23]. The lipid membrane was composed of abundant Polyunsaturated fatty acids (PUFAs) which are the targets of ROS. ROS steal electrons from PUFAs of a cell membrane and initiating lipid peroxidation. Peroxidation of PUFAs in sperm cell membranes can give to cell dysfunction with loss of membrane function and

integrity [24]. Degenerative of sperm membranes can cause reducing of membrane fluidity which makes a sperm die quickly and decrease of sperm viability [18].

### Sperm Abnormality

The orally given of MSG for 15 and 45 days both induced a significant elevated of the sperm abnormality over the control group values (22.39 and 27.92%, respectively) (Table 1). The percentage of sperm abnormality (both primary and secondary abnormal) was very significantly increased ( $p \leq 0.01$ ) in the MSG treatment group (M1 and M2) compared to the control group (Figure 1). Primary abnormal only occurred in the M2 group at 0.47%. The highest enhancement of secondary abnormal occurred in the M2 group (27.44%), followed by the M1 group (22.39%).

Sperm abnormality was divided into primary and secondary abnormality [25]. Primary abnormality is abnormality occurring during the spermatogenesis process in testes such as double head, double tail, round head, rudimentary tail and droplet cytoplasm [26]. Secondary abnormality arises during epididymal maturation, transit or ejaculation of sperm such as the bent neck, bent tail, curved tail, headless (normal sperm without head), tailless (normal sperm without tail), and coiled tail [10].

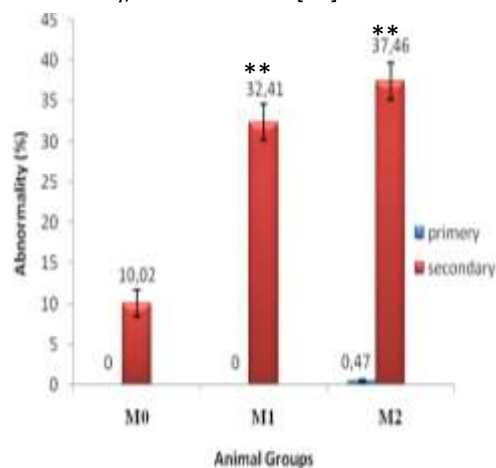


Figure 1. Effect of MSG treatment in the type of sperm abnormality. M0 (Control), M1 (treatment MSG for 15 days), M2 (treatment MSG for 45 days)

The primary abnormal was only found in the M2 group with double and round head characters (Table 3). The secondary abnormal types found in all type abnormal include the headless, bent neck, curved tail, coiled tail and tailless, while coiled tail in M2 group only. The bent neck character about 121 cells (M1 group) and 161 cells (M2 group) was the most significant

increase as compared control. It is showed that consumption of MSG at 4 mg.gBW<sup>-1</sup> dose can cause an increase of secondary abnormality and on long-term administrated of MSG lead to the formation of primary abnormality such as the double and round head characters.

**Table 3.** Character of sperm abnormality in male rats which treated 4mg.gBW<sup>-1</sup> MSG.

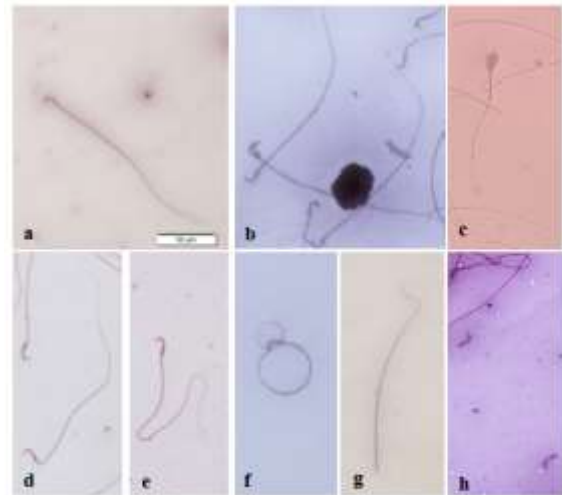
AT	AP	Character	M0	M1	M2
Primary	Head	Double Head			1
		Round Head			4
Secondary	Head	Headless	52	70	71
	Mid piece	Bent neck	32	153	193
		Coiled-tail			10
	Tail	Curved tail	12	64	56
		Tailless	24	86	72
	<b>Total number of normal sperm</b>			1097	797
<b>Total number of abnormal sperm</b>			120	373	407

