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# Research Article
# Effect of Giving Traditional Drinks Kameko and Pongasih from Southeast Sulawesi on Sperm Quality of Mice (*Mus musculus*)

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## Abstract

**Background and Objective:** The traditional beverage of Southeast Sulawesi is Kameko and Pongasih, which is a traditional alcoholic beverage. One of the bad effects of alcohol on reproductive health is the occurrence of infertility. This research aimed to determine the effect of consuming traditional beverages of Kameko and Pongasih on the quality of sperm of mice (*Mus musculus*). **Materials and Methods:** This research used a true experiment. The samples were some healthy mice (*Mus musculus*) aged 6-8 weeks as much 24 tails and divided into 4 groups. On the 36th day after the treatment was completed, the mice were terminated and prepared for microscopic sperm quality analysis. The quality of sperm includes motility, viability, concentration and morphology. The data test uses the Shapiro-Wilk Test, One-way ANOVA and *post hoc* LSD Test. **Results:** Kameko traditional beverage had a significant influence on sperm quality with motility, morphology, sperm concentration ($p = 0.000$) and viability ($p = 0.001$). Pongasih traditional beverage has a significant influence on sperm quality with motility parameter, morphology, sperm concentration ($p = 0.000$) and viability ($p = 0.001$). Kameko and Pongasih traditional beverages did not have a significant difference in sperm quality with motility parameter ($p = 0.463$), viability ($p = 1.000$), morphology ($p = 0.553$) and amount of concentration ($p = 0.714$). **Conclusion:** South East Sulawesi traditional beverages Kameko and Pongasih have effects on mice's (*Mus musculus*) sperm quality, but there was no difference in the group of mice which have been given Kameko and Pongasih.

Key words: Alcohol, traditional beverage, kameko, pongasih, sperm quality, mice, infertility

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

World Health Organization (WHO) in 2014, 50.1% of the total alcohol consumed was in the form of liquor. The most consumed types of drinks were beer at 34.8%, wine at 8.0% and "other" drinks only represented 7.1%[1]. The prevalence of alcohol drinkers in Indonesia based on Riskesdas data in 2018 reached 4.6% where there was an increase in alcohol users starting at the age of 15-24 years, namely 5.5% which then increased to 6.7% at the age of 25-34 years, but then decreases with age. The prevalence of alcohol drinkers in Southeast Sulawesi Province is 15 years old, which is 13.4% in urban areas and 14.1% in rural areas. In Southeast Sulawesi Province itself, the type of beverage that is most often drunk is wine (40.3%) followed by traditional drinks (38.5%). In Kendari City 3 out of 4 drinkers (73.4%) drink wine, while 3 out of 4 drinkers (79.2%) in Muna drink traditional drinks.

Southeast Sulawesi's most well-known traditional drinks are Kameko and Pongasih. These alcoholic drinks are spread throughout the Southeast Sulawesi Region. Kameko is usually widely found in the Muna Regency Area, Pongasih is mostly found in the Konawe Regency Area. Kameko is an alcoholic beverage fermented from palm tree sap. While pongasih is a drink that comes from the fermentation process of glutinous rice. This Kameko and Pongasih are often consumed by the community at traditional ceremonies as a tradition.

Alcohol use causes a decrease in the activity of enzymes that play a role in the synthesis of male reproductive hormones[2]. Alcohol also causes the hypothalamus and pituitary to fail to secrete GnRH (Gonadotrophin Releasing Hormone), FSH (Follicle Stimulating Hormone) and LH (Luteinizing Hormone)[3]. A decrease in GnRH will decrease the secretion of LH and FSH[4]. The function of FSH as a maintainer of the process of spermatogenesis through Sertoli cells and LH in Leydig cells both in growth and its function in secreting the hormone testosterone is also disturbed due to the influence of alcohol[5]. Delayed puberty, testicular atrophy, erectile dysfunction, gynecomastia, impaired spermatogenesis and infertility can also occur due to the negative influence of alcoholic beverages[6].

