



**SURAT KETERANGAN**  
Nomor : 1881/UN3.1.6/KP/2023

Yang bertanda tangan dibawah ini :

Nama : Prof. Dr. Mustofa Helmi Effendi, drh., DTAPH  
NIP : 196201151988031002  
Pangkat/Golongan : Pembina (Gol. IV/a)  
Jabatan : Wakil Dekan III

Dengan ini menerangkan bahwa :

Nama : Dr. Erma Safitri, drh., M.Si  
NIP : 196907231999032001  
Pangkat/Golongan : Penata Tk. I (Gol. III/d)  
Jabatan : Lektor

Telah melaksanakan penelitian dengan judul sebagai berikut :

No	Judul Karya Ilmiah	Tahun Pelaksanaan Penelitian
1	Growth Improvement of Gurame Fish ( <i>Osphronemus gouramy</i> ) Due to Insulin Like Growth Factor-I (IGF-I) from Local Pregnant Mare Serum.	2020
2	Diphyllobothriasis in Cats Fed Daily with Raw 'Mujair' fish in Surabaya, East Java, Indonesia	2019
3	The Combination Effect of Probiotic Prebiotic Lactic Acid Bacteria on Efficiency of Feed Usage on Broiler Chicken	2019
4	Effect of Green Tea Extract Supplementation in the Semen Extender on Post-Thaw Sperm Quality of Simmental Bulls	2018
5	Penambahan L-Arginin dalam Pengencer Susu Skim Kuning Telur Terhadap Viabilitas dan Motilitas Spermatozoa Sapi Limousin Post Thawing pada Semen Beku.	2016
6	Pengaruh Penambahan Glukosa Sebagai Sumber Energi Terhadap Viabilitas dan Motilitas Spermatozoa Sapi Madura dalam Pengencer Susu Skim Kuning Telur	2017
7	The Effectiveness of Honey in Physiological NACI to Maintain of Viability and Motility of Spermatozoa	2017

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Surabaya, 3 April 2023

Wakil Dekan III,

Prof. Dr. Mustofa Helmi Effendi, drh., DTAPH

NIP 196201151988031002



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Website: <http://www.fkh.unair.ac.id> ; e-mail: [info@fkh.unair.ac.id](mailto:info@fkh.unair.ac.id)

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Dear Erma Safitri,  
Universitas Airlangga

Once again, I would like to congratulate you on your successful paper submission. This letter is an official confirmation that your paper

**“The Effectiveness of Honey in Physiological NACL in Maintain of Viability and Motility of Spermatozoa”**

is **ACCEPTED** to be presented at The **VMIC – Veterinary Medicine International Conference 2017** in Biomedical Science Oral Session 9 on Thursday 13th of July 2017 and is submitted to be published in our proceeding in **KnowledgeE** publisher.

Be sure to follow us on our social media platforms, and keep an eye on our website, to make sure you remain up to date with our upcoming conference! I would like to wish you luck with the registration procedures, as well as all the preparations for the upcoming conference to be held from the 12th to the 13th of July, 2017.

Do not hesitate to contact us with any questions or concerns. You can do this on VMIC website <http://conference.unair.ac.id/index.php/vmic/vmic1>, via mail [vmic@fkh.unair.ac.id](mailto:vmic@fkh.unair.ac.id) or by phone/Whatsapp Ms.Martia Rani Tacharina +62 87852341377 .

Kind Regards,

Chairman,



**Prof. Dr. Fedik A. Rantam, DVM**





  
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## The Veterinary Medicine International Conference (VMIC)

12-14 July 2017

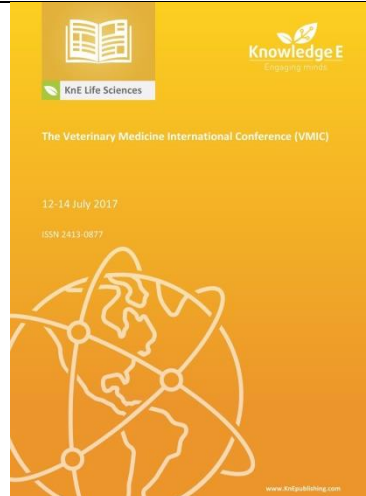
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# The Veterinary Medicine International Conference (VMIC)

