FINAL RESEARCH REPORT

INTERNATIONAL RESEARCH COLLABORATION OF MERCU BUANA YOGYAKARTA UNIVERSITY , INDONESIA AND CAPIZ STATE UNIVERSITY, PHILIPINES

THE QUALITY OF POST THAWING SPERMATOZOA OF INDIGENOUS INDONESIA CATTLE STORED USING ICE CUBE AND SALT AS AN ALTERNATIVE OF LIQUID NIROGEN

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APPROVAL PAGE

FINAL RESEARCH REPORT
INTERNATIONAL RESEARCH COLLABORATION OF
MERCU BUANA YOGYAKARTA UNIVERSITY
AND CAPIZ STATE UNIVERSITY,
PHILIPPINES

Title of Research	:	**The Quality of Post Thawing Spermatozoa of Indigenous Indonesia Cattle Stored using Ice Cube and Salt as an Alternative of Liquid Nitrogen**
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Yogyakarta, 22 November 2021

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IDENTITY AND GENERAL INFORMATION

1. **Research Title:**
   The Quality of Post Thawing Spermatozoa of Indigenous Indonesia Cattle Stored Using Ice Cube and Salt as an Alternative of Liquid Nirogen

2. **Researchers**

No	Name	Position	Expertise		Institution	Alocated Time (hour/week)
1	Ir. Ajat Sudrajat, S.Pt., M.Pt., IPP.	Head	Animal Technology		UMBY	20 hour/week
2	Nurul Hidayat, S.Pt., M.Sc.	Secretary	Animal Technology		UMBY	20 hour/week
3	Ir. Nur Rasminati, MP.	Treasurer	Animal Production		UMBY	20 hour/week
4	Ir. Setyo Utomo, MP.	Member	Animal Reproduction		UMBY	20 hour/week
5	Dr. Maryneth B. Barrios	Member	Veterinary Medicine/Animal Science		CAPZU	20 hour/week
6	Leah A. Ingalla	Member	Veterinary Medicine/Animal Science		CAPZU	20 hour/week

3. **Research Object:**
   Frozen semen of Pasundan cattle (from East Java, Indonesia)

4. **Time/Period :**
   Starts : March 2021
   Ends : December 2021

5. **The Proposed Budget to Directorate General of Research and Development**
   Cost from UMBY : Rp. 30.000.000,- (Php 109.133)
   Cost from CAPZU : Rp. 91.000.000,- (Php 311.408)

6. **Research Location**
   Laboratory of BIB Lembang, Bandung

Laboratory of Livestock Reproduction and Biotechnology of UMBY

7. **Research Partner**

   Capiz State University address Iloilo East Coast-Capiz Rd, Pilar, 5804 Capiz, Philipina.

8. **The Targeted Findings (explanation, method, theory, or anticipantion contributed to the field of study)**

   Find appropriate technology that is cheap and easy to apply, especially as a replacement for liquid nitrogen so that it does not depend on imports.

9. **The Basic Contribution to the Field of Study**

   Improve the science of reproduction technology, especially in local cattle up breeding and the application of artificial insemination technology using the thawing method. Contribute to government programs in breeding and increasing the population of cattle to achieve national beef self-sufficiency and the development of local cattle throughout the archipelago.

10. **The Targeted Journals:**

    International journal reputable (scopus)

11. **Plan for Intellectual Property Right, Book, Prototype or Other Targeted Outcomes, the Targeted Year or the Completion Year :**

    It is planned that this research model/method will be in intellectual property rights after publication at the end of 2021.

CONTENTS

ABSTRACT

The main obstacle in the implementation of Artificial Insemination (AI) is the absence of cheap and effective field tools or flasks that can maintain the quality of frozen sperm during AI implementation. So far, inseminators always use flasks with liquid nitrogen to bring frozen straw into the field. So that it is higher cost and ineffective. The purpose of this study was to determine the storage time of frozen sperm in a field thermos with ice cooling and table salt in various ratios of the effect on the quality of semen from Pasundan cattle from West Java. The research will be carried out from March to August 2021 at the Balai Inseminasi Buatan (BIB) Lembang, West Java and the Laboratory of Biotechnology and Reproduction, Universitas Mercu Buana Yogyakarta**.** The research used frozen semen straws in the form of mini straw from the Pasundan cattle breed, which was produced by the BIB Lembang. The research used an experimental method with a completely randomized design with a unidirectional pattern, namely ice and salt ratio with 3 replications. T0 for control, T1 for ice cubes + 30% salt, T2 for ice cubes + 40% salt and T3 for ice cubes + 50% salt. Each treatment is stored for 0, 2, 4, 6 hours. To determine the difference in motility in the treatment of the ratio of ice and salt in a field flask, analysis of variance was carried out. If there is a difference, continue with the Duncan's Multiple Range Test (DMRT) difference test. The use of storage media filled with liquid nitrogen had a higher motility rate ($p<0.05$) compared to a mixture of ice cubes and salt (T1, T2 and T3). The use of liquid nitrogen (T0) was higher viability ($p<0.05$) compared to a mixture of ice cubes and salt (T1, T2 and T3). However, the use of ice cubes and salt at all levels (T1, T2 and T3) showed no significant difference ($p<0.05$). Viability of spermatozoa decreased by 29.62%. The results showed that the average mass movement of spermatozoa ranged between good and sufficient categories, this could occur because treatment using salt and ice caused cold shock. Extreme temperature changes that occur, namely from a temperature of -196^0C (liquid nitrogen temperature) to the temperature of the ice cube and salt treatment in the thermos, which is -15^0C, make most of the spermatoza dead. T3 shown the best rwsults for the variable motility, viability and wave motion.

Key words : Pasundan Cattle, Ice cube, Salt, Quality of spermatozoa

INTRODUCTION

Background

The development of the livestock sub-sector plays a crucial role in supplying the nutritional needs of the Indonesian people, which in 2004 amounted to more than 220 million. The provision of nutritional needs derived from livestock products such as eggs, meat, and milk has not been fully provided domestically. Some products such as meat and milk still have to import from abroad (Anonymous, 2000). The development of the integrated livestock sub-sector at this time must be directed to the orientation of agrAIusiness and agro-industry. In line with this program, the use of superior seeds is necessary to ensure business continuity, both in productivity and business sustainability. The fulfillment of community needs for quality and sustainable livestock meat is really a solution to a problem that exists both at the community level and at the national level (Anonymous, 2000).

The appearance of livestock potential is fundamentally influenced by two main factors: genetic and environmental factors, including the overall maintenance management. It is known that the environment and adequate management handling or following the needs of the livestock will not give the expected production expression (quality or quantity) if the good genetic potential of livestock does not support it. Furthermore, vice versa, if the livestock has good genetic potential, it will not be expressed optimally if the maximum environment and management do not support it. Thus, these two factors should receive equal attention in the maintenance of livestock commodities. Raising livestock with high genetic value accompanied by good management will certainly provide optimal results both in terms of production and business efficiency (Syukur, DA, 2006).

Cows are livestock that is widely known by the general public, especially in rural areas. Cows in Indonesia are generally raised by farmers traditionally (from generation to generation), so that their development is fully in the midst of farmers (Utomo and Nur R., 2012). Beef cows are one of the resources for producing food in the form of meat, which has high economic value and is important for people's lives. A group of cattle can produce various kinds of needs, especially as a food ingredient in the form of meat and other by-products such as manure, leather, etc. (Sugeng, 2002). Furthermore, it is stated that based on their origins, cows are

broadly classified into 3 groups, namely: Indicus bosses (Zebu cattle = humps), Taurus bosses (European cows), and Bos Sondaicus (bull bulls / BAIos cows).

The need for animal protein from livestock in Indonesia is 5.4 g / capita/day. Based on these needs, Indonesians must consume 9.6 kg/cap/year of meat, 3.5 kg/cap/year of eggs, and 4.6 kg/capita/year of milk. The field's real conditions show that the Indonesian people can only meet animal protein consumption from livestock, an average of 3.47 g / capita/day (Harmadji, 1999). Conservation of genetic resources needs to be carried out with two considerations, namely the increasing demand for livestock products, especially in developing countries as the FAO predicts a 2-fold increase in demand for meat in the 30 years from 2000 - to 2030 and the increasing demand for milk is more of two times, as well as the rapid reduction in almost all over the world of genetic resources (germplasm) (Subandriyo, 2006).

The implementation of AI (Artificial Insemination) in Indonesia began nationally in 1972. Through this AI activity, it is hoped that it will improve the genetic quality of local livestock breeds, so it is hoped that the cattle owned by the Indonesian people can gradually improve their genetic quality. In the development of national nurseries, the problems faced are how to increase the number of livestock breeds in good quality, the linkage and interdependence of breeding actors to provide livestock seeds in the number, type, and quality according to needs (Bahri, 2006). At the beginning of the AI implementation in Indonesia (1972-1973), using liquid semen (fresh). AI with fresh semen was felt to be very slow in development, so in 1973 with the help of frozen semen from the British and New Zealand governments, the use of frozen semen in straw (plastic straw) packaging was introduced. Since the use of frozen straws, AI development is very rapid to date, especially after the operation of BBAI in Lembang and Singosari as a producer of frozen straw, especially superior cow semen.

The main obstacle in implementing AI is the absence of cheap and effective field tools/flasks that can maintain the quality of frozen sperm in a straw when going to AI right. So far, inseminators always use flasks with liquid NITROGEN in them to bring frozen straw to the field. So that costs are higher and less effective because liquid NITROGEN is expensive and has limited availability. So it is necessary to create a tool with cheap cooling material but has the

ability to maintain the quality of frozen sperm in a relatively long time. For this reason, research activity was carried out with the title:

“The Quality of Post Thawing Spermatozoa of Indigenous Indonesia Cattle Stored using Ice CubeAnd Salt As An Alternative of Liquid Nitrogen”

Research Objectives

This sttudy to determine the length of storage for frozen sperm in a field flask with ice and salt as a cooling agent in various ratios of the effect on semen quality.

Identification of the Problems

There are various ways for inseminators to carry the frozen straws of cow semen in the field when they are going to do AI. The implementation of AI in the field must use frozen sperm in a mini straw, which has excellent sperm quality to carry out fertilization. The decline in semen quality during the freezing process is low (-196°C) in liquid nitrogen and during storage is quite high at around 50%. This is worsened by the distance that is quite far from freezing with liquid nitrogen to the place of insemination, resulting in a decreased ability to fertilize.

One of the activities of spermatozoa is influenced by the temperature factor or the temperature of the environment. The normal spermatozoa activity occurs at 37 ° C and will increase twofold at a temperature of about 46 ° C, likewise at low temperatures, and their activity will decrease (Partodihardjo, 1992). This decrease in activity is the basic idea for spermatozoa storage both in the short and long term. Long-term spermatozoa storage decreases the temperature to -196 ° C using liquid nitrogen refrigerant, and this method has been the guide for long-term sperm storage activities. Long-term sperm storage constraints using liquid nitrogen are the processing technique and the large cost required, especially with the high cost of liquid nitrogen and the high processing costs. Not everyone can do it, especially farmers.

Semen for the AI process can be divided into two, namely frozen semen and liquid semen. The AI technique with frozen semen is relatively easy to do. The equipment needed is in the form of a speculum (in the shape of a duck's beak) to open the vagina, an Artificial Insemination (AI) Gun to "shoot" the semen into the cervix, and plastic sit to place the straw (frozen semen package). The problem faced by using frozen cow semen for AI is liquid nitrogen availability in all corners of the country. Transporting frozen semen to the AI location with a

flask containing liquid nitrogen is expensive and the minimal availability of liquid NITROGEN. This constraint is the background of this research activity, which aims to create a field flask as a container for frozen straws to the AI site using simple, inexpensive, and easily available materials in the field.

This decrease in sperm quality will be followed by the failure of AI in the field, which is indicated by the high S / C value. Actually, this problem has been resolved by the presence of a field flask that uses Liquid nitrogen. However, due to the relatively high price of liquid nitrogen and limited availability, the flask is less effective and wasteful. The existence of a device with cheap materials but has a high ability to store frozen sperm when brought to the field is needed.

Research Contribution

This research will usefull to Inseminator practitioners carrying out their duties so that sperm still has the optimal ability in the fertilization process after artificial insemination. The field flask made of ice and salt will increase the success of the AI in the field.