Alcohol is metabolized in the body to acetic acid. Alcohol can produce free radicals when consumed in excess. This is because alcohol metabolism by the Microsome Ethanol-Oxidising System (MEOS) will involve cytochrome P450 and CYP2E1 enzymes, in this process will produce molecules that become the forerunners of the formation of free radicals, namely Superoxide (O.2), Hydroxyl Radical (OH), Hydrogen Peroxide ($H_2O_2$)[7]. The catalase process also plays a role in the formation of oxidative stress. Alcohol directly damages the function of Superoxide Dismutase (SOD) so that the product of alcohol metabolism in the form of Superoxide Dismutation cannot be catalyzed into $O_2$ and $H_2O_2$[8]. So the molecules will continue to form. The ROS (reactive oxygen system) will bind to hydrogen atoms from other molecules, thus turning these molecules into highly active free radicals. If free radicals are in the body, it will harm reproductive health. This is thought to involve the function of the hypothalamus in secreting the hormone GnRH and the direct toxic effect of alcohol that interferes with the work of Leydig cells[8].

Clinical research proves about 50-80 million couples experience infertility in the world. Infertility in developing countries is higher, which is around 30%, compared to developed countries which is only 5-8%[9]. The prevalence of infertility in Asia is 30.8% in Cambodia, 10% in Kazakhstan, 43.7% in Turkmenistan and 21.3% in Indonesia[10]. In Indonesia, 10-20% of the 2 million patients experience infertility[11,12].

Based on risk factors, it shows the incidence of delay in conception (infertility) in married couples. Men who consume large amounts of alcohol are associated with low levels of the hormone testosterone, which will certainly interfere with sperm growth[13]. Marijuana is also known as one of the causes of impaired sperm growth, so stopping the use of marijuana and alcohol is a preventive measure for infertility[14]. Meanwhile, in other studies, it is stated that there is no relationship between alcohol consumption and damage to sperm quality which can lead to infertility, but this is still a problem by several researchers from the UK[15].

Therefore, as has been explained that the impact of alcohol consumption, especially ethanol, greatly affects reproductive health which can lead to infertility and some adverse side effects for the body. So the author wants to do a special study on the effect of Kameko and Pongasih on reproductive health by looking at sperm quality.

## MATERIALS AND METHODS

This research is a true experiment with a post-test under only a control group design. The samples were 24 adult male mice (*Mus musculus* L.) aged 6-8 weeks with a body weight of 20-30 kilograms. Mice were divided into 4 groups, namely the control group (K), ethanol treatment group ($P_1$), Kameko ($P_2$) and Pongasih ($P_3$). This research was conducted in the laboratory of the Faculty of Medicine, Universitas Halu Oleo, Kendari, Indonesia, from January to April, 2022.

**Experimental animal adaptation:** Experimental animals were adapted in the Experimental Animal Laboratory, Faculty of Medicine, the University of Halu Oleo for 7 days with food

and drink. The food given was 8 g/day and 8 mL of drink per day for each mouse. These experimental animals were placed in cages where each cage consisted of 1 adult male mouse.

**Research implementation**

**Giving treatment:** Experimental animals were given treatment in each group, namely the administration of ethanol ($P_1$), Pongasih ($P_2$) and Kameko ($P_3$).

**Mice surgery:** After 35 days of treatment, each mouse before surgery was terminated and then the surgical process was carried out, taking the testes and epididymis of mice. Both organs were placed in a petri dish containing 0.9% NaCl. Furthermore, the cauda epididymis was cut into small pieces until smooth while the cauda was pressed slowly until the secretion or epididymal fluid came out and stirred to obtain a homogeneous suspension of spermatozoa. The suspension obtained was analyzed microscopically at 37°C and the specimen was immediately examined no later than 60 min postoperatively.

**Spermatozoa motility test:** A 10 L sperm suspension was placed in an object glass and then closed using a cover glass after which it was observed under a microscope with a magnification of 10×40. The examination was carried out by observing in one field of view, then the ratio in percent was estimated between the spermatozoa with good, poor and advanced motility which is not good. The following motility categories are based on A (not moving), (B) fast-moving, (C) slow-moving and D (moving in place/dead). The percentage of motile spermatozoa was determined by adding up categories B and C divided by the total number of categories multiplied by 100%. With the criteria of normal value of good motility >50% and abnormal value of good motility 50%.

**Spermatozoa viability test:** The spermatozoa suspension was pipetted as much as 10 L, then placed on a slide, then added 10 L of 1% Eosin Y solution, then covered with a cover glass and observed under a microscope with 400 times magnification. The live spermatozoa are colourless, while the dead are red and the yield is in percentage form. The percentage of viable spermatozoa viability was calculated by dividing the number of dead sperm cells divided by the number of cells observed multiplied by 100%. The criteria for normal values are 75% live sperm and abnormal values <75% live sperm. Normal value criteria: 20 million/mL and abnormal value: <20 million/mL.