	<p><b>KnE Life Sciences</b> / The Veterinary Medicine International Conference (VMIC) VMIC—The Veterinary Medicine International Conference—is one of the world's leading conference focusing on a wide array of topics including Veterinary Medicine and Biomedical Science. It offers a stimulating venue for scientists, researchers, lecturers, general practitioners, and others to broaden their social scientific network. This conference aimed at improving human and public health by improving agricultural and food systems, advancing biomedical and comparative medical research, preventing and addressing zoonotic diseases, kit diagnostic, enhancing environmental and ecosystem health, and helping manage the 21st-century public health challenges.</p> <p><b>Conference date:</b> 12–14 July 2017 <b>Location:</b> Surabaya, East Java, Indonesia <b>Editors:</b> Sri Agus Sudjarwo, Fedik A. Rantam, rer. nat. Gunawan Indrayanto, Muchammad Yunus, Rimayant, Wiwik Misaco, Tita Damayanti Lestari, Mustofa Helmi Effendi, Dikky Eka Mandala Putranto, and Shafia Khairani <b>Organizer:</b> Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia <b>Sponsors:</b> Ministry of Research, Technology and Higher Education of the Republic of Indonesia, Universitas Airlangga, ROMINDO PRIMA VETCOM, Gadjah Mada University, Bogor Agriculture Institute, ILRI (International Livestock Research Institute), Kagoshima University, Miyazaki University, Faculty of Veterinary Science, Chulalongkorn University, College of Veterinary Medicine, Tarlac Agricultural University, Yamaguchi University, Erasmus MC, Kasetsart University, Wyndham Veterinary Clinic, GERBU – Germany, USAID <b>Published:</b> 29 November 2017 <b>ISSN:</b> 2413-0877</p>
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## Conference Paper

# THE EFFECTIVENESS OF HONEY IN PHYSIOLOGICAL NaCl TO MAINTAIN OF VIABILITY AND MOTILITY OF SPERMATOZOA

Elsa Agustina<sup>1</sup>, Herry Agoes Hermadi<sup>2</sup>, Hario Puntodewo S<sup>3</sup>, Tatik Hernawati<sup>2</sup>, Indah Norma Triana<sup>2</sup>, and Erma Safitri<sup>2</sup>

<sup>1</sup>Student of Veterinary Medicine Faculty Surabaya, 60115, Indonesia

<sup>2</sup>Veterinary Reproduction Department of Veterinary Medicine Faculty, Surabaya, 60115, Indonesia

<sup>3</sup>Veterinary Public Health Department of Veterinary Medicine Faculty, Surabaya, 60115, Indonesia

## Abstract

This research was conducted to determine whether there is influence of various concentrations of mead physiological saline as diluent on the viability and motility fat-tailed sheep during storage 5°C. This research is compiled using the trial completely randomized design (CRD). Differences in the treatment of thinning the honey in physiological saline are PO (0%), P1 (0.2%), P2 (0.4%), P3 (0.6%), P4 (0.8%). The treatment stored in a refrigerator at a temperature of 5°C and then observed every 0, 24, and 48 hours. Data were analyzed by ANOVA followed by Duncan's Multiple Range Test. The conclusions obtained from this research there is the effect of adding various concentrations of NaCl physiological mead on the viability and motility fat tailed sheep during storage 5°C and conscentration mead NaCl physiological to maintain viability and motility best tail sheep are treated P3 (0.6% solution = 0.6 ml honey in 99.4 ml physiological NaCl) at the time T24 (24 hours).