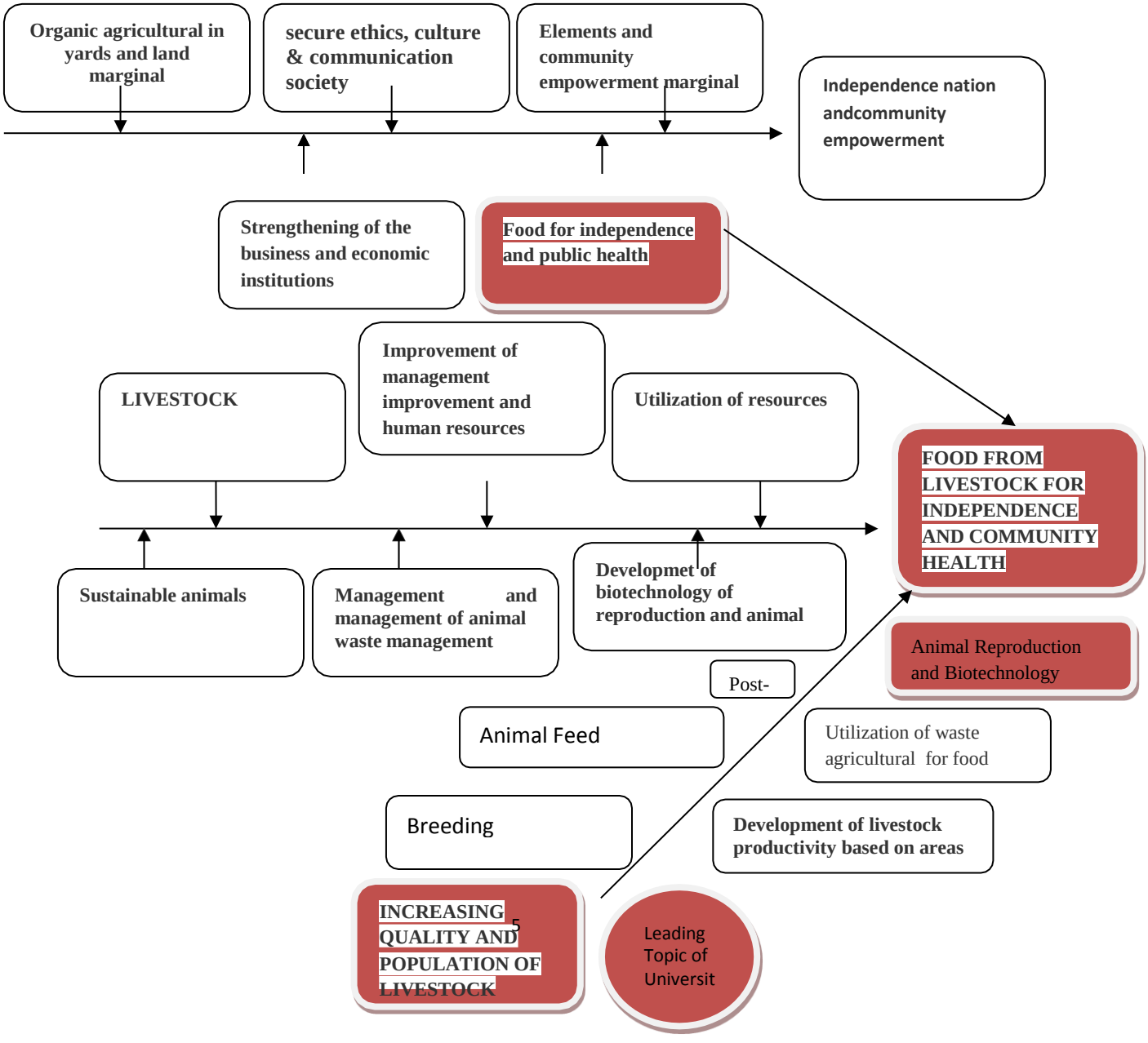
Table 1. Plan for target achievement

No	Type of output achievement		Target
	Category	Sub category	
1	Scientific article published in journal	International/reputable international	Yes
		Accredited National	No
		National ISSN	No
2	Invited speake inscientific meeting	International	No
		National	No
3	Visiting lecture	International	No
4	Hak Kekayaan Intelektual (HKI)	Paten	No
		Simple patent	No
		Copyright	No
		Trademarks	No
		Trade secrets	No
		Industrial Product Design	No
		Geographical Indications	No
		Protection of Integrated Circuit Topography	No
	Appropriate Technology		Yes
	Model / prototype / design / artwork / social engineering		Yes
	Textbook (ISBN) / report in book form		No
	Technology Readiness Level (TKT)		3

RENSTRA AND ***ROAD MAP OF*** HIGHER EDUCATION RESEARCH

This research proposal refers to the Strategic Plan (Renstra) or Research Master Plan (RIP) of Mercu Buana University, Yogyakarta 2012-2016. Non-superior research, national research, and applied research are increasingly directed within the scope of international research. UMBY's Leading Research is oriented to "national independence and people's empowerment." Following the Rector's Decree: 124 a / Sk / VI / 2012, RIP UMB Y for the years 2012 - 2016 determined 5 excellent types of research, namely; (1) food security; Food for self-reliance and public health, (2) psychology and community empowerment (3) sustainable agriculture (4) business, cooperatives and MSMEs (5) cultural and community ethics.

Furthermore, the UMBY leading research Road Map and Animal Husbandry Road Map are listed in the following fishbone description:



LITERATURE REVIEW

Cattle

cattle are widely known by the general public, especially in rural areas. Cows in Indonesia are generally raised by farmers traditionally (from generation to generation) so that their development is fully in the farmers' hands (Utomo and Nur R., 2013). cattle are one of the resources for producing food in the form of meat, which has high economic value and is important for people's lives. A group of cattle can produce various kinds of needs, especially as a food ingredient in the form of meat and other by-products such as manure, leather, etc. (Utomo and Nur R, 2012). Furthermore, it is stated that based on their origin, cows are broadly classified into 3 groups, namely: Indicus bosses (Zebu cattle = humps), Bos Taurus (European cows), and Bos Sondaicus (Bull / Bos BAIos cows).

The systematics of cattle classification, according to Reksohadiprojo (1984) and Blakely and Bade (1992), are as follows:

Kingdom : Animalia
Phyllum : Chordata
Class : Mamalia
Ordo : Ungulata
Sub Ordo : Artiodactyla
Familia : Bovidae
Sub Familia : Bovinae
Genus : Bos
Species : Bos Taurus, Bos Indicus, Bos Sondaicus.

Several tropical cattle breeds are quite popular and are widely available in Indonesia, namely Bali cattle, Madura cattle, Ongole cows, American Brahman cattle, etc. (Sugeng, 2003).

1. Bali cattle (Bos sondaicus = Bos BAIos = Banteng Wagner)

   Are native Indonesian cattle descended from Bos Banteng or from the descendants of Bos Sondaicus who have experienced domestication (Siregar, 2003). Bali cow is unique because it is the only type of bull that is bred. This breed of cattle is used as working cattle, including the type of meat with a high percentage of meat (Reksohadiprojo, 1985).

2. Madurese cattle Cattle are the result of a cross between the Sondaicus boss and the Indicus boss. Location of distrAIution in P. Madura and East Java. In Madura, these cows are kept purely. These cows include meat and work (Siregar, 2003).
3. Ongole Cattle (Bos Indicus = Nellore)
   Ongole cattle originate from the Bos Indicus descendants who entered Indonesia from the IX X century and developed well in Sumba Island. Ongole cows are often crossed with female Javanese cows, which eventually resulted in a breed of PO cattle named SO (Sumba Ongole) (Murtidjo, 1990; Siregar, 1990).
4. 4. Brahman Cattle Brahman
   Cattle are breeds of beef cattle that grow well in poor grazing areas and respond well to caged feed (Reksohadiprojo, 1985).

Frozen Cement Frozen

Semen in liquid nitrogen at a temperature of -196 ° C aims to maintain spermatozoa life by reducing metabolic activity to zero metabolic conditions (Djanuar, 1985). Furthermore, it is stated that if it is to be used for AI, it must go through a thawing process so that the results can be optimal. During the freezing process, the cow's sperm will experience an average of 50% death, so the dosage once AI must be increased 2 times compared to AI with fresh semen (Toelihere, 1985). The frozen semen process begins with cement storage using the Artificial Vagina (VB) method in general, then tests for macroscopic and microscopic quality, then calculates the concentration to determine the AI dose and the total number of spermatozoa/ml., Then dilution, glycerolization, equilAIration, filling sealing, pre-freezing and then frozen in liquid NITROGEN gradually (Hafez, 1985). The use of frozen semen in the field must go through a thawing process, which in principle is to gradually increase the temperature from -196°C to room temperature, which will then be AI in the cervix. Generally, thawing is carried out in ice water, tap water in a period of 15-35 seconds.

Characteristics of cow sperm

The volume of sperm in cows is influenced by the breed of the cow, age, and bodyweight associated with bulls' reproduction process. The average cow's sperm has a concentration of 1200 million cells/ml or 1.2 billion / ml of sperm with a range of 400 million to 2 billion

cells/ml. The mean sperm concentration of Brahman bulls was around 1.284 + 97.66. 109 cells/ml with a range of 1.283 - 2.087. 109 cells / ml, up to 1,700 + 333.84. 109 cells/ml. (Sumediana, *et al*., 2007). Sperm volume is the number of milliliters of semen per ejaculate (Toelihere, 1985). The average volume of semen produced by Brahman bulls is around 2.38 - 5.74 ml with a range of 2.60 - 10.20 ml (Sumediana *et al*., 2007). Meanwhile, Hafez (1985) made a range of semen volume between 5-8 ml/ejaculate.

Sperm Motility Sperm

Motility is a determinant of the feasAIility of sperm quality in terms of its fertilization ability. Motility after freezing at low temperatures indicates its quality in carrying out fertilization activity. Motility is used as a guide in assessing sperm quality (Nugroho, 2003). Motility of spermatozoa is used as an assessment of spermatozoa's ability to fertilize an egg, so that motility has an important role in the process of fertilizing an egg (Widiastuti, 2001).

The spermatozoa motility can be observed by dropping a drop of sperm on a glass object and then observing the individual movements under a microscope with a magnification of 400 times. Motility will be shown in the presence of a unidirectional movement, fast individual progressive movements appear. It is preferable to observe motility at 37 ° C (Djanuar, 1985). Furthermore, it is stated that motility is influenced by ambient temperature, chemicals, urine, frequency of ejaculation, and first ejaculation.

One of the activities of spermatozoa is influenced by the temperature factor or the temperature of the environment. The normal spermatozoa activity occurs at 37°C and will increase twofold at a temperature of about 46 ° C, likewise at low temperatures, and their activity will decrease (Partodihardjo, 1992). This decrease in activity is the basic idea for spermatozoa storage both in the short and long term. Long-term spermatozoa storage decreases the temperature to -196°C using liquid nitrogen refrigerant, and this method has been the guide for long-term sperm storage activities. Long-term sperm storage constraints using liquid nitrogen are the processing technique and the large cost required, especially with the high cost of liquid nitrogen and the high processing costs. Not everyone can do it, especially farmers/groups.

The insemination equipment consists of a speculum made of glass pipe *pyrex* measuring 18 cm in length with a diameter of 2 cm, one insemination pipette made of 1 ml glass, and a scale. The insemination pipette is connected with a small rubber hose and then connected to a

syringe. As a lighting device, you can use a small spotlight, a headlight, or simply a flashlight, or if you are used to it without using a lamp, AI can easily do it. Insemination should be carried out on the second day of the estrous period between 12-18 hours after the first visAIle lust. The spermatozoa that we input will survive about 20 hours in the female genital tract.

Semen Dilution

The function of diluents is to provide nutrients as a source of energy for spermatozoa, protect spermatozoa from cold shock, provide buffer material (buffer), maintain isotonic conditions, prevent sperm growth and increase volume. Whereas the requirements for sperm diluents are cheap, simple, practical to make but have high preservation power, the diluent must contain elements that are almost the same physical and chemical properties as cement. It must not contain toxic substances (poison) or good in nature. For the life of spermatozoa and ovum (not inhAIiting fertilization), it must remain isotonic, and the diluent must still ensure ease in testing the quality of semen (Feradis, 2010).

A good diluent must have isotonic osmotic pressure and be able to maintain it during storage, provide the mineral balance needed by spermatozoa, provide food for spermatozoa for their metabolic processes, have lipoproteins or lecithin to protect spermatozoa against cold shock, provide a buffer, are a source of reducing material to protect cellular enzymes containing sulfhydryl, free from germs. Diluents commonly used to dilute semen include diluent tris, lactose, and milk (Salisbury and Van Demark (1985).

Coconut water is produced in many tropical countries as a diluent for sperm, replacing milk as a diluent. Sources of food for spermatozoa are understandable, but it still needs to be studied/researched as a buffer. Coconut water can be added to egg yolk in the same ratio as in egg yolk-citrate diluent (Feradis, 2010). Apart from coconut water, there is also plenty of coconut juice, which contains sugar as a nutrition source for spermatozoa.

Egg yolks are useful for cooling sperm to close to 0C, and egg yolks will protect spermatozoa when the semen is diluted and during the process of freezing and thawing again (Salomon and Maxwell, 1995). Egg yolks contain lipoproteins and lecithin, which can coat the plasma membrane of cells to maintain da n protects the integrity of the sperm cell lipoprotein sheath and protects cold temperatures during the freezing process (Toelihere, 1985).

Sperm storage methods

In the development of national nurseries, the problem faced is how to increase the number of livestock breeds in good quantity and quality (Bahri, 2006). One of the activities of spermatozoa is influenced by the temperature factor or the temperature of the environment. The normal spermatozoa activity occurs at 37 ° C and will increase twofold at a temperature of about 46 ° C, likewise at low temperatures, and their activity will decrease (Partodihardjo, 1992). This decrease in activity is the basic idea for spermatozoa storage both in the short and long term. Long-term spermatozoa storage decreases the temperature to -196 ° C using liquid nitrogen refrigerant, and this method has been the guide for long-term sperm storage activities. Long-term sperm storage constraints using liquid nitrogen are the processing technique and the large cost required, especially with the high cost of liquid nitrogen and the high processing costs. Not everyone can do it, especially farmers/groups.