**Examination of the number of spermatozoa concentrations:** First, the sperm must be diluted before examination in the counting chamber. The dilution was carried out according to the number of sperm in the visual field. By using a leukocyte thoma pipette, for the 20 times dilution, sperm was sucked up to the 0.5 mark and then Eosin Y solution (dissolved in 100 mL of distilled water) was sucked up to 11. Then shake the pipette vertically to make the mixture homogeneous, then shake the pipette according to the number eight (shake several times until the mixture is completely homogeneous). For examination in the counting chamber (Hemocytometer Improved Neubauer), the first drop is discarded. Next, put the drops in the counting room and wait a few minutes until all the counting rooms are filled with a mixture of sperm. Examination using a microscope magnification 400 or 450 times. If N (number of spermatozoa) is obtained in this middlebox, then the concentration was N x 200,000/mL (if the dilution is 20 times) and Nx 100,000/mL (if the dilution is 10 times). Normal value criteria: 20 million/mL and abnormal value: < 20 million/mL.

**Spermatozoa morphology examination:** The morphological examination can be observed on the smear preparation by dripping one drop of 10 L of sperm suspension on the object glass slightly to the edge. After making the sperm smear, the preparation was dried in air for 5 min. Then the preparation was fixed with 96% methanol for 10 min and dried again. Then drip 5% Giemsa solution, let stand for 30 min, then rinsed with distilled water. It was left to dry and observed for morphological abnormalities with a microscope with 1000 times magnification. Observation results are expressed in percentages. Criteria for normal value 30% and abnormal value: <30%.

**Statistical analysis:** Statistical analysis using One-way ANOVA Test and *post hoc* Test.

## RESULTS

Based on the results of the normality test with the weight measurement data of mice before and after treatment using the Shapiro-Wilk normality test, it was assumed that the data were normally distributed with a p>0.05 (Table 1). This indicates that the data has a significant difference due to differences in the treatment of the mice sample so the data produced by each treatment group was significantly different. However, the weight normality test per treatment group (not the weight of all treatments) obtained data that were normally

Table 1: Normality test of mice body weight measurement before and after treatment

| | Mice body weight (g) | | | |
|---|---|---|---|---|
| Groups | Before±SD | p-value | After±SD | p-value |
| K | 22.89±1.38 | 0.895 | 33.96±2.4 | 0.292 |
| $P_1$ | 21.89±1.14 | 0.377 | 37.04±3.42 | 0.780 |
| $P_2$ | 22.76±1.55 | 0.830 | 36.92±4.64 | 0.408 |
| $P_3$ | 22.01±1.25 | 0.614 | 35.43±3.44 | 0.978 |

Table 2: Normality test for sperm quality of adult male mice

| | Sperm quality | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Motility | | | | Viability | | | | Concentration | | | | Morphology | | | |
| Groups | N | % | T | % | N | % | T | % | N | % | T | % | N | % | T | % |
| K | 6 | 100 | 0 | 0 | 6 | 100 | 0 | 0 | 6 | 100 | 0 | 0 | 6 | 100 | 0 | 0 |
| $P_1$ | 0 | 0 | 6 | 100 | 0 | 0 | 6 | 100 | 0 | 0 | 6 | 100 | 0 | 0 | 6 | 100 |
| $P_2$ | 0 | 0 | 6 | 100 | 0 | 0 | 6 | 100 | 0 | 0 | 6 | 100 | 0 | 0 | 6 | 100 |
| $P_3$ | 0 | 0 | 6 | 100 | 0 | 0 | 6 | 100 | 0 | 0 | 6 | 100 | 0 | 0 | 6 | 100 |

distributed. And the results of the normality test which included sperm analysis based on parameters, namely motility, viability, total concentration and sperm morphology in mice, obtained data with a normal distribution with a percentage of 100% (Table 2).

