LEVERAGE FACTORS OF ECOLOGICAL SUSTAINABILITY ON EPIDIDYMAL SPERMATOZOA UTILIZATION FROM INDONESIAN CATTLE IN RUMINANT SLAUGHTERHOUSES

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Abstract

The utilization of epididymal spermatozoa of original Indonesian cattle in the ruminant slaughterhouse (Rumah Potong Hewan-Ruminansia or RPH-R) service business unit is still not widely carried out, and is partial, and has not described sustainability in a harmonization manner that includes environmental, social, economic, regulatory and technological dimensions. The utilization of epididymal spermatozoa in the management of the RPH-R service business unit produced is still limited in its sustainability in the ecological dimension. This paper is based on research that aims to analyze the leverage factors for the utilization of epididymal spermatozoa in the management of the RPH-R service business unit. Sustainability status of epididymal spermatozoa was analyzed using the R*ap-CattleSlaughterhouse* method with the *Multi Dimentional Scaling (MDS)* technique*, leverage* analysis*,* dan *Monte Carlo* analysis, It was started with the preparation of diluent, testicular/epididymal collection, organ preparation, spermatozoa collection, semen evaluation, semen processing, freezing and spermatozoa quality testing after freezing. Epididymal samples were taken from RPH-R in Bogor, Yogyakarta, and Bali in 2019. The results showed that the sustainability of the epididymal spermatozoa in native Indonesian animals (cattle) at RPH-R in Indonesia includes ecological dimension. The sustainability index of the ecological dimension indicates a sustainable status (> 50%) which includes 9 attributes, namely testicular physique, fresh semen pH, semen color, semen consistency, spermatozoa motility, spermatozoa movement, spermatozoa concentration, spermatozoa viability, and spermatozoa morphology. Of the 9 attributes that function as leverage, the most important is the movement and concentration of epididymal spermatozoa.

Keywords: Sustainability, Epididymal Spermatozoa, Cattle, Ruminant-Slaughterhouse, Multi-Dimensional Scaling

1 INTRODUCTION

The attribute of animal health status in the ecological dimension in the management of Ruminant Slaughterhouse (RPH-R) is one of the leverage attributes with a Root Mean Square (RMS) value of 5.08. This means that these attributes have a major influence on the sustainability index of the ecological dimension in the sustainable RPH-R service business management model. In the same way, the attribute of semen salvage and preservation technology with an RMS value of 3.95 is also one of the attributes included in the technology dimension sustainability index in the sustainable RPH-R service business management model (MDD Maharani Eriyatno et al., 2015). These two attributes, both animal health status and semen preservation technology, have a high correlation in the utilization of the epididymal spermatozoa of large ruminant cattle in RPH-R in supporting food sovereignty in Indonesia.

Prohibition of slaughtering productive female animals has been promoted by the government, so that the slaughter of female animals can be more controlled. Unfortunately, the rules for slaughtering male animals have not been regulated, so that many productive bulls that have good performance are wasted, whereas in the testes, especially in the epididymis, there is a source of genetic material that can still be utilized. One source of genetic material for native Indonesian animals is found in RPH-R, which is managed by the government in the form of Regional Technical Implementation Units (UPTD), Regional Owned Enterprises (BUMD) and private companies. The institutions, in carrying out its performance, requires innovation and creativity as well as science and technology in managing the animal slaughter service business in a sustainable manner. Utilization of the structure of animal genetic material after slaughter is one of the opportunities for innovation, creativity, and science and technology, which can be implemented.

The genetic material structure of animals or livestock, whether for breeding or re-cultivation purposes, in captivity for wild or non-wild animals, can be lost at any time, which is caused, among others, by: (1) death, intentionally due to slaughter at the RPH-R, (2) unexpected death, (3) low libido, and (4) occurrence of reproductive disorders. Efforts that can be made in re-preserving the genetic material because it is wasted (gamete rescue), a rescue and preservation of the genetic material structure of male animals that have died after being slaughtered at the RPH-R, is by utilizing epididymal spermatozoa.