**Keywords:** Fat-Tailed Sheep, Honey, Motility, Physiological NaCl, Spermatozoa, Viability.

## 1. Introduction

The efforts to increase local livestock production are necessary to support the demand for meat consumption. Fat-tailed sheep is one of the genetic resources of livestock that has the potential to be developed in the provision of meat. Sheep is one type of livestock that plays a large role in the life of farmers, because sheep are suitable for cultivation at the village level. Fat-tailed sheep have several advantages such as the ability to give birth to twins [1], fast adult age, good reproduction rate and high

Corresponding Author:

Erma Safitri

erma-s@fkh.unair.ac.id

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adaptability to the environment [2]. The population of sheep in Indonesia reaches 16,509.33 tails per year [3].

One of the many ways to increase livestock productivity is by introducing and implementing artificial insemination technology (AI). AI is a way of inserting spermatozoa into the female reproductive organs with a specific tool with the help of human, including semen collect, assessment, dilution, until the result of Artificial Insemination. AI technology in sheep and goats is still less widely applied in Indonesia. Due to various obstacles, such as timeliness, insemination techniques, and semen quality. The quality of semen is determined by several factors such as preservation techniques (storage-management) and the diluent composition used [4].

Semen dilution is an attempt to increase the volume of semen, reduce spermatozoa density and maintain the survival of spermatozoa up to a certain time under conditions of storage below or above freezing. Dilution and storage of semen is an attempt to maintain spermatozoa fertility over a longer period to extend the viability, motility, and fertility of spermatozoa [5].

Some commonly used diluents are egg yolks, milk, coconut water. Other ingredients are often used for the dilution of the semen NaCl solution. NaCl solution gives buffer properties (maintaining pH = 7) at room temperature and isotonic with liquid cell. Storage of semen with a physiologic NaCl diluent solution can only be used no more than 60 minutes after shelter because it lacks the energy source required by the spermatozoa. It is necessary to add other ingredients that provide energy or nutrients so as to prolong the viability of spermatozoa and spermatozoa motility in storage media [4].

The energy required by the spermatozoa is provided by simple sugars (monosaccharides). Honey contains simple sugars (monosaccharides) needed by spermatozoa to keep alive. Honey as an addition of energy ingredients or nutrients from physiologic NaCl diluents is expected to support the survival and movement of spermatozoa in the storage process [6].

Various studies have been conducted to determine the effect of adding honey to the diluents of various animal species such as to dilute semen etawa goat breed [7], comet fish [8], catfish [9], turkey [10]. Until now the effect of adding honey to dilute semen sheep has never been done. This study aims to determine the effect of adding various concentrations of honey in physiological NaCl to viability and motility of fat-tailed sheep spermatozoa during storage of 5 °C.



## 2. Materials and Methods

### 2.1. Design Research

The diluent used in this study was the honey diluent in physiological NaCl with various concentrations including P<sub>0</sub> (0%), P<sub>1</sub> (0.2%), P<sub>2</sub> (0.4%), P<sub>3</sub> (0.6%), and P<sub>4</sub> (0.8%) are repeated 4 times. Concentration of diluent solution in% units equivalent to ml / 100ml: P<sub>0</sub> (control) = 0 ml of honey in 100 NaCl Physiological; P<sub>1</sub> (0.2% solution) = 0.2 ml of honey in 99.8 ml NaCl Physiological; P<sub>2</sub> (0.4% solution) = 0.4 ml of honey in 99.6 ml NaCl Physiological; P<sub>3</sub> (0.6% solution) = 0.6 ml of honey in 99.4 ml NaCl Physiological; P<sub>4</sub> (0.8% solution) = 0.8 ml of honey in 99.2 ml NaCl Physiological.

Spermatozoa are collected using an artificial vagina. Spermatozoa that have been accommodated macroscopic and microscopic examination. Macroscopic observations were observed in volume, color, odor and pH of spermatozoa. Microscopic observations include determination of spermatozoa concentration, determination of live percentage (viability) of spermatozoa and motility of spermatozoa. Sperm dilution is done by using sperm ratio: diluent = 1: 9. Observations in every 0, 24, and 48 hours.

### 2.2. Design Research and Data Analysis

The design used Completely Randomized Design (RAL) with five treatments with four repetitions. The data obtained were analyzed using ANOVA (Analysis of Variant). If there is a significant effect then proceed with Duncan's test with a significant level of 5% [11].