**Effect of giving traditional kameko drinks on the quality of mice's sperm:** There was an influence of traditional alcoholic beverages from Southeast Sulawesi Kameko on sperm quality with parameters of motility, viability, morphology and total sperm concentration in the control group, with the One-way ANOVA Test, the p = 0.000 (p<0.05) means that H0 was rejected, then followed by the *post hoc* Test, then a constant value was obtained where the comparison between the group of mice without treatment and the group given Kameko with parameters of motility, morphology, total sperm concentration obtained p = 0.000 and viability p = 0.001 (p<0.05) (Table 3). This means that there was an effect of giving Kameko on the sperm quality of mice. In the Kameko group and the ethanol group, a constant value was obtained with the motility parameter p = 0.033, viability p = 0.122, morphology p = 0.869 and total sperm concentration p = 0.160 (p>0.05) (Table 3), so that, H0 was accepted and there was no difference in the administration of Kameko on quality. Sperm in mice given ethanol motility, morphology and total sperm concentration obtained p = 0.000 and viability p = 0.001 (p<0.05) (Table 3), so H0 was rejected and there was an effect of giving pongasih on the sperm quality of mice. The ethanol group and the untreated mice group with parameters of motility, viability, morphology and total sperm concentration obtained a constant value of p = 0.000 (p<0.05) (Table 3), so H0 was rejected and found. For the pongasih group and the ethanol group, a constant value was obtained with motility parameters p = 0.138, viability p = 0.122, morphology p = 0.450 and total sperm concentration p = 0.290 (p>0.05) (Table 3) so that H0 was accepted and there was no difference in the administration of Kameko on quality of sperm in ethanol-treated mice.

**Effect of giving pongasih traditional drinks on sperm quality of mice (*Mus musculus*):** The influence of traditional Southeast Sulawesi alcoholic beverages Pongasih on sperm quality with parameters motility, viability, morphology and total sperm concentration in the control group, with the One-way ANOVA Test, obtained p = 0.000 (p<0.05), then a constant value was obtained where the comparison between the control group and the group that was given pongasih with parameters of motility, morphology, total sperm concentration obtained p = 0.000 and viability p = 0.001 (p<0.05) (Table 4) so that H0 was rejected and there is an effect of giving pongasih on the sperm quality of mice. The ethanol group and the untreated mice group with parameters of motility, viability, morphology and total sperm concentration obtained a constant value of p = 0.000 (p<0.05) (Table 4), so H0 was rejected and found. For the pongasih group and the ethanol group, a constant value was obtained with motility parameters p = 0.138, viability p = 0.122, morphology p = 0.450 and total sperm concentration p = 0.290 (p>0.05) (Table 4) so that H0 was accepted and there was no difference in the administration of Kameko on quality of sperm in ethanol-treated mice).

**Difference between giving Kameko and Pongasih to the quality of sperm of mice (*Mus musculus*):** The difference between traditional alcoholic drinks from Southeast Sulawesi, Kameko and Pongasih on sperm quality with parameters of motility, viability, morphology and total sperm concentration

Table 3: Effect of giving kameko on sperm quality in mice (*Mus musculus*)

| | Sperm quality parameters (p-value) | | | |
|---|---|---|---|---|
| Groups | Motility | Viability | Morphology | Concentration |
| K | 0.000 | 0.001 | 0.000 | 0.000 |
| $P_1$ | 0.033 | 0.122 | 0.869 | 0.160 |
| $P_2$ | 0.000 | 0.001 | 0.000 | 0.000 |

Table 4: Effect Of giving pongasih on sperm quality of mice (*Mus musculus*)

| | Sperm quality parameters (p-value) | | | |
|---|---|---|---|---|
| Groups | Motility | Viability | Morphology | Concentration |
| K | 0.000 | 0.001 | 0.000 | 0.000 |
| $P_1$ | 0.138 | 0.122 | 0.450 | 0.290 |
| $P_3$ | 0.000 | 0.001 | 0.000 | 0.000 |

Table 5: Differences in giving Kameko and Pongasih to mice sperm quality (*Mus musculus*)

| | Sperm quality parameters (p-value) | | | |
|---|---|---|---|---|
| Groups | Motility | Viability | Morphology | Concentration |
| $P_2$ | 0.463 | 1.000 | 0.553 | 0.714 |
| $P_3$ | 0.463 | 1.000 | 0.553 | 0.714 |

using the *post hoc* Test, obtained constant values, namely motility p = 0.463, viability p = 1.000, morphology p = 0.553 and total sperm concentration p = 0.714 (p>0.05) (Table 5), so that H0 is accepted, this indicates that there is no significant difference in the group of mice given Kameko and the group of mice given pongasih on the sperm quality of mice.

## DISCUSSION

The quality of the mice sperm in question is motility (sperm movement), viability (number of live sperm), total sperm concentration and normal sperm morphology (shape) observed microscopically. The samples from this study were 24 healthy adult male mice, aged 6-8 weeks weighing 20-30 g. The results of sperm analysis of adult male mice showed that in the control group, all six samples were normal. Meanwhile, in the treatment group, in this case, ethanol was given as a positive control and Kameko and Pongasih were not found to have normal sperm quality.