Several research results provide information that the utilization of epididymal spermatozoa, which aims for Artificial Insemination (IB), and In vitro Fertilization (FIV), still shows the ability to produce offspring or children. Currently, the utilization of spermatozoa originating from the cauda epididymis has not received serious attention even though it has been proven to be able to fertilize oocytes. Therefore, an agroecological policy and strategy is needed in the directive, strategic, operational and technical hierarchy in managing the RPH-R service business in a sustainable manner so that the management of saving and preserving the semen of native Indonesian animals can be achieved through the use of spermatozoa epididymis as one of the real actions to support food sovereignty in Indonesia (Maharani et al., 2017).

Bali and Madura cattle are native Indonesian cattle that have many superiorities, including being easy to adapt to various types of feed and environmental conditions. Bali cattle can adapt to seasonal changes as evidenced by data on weaning weight, yearly weight, and good reproductive conditions (Suranjaya et al., 2010). Bali cattle are one of the types of beef cattle that have a fairly high carcass percentage, namely 51% (Hafid et al., 2018) to 54% (Suryanto et al., 2017). Bali cattle also have the best productivity levels with low input values and good environmental stress resistance (Sutarno & Setyawan, 2016). Bali beef has good quality with low fat content (Sutarno & Setyawan, 2016). The reproductive efficiency of Bali cattle is classified as good with a service per conception (S/C) value of 1.76 to 1.84 (Gunawan Muhammad et al., 2017) with a conception rate (CR) of 80.00 - 86.67% (Mardiansyah et al., 2016).

Spermatozoa that come out of the testes and enter the epididymis are non-functional spermatozoa because of their low motility and ability to fertilize oocytes (Cornwall & von Horsten, 2007). Incompetent spermatozoa produced by the testes undergo a process of maturation and storage in the ductus epididymis (Cooper, 2011). Spermatozoa in each part of the epididymis have different characteristics because there are morphological changes, especially in the head and migration of cytoplasmic droplets (Cooper, 2011).

During the journey from the head to the body, cytoplasmic droplets move, from the head to the tail intermediate then will be released from the spermatozoa either through the fluid in the cauda epididymis, during or after ejaculation, depending on the species. In addition, changes in the pattern or integrity of the acrosome also occur with the transit of spermatozoa in the epididymis. The study conducted by (Varesi et al., 2013). showed that spermatozoa located in the cauda epididymis had acrosomal integrity and acrosome normality that were higher than those in the head and corpus. Furthermore, the proportion of normal spermatozoa morphology also increased significantly from the head to the cauda epididymis.

According to (Gatti et al., 2004) spermatozoa motility has also developed in the head of the epididymis. A small number of spermatozoa in the head of the epididymis began to show the ability to swim progressively and recognize oocytes (Aitken et al., 2007). The concentration of spermatozoa in the head is 25-50 x 10^6 (Senger, 2005). Spermatozoa in the head of the epididymis have cytoplasmic droplets in the proximal part (Hopper Richard M., 2015).

Characteristics of spermatozoa continue to develop from the head of the epididymis before reaching optimal levels in the distal cauda. The location of cytoplasmic droplets of spermatozoa in the corpus epididymis has translocated to the middle area in the indentation of the flagellum (Hopper Richard M., 2015). The concentration of spermatozoa in the corpus is 8-25 x 10^9 (Senger, 2005). According to (Gatti et al., 2004), spermatozoa in this section already have the ability to do zone bonding. Spermatozoa in this section have also shown some fertility expressions (Senger, 2005).

The concentration of spermatozoa found in the cauda epididymis is 10-50 x 10^9 (Senger, 2005). The location of cytoplasmic droplets of spermatozoa in the cauda epididymis has migrated more distally (Hopper Richard M., 2015). Spermatozoa in this section have high disulfide cross-linking, and have shown normal motility (Senger, 2005). The ability to fuse with the oolemma has been obtained by spermatozoa in the cauda epididymis (Gatti et al., 2004).

Diluent is a material that is added to semen to increase the volume of semen, reduce the concentration of spermatozoa and maintain the viability of spermatozoa for a certain time. A semen diluent solution that has a more complete chemical composition will provide a good function for the diluted semen, because the substrates in the diluent are needed by spermatozoa to maintain their life (Ridwan, 2009).