Artificial Insemination Artificial

Insemination can use fresh cement, the cement that has been diluted with a suitable diluent, or cement that has been frozen. The advantages of using frozen semen include The possAIility of semen being stored for a long time, allows selective mating anywhere and at any time, prevents sexually transmitted diseases in female animals. The number of males required is minimized.

Cement for AI can be divided into two, namely frozen cement and liquid cement. The AI technique with frozen semen is relatively easy to do. The equipment needed is in the form of a speculum (in the shape of a duck's beak) to open the vagina, an Artificial Insemination (AI) Gun to "shoot" the cement into the cervix, and plastic sit to place the straw (frozen semen package).
The problem faced by using frozen sheep semen for AI is liquid nitrogen availability in all corners of the country. This constraint is the background of the holding of this research activity, which aims to make it easier to obtain cement that can be used for AI throughout Indonesia using only diluents available in each region and using storage tools that are scattered throughout the country in the form of refrigerators (refrigerators) and easy. Implemented by every community.

The research roadmap includes determining the length of storage at 5oC as measured at 50% motility, which will then be used for AI in sheep. Whereas in the second year, all treatments will be AI in sheep based on the motility of 50% achieved at a certain time (hours) the results of the study year - 1 (first).

Furthermore, the research roadmap is as follows:

THE EFFECT OF A MIXTURE OF ICE AND KITCHEN SALT AS A PRESERVATIVE IN A FIELD QUALITY OF SPERMATOZOA FLASK ON THE FROZEN CEMENT THAWING

⇩

"THE QUALITY OF POST THAWING SPERMATOZOA OF INDIGENOUS INDONESIA AND PHILIPINES CATTLE USING ICE CUBE AND SALT AS AN ALTERNATIVE OF LIQUID NITROGEN"

MATERIALS AND METHODS

Time and Place of Research

The research will be carried out from March to August 2021 at the BIB Lembang Bandung, West Java Province and at the Laboratory of Biotechnology and Reproduction, UMBY.

Materials and Methods

Materials and tools

The research used frozen cement straws in the form of mini straw from the Pasundan cattle breed, which was produced by the BIB Lembang Beef Cattle Livestock, West Java. The research used an experimental method with a completely randomized design with a unidirectional pattern, namely ice and salt ratio with 3 replications. A total of 60 mini frozen cement straws, while the tools used include ice flasks, water jacket tubes, thermometers, microscopes, glass objects, cover glass, heating tables, and stationery.

The research method used an experimental method with the basic completely randomized design with a unidirectional pattern, namely the ratio of ice cube vs. salt. The research will be repeated 3 times (straw).

How it Works : A total of 60 mini straws of frozen semen from Pasundan cattle were distributed in 4 flasks with a capacity of 750 ml made of stainless which were filled with nitrogen as T0 (control), T1: ice cubes + 20% salt, T2: ice cubes + 25% salt and T3: ice cubes + 30% salt. The straw is put into the water jacket in a large test tube then placed in the thermos standing up. Each flask contains 15 straws, which will be checked for motility (%) every 1 hour until the 5th hour of storage. Motility examination on the heating table using a microscope with a magnification of 10 x 40 times by looking at the spermatozoa object compared to spermatozoa that are not moving or moving but not progressive forward.

The schedule for the implementation activity

This researchis planned for March to September 2020, with the following schedule. :

No	Description ofactivities	Times (Month)					
		April	May	June	July	August	September
1	administrative preparationand material tools and permits						
2	Procurement of research materials						
3	Research implementation						
4	Preparation of reports						
5	Seminar / publication						

RESULTS AND DISCUSSION

1. Post Thawing Motility (%)

Motility is the movement of spermatozoa that can be used as a reference in assessing the quality of spermatozoa for artificial insemination (Bintara, 2011). The results of the complete examination of sperm motility of Pasundan cattle and BIB production in Lembang are presented in Table 1.

Table 1. Spermatozoa motility of pasundan cattle (%).

Treatment	Storage times (hours) (%)				Average
	0	2	4	6	
T0	50.00 ±10.00	67.50 ± 2.50	58.33 ± 2.88	48.33 ± 2.88	**56.04 ± 9.13[c]**
T1	50.83 ± 10.10	30.00 ± 5.00	16.66 ± 2.88	11.66 ± 2.88	**27.29 ±16.63[a]**
T2	52.50 ± 2.50	30.00 ± 17.32	18.33 ± 2.88	13.33 ± 5.77	**28.54 ± 17.66[a]**
T3	51.66 ± 9.46	40.83 ± 2.88	28.33 ± 5.20	24.16 ± 5.20	**36.25 ± 12.40[b]**
Average	**51.25 ± 7.42[c]**	**42.08 ±17.83 [b]**	**30.41 ± 17.73[a]**	**24.37 ±24.31[a]**	

Notes:* a,b,c Different superscripts in the same line show significant differences (P<0.05).
**(T0= nitrogen, T1= ice cube + 30% salt, T2= ice cube + 40% salt, T3= ice cube + 50% salt)

The average percentage of spermatozoa motility can be seen in Table 1. Based on these results, it shows that the media and storage time have a significant effect (P<0.05) on spermatozoa motility in T0 and T3 treatments, while T1 and T2 treatments are not significantly different (P>0 ,05). The use of storage media filled with liquid nitrogen had a higher motility rate (P<0.05) compared to a mixture of ice cubes and salt (T1, T2 and T3). This is because when the straw is stored with liquid dinitrogen, the metabolism of spermatozoa cells can be said to stop, the metabolism of spermatozoa cells will increase along with the increase in temperature. Storage of semen in media with temperatures ranging from 3-4°C, metabolism is still running but

very slowly, because it is far below the physiological temperature (38°C). The longer the storage time, the more use of nutrients between spermatozoa as a result of ongoing metabolism, this causes the availability of nutrients to be depleted. A large number of spermatozoa will accelerate the use of nutrients for the purposes of spermatozoa metabolism, so that nutrients will be quickly depleted and the production of ATP (adenosine triphosphate) in the mitochondria will be inhibited so that it can affect the motility of spermatozoa (Hayati, 2011).

The results of the research on the motility of the sperm of Pasundan cattle stored in a thermos with a storage medium of a mixture of salt and ice cubes showed that the best average motility that entered the SNI standard (Indonesian National Standard) was 40.83% in the T3 treatment (ice cubes + 50% salt) for a long time. two hours of storage, while the other motility averages were still below the SNI standard or their motility was below 40% except at zero hours of storage. This is because treatment using a mixture of salt and ice cubes causes cold shock. Changes in temperature that occurred, namely from -196OC (liquid nitrogen temperature) to the temperature of ice cubes and salt treatment in the thermos, namely ± -15OC, made most of the spermatoza die and their motility decreased. According to Sonjaya et al., (2005) stated that the biggest factors that affect the decrease in sperm quality due to cold shock are temperature and pH.

Garner and Hafez (2000) stated that the motility of bovine spermatozoa ranged from 40-75%. Differences in spermatozoa motility can be caused by age in cattle, 1.5 years old has lower motility than 2 years old, this is because in 2 year old cows the primary and secondary reproductive organs are optimal (Azzahra et al., 2016). Meanwhile, according to Herdis (2005) states that the motility of spermatozoa is influenced by differences in the breed of livestock and the time of examination. Another factor that can affect motility is feed (Zulfan, 2008).

2. Viability (%)

The results of the examination of spermatozoa viability of Pasundan cattle and BIB production in Lembang are presented in full in Table 3.

Table 3. Viability (%)

Treatment	Storage times (hours) (%)				Average
	0	2	4	6	
T0	79.33 ± 2.56^{h}	74.05 ± 6.05^{gh}	71.24 ± 12.78^{gh}	67.36 ± 10.31^{gh}	**72.99 ± 8.81^{e}**
T1	72.16 ± 5.48^{gh}	58.05 ± 20.56^{gh}	52.42 ± 24.01^{g}	26.35 ± 12.72^{f}	**52.24 ± 22.73^{d}**
T2	71.50 ± 2.17^{gh}	67.66 ± 8.31^{gh}	20.17 ± 11.82^{f}	20.17 ± 11.82^{f}	**47.64 ± 25.42^{d}**
T3	58.67 ± 4.73^{gh}	65.07 ± 11.14^{gh}	29.83 ± 6.11^{f}	19.91 ± 9.25^{f}	**43.37 ± 21.01^{d}**
Average	**70.41 ± 8.48^{c}**	**66.21 ± 12.43^{c}**	**46.18 ± 22.81^{b}**	**33.44 ± 22.69^{a}**	

Notes:* a,b,c Different superscripts in the same line show significant differences (P<0.05).
**(T0= nitrogen, T1= ice cube + 30% salt, T2= ice cube + 40% salt, T3= ice cube + 50% salt)
*** a,b,c Different superscripts in the same column show significant differences (P<0.05)
d,e Different superscripts on the same line show significant differences (P<0.05)
f,g,h Different superscripts in the same column and line show significant differences (P<0.05)

The average percentage of live spermatozoa from the results of the study can be seen in Table 3. Based on these results, it shows that the media and storage time have a significant effect (P<0.05) on live spermatozoa. The use of liquid nitrogen (T0) was higher (P<0.05) compared to a mixture of ice cubes and salt (T1, T2 and T3). However, the use of ice cubes and salt at all levels (T1, T2 and T3) showed no significant difference (P<0.05). Viability of spermatozoa decreased by 29.62%. This percentage decrease occurred when using ice cubes and salt media (T1, T2 and T3). The percentage of spermatoza viability decreased due to damage to the plasma membrane and acrosome membrane due to the influence of cold shock. The temperature of the frozen cement storage media used in this study was different, namely M0 (-196 0C). This is in

accordance with Septiani et al. (2017) that the metabolic rate and motility of spermatozoa will decrease at a temperature of 3-5 0C. Viability is the vitality of spermatozoa as an indicator of spermatozoa quality. The viability of diluted frozen semen has at least 60%-75% live spermatozoa (Garner and Hafez, 2000).

The storage time of 0 and 2 hours was higher ($P<0.05$) than 4 and 6 hours. However, the storage time of 4 hours tended to be better ($P<0.05$) than 6 hours. Based on the results of the study, the length of storage time decreased the viability of spermatozoa by 36.70%. The longer the storage time, the decrease in viability is also high. Spermatozoa quality is said to be good if it has a high number of live spermatozoa and <15% dead spermatozoa (Bintara, 2011).

The results of the analysis of variance showed that there was a significant interaction between the media and storage time ($P<0.05$). This shows that the media and the length of storage time affect each other's viability. The use of M0 with a shelf-life of 0 hours showed the highest viability compared to other treatments and T3 with the lowest curing time of 6 hours ($P<0.05$). The use of T1 with a storage time of 2 hours tends to be better than T2 and T3 although it is not significant.

Likewise with the long storage time of 6 hours. The percentage of viable spermatozoa is determined by the intact plasma membrane. The plasma membrane functions to protect spermatozoa organelles and transport electrolytes for spermatozoa metabolism. Damaged plasma membrane can affect the physiological function and metabolism of spermatozoa, causing spermatozoa to die. Intact plasma membrane has a correlation with spermatozoa motility, the more intact spermatozoa plasma membrane, high motile spermatozoa (Azzahra et al., 2016). In this study, the decrease in viability occurred because the spermatozoa experienced cell damage, namely changing the structure of the plasma membrane phospholipids and disrupting the

permeability function of the cell membrane. If there is damage to the membrane, the metabolic process will be disrupted, the synthesis of Adenosine Tri Phosphate (ATP) does not work well and results in a decrease in the viability of spermatozoa.

3. Wave Motion

Spermatozoa mass movement is the active movement of spermatozoa by showing undulating and massive mass movements. The mass movement scores consist of very good (3/+++), good (2/++), moderate (1/+), and bad (-) (Tambing et al., 2001). The results of the examination of the mass movement of the spermatozoa of Pasundan cattle and BIB production in Lembang are presented in Table 2.

Table 2. Mass motion of Pasundan cattle spermatozoa.

Treatment	Storage times (hours) (%)				Average
	0	2	4	6	
T0	2.00 ± 0.00	2.16 ± 0.28	1.83 ± 0.28	1.83 ± 0.28	**1.95 ± 0.25^{b}**
T1	2.00 ± 0.00	1.16 ± 0.28	1.00 ± 0.00	1.16 ± 0.28	**1.33 ± 0.44^{a}**
T2	2.33 ± 0.28	1.33 ± 0.28	1.16 ± 0.28	1.33 ± 0.28	**1.54 ± 0.54^{a}**
T3	2.00 ± 0.50	1.66 ± 0.57	1.33 ± 0.28	1.16 ± 0.28	**1.54 ± 0.49^{a}**
Average	**2.08 ± 0.28^{b}**	**1.58 ± 0.51^{a}**	**1.33 ± 0.38^{a}**	**1.37 ± 0.37^{a}**	

Notes:* a,b,c Different superscripts in the same line show significant differences (P<0.05).
**(T0= nitrogen, T1= ice cube + 30% salt, T2= ice cube + 40% salt, T3= ice cube + 50% salt)

The results showed that the average mass movement of spermatozoa ranged between good and sufficient categories, this could occur because treatment using salt and ice caused cold shock. Extreme temperature changes that occur, namely from a temperature of -196 ^{0}C (liquid nitrogen temperature) to the temperature of the ice cube and salt treatment in the thermos, which

is ±-15 ^{0}C, make most of the spermatoza dead. The biggest exogenous factors that affect the decrease in sperm quality due to cold shock are temperature and pH (Sonjaya et al., 2005).