**Notes:** AT = Abnormal Type, AP = Abnormal Position, M0 = Control, M1 = treatment MSG for 15 days, M2 = treatment MSG for 45 days.

The characteristics of sperm abnormality that occur in this research include double head, round head, bent neck, curved tail, coiled-tail, headless, and tailless. Abnormal sperm has different forms compared to normal sperm (Fig. 2). The normal sperm has a curved pointed head shape like a hook with a long straight tail (Fig. 2a). Double head sperm has 2 heads attached to one tail (Fig. 2b). The round head sperm type has a rounded head shape, not like a hook (Fig. 2c). Bent neck sperm has bent form in midpiece of sperm so the head point to approach the tail (Fig. 2d). Curved tail sperm has unstraight of the tail (Fig. 1e), the contrast to coiled-tail form has a circular tail (Fig. 2f). The headless character is normal sperm without a head (Fig. 2g), while the tailless is normal sperm without a tail (Fig. 2h).

High level of glutamate in the body can increased intracellular calcium level and cause the excessive influx of calcium ions into the mitochondria. It consequently increases the production of ROS [27]. ROS have the ability to directly attack of the purine and pyrimidine bases and degradation of sperm DNA (DNA damage). The DNA damage occurred via cross-links, single and double-strand DNA breaks, and chromosomal rearrangements [28]. DNA damage during specific stages of spermatogenesis can cause inhibition of sperm maturation [18]. DNA fragmentation can inhibit the process of

spermiation. It will induce the formation of primary abnormality sperm [29]. While secondary abnormality also occurs because Of hyperviscosity semen in the epididymis. Hyperviscosity caused by the functional changes of the accessory gland due to a decrease in SOD [30]. So, MSG was increase abnormal of the sperm [31].



**Figure 2.** Effect administration of MSG in the character of sperm abnormality in adult male rats. Normal sperm (a), Double head (b), Round head (c), Bent neck (d), Curved tail (e), Coiled tail (f), Headless (g), and tailless (h).

### Sperm Concentration

The sperm concentration between the control and experimental group showed no significant differences ( $p > 0.05$ ). The treatment groups (M1 and M2) with orally administered of 4mg.gBW<sup>-1</sup> MSG showed a reduction of sperm concentration by 21.25% and 31.25% respectively compared the control group. The use of MSG for the long term reduce sperm concentration.

The treatment of MSG could increases of Malondialdehyde (MDA) level in testes, epididymis, and accessory gland via [27,32]. MDA is the result of lipid peroxidation process which used as indicated of ROS formation [28]. The formation of ROS initiated the impairment of mitochondrial membrane fluidity, promote a membrane fusion and then decrease of ionic channels and inactivation of membrane enzymes activity [30]. Moreover, ROS can lead to DNA mutation or fragmentation, and protein oxidation. It will cause DNA damage, cell cycle irregularity, DNA repair or replication disorder and gene mutations of cells. The abnormal cell initiated the apoptotic and necrotic process [31]. The process of apoptotic and necrotic on damaged and inactive cells causes reduced sperm production in semen [32].

## CONCLUSION

The results indicate that adult male rats treatment with the dose 4 mg.gBW<sup>-1</sup> of MSG had deterioration of semen quality. The deterioration of semen quality involves reduced sperm motility, sperm viability, and sperm concentration. Moreover, MSG consumed in the long term was significantly increased sperm abnormality especially on the number of primary abnormal. Therefore, reconsidering the use of MSG as an enhancer for the taste of food is very important.

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