The commonly used semen diluent in Indonesia is Tris egg yolk which consists of Tris Hydroxy methyl aminomethane, citric acid, fructose, egg yolk, penicillin, streptomycin, and aquabidest. Tris together with citric acid acts as a buffer to maintain pH changes due to the formation of lactic acid from spermatozoa metabolism and plays a role in maintaining osmolarity pressure and electrolyte balance (Siswanto, 2006). Egg yolk contains lipoproteins and lecithin which can maintain and protect the integrity and lipoprotein envelope of spermatozoa cells (Baharun et al., 2017).

Tris egg yolk diluent was able to maintain semen quality in Pasundan cattle as well as commercial diluents (Baharun et al., 2017). The content of fructose in the egg yolk Tris diluent serves as an energy source so that spermatozoa can keep moving. Fructose plays a role in producing energy in the form of ATP which contains energy-rich organic phosphate and will be used for the contraction of fibrils for the movement of spermatozoa. (D. Hartanti et al., 2012) added that the viability of spermatozoa using egg yolk Tris diluent was higher in various observation intervals, after dilution in Brebes Java cattle.

2 PROBLEMS

Previous research related to the problem of Utilization of Epididymal Spermatozoa was mostly carried out on rare or protected animals or livestock. On the other hand, the utilization of Spermatozoa Epididymis of Original Indonesian Cattle in the RPH-R service business unit is still not widely carried out and is partial, and has not described sustainability in a harmonization manner that includes environmental, social, economic, regulatory and technological dimensions.

The utilization of Epididymal Spermatozoa in the management of the RPH-R service business unit produced is still limited in its sustainability in the ecological dimension. In terms of the methodology used in the previous study, there was no analysis of the Utilization of Epididymal Spermatozoa that already existed at that time, and there was still no feedback process on the results achieved.

Some of the problems in the Utilization of Epididymal Spermatozoa in the management of the RPH-R service business unit include:

1) The behavior of the Millennial Generation of Service Users who play an active role shows a lack of harmonization so that it contains the potential for conflict between fellow Service Users, thus making it difficult to implement the Utilization of Epididymal Spermatozoa.

2) The Managers are not firm in regulating the infrastructure and slaughtering processes at RPH-R locations, thereby inviting potential conflicts between Service Users and Managers, among Managers, among Service Users, as well as between animal or livestock traders.

3) RPH-R which has been built by the Government, Private and Regional Companies cannot provide guarantees to Service Users who are willing to sell genitals containing Epididymal Spermatozoa and use them to recycle animals or livestock ready for slaughter.

4) The function of RPH-R as one of the producers of Regional Original Income (PAD) is more dominant than other functions (where the slaughter of livestock is carried out correctly, where the inspection of livestock before slaughter or ante mortem is carried out and inspection of meat or post mortem to prevent transmission livestock disease to humans, a place to detect and monitor livestock diseases found during ante mortem and post mortem examinations for the prevention and eradication of infectious livestock diseases in the area of origin of livestock, a place to carry out selection, control the slaughter of large productive horned female cattle, as well as a place for animal recycling or cattle after slaughter).

5) There is no legislation that directs the use of Spermatozoa Epididymis in the management of the RPH-R service business unit to make it sustainable.

From the description above, some research questions are as follows:

1) What are the leverage factors that play an important, appropriate, necessary, and influential role in the utilization of Spermatozoa Epididymis in the management of the RPH-R service business unit to make it sustainable, to be used as a basis?

2) How to measure the status or sustainability index of the use of Spermatozoa Epididymis in the management of the RPH-R service business unit to make it sustainable?

3 OBJECTIVES

The general objective of this research is to formulate leverage factors for the utilization of Spermatozoa Epididymis in the management of the RPH-R service business unit so that it is sustainable, while the specific objectives of this study are:

1) Analyzing and measuring important and necessary variables to develop sustainable use of Epididymal Spermatozoa in the management of the RPH-R service business unit.

2) Analyzing and measuring leverage variables in the sustainability of the use of Spermatozoa Epididymis in the management of the RPH-R service business unit.