Cold shock that occurs due to changes in extreme temperatures and storage time in the treatment causes the metabolism of spermatozoa to run very quickly, causing the energy for locomotion to decrease and ultimately causing the spermatozoa to die. Spermatozoa will use energy for locomotion produced by accessory glands (Azzahra et al., 2016). Accessory glands will secrete plasma semen by producing several kinds of nutrients such as glucose, fructose, sucrose, citric acid, protein, potassium, sorbitol, insitol and glycerylphosphoryl-choline (GPC) which function as energy substrates for spermatozoa including sperm motility (Sujoko, 2009).

Cold shock that occurs also increases the production of lactic acid in the sperm to be high as a result of the rapid metabolic process. The high content of lactic acid in sperm will result in increased damage to the membrane that affects metabolism and the energy produced. The high amount of lactic acid will affect the increase in the osmotic pressure of the semen plasma thereby reducing the permeability of the spermatozoa membrane and increasing membrane damage. Damage to the spermatozoa membrane will affect the metabolic process and decrease the formation of energy. The reduced energy will greatly affect the activity and mobility of spermatozoa (Samsudewa et al., 2006). This is what causes the large number of dead spermatozoa. It can be concluded that the storage of spermatozoa in a thermos with salt and ice treatment was not able to maintain the mass movement of spermatozoa significantly.

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APPENDICES

Appendix 1. Support for research support facilities and infrastructure

Supporting facilities and infrastructure for research at the UMBY campus and at the CAPZU are sufficient, however, for some sample testing and preparation of research materials, the partners will collaborate with the partners while the partners are labs. Reproductive Physiology, Faculty of Animal Science UGM, BPBPTDK Yogyakarta Yogyakarta and UPTD of Ciamis Beef Cattle Breeding Development Center, West Java.

Appendix 2. Organizational structure of the research team and task development

No	Name	Position	Field of expertise	Agency of origin	Time allocation	Job Description
1	Ir. Ajat Sudrajat, S.Pt., M.Pt., IPP.	Head	Animal Technology	UMBY	20 hours / week	Carry out coordination and as the person in charge of research
2	Nurul Hidayat, S.Pt., M.Sc.	Secretary	Animal Technology	UMBY	20 hours / week	Carrying out administrative and documentation tasks as well as making reports and coordinating with the chairman and the research team
3	Ir. Nur Rasminati, MP.	Treasurer	Animal Production	UMBY	20 hours / week	Carry out financial treasury, record income and expenditure of funds and reporting
4	Ir. Setyo Utomo, MP.	Member	Animal Reproduction	UMBY	20 hours / week	Assisting the implementation of the research process
5	Dr. Maryneth B. Barrios	Member	Animal Reproduction	UMBY	20 hours / week	Assisting the implementation of the research process

6	Salvacion J. Legaspi	Member	Animal Reproduction	UMBY	20 hours / week	Assisting the implementation of the research process

ATTACHMENT

Attachment 4. Biodata of the chairman and members of the proposing team
Chief Researcher

A. Personal Identity

1.	Full name (with title)	Ir. Ajat Sudrajat, S.Pt., M.Pt., IPP.
2.	Gender	Male
3.	Functional Position of	Teaching Staff
4.	NIP / NIK / Other Identity	201739
5.	NIDN	0512059501
6.	Place and Date of Birth of	Ciamis, May 12, 1995
7.	E-mail	sudrajatajat135@mail.ugm.ac. en ajat@mercubuana-yogya.ac.id sudrajatajat135@gmail.com
8.	Telephone Number / HP	082240054084
9.	Office Address of	Animal Husbandry Study Program, Faculty of Agroindustry, University of Mercu Buana Yogyakarta Jl. Wates Km. 10 Yogyakarta 55753
10.	Telephone / Fax Number	(0274) 6498211, 6494212 / (0274) 6498213
11.	E-mail	mercubuana-yogya.ac.id
12.	Graduates who have earned	S-1 =… people; S-2 =… people; S-3 =… people
13.	Subjects taught in	1. Dairy Production Animal Husbandry 2. Management Integrated Agricultural Field Lecture, 3. Practicum Counseling and Communication Animal 4. Practicum Reproduction 5. Inseminator 6. Practicum Practicum Poultry Production Management Poultry 7. Practicum Production Science

B. Education History

	S1	**S2**	**Profession**
Name College	**Mercu Buana**	**Jenderal Soedirman**	**Gadjah Mada University**

	University of Yogyakarta (UMBY)	**University (UNSOED) Purwokerto**	**(UGM) Yogyakarta**
Field of	Animal Science	Animal Science	Animal Industry Engineer
Graduation Year	2013-2017	2017-2019	2019-2020
Thesis Title / Final Project / Thesis / Dissertation	Effect of Supplementation Turmeric and Cinnamon Spices on the Development of Male Quail Reproductive Organs	Factors Affecting the Production and Quality ofCow Milk *Holstein Friesian* in KPBS Pangalengan Kab. Bandung	Productivity of Etawah Crossbreed Goat (PE) in Kaligesing Animal Park Integrated Livestock Breeding and Breeding Center, Central Java Province Livestock and Animal Health Service.
Name of Supervisor / Promoter	1. Ir. FX. Suwarta, MP. 2. drh. Anastasia Mamilisti Susiati, MP.	1. Prof. Ir. Dadang Mulyadi Saleh, MS., M.Agr. Sc.,Ph.D. 2. Dr.Ir. Efka Aris Rimbawanto, MP. 3. Dr.Agr., Ir. Yusuf Subagyo, MP.	1. Prof. Ir. I Gede Suparta Budisatria, M.Sc., Ph.D., IPU., ASEAN Eng. 2. Dr. Ir. Sigit Bintara, M.Sc., IPM., ASEAN Eng.

C. Work History

No	**Work Unit**	**Starting from ... to ...**	**Position**
1.	Department of Agriculture Kab. Purworejo (UPSUS SIWAB KEMENTAN-UGM Program)	April sd. August 2017	Field extension assistant (PPL)
2.	Fac. Agroindustry	2017 - 2020	Staff. Education
3.	Fac. Agroindustry	2020 - Present	Lecturer

D. Research Experience in the Last 4 Years

No.	Year	Title Research	Funding	
			Source *	Qty (million Rp)
1.	2017	Effect of Turmeric and Cinnamon Spices Supplementation on the Development of Male Quail Reproductive Organs.	DIKTI	60
2.	2019	Factors Affecting the Production and Quality	KPBS	3.5

		ofCow Milk *Holstein Friesian* at KPBS Pangalengan Kab. Bandung.	Pangalengan Bandung	
3.	2020	The Effect of a Mixture of Ice and Kitchen Salt as a Preservative in a FieldQuality ofSpermatozoa Flask on theFrozen Cement Thawing.	UMBY	8
4.	2020	Parent RamProductivity Peranakan Etawah (PE) in Taman Kaligesing Livestock and Livestock Breeding Center for Integrated Aquaculture Department of Animal Husbandry and Animal Health of Central Java province	Mandiri and Wildlife Livestock Kaligesing	3
5.	2020	hazard identification, risk and accident prevention Work (Case Study at Kaligesing Animal Park, Center for Integrated Livestock Cultivation and Breeding, Central Java Province Livestock and Animal Health Service	and Kaligesing Animal Park	3

E. Experience of Community Service in the Last 4 Years

No.	Year	Title Community Services	Funding	
			Source *	Quantity (million US $)
1.	2020	Revenue Improvement Society Through Farming Livestock Goat and Sheep in Hamlet Dampit I and II Dampit District Windusari, Magelang	UMBY AND SELF	5.5

F. Publication of scientific articles in the journal in 4 years Last

No.	Title of Scientific Article	Name of Journal	Volume / Number / Year
1.	Factors Affecting Production and Quality of Cow Milk *Holstein Friesian* at KPBS Pangalengan Bandung District.	Journal of Animal Production	Submited 2019

G. Speakers of Scientific Seminar (*Oral Presentation*) in the Last 4 Years

No.	Name of Scientific Meeting / Seminar	Title of Scientific Article	Time and Place
1.	Seminar on	Effect of Turmeric and Cinnamon Supplementation	(3 February 2017)

	Research Results The	on the Development of Male Quail Reproductive Organs.	Fac. UMBY Agroindustry
2.	Seminar on Research Result	Factors Affecting Production and Quality ofCow Milk *Holstein Friesian* at KPBS Pangalengan Bandung District.	(August 2019) Faculty of Animal Science UNSOED
3	Seminar on the Results of Research on	Productivity of Etawah Crossbreed Goats (PE) at Kaligesing Animal Park, Center for Integrated Livestock Cultivation and Breeding, Central Java Province Animal Husbandry and Animal Health ServiceScience	(18 December 2020) Faculty of Animal Science UGM
4	Seminar on Research Results	Identification Potential Hazards, Risks and Prevention of Occupational Accidents (Case Study at Kaligesing Animal Park, Integrated Livestock Breeding and Breeding Center, Central Java Province Animal Husbandry and Animal Health Service)of Animal Science	(18 December 2020) Faculty of Animal Science UGM

H. Book Works in the Last 4 Years

No	Title ofBook	Year	Number of Pages	Publisher
1.	Occupational Health and Safety and Environment (K3L) Book for Animal Husbandry	2020	180	UGM PRESS (On Process)
2.				

I. IPR Acquisition in the Last 4 Years

No	Title / Theme IPR	Year	Type	P / ID Number
1.				
2.				

J. Experience Formulating Public Policy / Other Social Engineering in the Last 7 Years

No.	Title / Theme / Other Types of Social Engineering That Have Been Implemented	Year	Place of Application for	Community Response
1.				
2.				

K. Award in the last 4 years (from government, association or other institution)

No.	Type of Award	Institution Awarding	Year	No.
1.	Outstanding Graduates of	UNSOED	2019	B / 001 / UN.23.09 / PP.06.01 / 2019
2.	1st place inLearning Vidio	UMBY	2020	1/01 / B. ACADEMIK /

	Competition			UMBY / XI / 2020
3.	Best Graduates of the Second Period Engineering Professional Program	UGM	2021	243 / J01.1.25 / PASCA / 2021

All data that I have entered and included in this biodata are true and can be accounted for legally. If in the future there is a disagreement with reality, I can accept sanctions.

Yogyakarta, 26 January 2021
Composers,

Ir. Ajat Sudrajat, S.Pt., M.Pt., IPP.

Biodata of Members (1)

A. Personal Identity

1.	Full name (with title)	Ir. Nur Rasminati, MP
2.	Gender	~~M~~/ F
3.	Functional Position of	Head Lector
4.	NIP / NIK / Other Identity	900087
5.	NIDN	0506036401
6.	Place and Date of Birth	Magelang, March 6, 1964
7.	E-mail	rasminati @ mercubuana- yogya.ac.id nurrasminati@yahoo.co.id
8.	Phone Number / HP	085878578282/081804120389
9.	Office Address for	Animal Husbandry Study Program, Faculty of Agroindustry, University of Mercu Buana Yogyakarta Jl. Wates Km. 10 Yogyakarta 55753
10.	Telephone / Fax Number	(0274) 6498211, 6494212 / (0274) 6498213
11.	E-mails	-
12.	Graduates who have earned	S-1 = 158 people; S-2 =… people; S-3 =… people
13.	Subjects taughtBeef	8. inCattle Production Science 8. ManagementCattle 8. of BeefFeedlot Technology 8. Comprehensive Project of Animal Husbandry Industry 8. Introduction to Animal Science 8. FieldI 8. StudyField Study II

B. Education Background

	S1	**S2**	**S3**
Name of College	University of Diponegoro Semarang	Gadjah Mada University Yogyakarta	
FieldScience	Animal	Animal Science	
Sign-Pass Year	1982-1987	1997-1999	
Title Thesis / Thesis / Dissertation	digestibility in Vitro Imperata (Imperata cylindrica (L. Beauv) in Various Age treatment Cutting with H_2SO_4	performance of Local Sheep Males In the Feeding of Rice Leaf Shoots and Elephant Grass.	
Name of Supervisor / Promoter	3. Ir. Widiyanto, SU. 3. Ir. Sunarso, MS.	1. Dr. Ir. Nono Ngadiyono, MS. 2. Dr. Ir. Kustono, M.Sc.	