## 3. Results

### 3.1. Fresh Sperm Quality of Fat-tailed sheep

Initial inspection of fresh semen quality is very important to know as the determination of semen feasibility to be processed further. The sheep semen to be diluted is first examined macroscopically and microscopically. Macroscopic examination results obtained an average volume of 0.8 ml of semen with a dense white color, the distinctive smell of sheep semen, semen pH 7 and the consistency of viscous semen. Microscopic examination of fresh sperm of fat-tailed sheep obtained average semen concentration of 1.380 million / MI (10<sup>6</sup>), with 95% spermatozoa viability, progressive



**Figure 1:** Test of Spermatozoa Viability (400X magnification). Live spermatozoa with white head (a) and dead spermatozoa with red head (b).

sperm motility (+++), and individual motility 80%. Based on the results of macroscopic and microscopic fresh sperm fat tailed sheep are still worthy of further research.

### 3.2. Spermatozoa Viability of Fat-tailed sheep

The percentage of viability is determined by the absorption of the eosin dye mixed in the sperm. Spermatozoa is calculated by calculating transparent (colorless) spermatozoa in the staining process with eosin-negrosin. The living spermatozoa will not be colored by the dye, while the dead spermatozoa on the head will be red-purple. Examination was observed using a microscope with 400X magnification. A total of 4 times a field of view.

The result of statistical analysis from variance analysis shows that the value of F test sig for diluent solution, storage time and also interaction between dilution solution and storage time is 0.00, because it is smaller than sig level. 0.05, it can be said that the effect of dilution dilution interaction and real time observation on viability of spermatozoa. Furthermore, Duncan Distance Test is used to find out which interactions provide the highest percentage of motility during storage.

The study showed that all parameters of semen quality observed showed a significant decrease in the quality of semen ( $p < 0,05$ ) after storage of  $T_0$ ,  $T_{24}$ , and  $T_{48}$ . This situation indicates that during processing and storage there are physical and biochemical changes of the spermatozoa used. The process of storing spermatozoa at 5°C causes a decrease in viability, because during storage at 5°C the spermatozoa retain metabolism to maintain life against cold. The metabolism of spermatozoa will produce a by-product of lactic acid which will be toxic to spermatozoa [12].

TABLE 1: Mean and Standard Deviation Percentage of Spermatozoa Viability of Fat-tailed Sheep.

Jam Ke-	Mean and Standard Deviation				
	Po	P1	P2	P3	P4
Ho	86.25 <sup>a</sup> ± 1.50	84.75 <sup>a</sup> ±3.68	85.50 <sup>a</sup> ±1.00	87.00 <sup>a</sup> ± 1.41	85.50 <sup>a</sup> ±1.00
H24	7.50 <sup>a</sup> ± 5.00	40.00 <sup>b</sup> ±8.16	48.25 <sup>c</sup> ±2.36	55.50 <sup>c</sup> ±3.31	53.75 <sup>c</sup> ±4.78
H48	0.00 <sup>a</sup> ± 0.00	5.00 <sup>a</sup> ±4.08	20.00 <sup>b</sup> ±7.07	27.50 <sup>b</sup> ± 6.45	22.50 <sup>b</sup> ±5.00

Different superscript in the same column shows a marked difference (p <0.05).