4 ADVANTAGES

The results of this study are expected to contribute in the form of suggestions to various parties and partners, namely:

1) For the central government, the results of this research can be used as material to develop guidelines for the sustainability of the use of Spermatozoa Epididymis in the management of RPH-R service business units in Indonesia.
2) For local governments, this information can be used as material for formulating a gamet rescue program on the sustainability of the RPH-R service business unit management as a source of PAD.
3) For business unit actors, the sustainable use of Epididymal Spermatozoa in the management of the RPH-R service business unit can be a reference for improving performance and participating in sustainable development and food sovereignty.

5 NOVELTY

Previous studies related to the use of Spermatozoa Epididymis are still limited to rare or protected animals or livestock. Utilization of Spermatozoa Epididymis in the management of the RPH-R service business unit is still limited to the ecological and technological dimensions, and has not considered the need for harmonization in the economic, social, and regulatory dimensions.

The novelty of this research is the formulation of leverage factors for the sustainability of the use of Spermatozoa Epididymis in the management of the RPH-R service business unit, by incorporating the integration of several dimensions, namely the ecological, economic, social, regulatory, and technological dimensions.

6 METHODOLOGY

Epididymal Sampling

Epididymal samples were taken from RPH-R in Bogor, Yogyakarta, and Bali in 2019. The research started from preparing diluents, testicular/epididymis collection, organ preparation, sperm collection, semen evaluation, semen processing, freezing and testing the quality of spermatozoa after freezing.

Preparation of Diluent

All chemicals used were Merck products (Germany), and antibiotics (Meiji Seika Pharma, Japan). Tris basic diluent composition (buffer) is presented in Table 1.

Table 1. *Tris Buffer Composition*

Composition	Amount
Tris (*hydroxymethyl*) aminomethan (g)	3.03
Citric acid (g)	1.78

Fructose (g)	1.25
Aquabidest (ml)	*100*

The buffer was then added with 20% egg yolk from the total volume of the diluent, 6% glycerol and 1000 IU/ml antibiotics and 1 mg/ml streptomycin. The diluent is frozen and thawed at the time of use.

Testicular/Epididymal Collection

Testicular/epididymal collections were carried out at RPH Bogor, Yogyakarta and Bali. The testes and epididymis were taken from males that was just slaughtered 15 minutes before.

Organ Preparation

The testes were prepared up to the vas deferens, both ends of the sections were ligated to prevent the release of spermatozoa. The connective tissue around the organ is removed carefully to avoid bleeding. Organs were taken and immersed in physiological NaCl solution (0.9% NaCl). The testes and epididymis were measured for parts.

Spermatozoa Collection

Spermatozoa were collected from dexter and sinister. The head, body and cauda were incised alternately using a scalpel. Each part of the epididymis is flushed with a cement diluent solution. The white liquid that comes out of each part of the epididymis is collected in a petri dish.

Semen Evaluation

Semen evaluation was carried out only microscopically for spermatozoa motility, spermatozoa concentration, spermatozoa viability and spermatozoa morphology. The assessment is carried out in the following manner:

a. *Spermatozoa Motility*

Motility assessment can be done by dripping cement and physiological NaCl on a glass object with a ratio of 1:4 or 1:5 and warmed with a heating table. The semen was then observed using a microscope at a magnification of 400 times by observing the movement of the spermatozoa. Observations were made in 10 fields of view and graded in %.

b. *Spermatozoa Viability*

Spermatozoa viability was tested using eosin-nigrosine staining. The cement was mixed with eosin-nigrosine (1:3) dye. The mixture of cement and eosin-nigrosine was prepared for smears and dried on a heating table for 10 seconds. The preparations were observed under a microscope with a magnification of 40 × 10. Spermatozoa were observed in 10 fields of view or with a sperm count of at least 200 cells. The live spermatozoa do not absorb the color (transparent) and the dead will absorb the red color on the head. Spermatozoa were counted by dividing the number of live spermatozoa by the total number of spermatozoa counted multiplied by 100%, with the formula:

$$\text{Spermatozoa viability} = \frac{\Sigma \text{ live spermatozoa}}{\Sigma \text{ total spermatozoa}} \times 100\%$$

c. *Spermatozoa concentration*

It was carried out using a counting chamber, carried out by diluting cement in a formal saline solution with a ratio of 1:200 (5 µl semen: 995 µl *formal saline*). Observations were made using a microscope with a magnification of 400 times. Calculations are carried out on 5 counting chamber boxes, each of which has 16 small boxes. The concentration calculation was done by multiplying the number of spermatozoa in 5 boxes by 10^7.