C. Job History

No	Work Unit	Starting from year ... to ...	Position
1.	Agriculture Faculty	1995 - 1997	Secretary of Animal Husbandry Department
2.	Agriculture Faculty	2000 - 2004	Secretary of Animal Husbandry Department
3.	Agriculture Faculty	2004 - 2006	Head of Animal Production Lab
4.	LPPM & Bureau UPT	5 April 2008 - 2012	Head of Library
5.	Fac. Agroindustry	2012 - 2016	Head of Animal Husbandry Study Program
6.	Fac. Agroindustry	2016 - 2020	Head of Animal HusbandryProgram

D. Study Research Experience in the Last 5 Years

No.	Year	Title Research	Funding	
			Source *	Qty (million Rp)
1.	2015	Performance Evaluation and Development Potential of Local Sheep in Kaliangkrik District of	UMB Yogya	1.25
2.	2016	Growth of Sheep Given Fermented Feed with Starters and Local Nutrients Source of	UMB Yogya	1.5
3 .	2016	Quality Sheep Genetics and Reproductive performance of Local Owned Poor Households (RTM) in Pakis subdistrict, Magelang	Mandiri	4,725
4.	2017	Beef CattleIntegrated Development in Poor Rural Areas Pakis subdistrict	UMB Yogya	4
5.	2018	Effect of temperature Packaging Aluminum Foil and Save Local Sheep Cement on Sperm Quality and Pregnancy Rate	PTUPT Th. I DIKTI TA. 2018	50
6.	2019	Effect of Aluminum Foil Packaging and Storage Temperature of Local Sheep Cement on Sperm Quality and Pregnancy Rate	of PTUPT Th. II DIKTI TA. 2019	158

E. Experience of Community Service in the Last 5 Years

No.	Year	Title of Community Service	Funding	
			Source *	Qty

				(million Rp)
1.	2015	IbW Pakis District for Poverty Alleviation	Program IbW TH. I DP2M Dikti TA. 2015	100
2.	2016	IbW Pakis District for Poverty Alleviation	Program IbW TH. II DP2M Dikti TA. 2016	100
3.	2016	IbM Banyusidi Village IbM	DP2M Dikti Program TA. 2016	36
4.	2017	IbM Desa Ketundan IbM	DP2M Dikti Program TA. 2017	43.5
5.	2017	IbW Pakis District for Poverty Alleviation	IbW TH Program. III DP2M Dikti TA. 2017	100
6.	2018	Application of Feed Technology to Increase the Productivity of Sheep for the Community of Ngemplak and Sreyal Hamlets, Ngemplak Village, Kec. Windusari, Magelang	UMBY Fund	5
7.	2019	PPM Tempak Village Through the Application of*Technofeeding*Goatfor Poverty Alleviation	UMBY Fund	9,125

F. Publication of Scientific Articles in Journals in the Last 5 Years

No.	Title of Scientific Article	Name of Journal	Volume / Number / Year
1.	Effect of complete feed made from local raw materials on the growth of sheep.	Proceedings of the national seminar: Development of Animal Husbandry Based on Local Resources to Face the ASEAN Economic Community. Fak. UNSOED Animal Husbandry, Purwokerto	ISBN: 978-602-1004-09-8 p. 83: 88 September, 2015
2.	Productivity of beef cattle on the slopes of Merapi, Dukun District, Magelang.	Proceedings of the national seminar: Development of Animal Husbandry Based on Local Resources to Face the ASEAN Economic Community. Fak. UNSOED Animal Husbandry, Purwokerto	ISBN: 978-602-1004-09-8 p. 359: 365 September, 2015
3.	Performance Evaluation and Development Potential of Local Sheep in Kec.	Proceedings of the National Seminar on Animal Husbandry II. Master Program in Animal Science, Fac. Animal Husbandry and Agriculture, UNDIP, Semarang, 12 May 2016	ISBN: 978-602-72086-2-9 Page: 663-670, 12 May 2016

	Kaliangkrik		
4.	Potential of Sheep Development in Gunungkidul Regency, Yogyakarta	Proceedings of the National Seminar on Animal Resurrection II. Master Program in Animal Science, Fac. Animal Husbandry and Agriculture, UNDIP, Semarang, 12 May 2016	ISBN: 978-602-72086-2-9 Page: 671-679, 12 May 2016
5.	Potential of Goat Development in Gunungkidul Regency, Yogyakarta	Proceedings of the National Seminar on Animal Resurrection II. Master Program in Animal Science, Fac. Animal Husbandry and Agriculture, UNDIP, Semarang, 12 May 2016	ISBN: 978-602-72086-2-9 Page: 792-801, 12 May 2016
6.	Science and Technology for Banyusidi Village Communities for Poverty Alleviation through Kampung Chicken Agribusiness,	**Abdimas Journal** Institute for Research and Community Service (LP2M) Semarang State University	[p-ISSN: 1410-2765 | e-ISSN 2503-1252] Vol 20, No 2 (2016) December, 2016 pp. 113 - 118
7.	Poverty Alleviation through the Village Chickens Babonization System in the IbW Program, Pakis Magelang District.	Journal of INOTEK Univ. Negeri Yogyakarta	Vol 21 No.2, August 2017 ISSN: 1411-3554 Page: 96-100
8.	Evaluation of Performance and Potential Development of Local Sheep in Kaliangkrik District	Journal of Agricultural Science and Technology A http://www.davidpublisher.com	Vol. 7, No. 7, July 2017 (Serial Number 65) ISSN 2161-6256 DOI: 10.17265 / 2161-6256 / 2017.07.006 pp. 501-506
9.	The Development Potential of Goat for Poverty Reduction in Windusari Subdistrict, Magelang Regency	Food Science Conference 2018, LPPM UMBY https://iopscience.iop.org/article/10.1088/1755-1315/379/1/012009	IOP Conference Series: Earth and Environmental Science 379 (1) 012009

10.	Application of Sheep Technobreeding and Technofeeding for the Community of Ngemplak Village, District, Magelang	Book Chapter National Seminar: Working for the Country: Implementing Technology for Community Empowerment Towards a Society Era 5.0. The 2019	MBridge Press ISBN: 978-623-7587-34-7 p. 123-134Male
11.	The Influence of LocalSheep and Different Packaging on 5ºC Storage on Sperm Motility	Book Chapter National Seminar: Working for the Country: Technology Implementation for Community Empowerment Towards a Society Era 5.0. The 2019	MBridge Press ISBN: 978-623-7587-34-7 p. 257-270
12.	Integrated Beef Cattle Development in Poor Village Areas, Pakis District	Book Chapter National Seminar: Working for the Country: Implementing Technology for Community Empowerment Towards a Society Era 5.0. The 2019	MBridge Press ISBN: 978-623-7587-34-7 p. 271-288
13.	Increasing Goat Productivity Through Feed Technology in the PKH Group of Tempak Village, Candimulyo, Magelang	Dharma Bakti Journal - LPPM IST Akprind Yogyakarta. https://ejournal.akprind.ac.id/index.php/dharma	LPPM IST Akprind ISSN: 2614-2929. Vol. 3 No 1, April 2020 p. 1-8
14.	Application of *Technobreeding* Goat Livestockfor the People of Tempak Village, Candimulyo, Magelang	Dharma Bakti Journal - LPPM IST Akprind Yogyakarta. https://ejournal.akprind.ac.id/index.php/dharma	LPPM IST Akprind ISSN: 2614-2929. Vol. 3 No 1, April 2020 p. 9-16

G. Presenter Scientific Seminar (*Oral Presentation*) in the Last 5 Years

No.	Name of Scientific Meeting / Seminar	Title of Scientific Article	Time and Place
1.	Presentation at the National Seminar on Local Resource-	The Effect of Complete Feed With	Fapet. UNSOED Purwokerto,

	Based Animal Husbandry Development to Face the ASEAN Economic Community (MEA)	Local Raw Materials onSheep Growth	30 - 31 May 2015
2.	Presenter at the National Seminar on Local Resource Based Animal Husbandry to Face the ASEAN Economic Community (MEA)	Productivity of beef cattle on the slopes of Merapi, Dukun District, Magelang	Fapet. UNSOED Purwokerto, 30 - 31 May 2015
3.	Presenters at the National Seminar on Awakening of Animal Husbandry II	Performance Evaluation and Potential Development of Local Sheep in Kec. Kaliangkrik	Master of Animal Science Study Program, Faculty of Animal Science. Animal Husbandry and Agriculture, UNDIP, Semarang, 12 May 2016
4.	Presenter at the National Seminar on the Awakening of Animal Husbandry II the	Potential of Sheep Development in Gunungkidul Regency, Yogyakarta	Study Program of Master of Animal Science, Fac. Animal Husbandry and Agriculture, UNDIP, Semarang, 12 May 2016
5.	Presenter at the National Seminar on the Awakening of Animal Husbandry II of the	Potential of Goat Development in Gunungkidul Regency, Yogyakarta	Master of Animal Science Study Program, Fac. Animal Husbandry and Agriculture, UNDIP, Semarang, 12 May 2016
6.	Presenters at the National Seminar: Working for the Country: Implementing Technology for Community Empowerment Towards a Society Era 5.0. In 2019,	Integrated Beef Cattle Development in Poor Village Areas, PakisDistrict,	FikomUMBY 31 October 2019

H. Book works in the last 5 years

No	Title ofBook	Year	Number of Pages	Publishers
1.	Animal Reproduction Biotechnology	2018	181	Mbridge Press
2.	Introduction to Animal Science	2018	156	Mbridge Press Acquired

I. IPR in the last 5–10 years

No	Title / Theme of IPR	Year	Type	P / ID Number
1.	Animal Reproduction Biotechnology	2018	Textbook	000129849
2.	Introduction to Animal Science	2018	Textbook	000132328
3.	Evaluation of Performance and Potential	2019	Copyright	000159604Materials

	Development of Local Sheep in Kaliangkrik District.			on
4.	Effect of Complete Feed With Local RawSheep Growth.	2019	Copyright	000159603

J. Experience Formulating Public Policy / Other Social Engineering

No.	Titles / Themes / Other Types of Social Engineering That Have Been Implemented	Year	Place of Application of	Community Response
1.	Become a Consultant in the Agricultural Sector Policy Analysis of Yogyakarta Province 2011	2011	Areas affected by the eruption of Merapi Kab. Sleman	Good
2.	Become a Consultant for Animal Husbandry Development Master Plan inGunungkidul Regency	2016	District. Gunungkidul	Baik
3.	Become a member of the Regional Research Council of Magelang Regency	2016 - 2018	Kab. Magelang	Good
4.	Become a member of Poverty Alleviation Kab. Magelang	2017-2018	District Region. Magelang	Good

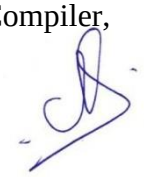
K. Award in the last 10 years (from government, association or other institutions)

No.	Type of Award	Institution Awarding	Year	No.
1.	Service for 25 years of	UMBY	2015	743 / A.29 / Rek / X / 2015
2.	Best Service III	UMBY	2016	1348 / C.01 / Rek / IX / 2016
3.	Best Service II	UMBY	2017	193 / LPPM / UMBY / IX / 2017

All data that I have filled in and included in this biodata are true and can be accounted for legally. If in the future there is a disagreement with reality, I can accept sanctions.
This is the biodata that I actually made to fulfill one of the requirements in submitting the PPM Proposal for 2021

Yogyakarta, 24 January 2021

Compiler,



Ir. Nur Rasminati, MP

Biodata of Members (2)

A. Personal Identity

1.	Full name (with title)	Ir. Setyo Utomo, MP
2.	Gender	M /~~F~~
3.	Functional Position of	Head Lector
4.	NIP / NIK / Other Identity	19671216 1992031 004
5.	NIDN	0016126701
6.	Place and Date of Birth of	Purbalingga, December 16, 1967
7.	E-mail	esutama_set @ yahoo.com
8.	Phone Number / HP	082138454463/081804120398
9.	Office Address of	Animal Husbandry Study Program, Faculty of Agro-industry, University of Mercu Buana Yogyakarta Jl. Wates Km. 10 Yogyakarta 55753
10.	Phone / Fax Number	(0274) 6498211, 6494212 / (0274) 6498213
11.	E-mail	-
12.	Graduates who have earned	S-1 = 180 people; S-2 =… people; S-3 = ... people
13.	Subjects taught are	1. Basic Animal BreedingAnimal 2. ManagementBreedingAnimal 3. Biogas Technology 4. ReproductionAnimal 5. Introduction toScience Animal 6. Reproduction Biotechnology

B. Education Background

	S1	S2	S3
College Name of	Jenderal Soedirman University	Gadjah Mada University Yogyakarta	
Field of Science	Animal Production	Animal Science	
Year of Entry - Passed	1986-1991	1994-1997	
Title of Thesis / Thesis / Dissertation	Effect of nitrogen fertilizer dosage on ash and coarse fiber content of King Grass (*Pennisetum phurpureophoides*)	Effect of *Beltsville Poultry Semen Extender* (BPSE) on Fertility and Hatchability of Chicken	
Advisor / Promoter Name	1. Ir .Pudjiarti, SU. 2. Ir.Tri Raharjo, MS. 3. Ir.Bahrun	1. Dr.Ir. Kustono, M.Sc. 2. Dr. Ir. Tri Yuwanta, SU., DEA.	