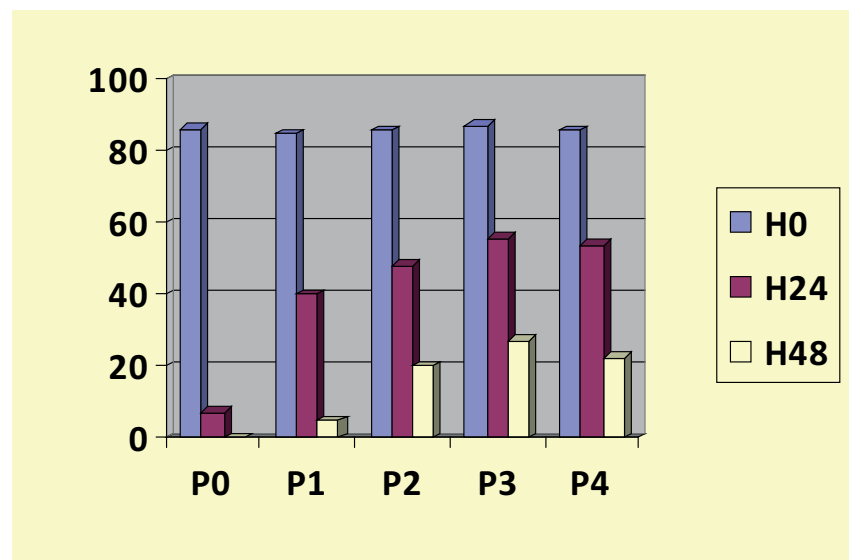


Figure 2: Mean and Standard Deviation Percentage of Spermatozoa Viability of Fat-tailed Sheep.

### 3.3. Spermatozoa Motility of Fat-tailed Sheep

The percentage of motility is a representation of progressive spermatozoa activity (forward motion). The ultimate goal of dilution is that it can be used for artificial insemination activities, the progressive spermatozoa becomes an absolute standard [13]. This examination was observed using a microscope with 400X magnification. This test is focused on observing the percentage of the number of active spermatozoa in each field of view. Observation of sperm motility is determined by the number of spermatozoa moving from a field of view and percentage.

Data Tables and figures show that on observation of To (clock o) there is no significant difference between treatment Po (83.25%), P1 (81.25%), P2 (82.50%), P3 (83.00%), and P4 (82.00%). Observation of T24 (24th hour) there was a significant difference between treatments (p <0.05). The highest motility percentage for T24 was obtained at treatment P4 (55%) which was significantly different with Po (3.75%). Observation of T48 (48th hour) there was a significant difference between treatments

TABLE 2: Mean and Standard Deviation of Spermatozoa Motility Percentage of Fat-tailed Sheep.

Jam Ke-	Mean and Standard Deviation				
	Po	P1	P2	P3	P4
Ho	83.25 <sup>a</sup> ± 3.94	81.25 <sup>a</sup> ± 2.50	82.50 <sup>a</sup> ± 2.88	83.00 <sup>a</sup> ± 3.55	82.00 <sup>a</sup> ± 4.00
H24	3.75 <sup>a</sup> ± 2.50	20.00 <sup>b</sup> ± 4.08	45.00 <sup>c</sup> ± 4.08	51.25 <sup>c</sup> ± 6.29	55.00 <sup>cd</sup> ± 7.07
H48	0.00 <sup>a</sup> ± 0.00	18.75 <sup>b</sup> ± 2.09	20.00 <sup>b</sup> ± 4.08	31.25 <sup>bc</sup> ± 2.50	36.25 <sup>c</sup> ± 7.50

*Superscript yang berbeda pada kolom yang sama menunjukkan perbedaan yang nyata (p < 0,05).*