d. *Spermatozoa Morphology*

Spermatozoa morphology was tested using eosin-nigrosine staining. The procedure for making preparations is the same as that for observing spermatoza viability. The preparations were observed under a microscope with a magnification of 40 × 10. Spermatozoa were observed in 10 fields of view or with a sperm count of at least 200 cells. Spermatozoa morphology testing was conducted to see the normality and abnormality of spermatozoa. Spermatozoa were counted by dividing the number of abnormal spermatozoa by the total number of spermatozoa counted multiplied by 100%, with the formula:

$$\text{Spermatozoa abnormality} = \frac{\Sigma \text{ abnormal spermatozoa}}{\Sigma \text{ total spermatozoa}} \times 100\%$$

Semen Processing

Semen/spermatozoa are mixed with diluent. Dilution can be calculated by the following equation:

$$\text{Dilution volume (ml)} = \frac{V \times M \times K}{\text{IB dose}} \times 0.25$$

Descripition: V = Semen volume (ml)
M = Spermatozoa motility (%)
K = Spermatozoa concentration (million/ml)
IB dose = 25 million

Freezing and Spermatozoa Quality Testing after Freezing

Semen was packed in mini straws (0.25 ml) and then equilibrated at 5 °C for four hours. The freezing process was carried out using styrofoam measuring 38 × 27 × 27 by storing a 6 cm high straw rack of liquid nitrogen (evaporated) for ten minutes. Frozen semen was stored in liquid nitrogen (-196^{o}C) for evaluation. The parameters of frozen semen examination include motility, viability, the same as in fresh semen.

Furthermore, several attributes of the ecological dimensions of the utilization of epididymal spermatozoa are discussed using the DELPHI method, using controlled, iterative and feedback information collection. To determine the leverage factors and the sustainability status of the ecological dimension of the utilization of spermatozoa epididymis, methods and stages of sustainability through the Multi Dimensional Scaling (MDS) method.

Sustainability Analysis Methods and Stages

In assessing the sustainability status of the Utilization of Epididymal Spermatozoa native to Indonesian cattle in Ruminant Slaughterhouses (RPH-R), the Rap-GametRescue method was used, which had been modified from the Rapfish program with MDS techniques, such as in the fisheries system (Fauzi & Anna, 2005) design of a sustainable beef cattle farming system to support the implementation of regional autonomy in South Bengkulu Regency (Mersyah, 2005), a sustainable dairy farming agribusiness model in tourism areas in Bogor Regency (Ridwan, 2006). The MDS method is used to design models, analyze, and design sustainable operational management. Non-metric MDS techniques have been used to describe the structure of bacterial communities and microbial populations as well as ecological communities at the RPH-R Macelo La Muda in Guaynabo, Puerto Rico (Godoy-Vitorino Filipa et al., 2012). At first Rapfish was developed by the Fisheries Centre, University of British Columbia or UBC Canada (Fauzi & Anna, 2005). The principle of the application of this analysis tool is indicator-based with an MDS-based settlement approach.

(Kavanagh, 2001) recommends five stages that must be passed in the Rapfish procedure, namely: (1) determination of indicators as assessment criteria and identification of current conditions, (2) assessment or score of each indicator, (3) ordination of each indicator, (4) Monte Carlo analysis and sensitivity, and (5) sustainability analysis. Meanwhile, according to (Fauzi, 2012) the procedure for using Rap-GametRescue is as follows: (1) attribute review includes various categories and scoring; (2) identification and definition of attributes; (3) scoring to construct reference points for good and bad; (4) Multi Dimensional Ordination for each attribute; (5) Monte Carlo Simulation, (6) Leverage Analysis; (7) Sustainability analysis.