C. Research Experience In The Last 5 Years

No.	Year	Title of Research	Funding	
			Source *	Qty (million Rp)
1.	2015	Selection of Substitute Parent Candidates for Local Sheep Based on Records ofWeight Weaning, Reproduction Records and Parent Litters in Munggangsari Village, Kaliangkrik.	UMB Yogya	1,25
2.	2016	Genetic Quality and Reproduction Performance of Local Sheep owned by Poor Households (RTM) inDistrict, Magelang	PakisUMBY	4,725
3.	2017	Potential Development of Goats for Poverty Alleviation in Kec. Windusari, Magelang	UMBYStorage	4,20
4	2018	Effect of Aluminum Foil Packaging andTemperature of local sheep semen on Sperm Quality and Pregnancy Rate (PTUPT) year 1 ofStorage	Research and Technology DIKTI	50.00
5.	2019	Effect of Aluminum Foil Packaging andTemperature of local sheep semen on Quality sperm and pregnancy rate (PTUPT) year 2	Research and Technology DIKTI	158.00

D. Experience of Community Service in the Last 5 Years

No.	Year	Title of Community Service	Funding	
			Source *	Qty (million Rp)
1.	2015	IbW Kaliangkrik District (Year III, Chair)	DP2M Dikti TA. 2015	100
2.	2016	IbW Pakis District for Poverty Alleviation	Program IbW TH. II DP2M Dikti TA. 2016	100
3.	2017	IbW Windusari District 1st year (chairman)	IbW TH Program. II DP2M Dikti TA. 2017	135
4.	2018	Awareness of the Importance of Sheep Reproductive Management and Breeding Techniques for the Community of Ngemplak and Sreyal Hamlets, Ngemplak Village, Kec. Windusari, Magelanng.	UMBY	5
5.	2019	Implementation of Breeding Technology (*Technobreeding*Goat) for Poverty Alleviation in	UMBY	10.55

		Tempak Village, Kec. Candimulyo, Magelang		

E. Publication of Scientific Articles in Journals in the Last 5 Years

No.	Title of Scientific Article	Name of Journal	Volume / Number / Year
1.	Productivity of Beef Cattle on the Slopes of Merapi, Kec. Dukun Magelang	Proceedings of the National Seminar on Animal Husbandry Development Based on Local Resources to Face the ASEAN Economic Community (AEC) Fapet. UNSOED Purwokerto, 30 - 31 May 2015	ISBN: 978-602-1004-09-8
2.	The potential for sheep development in Gunungkidul district (KETUA) (p: 251)	Proceedings of the National Seminar on Animal Husbandry II, Master Program in Animal Science UNDIP, 12 May 2016	ISBN: 978-602-72086-2-9
3.	Performance Evaluation and Development Potential of Local Sheep in Kaliangkrik District (member) (p: 243)	Proceedings of the National Seminar on Animal Husbandry II, Masters Study Program in Animal Science UNDIP, May 12 2016	ISBN: 978-602-72086-2-9
4.	Science and technology for the Banyusidi Village Community for Poverty Alleviation through Kampung Chickens Agribusiness.	**Journal of Abdimas** Institute for Research and Community Service (LP2M) State University	[p-ISSN: 1410-2765 | e-ISSN 2503-1252] Vol 20, No 2
5.	Poverty Alleviation through the Babonization System of native chickens in the IbW program, Pakis Magelang District (Member)	Journal of INOTEKS Publisher LPPM UNY Volume 21, No. 2, August 2017	ISSN: 14411-3554
6.	Evaluation of Performance and Potential Development of Local Sheep in Kaliangkrik District	Journal of Agricultural Science and Technology A http://www.davidpublisher.com	Vol. 7, No. 7, July 2017 (Serial Number 65) ISSN 2161-6256 DOI: 10.17265 / 2161-6256 / 2017.07.006 pp. 501-506
7.	Implementation of Technobreeding and	Jurnal Dharma Bhakti IST AKPRIND,	https://journal.akprid.ac.id 1/4/2018

	Techofeeding based on local wisdom		
8.	The Development potential of goat for poverty reduction in windusari subdistrict, Magelang regency	IOP Conferences Series: Earth and Environmental Science 379 (1) 012009	Indexed Scopus Year 2019
9.	Application of technology and technology for sheep farming for the community of Ngemplak village, Windusari sub-district, Magelang	Semnas PPM LPPM UMBY, Work for the country: Implementation of technology for community empowerment towards a society era 5.0	Bookchapter publisher Mbridge Press ISBN

F. Speaker on Scientific Seminar (*Oral Presentation*) in the Last 5 Years

No.	Name of Scientific Meeting / Seminar	Title of Scientific Article	Time and Place
1.	National Seminar on the results of 2015 research on	Beef Cattle Productivity on the Slopes of Merapi, Dukun Magelang District,	Faculty of Animal Husbandry, UNSOED.
2.	Semnas Unsoed 2015Materials on	Influence of *Complete Feed with* Local RawSheep Growth,	Faculty of Animal Husbandry, UNSOED.
3.	National Seminar on Animal Awakening II for Master Program in Animal Science UNDIP, 12 May 2016	Potential Development of Sheep in Gunungkidul Regency (p; 251)	UNDIP Animal Science Masters Study Program, Semarang, 2016
4.	National Seminar on Research Results at Undip, 2016	Potential Development of Goats in Gunungkidul Regency	Post-Graduate UNDIP, Semarang, 2016
5.	UMBY International Seminar	The development potential of goat for poverty reduction in Windusari subdistrict, Magelang regency	UMBY Yogyakarta, 2018
6.	Presenters at the National Seminar: Working for the Country: Implementing Technology for Community Empowerment Towards Society Era 5.0. In 2019, the	Influence of the Nation of Local Rams and Different Packaging on 5ºC Storage on Sperm Motility of	Fikom UMBY 31 October 2019
7.	Presenters at the National Seminar: Working for the Country:	Implementation of Sheep Technobreeding and	Fikom UMBY 31 October 2019

	Implementation of Technology for Community Empowerment Towards a Society 5.0 Era. Year 2019	Technofeeding for the Community of Ngemplak Village, District, Magelang	

G. Book work for the last 5 years

No	Title of Book	Year	Number of Pages	Publisher
1.	BIOTECHNOLOGY ANIMAL REPRODUCTION	2018	181	OF Mbridge Press UMBY No.ISBN: 978 -602-52470-3-3
2.	INTRODUCTION TO ANIMAL SCIENCE	2018	155	Mbridge Press UMBY No.ISBN: 978-602-5247-0-19

H. Acquisition of IPR in the last 5-10 Years

No	Title / Theme IPR	Year	Type	Number P / ID
1.	POTENTIAL FOR THE DEVELOPMENT oF SHEEP IN THE DISTRICT GUNUNGKIDUL YOGYAKARTA, May 12, 2016 Publication	2019	Copyright	000 159 600
2	THE POTENTIAL DEVELOPMENT oF GOAT FOR POVERTY REDUCTION IN Windusari Subdistrict, Magelang REGENCY, publication 14 November 2018, Yogyakarta	2019	Copyright	000 159 601
3	Evaluation of Performanceand potential development of Local sheep ink Publication 6 July 2017 in New York	2019	Copyright	000159604
4	EFFECT OF LOCALCOMPLETE FEED ON SHEEP GROWTH, Publication 7 September 2015 in Purwokerto	2019	Copyright	000159603

I. Experience Formulating Other Public / Social Engineering Policies in the Last 5 Years

No.	Titles / Themes / Other Types of Social Engineering That Have Been Implemented	Year	Place for	Community Response
1. The	Head of the Formulating Team at the Animal Husbandry Department for policy input from the Director General of Animal Husbandry regarding Bachelors in Village	2003	All Indonesia	Good
2.	DevelopmentConsultation for the preparation of Master Plan for Animal Husbandry Development in Gunungkidul	2016	, Gunungkidul Regency	both

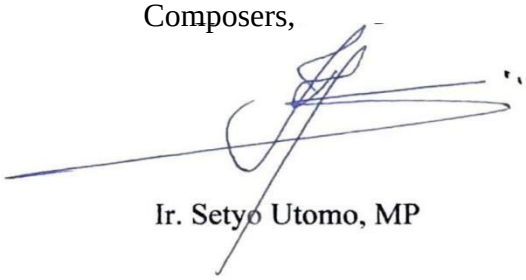
J. Awards in the last 10 years (from government, association or other institutions)

No.	Type of Award	Institution Awarding	Year	No.

1.	Lecturer with Level I Wangsa Manggala University Yogyakarta	Rector of UNWAMA	2002	781 / A.03 / Rek / X / 02
2.	Exemplary Lecturer I Kopertis Region V Yogyakarta	Kopertis Region V	2002	1518B / KOP.V / B.1 / VIII / 2002
3.	Satya Karya Satya XX Award for the	President of the Republic of Indonesia	2012	62 / TK / TAHUN 2012

All data that I have entered and included in this biodata are true and can be accounted for legally. If in the future there is a disagreement with reality, I can accept sanctions.
Thus, I made this biodata actually to fulfill one of the requirements in submitting a PPM proposal in 2021.

Yogyakarta, January 24, 2021
Composers,



Ir. Setyo Utomo, MP

Biodata of Members (3)

A. Personal Identity

1.	Full name (with title)	Nurul Hidayat, S.Pt., M.Sc.
2.	Gender	Male
3.	Functional Position of	Asisten
4.	NIP / NIK / Other Identity	-
5.	NIDN	
6.	Place and Date of Birth of	Selakau, April 15, 1995
7.	E-mail	Nurulhidayat95@mail.ugm.ac. Id Nurulhidayatuul@gmail.cim
8.	Telephone Number / HP	+62 895 2016 5474
9.	Office Address of	Animal Husbandry Study Program, Faculty of Agroindustry, University of Mercu Buana Yogyakarta Jl. Wates Km. 10 Yogyakarta 55753
10.	Telephone / Fax Number	(0274) 6498211, 6494212 / (0274) 6498213
11.	E-mail	-
12.	Graduates who have earned	S-1 =… people; S-2 =… people; S-3 =… people
13.	Subjects taught in	

B. Education History

	S1	**S2**
Name College	**Mercu University Buana Yogyakarta (UMBY)**	**Universitas Gadjah Mada**
Field of	Animal Science	Master of Animal Science
Graduation Year	2013-2017	2018-2019
Thesis Title / Final Project / Thesis / Dissertation	Effect of Fermented Palm Kernel Cake by Candida utilis in the Ratio on Performance of Local Lying Duck	Effect of Dayak Onions (Eleutherine palmifolia L.Merr) and Storage Time on pH, Chemical Compotition, Sensory Quality, DPPH Value, Total Microbes, and Peroxides Value of Rejected Duck Nugget
Name of Supervisor / Promoter	Ir. Sonita Rosningsih, M.S. Dr. Ir. Sundari, M.P.	Ir. Rusman, M.P., Ph.D Ir. Edi Suryanto, M.Sc., Ph.D., IPU., ASEAN Eng.

C. Work History

No	Work Unit	Starting from ... to ...	Position
1.			
2.			
3.			

D. Research Experience in the Last 4 Years

No.	Year	Title Research	Funding	
			Source *	Qty (million Rp)
1.	2017	Effect of Fermented Palm Kernel Cake by Candida utilis in the Ratio on Performance of Local Lying Duck	DIKTI	70
2.	2019	Effect of Dayak Onions (Eleutherine palmifolia L.Merr) and Storage Time on pH, Chemical Compotition, Sensory Quality, DPPH Value, Total Microbes, and Peroxides Value of Rejected Duck Nugget	Self Funded	20

E. Experience of Community Service in the Last 4 Years

No.	Year	Title Community Services	Funding	
			Source *	Quantity (million US $)
1.				

F. Publication of scientific articles in the journal in 4 years Last

No.	Title of Scientific Article	Name of Journal	Volume / Number / Year
1.	**The Effect of Fresh Dayak Onion (*Eleutherine palmifolia* L. Merr) and Storage Time on Rejected-Duck Nuggets**	Proceedings of The International Conference on Environmentally Sustainable Animal Industry (ICESAI)	2020

G. Speakers of Scientific Seminar (*Oral Presentation*) in the Last 4 Years

No.	Name of Scientific Meeting / Seminar	Title of Scientific Article	Time and Place
1.	Proceedings of The International Conference on Environmentally Sustainable Animal Industry (ICESAI)	**The Effect of Fresh Dayak Onion (*Eleutherine palmifolia* L. Merr) and Storage Time on Rejected-Duck Nuggets**	Malang, 18-19 November 2020

H. Book Works in the Last 4 Years

No	Title ofBook	Year	Number of Pages	Publisher
1.				
2.				

I. IPR Acquisition in the Last 4 Years

No	Title / Theme IPR	Year	Type	P / ID Number
1.				
2.				

J. Experience Formulating Public Policy / Other Social Engineering in the Last 7 Years

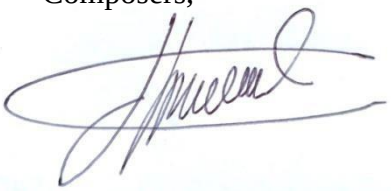
No.	Title / Theme / Other Types of Social Engineering That Have Been Implemented	Year	Place of Application for	Community Response
1.				
2.				

K. Award in the last 4 years (from government, association or other institution)

No.	Type of Award	Institution Awarding	Year
1.	The Best Graduate with High GPA from Animal Science, UMBY.	UMBY	2017
2.	The Fastes Graduate from Animal Science, UGM	UGM	2019
3.	Delegate from The Country of Ghana on Global Milenial Model United Nation	MUN	2020

All data that I have entered and included in this biodata are true and can be accounted for legally. If in the future there is a disagreement with reality, I can accept sanctions.

Yogyakarta, 26 January 2021
Composers,



Nurul Hidayat, S.Pt., M.Sc.

Attachment 4. Statement letter from the head of proposer

I, which is signed below:

Name : Ir. Ajat Sudrajat, S.Pt., M.Pt., IPP.
NIDN 0512059501
Rank / Group : IIIb
Functional Position : Lecturer

I hereby declare that my research proposal is entitled:
The Quality of Post Thawing Spermatozoa of Indigenous Indonesian and Philippines Cattle using Ice Cube and Salt as an Alternative of Liquid Nitrogen proposed in the joint venture international research in 2021 is original and has never been financed by other institutions / funding sources. If in the future there is a discrepancy with this statement, then I am ready to accept the consequences in accordance with the applicable provisions and return all research costs.