The results of the One-way ANOVA Test with a significance of 5% showed that all groups that had been treated with adult male mice could affect sperm quality. The univariate statistical test showed that the administration of ethanol, Kameko and Pongasih to adult male mice could affect sperm quality. This is thought to be due to a hormonal response as a form of body reaction as a result of the direct alcohol metabolism reaction to the body of the mice. In addition to suspected hormonal disturbances due to alcohol administration[16]. According to Finelli *et al.*[17] during Alcohol administration there will be a decrease in enzyme activity that plays a role in the process of spermatogenesis[17]. In alcohol metabolism, free radicals are formed. According to Asadi *et al.*[18], said that free radicals will cause defective germ cells and DNA mutations in sperm, so programmed cell death (apoptosis) will occur and as a result, will disrupt the process of spermatogenesis[18].

The results of observing and calculating the sperm motility of mice found a decrease in the percentage of sperm motility in mice after receiving treatment according to each group of mice. This result was following the statement of Adamczewska *et al.*[19] and Whirledge *et al.*[20] stated that increasing stress in mice can cause process barriers at the hypothalamic level and cause hormonal disturbances, failing Leydig cells to secrete the hormone testosterone. As a result, there is a disruption in the process of sperm maturation in the epididymis, especially disturbances in the glycolysis process. According to Mukai and Okuno[21] and Marín-Briggiler *et al.*[22], this glycolysis process will produce energy in the form of Adenosine Tri Phosphate (ATP). ATP is used by sperm as an energy source so that it can remain motile and at the same time maintain its vitality. The source of energy used in sperm motility is Adenosine Tri Phosphate (ATP). If the energy generated from the glycolysis process is low, sperm motility will decrease[23,24].

In observation of sperm that had been stained using 1% Eosin, microscopically it was found that there were live sperm that was clear white and dead sperm that were red in each treatment. This result was following the statement of Alahmar[25] which states that live sperm have an intact plasma membrane and are marked with a white head, while dead

sperm are marked by a redhead. This was thought to be due to damage to the plasma membrane of dead sperm which causes the sodium pump to no longer function properly to regulate the circulation of substances to and from the cell so that 1% Eosin dye enters the cell and remains inside and stains the sperm red. especially on the head[25].

The results of the sperm viability analysis of mice found that all samples in the control group had normal sperm viability (100%). From the observations of the treatment group on mice, it was found that the sperm viability of normal mice was not found and based on the results of the calculation of viability, it was found that the longer the administration of alcohol, the lower the percentage of viability. This was presumably due to the administration of alcohol which can cause a decrease in the FSH hormone which plays a very important role in the function of Sertoli cells, thus disrupting metabolic processes that result in sperm death. According to Gashti *et al.*[26], this was because Sertoli cells function as a regulator, supporters and protectors for developing sperm.

The results of the observation of the amount of sperm concentration studied showed that there was no normal concentration. Wang *et al.*[27], that the amount of sperm concentration produced depends on the process of spermatogenesis in the seminiferous tubules. In the samples that experienced a decrease in the number of sperm concentrations, it was suspected that it was due to the effects of stress caused by the treatment given to mice, thus activating the hormonal response which caused a decrease in the hormones LH, FSH and testosterone.

The results of the analysis of the treated group had abnormal sperm morphology. It was suspected that various disturbances of the spermatogenesis process occurred before the formation of the head, neck and tail, especially in the process of spermiogenesis in the epididymis as a result of free radicals. In the epididymis, sperm undergoes a series of morphological, functional, size, shape and ultrastructural changes based on the statement of di Guardo *et al.*[28]. The change in sperm shape was caused by a decrease in testosterone following the statement of di Guardo *et al.*[28] who say that this disorder can be caused by hormones, free radicals and chemicals. Functionally the epididymis depends on testosterone in the process of these changes so that if testosterone levels decrease, it will cause abnormal sperm morphology. This study found that there is an effect of giving traditional Southeast Sulawesi drinks Kameko and Pongasih on sperm quality. This research is a reference for the development of traditional medicine.

## CONCLUSION

There is an effective positive of giving traditional Southeast Sulawesi drinks Kameko and Pongasih on sperm quality and there is no difference in mice (*Mus musculus* L.) given Kameko and given Pongasih on sperm quality.

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