Each indicator on each criterion is given a score based on the scientific judgment of the scorer. The score ranges from 0-3 or 0-4, depending on the state of each indicator which is interpreted from bad (0) to good (3) or (4). The score value of each indicator is analyzed multidimensionally to determine one or several points that reflect the position of sustainability of the five dimensions of the utilization of Spermatozoa Epididymis native to Indonesia in Ruminant Slaughterhouses (RPH-R) which are assessed against two reference points, namely the good point and bad point. According to (Kavanagh P. & Pitcher, 2004), scores were analyzed by Rap-GametRescue to determine sustainability status as shown in Table 2.

Table 2. *Category Index and Sustainability Status*

No.	Index score	Category
1	0,00 - 24,99	Poor (unsustainable)
2	25,00 - 49,99	Less (less sustainable))

3	50,00 - 74,99	Moderate (moderately sustainable)
4	75,00 - 100,00	Good (sustainable)

Source: Kavanagh and Pitcher (2004)

The sustainability index value of each dimension criterion for the utilization of Spermatozoa Epididymis of native Indonesian cattle at Ruminant Slaughterhouses (RPH-R) which includes ecological, economic, social, regulatory and technological dimensions is visualized in the form of a kite diagram. The most sensitive indicator that contributes to the sustainability index of the use of Spermatozoa Epididymis native Indonesian cattle in Ruminant Slaughterhouses (RPH-R), is shown through sensitivity analysis by looking at the shape of the change in Root Mean Square (RMS) ordinance on the x-axis. In this case, the greater the change in the RMS value, the more sensitive the indicator is in the sustainability of the use of Spermatozoa Epididymis native to Indonesia in Ruminant Slaughterhouses (RPH-R).

Rap-GametRescue is a statistical technique with an MDS approach, providing stable results compared to other multivariate analysis methods. MDS is essentially a perceptual mapping that relies on Euclidian Distance between one dimension and another. In MDS, the attribute or measure to be measured can be mapped within the Euclidian distance where objects perceived to have the same characteristics are considered to have the closest Euclidian distance. On the other hand, objects with different characteristics are said to have dissimilarities so that the difference between the two can be measured in the perception distance which is translated into a perception index such as a sustainability index. The distance determination technique is based on Euclidian Distance with the following formula:

$$d_{1,2} = \sqrt{(X_1 - X_2)^2 + (Y_1 - Y_2)^2 + (Z_1 - Z_2)^2 + \ldots} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Description :
$d_{1,2}$ = Euclidian distance
X, Y, Z = Attribute
1,2 = Observation

The euclidian distance between the two points ($d_{1,2}$) then in the MDS, is projected into a two-dimensional euclidian distance ($\dot{D}_{1,2}$) based on the regression formula in the following equation:

$$D_{1,2} = a + b\,D_{1,2} + c \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Description :
a = intercept
b = slope
c = error

In MDS, two points or the same object are mapped in a single point that is close to each other. The technique used is the ALSCAL algorithm and is easily available in almost every statistical software (SPSS and SAS). Rap-gametRescue in principle makes iterations of the regression process so that the smallest e value is obtained and tries to force the intercept in the equation to be equal to 0 (a=0). The iteration stops if the stress is < 0.25 (Byongho Choe, 2001). For attributes as much as m, stress can be formulated in the following equation:

$$stress = \sqrt{\frac{1}{m}\sum_{k=1}^{m}\left(\frac{\sum_i\sum_j\left(D_{ijk}^2 - d_{ijk}^2\right)^2}{\sum_i\sum_j d_{ijk}^2}\right)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(3)$$

The stress value is shown in Table 3.

Table 3. Stress Value

No.	Stress value	Suitability
1	➢ 20 %	Poor
2	(10 - 20) %	Moderate
3	(5 - 10) %	Good
4	(2,5 - 5) %	Very good

Source: (Kavanagh P. & Pitcher, 2004)

Through the rotation method, the position of the sustainability point can be visualized through the horizontal and vertical axes, with the sustainability index value given a score of 0 percent (bad) and 100 percent (good). If the system under study has a sustainability index value greater than or equal to 50 percent, then the system is said to be sustainable, and if the index value is less than 50 percent, the system is said to be unsustainable. The illustration of determining the sustainability index in the ordinance scale is at two extreme points of bad (0 percent) and good (100 percent). From the results of the analysis, the effect of error is obtained, which can be caused by various things, such as errors in scoring, misunderstanding of the attributes or imperfect conditions of the research location, variations in scores due to differences in opinions or judgments by observers, repeated MDS analysis processes, errors in data input or missing data, and high stress values.