Thus this statement is made truthfully and truthfully.

Yogyakarta, 28 January 2021
Who make the statement

Ir. Ajat Sudrajat, S.Pt., M.Pt., IPP.
NIDN: 0512059501

STATEMENT OF COOPERATION AVAILABILITY

I the undersigned:

Name	: Dr. Maryneth B. Barios
Address	: Capiz, Philippines
Institution	: Faculty of Animal Husbandry Capiz State University, Philippines
No. HP	: +639516467629

Hereby declare that I am willing to join the research team with the research team from Mercu Buana University Yogyakarta, Indonesia.

Name of chairman	: Ir. Ajat Sudrajat, S.Pt., M.Pt., IPP.
NIDN	0512059501
Institution	: Animal Husbandry Study Program Mercu Buana University Yogyakarta

In carrying out a joint research with the title:

"**The Quality of Post Thawing Spermatozoa of Indigenous Indonesian and Philippines Cattle using Ice Cube and Salt as an Alternative of Liquid Nitrogen**"

This statement is made truthfully and with actually without coercion from anyone.

Capiz Philipines, 28 January 2021

Who make the statement

Dr. Maryneth B. Barios

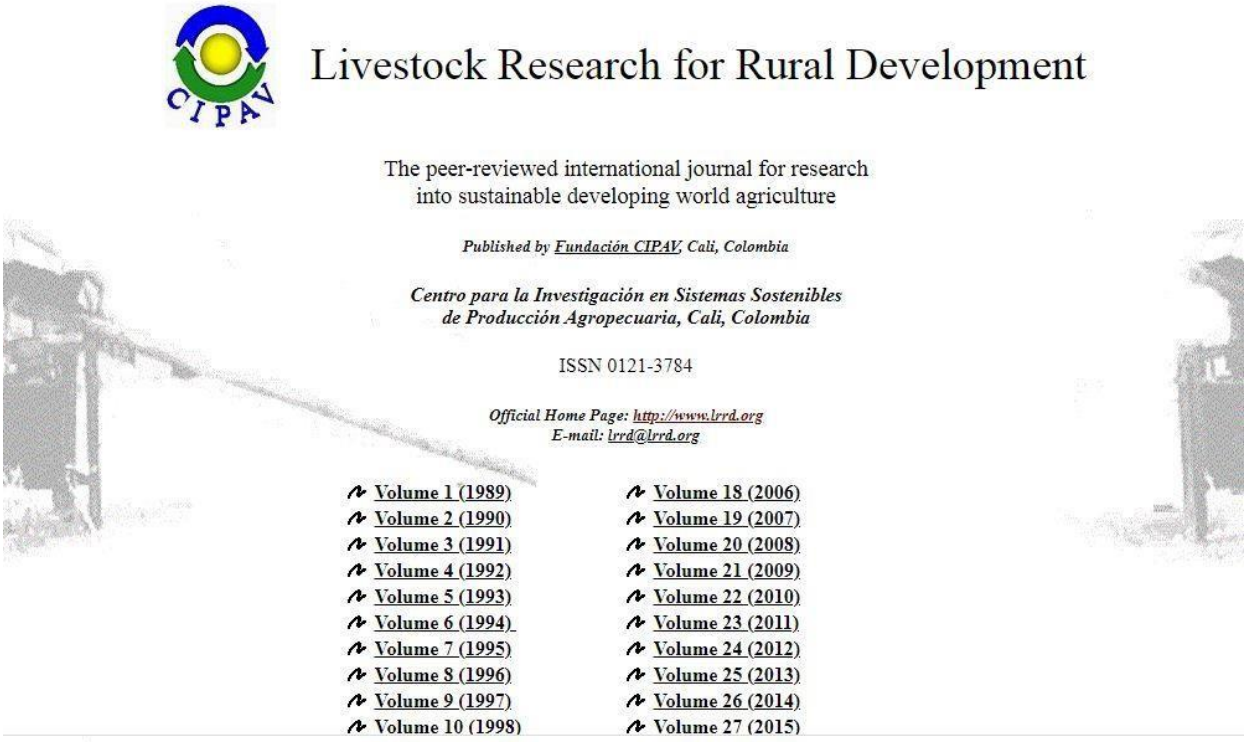
Attachment 5. Activity Documentation

Materials



1. Spermatozoa of Pasundan Cattle
2. Salt (NaCL 99%)
3. Ice cube
4. Eosin Nigrosin 2%

Attachment 6. Submission



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ISSN 0121-3784

Official Home Page: http://www.lrrd.org
E-mail: lrrd@lrrd.org

Ajat Sudrajat <ajat@mercubuana-yogya.ac.id> 16.42 (0 menit yang lalu)
kepada reg.preston

Dear Professor T R Preston, Ph.D., D.Sc.
Senior Editor in LRRD
in Colombia

Good afternoon. We hope Prof. T R Preston, Ph.D., D.Sc., is always healthy, happy doing the activity every day. I am Ajat Sudrajat from the Faculty of Agoindustry, Universitas Mercu Buana Yogyakarta, Yogyakarta, Indonesia. I would like to submit our paper with entitle "The Quality of Post Thawing Spermatozoa of Indigenous Indonesia Cattle Using Ice Cube and Salt As an Alternative Of Liquid Nitrogen".
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In our study, we use ice cube and salt as an alternative of liquid nitrogen. Especially reduces costs in Indonesia. Based on the results, we found that ice cube + 50% salt in 2 hours show good result.
However, ice cube + 50% salt is unable to replace control as alternative of liquid nitrogen.
We hope our paper can be Accepted and Published in Livestock Research for Rural Development

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Kind Regards,
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The quality of post thawing spermatozoa of indigenous Indonesia cattle stored usingice cube and salt as an alternative of liquid nirogen

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Abstract

The main obstacle in the implementation of Artificial Insemination (AI) is the absence of cheap and effective field tools or flasks that can maintain the quality of frozen sperm during AI implementation. So far, inseminators always use flasks with liquid nitrogen to bring frozen straw into the field. So that it is higher cost and ineffective. The purpose of this study was to determine the storage time of frozen sperm in a field thermos with ice cooling and table salt in various ratios of the effect on the quality of semen from Pasundan cattle from West Java. The research will be carried out from March to August 2021 at the Balai Inseminasi Buatan (BIB) Lembang, West Java and the Laboratory of Biotechnology and Reproduction, Universitas Mercu Buana Yogyakarta. The research used frozen semen straws in the form of mini straw from the Pasundan cattle breed, which was produced by the BIB Lembang. The research used an experimental method with a completely randomized design with a unidirectional pattern, namely ice and salt ratio with 3 replications. T0 for control, T1 for ice cubes + 30% salt, T2 for ice cubes + 40% salt and T3 for ice cubes + 50% salt. Each treatment is stored for 0, 2, 4, 6 hours. To determine the difference in motility in the treatment of the ratio of ice and salt in a field flask, analysis of variance was carried out. If there is a difference, continue with the Duncan's Multiple Range Test (DMRT) difference test. The use of storage media filled with liquid nitrogen had a higher motility rate ($p<0.05$) compared to a mixture of ice cubes and salt (T1, T2 and T3). The use of liquid nitrogen (T0) was higher viability ($p<0.05$) compared to a mixture of ice cubes and salt (T1, T2 and T3). However, the use of ice cubes and salt at all levels (T1, T2 and T3) showed no significant difference ($p<0.05$). Viability of spermatozoa decreased by 29.62%. The results showed that the average mass movement of spermatozoa ranged between good and sufficient categories, this could occur because treatment using salt and ice caused cold shock. Extreme temperature changes that occur, namely from a temperature of -196^0C (liquid nitrogen temperature) to the temperature of the ice cube and salt treatment in the thermos, which is -15^0C, make most of the spermatoza dead. T3 shown the best rwsults for the variable motility, viability and wave motion.

Key words : Pasundan Cattle, Ice cube, Salt, Quality of spermatozoa

Introduction

The development of the livestock sub-sector plays a crucial role in supplying the nutritional needs of the Indonesian people, which in 2004 amounted to more than 220 million. The provision of nutritional needs derived from livestock products such as eggs, meat, and milk has not been fully provided domestically. Some products such as meat and milk still have to import from abroad (Anonymous, 2000). The development of the integrated livestock sub-sector at this time must be directed to the orientation of agrAIusiness and agro-industry. In line with this program, the use of superior seeds is necessary to ensure business continuity, both in productivity and business sustainability. The fulfillment of community needs for quality and sustainable livestock meat is really a solution to a problem that exists both at the community level and at the national level (Anonymous, 2000).

The appearance of livestock potential is fundamentally influenced by two main factors: genetic and environmental factors, including the overall maintenance management. It is known that the environment and adequate management handling or following the needs of the livestock will not give the expected production expression (quality or quantity) if the good genetic potential of livestock does not support it. Furthermore, vice versa, if the livestock has good genetic potential, it will not be expressed optimally if the maximum environment and management do not support it. Thus, these two factors should receive equal attention in the maintenance of livestock commodities. Raising livestock with high genetic value accompanied by good management will certainly provide optimal results both in terms of production and business efficiency (Syukur, 2006).

Cows are livestock that is widely known by the general public, especially in rural areas. Cows in Indonesia are generally raised by farmers traditionally (from generation to generation), so that their development is fully in the midst of farmers (Utomo and Nur R., 2012). Beef cows are one of the resources for producing food in the form of meat, which has high economic value and is important for people's lives. A group of cattle can produce various kinds of needs, especially as a food ingredient in the form of meat and other by-products such as manure, leather, etc. (Sugeng, 2002). Furthermore, it is stated that based on their origins, cows are broadly classified into 3 groups, namely: Indicus bosses (Zebu cattle = humps), Taurus bosses (European cows), and Bos Sondaicus (bull bulls / BAIos cows).

The need for animal protein from livestock in Indonesia is 5.4 g / capita/day. Based on these needs, Indonesians must consume 9.6 kg/cap/year of meat, 3.5 kg/cap/year of eggs, and 4.6 kg/capita/year of milk. The field's real conditions show that the Indonesian people can only meet animal protein consumption from livestock, an average of 3.47 g / capita/day (Harmadji, 1999). Conservation of genetic resources needs to be carried out with two considerations, namely the increasing demand for livestock products, especially in developing countries as the FAO predicts a 2-fold increase in demand for meat in the 30 years from 2000 to 2030 and the increasing demand for milk is more of two times, as well as the rapid reduction in almost all over the world of genetic resources (germplasm) (Subandriyo, 2006).

The implementation of AI (Artificial Insemination) in Indonesia began nationally in 1972. Through this AI activity, it is hoped that it will improve the genetic quality of local livestock

breeds, so it is hoped that the cattle owned by the Indonesian people can gradually improve their genetic quality. In the development of national nurseries, the problems faced are how to increase the number of livestock breeds in good quality, the linkage and interdependence of breeding actors to provide livestock seeds in the number, type, and quality according to needs (Bahri, 2006). At the beginning of the AI implementation in Indonesia (1972 to 1973), using liquid semen (fresh). AI with fresh semen was felt to be very slow in development, so in 1973 with the help of frozen semen from the British and New Zealand governments, the use of frozen semen in straw (plastic straw) packaging was introduced. Since the use of frozen straws, AI development is very rapid to date, especially after the operation of BBAI in Lembang and Singosari as a producer of frozen straw, especially superior cow semen.

The main obstacle in implementing AI is the absence of cheap and effective field tools/flasks that can maintain the quality of frozen sperm in a straw when going to AI right. So far, inseminators always use flasks with liquid nitrogen in them to bring frozen straw to the field. So that costs are higher and less effective because liquid nitrogen is expensive and has limited availability. So it is necessary to create a tool with cheap cooling material but has the ability to maintain the quality of frozen sperm in a relatively long time.

Materials and Methods

Time and Place of Research

The research will be carried out from March to August 2021 at the Laboratory of Biotechnology and Reproduction UMBY, BIB Lembang and the Animal Reproduction Laboratory, UGM.

Materials and tools

The research used frozen cement straws in the form of mini straw from the Pasundan cattle breed, which was produced by the BIB Lembang Beef Cattle Livestock, West Java. The research used an experimental method with a completely randomized design with a unidirectional pattern, namely ice and salt ratio with 3 replications. A total of 432 mini frozen cement straws, while the tools used include ice flasks, water jacket tubes, thermometers, microscopes, glass objects, cover glass, heating tables, and stationery.

The research method used an experimental method with the basic completely randomized design with a unidirectional pattern, namely the ratio of ice cube vs. salt. The research will be repeated 3 times.

How it Works : A total of 432 mini straws of frozen semen from Pasundan cattle were distributed in 48 flasks with a capacity of 750 ml made of stainless which were filled with nitrogen as T0 (control), T1: ice cubes + 30% salt, T2: ice cubes + 40% salt and T3: ice cubes + 50% salt. The straw is put into the water jacket in a large test tube then placed in the thermos standing up. Each flask contains 9 straws, which will be checked for motility (%) every 0, 2, 4, 6 hours of storage. Motility examination on the heating table using a microscope with a magnification of 10 x 40 times by looking at the spermatozoa object compared to spermatozoa that are not moving or moving but not progressive forward.

Data Analysis

To determine the difference in motility in the treatment of the ratio of ice and salt in a field flask, analysis of variance was carried out. If there is a difference, continue with the Duncan's Multiple Range Test (DMRT) difference test.