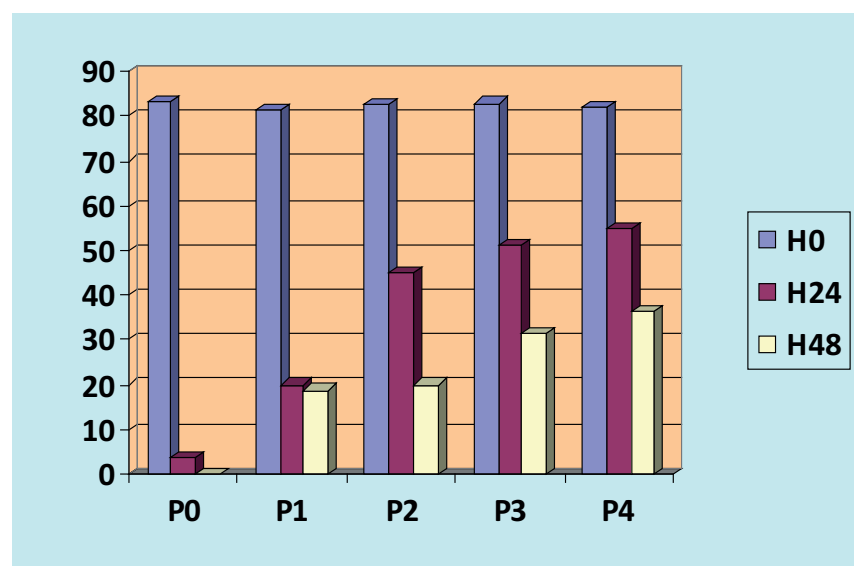


Figure 3: Mean and Standard Deviation of Spermatozoa Motility Percentage of Fat-tailed Sheep.

(p < 0.05), with the highest percentage at P4 (36.25%) which was not significantly different with P3 (31.25%), but significantly different P2 (20.00%) and P1 (18.75%). At T48 the treatment of Po (control) had no motility.

#### 4. Discussion

The percentage of viability of spermatozoa on observation can still last up to 48 hours, but the result of viability at T48 is 40% observation so as not to meet the minimum standard of viability for AI. According to [14] the survival spermatozoa viability test is 50% minimum for AI. Spermatozoa are able to live after exit from the testes only in the range of 1-2 hours [15]. The average percentage of Fat-tailed sheep spermatozoa viability in this study had a live percentage of more than 50% in P3 treatment (55.5%) and P4 (53.75%) on T24 observations (Table 1). The process of spermatozoa storage is done by addition of diluent solution which can spermatozoa survive by providing

nutrients as an energy source so that spermatozoa functionality can be maintained [16] (Sunarma et al., 2007). The main ingredient used by spermatozoa as a source of energy from outside the testes is fructose that is converted into lactic acid and energy with the help of the fructolysin enzyme in the process of glycolysis [12]. Reference Labetubun and Shiva, (2011) states that reducing sugar can be metabolized by spermatozoa to produce energy in the form of ATP. Furthermore, spermatozoa utilize ATP as a source of energy in maintaining viability [17].

The good spermatozoa motility is seen by looking at the progressive motion of the spermatozoa. The ability of the spermatozoa propels itself forward because of the contractile substance in the central part of the spermatozoa which is passed on to the rest of the tail. Normal spermatozoa motility shows spermatozoa movements coming forward simultaneously caused by a tail motion that leads left and right. Rapid and powerful tail movements will be able to push the spermatozoa into the egg for fertilization or fertilization [18].

The storage of spermatozoa after dilution is carried out at 5°C, intended for spermatozoa metabolism to be minimized thus saving energy. The goal is to minimize spermatozoa metabolism so that when used spermatozoa still have enough energy to support during the trip within the female reproductive tract [20]. According to Susilawato et al., 1999 quoted from [20] the decline in spermatozoa motility rate is due to the reduced spermatozoa energy due to the ongoing metabolic processes and is influenced by the dilution of semen that can cause damage to the plasma membrane. This statement is supported by Beraden and Fuquay (1984) quoted from [20] that spermatozoa motility in semen storage has decreased due to the continuous metabolic processes running during storage resulting in reduced energy, resulting in sperm motility increasingly decreasing. Metabolism continually causes the accumulation of lactic acid which causes decreased motility [21].

Conditions when spermatozoa are outside the testes, spermatozoa need nutrients to survive. This study used honey as a nutrient in spermatozoa. Honey mainly contains glucose and fructose that are used as a source of energy for the survival and motility of spermatozoa. The continued decomposition of ATP into ADP in mitochondria will produce energy for sperm motility [22].



## 5. Conclusion

Based on the research that can be concluded: There is an effect of adding various concentrations of honey solution in physiological NaCl to viability and motility of fat-tailed sheep spermatozoa during storage at 5°C and concentration of honey solution in physiological NaCl which can maintain viability and motility of fat-tailed sheep spermatozoa. The best treatment was P<sub>3</sub> (0.6% soluble = 0.6 ml of honey in 99.4 ml of physiological NaCl) at the time of T<sub>24</sub> (24h).

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