Leverage Analysis

Leverage analysis is needed to determine the effect of stability if one attribute is omitted when ordination is carried out. The results of the Leverage analysis show the percent change in the root mean square (RMS) of each attribute. The higher the RMS value means the greater the attribute role in sustainability level (Kavanagh P. & Pitcher, 2004). It means the attribute with the highest percentage is the most sensitive attribute to sustainability

Monte Carlo Analysis

To evaluate the effect of error on the estimation of the ordinance value, Monte Carlo analysis is used, which is a statistical simulation method to evaluate the effect of random error on the estimation process, as well as to evaluate the actual value.

The output of the Rap-GametRescue analysis is a sustainability index from 0-100 which is displayed in the ordinance and leveraging indicators. The sustainability index is grouped into 4 categories, namely: 0-25 (poor or unsustainable); 25.01-50 (less sustainable); 50.01-75 (moderately sustainable); 75.01-100 (good or very sustainable).

7 RESULTS

The results of the sustainability study on the ecological dimensions of the utilization of epididymal spermatozoa in native Indonesian animals include the following attributes: (1) testicular physique, (2) spermatozoa motility, (3) semen color, (4) fresh semen pH, (5) semen concentration; (6) semen consistency, (7) spermatozoa viability, (8) spermatozoa morphology, (9) spermatozoa movement, (10) morphology, and (11) climate elements, especially air temperature. Supporting data for the nine attributes on the ecological dimension of sustainable use of spermatozoa epididymis is indicated by the physical condition of the testes and epididymis described in the morphometry of the testis and epididymis which includes 4 parameters.

1. Physical Testis and Epididymis of Bali Cattle

The testes and epididymis of Bali cattle obtained from Bubulak Integrated RPH Bogor City weighed between 220 and 235 g, with a length of 9.5 to 10 cm (Table 4).

Table 4. *Testis and Epididymis Morphometry of Bali Cattle from Bubulak Integrated RPH, Bogor City*

No.	Parameter	Right	Left
1	Testicle length (cm)	10±0,8	9.5±1.2
2	Testicular circumference (cm)	13±1,2	14±1.1
3	Epididymis		
	a. Caput length (cm)	6±0,15	6±0,14
	b. Caput width (cm)	5.5±0,18	6±0,17
	c. Corpus length (cm)	9±0,3	9±0,2
	Cauda length (cm)	8,2±0,5	11±0,45
4	Weight of testicles and epididymis (g)	220±5,8	235±4.3

Anatomically, the testes and epididymis have a normal shape, good consistency. The epididymis stands out clearly, this can be interpreted that the testes as gametes producers and the epididymis as a storage/storage place for spermatozoa function properly (Figure 1).

Figure 1. Testis and Epididymis of Bali Cattle from Bubulak Integrated RPH, Bogor City

2. Fresh Semen Quality from Epididymis

Spermatozoa of Bali cattle obtained from abattoirs showed a fairly good quality, which was milky white in color. Spermatozoa motility ranged from 30 to 75%, viability between 84.69 to 87.18%. **Spermatozoa concentration** is quite high between 2075 to 3050 million/ml. Primary spermatozoa abnormalities (on the head) ranged from 8.88 to 9.86%. The secondary abnormality was quite high, reaching 80%, namely the presence of residual cytoplasmic granules or cyto plasmic droplets (Figure 2).