Results and Discussion

Post thawing motility

Motility is the movement of spermatozoa that can be used as a reference in assessing the quality of spermatozoa for artificial insemination (Bintara, 2011). The results of the complete examination of sperm motility of Pasundan cattle and BIB production in Lembang are presented in Table 1.

Table 1. Spermatozoa motility of Pasundan cattle

Treatment	Storage time (hours)				Average
	0	2	4	6	
T0	50.00 ±10.00	67.50 ± 2.50	58.33 ± 2.88	48.33 ± 2.88	56.04^{c} ± 9.13
T1	50.83 ± 10.10	30.00 ± 5.00	16.66 ± 2.88	11.66 ± 2.88	27.29^{a} ±16.63
T2	52.50 ± 2.50	30.00 ±17.32	18.33 ± 2.88	13.33 ± 5.77	28.54^{a} ± 17.66
T3	51.66 ± 9.46	40.83 ± 2.88	28.33 ± 5.20	24.16 ± 5.20	36.25^{b} ± 12.40
Average	51.25^{z} ± 7.42	42.08^{y} ±17.83	30.41^{x} ± 17.73	24.37^{x} ±24.31	

T0 for nitrogen, T1 for ice cube + 30% salt, T2 for ice cube + 40% salt, and T3 for ice cube + 50% salt

^{abc} different superscript in the same column show significant differences ($p<0.05$)

^{xyz} different superscript in the same line show significant differences ($p<0.05$)

Based on these results, it shows that the media and storage time have a significant effect ($p<0.05$) on spermatozoa motility in T0 and T3 treatments, while T1 and T2 treatments are not significantly different ($p>0.05$). The use of storage media filled with liquid nitrogen had a higher motility rate ($p<0.05$) compared to a mixture of ice cubes and salt (T1, T2 and T3). This is because when the straw is stored with liquid nitrogen, the metabolism of spermatozoa cells can be said to stop, the metabolism of spermatozoa cells will increase along with the increase in temperature. Storage of semen in media with temperatures ranging from 3-4°C, metabolism is still running but very slowly, because it is far below the physiological temperature (38°C). The longer the storage time, the more use of nutrients between spermatozoa as a result of ongoing metabolism, this causes the availability of nutrients to be depleted. A large number of spermatozoa will accelerate the use of nutrients for the purposes of spermatozoa metabolism, so that nutrients will be quickly depleted and the production of ATP (adenosine triphosphate) in the mitochondria will be inhibited so that it can affect the motility of spermatozoa (Hayati, 2011).

The results of the research on the motility of the sperm of Pasundan cattle stored in a thermos with a storage medium of a mixture of salt and ice cubes showed that the best average motility that entered the SNI standard (Indonesian National Standard) was 40.83% in the T3 treatment

(ice cubes + 50% salt) for a long time. two hours of storage, while the other motility averages were still below the SNI standard or their motility was below 40% except at zero hours of storage. This is because treatment using a mixture of salt and ice cubes causes cold shock. Changes in temperature that occurred, namely from -196℃ (liquid nitrogen temperature) to the temperature of ice cubes and salt treatment in the thermos, namely -15℃, made most of the spermatoza die and their motility decreased. According to Sonjaya et al., (2005) stated that the biggest factors that affect the decrease in sperm quality due to cold shock are temperature and pH.

Garner and Hafez (2000) stated that the motility of bovine spermatozoa ranged from 40-75%. Differences in spermatozoa motility can be caused by age in cattle, 1.5 years old has lower motility than 2 years old, this is because in 2 year old cows the primary and secondary reproductive organs are optimal (Azzahra et al., 2016). Meanwhile, according to Herdis (2005) states that the motility of spermatozoa is influenced by differences in the breed of livestock and the time of examination. Another factor that can affect motility is feed (Zulfan, 2008).

Viability of spermatozoa

The results of the examination of spermatozoa viability of Pasundan cattle and BIB production in Lembang are presented in full in Table 2.

Table 2. Viability of spermatozoa (%)

Treatment	Storage time (hours)				Average
	0	2	4	6	
T0	79.33^{h}±2.56	74.05^{gh}±6.05	71.24^{gh}±12.78	67.36^{gh}±10.31	72.99^{e}±8.81
T1	72.16^{gh}±5.48	58.05^{gh}±20.56	52.42^{g}±24.0	26.35^{f}±12.72	52.24^{d}±22.73
T2	71.50^{gh}±2.17	67.66^{gh}±8.31	20.17^{f}±11.82	20.17^{f}±11.82	47.64^{d}±25.42
T3	58.67^{gh} ±4.73	65.07^{gh}±11.14	29.83^{f}±6.11	19.91^{f}±9.25	43.37^{d}±21.01
Average	70.41^{c}±8.48	66.21^{c}±12.43	46.18^{b}±22.81	33.44^{a}±22.69	

T0 for nitrogen, T1 for ice cube + 30% salt, T2 for ice cube + 40% salt, and T3 for ice cube + 50% salt

^{abc} different superscript in the same line show significant differences ($p<0.05$)

^{de} different superscript in the same column show significant differences ($p<0.05$)

^{fgh} different superscript in the same line and column show significant differences ($p<0.05$)

Based on these results, it shows that the media and storage time have a significant effect ($p<0.05$) on live spermatozoa. The use of liquid nitrogen (T0) was higher ($p<0.05$) compared to a mixture of ice cubes and salt (T1, T2 and T3). However, the use of ice cubes and salt at all levels (T1, T2 and T3) showed no significant difference ($p<0.05$). Viability of spermatozoa decreased by 29.62%. This percentage decrease occurred when using ice cubes and salt media (T1, T2 and T3). The percentage of spermatoza viability decreased due to damage to the plasma membrane and acrosome membrane due to the influence of cold shock. The temperature of the frozen cement storage media used in this study was different, namely M0 (-196℃). This is in accordance with Septiani et al. (2017) that the metabolic rate and motility of spermatozoa will decrease at a temperature of 3 to 5℃. Viability is the vitality of spermatozoa as an indicator of

spermatozoa quality. The viability of diluted frozen semen has at least 60 to 75% live spermatozoa (Garner and Hafez, 2000).
The storage time of 0 and 2 hours was higher ($p<0.05$) than 4 and 6 hours. However, the storage time of 4 hours tended to be better ($p<0.05$) than 6 hours. Based on the results of the study, the length of storage time decreased the viability of spermatozoa by 36.70%. The longer the storage time, the decrease in viability is also high. Spermatozoa quality is said to be good if it has a high number of live spermatozoa and <15% dead spermatozoa (Bintara, 2011).
The results of the analysis of variance showed that there was a significant interaction between the media and storage time ($p<0.05$). This shows that the media and the length of storage time affect each other's viability. The use of M0 with a shelf-life of 0 hours showed the highest viability compared to other treatments and T3 with the lowest curing time of 6 hours ($p<0.05$). The use of T1 with a storage time of 2 hours tends to be better than T2 and T3 although it is not significant.

Likewise with the long storage time of 6 hours. The percentage of viable spermatozoa is determined by the intact plasma membrane. The plasma membrane functions to protect spermatozoa organelles and transport electrolytes for spermatozoa metabolism. Damaged plasma membrane can affect the physiological function and metabolism of spermatozoa, causing spermatozoa to die. Intact plasma membrane has a correlation with spermatozoa motility, the more intact spermatozoa plasma membrane, high motile spermatozoa (Azzahra et al., 2016). In this study, the decrease in viability occurred because the spermatozoa experienced cell damage, namely changing the structure of the plasma membrane phospholipids and disrupting the permeability function of the cell membrane. If there is damage to the membrane, the metabolic process will be disrupted, the synthesis of Adenosine Tri Phosphate (ATP) does not work well and results in a decrease in the viability of spermatozoa.

Wave motion

Spermatozoa mass movement is the active movement of spermatozoa by showing undulating and massive mass movements. The mass movement scores consist of very good (3/+++), good (2/++), moderate (1/+), and bad (-) (Tambing et al., 2001). The results of the examination of the mass movement of the spermatozoa of Pasundan cattle and BIB production in Lembang are presented in Table 3.

Table 3. Mas motion of Pasundan cattle spermatozoa

Treatment	Storage time (hours)				Average
	0	2	4	6	
T0	2.00 ± 0.00	2.16 ± 0.28	1.83 ± 0.28	1.83 ± 0.28	1.95 ± 0.25^{b}
T1	2.00 ± 0.00	1.16 ± 0.28	1.00 ± 0.00	1.16 ± 0.28	1.33 ± 0.44^{a}
T2	2.33 ± 0.28	1.33 ± 0.28	1.16 ± 0.28	1.33 ± 0.28	1.54 ± 0.54^{a}
T3	2.00 ± 0.50	1.66 ± 0.57	1.33 ± 0.28	1.16 ± 0.28	1.54 ± 0.49^{a}
Average	2.08^{y} ± 0.28	1.58^{x} ± 0.51	1.33^{x}± 0.38	1.37^{x}± 0.37	

T0 for nitrogen, T1 for ice cube + 30% salt, T2 for ice cube + 40% salt, and T3 for ice cube + 50% salt
^{ab} different superscript in the same line show significant differences ($p<0.05$)
^{xy} different superscript in the same column show significant differences ($p<0.05$)

The results showed that the average mass movement of spermatozoa ranged between good and sufficient categories, this could occur because treatment using salt and ice caused cold shock. Extreme temperature changes that occur, namely from a temperature of -196^{0}C (liquid nitrogen temperature) to the temperature of the ice cube and salt treatment in the thermos, which is -15^{0}C, make most of the spermatoza dead. The biggest exogenous factors that affect the decrease in sperm quality due to cold shock are temperature and pH (Sonjaya et al., 2005).

Cold shock that occurs due to changes in extreme temperatures and storage time in the treatment causes the metabolism of spermatozoa to run very quickly, causing the energy for locomotion to decrease and ultimately causing the spermatozoa to die. Spermatozoa will use energy for locomotion produced by accessory glands (Azzahra et al., 2016). Accessory glands will secrete plasma semen by producing several kinds of nutrients such as glucose, fructose, sucrose, citric acid, protein, potassium, sorbitol, insitol and glycerylphosphoryl-choline (GPC) which function as energy substrates for spermatozoa including sperm motility (Sujoko, 2009).

Cold shock that occurs also increases the production of lactic acid in the sperm to be high as a result of the rapid metabolic process. The high content of lactic acid in sperm will result in increased damage to the membrane that affects metabolism and the energy produced. The high amount of lactic acid will affect the increase in the osmotic pressure of the semen plasma thereby reducing the permeability of the spermatozoa membrane and increasing membrane damage. Damage to the spermatozoa membrane will affect the metabolic process and decrease the formation of energy. The reduced energy will greatly affect the activity and mobility of spermatozoa (Samsudewa et al., 2006). This is what causes the large number of dead spermatozoa. It can be concluded that the storage of spermatozoa in a thermos with salt and ice treatment was not able to maintain the mass movement of spermatozoa significantly.

Conclusion

T3 shown the best rwsults for the variable motility, viability and wave motion.

Conflict of interest

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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Attachment 7. News in media online

UMBY Gelar Joint Research Progress Report dengan CAPSU

Kamis (25/03/2021), Pusat Penelitian Pengabdian dan Kerjasama kepada Masyarakat (P3MK) Universitas Mercu Buana Yogyakarta (UMBY), khususnya pada Bagian Kerjasama Luar Negeri menyelenggarakan kegiatan *Joint Research Progress Report* yang membahas laporan dari masing-masing team *Joint Research* antara UMBY dan Capiz State University (CAPSU). Acara ini diselenggarakan secara online melalui zoom meeting, pukul 13.00 – 16.00 WIB. Acara ini dihadiri sejumlah 39 dosen dari UMBY dan CAPSU.

Acara dibuka oleh sambutan dari Kepala Bagian Kerjasama Luar Negeri dan juga Sambutan Rektor UMBY, kemudian arahan dari *vice president of international affair* yaitu Dr. Mary Martellino yang mengingatkan tentang deadline penelitian dan tujuan dari penelitian yang didampingi Kepala Pusat Penelitian dan Pengembangan yaitu Dr. Eng. Efren Linan.

with link : http://mercubuana-yogya.ac.id/berita-6938-umby-gelar-joint-research-progress-report-dengan-capsu

with link: https://www.timesindonesia.co.id/read/news/354020/umby-kolaborasi-capiz-state-university-filipina-riset-pengembangan-bioteknologi-reproduksi-ternak

peternakan.mercubuana-yogya.ac.id/2021/06/18/prodi-peternakan-umby-dan-capiz-stat

Tim Peneliti joint research International UMBY-CAPZU Filipina

Prodi Peternakan Universitas Mercu Buana Yogyakarta (UMBY) dan Capiz State University (CAPZU) Filipina menjalin kerjasama riset internasional untuk pengembangan bioteknologi reproduksi ternak. Kolaborasi riset ini dilakukan di Indonesia dan di Filipina. Pada saat pertemuan perdana dengan pihak CAPZU Filipina, Ir. Ajat Sudrajat, S.Pt.,

with link : http://peternakan.mercubuana-yogya.ac.id/2021/06/18/prodi-peternakan-umby-dan-capiz-state-university-capzu-filipina-jalin-kolaborasi-riset-pengembangan-bioteknologi-reproduksi-ternak/