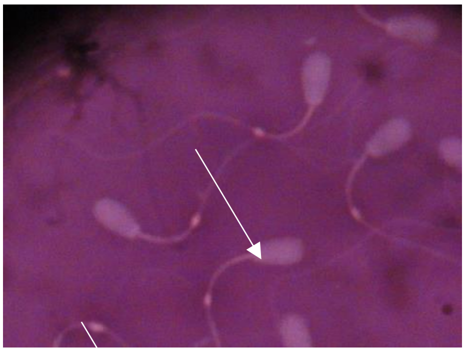

Figure 2. Secondary Abnormalities, Cytolasmic Droplets (arrows) from Cow Spermatozoa from the Epididymis of Bali Cattle

3. Quality of Frozen Bali Cow Semen from Epididymis in Egg Yolk Tris Diluent

The motility attributes of spermatozoa originating from the epididymis on the ecological dimension of the sustainability of the use of spermatozoa epididymis native Indonesian (cows) are shown in the data in Table 5. Moreover, the ecological sustainability of the utilization of epididymal spermatozoa is indicated by the spermatozoa viability attribute (Table 6).

Table 5. *Spermatozoa motility from Bali cattle epididymis after being frozen in Egg Yolk Tris Diluent.*

Repetition	Right epididymis	Left epididymis
1	25	40
2	20	40
3	20	40
4	20	40
5	20	35
6	20	40
7	15	35
8	15	40
9	20	40
10	20	40

Table 6. *Viability of Spermatozoa from Bali Cattle Epididymis after Freezing in Egg Yolk Tris Diluent*

Repetition	Right epididymis	Left epididymis
1	63,31	81,34
2	69,34	83,72
3	66,67	75,11
4	75,7	81,95
5	66,02	74,51
6	66,67	83,33
7	60,1	70,75
8	68,12	85,71
9	61,1	77,35
10	74,43	72,22

11	45	45
12	45	50
13	50	50
14	50	50
15	45	50
16	50	50
17	50	50
18	50	45
19	45	50
20	50	50
Average	33,75	44
SD	14,85	5,52

11	82,63	84,03
12	78,54	87,00
13	80,63	86,52
14	83,56	88,89
15	83,2	84,02
16	73,58	83,74
17	89,47	68,66
18	73,43	81,75
19	82,16	81,89
20	79,69	84
Average	73,92	80,82
SD	8,45	5,73

Furthermore, the sustainability of the ecological dimension of the use of spermatozoa epididymis of native Indonesian animals (cows) which includes the attributes of color, pH, consistency and concentration of semen can be seen in the description of the results of the characteristics of Bali cattle (Table 7).

Results of Observing the Characteristics of Bali Cattle

The results of observations of the use of spermatozoa epididymis in native Indonesian cattle in the laboratory are shown by the table of characteristics of fresh semen from the cauda epididymis as shown in Table 7.

Table 7. *Characteristics of Cattle Data in Bali in 2019*

Characteristics	**Male**			
	1		**2**	
Epididymis	Right	Left	Right	Left
Semen color	Milky white	Milky white	Milky white	Milky white
Semen pH				
Consistency	Thick	Thick	Thick	Thick
Spermatozoa motility (%)	**50**	**55**	**65**	**65**
Spermatozoa concentration (mill/ml)	**2230**	**2150**	**2430**	**2350**
Spermatozoa vability (%)	**58,5**	**62,5**	**73,2**	**74,8**
Abnormal spermatozoa morphology (%)	25	23	15	16,5
Testicular physique				
Semen concentration				

The Table of Characteristics of Bali Cattle Data shows that of the 9 attributes of animal use after slaughter at the Slaughterhouse illustrates that the sustainability of the ecological dimension of the utilization of spermatozoa epididymis tends to be sustainable.

8 CONCLUSIONS

The sustainability of the use of epididymal spermatozoa in native Indonesian animals (cows) at RPH-R in Indonesia includes an ecological dimension. The sustainability index of the ecological dimension indicates a sustainable status (> 50%) which includes 9 attributes, namely testicular physique, pH, color, consistency, motility, spermatozoa movement, spermatozoa concentration, viability, morphology. Of the 9 attributes that function as leverage, the most important is the **movement** and **concentration** of epididymal spermatozoa. The attributes of leverage on the ecological dimension are then worthy of being proposed as standards for government work units that have the authority and affairs related to matters of agricultural choice, namely animal food sovereignty.

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