BULETIN VETERINER UDAYANA

pISSN 2085-2495
eISSN 2477-2712

Received: 1 November 2025; Accepted: 1 December 2025; Published: 6 December 2025

QUALITY OF LANDRACE PIG SEMEN DILUTED WITH COCONUT WATER, EGG YOLK AND PURPLE SWEET POTATO ETHANOL EXTRACT

Kualitas Semen Babi *Landrace* yang Diencerkan Air Kelapa dan Kuning Telur dengan Penambahan Ekstrak Etanol Ubi Ungu

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How to cite: Viana LMJ, Bebas W, Trilaksana IGNB, Pemayun TGO, Laksmi DNDI, Susari NNW. 2025. Quality of landrace pig semen diluted with coconut water, egg yolk and purple sweet potato ethanol extract. *Bul. Vet. Udayana.* 17(6): 1867-1880. DOI: https://doi.org/10.24843/bulvet.2025.v17.i06.p10

Abstract

The success of artificial insemination in livestock depends on the quality and quantity of semen used in order to maintain a quality of semen itself. Hence, an effort to minimize the declining of spermatozoa quality during preservation is made by diluting semen using a diluent that contains nutrients in the right proportion between the diluent and semen.This study aims to determine the effect of adding an ethanol extract obtained from purple sweet potato to a coconut water egg yolk-based diluent on the quality of Landrace pig semen. This research was carried out based on an experimental laboratory study with a Completely Randomized Design (CRD) using a selected Landrace pig semen, which was taken from a pig. The research was also conducted in order to observe variables which included motility, viability, abnormalities, intact plasma membranes, and intact acrosomal membranes. The research used four specific treatments such as: P0 (BTS® as control), P1 (coconut water), P2 (coconut water and egg yolk), and P3 (coconut water, egg yolk + purple sweet potato ethanol extract), with six replications for each treatment. The data were analyzed using analysis of variance (ANOVA). If significant differences were found ($p<0.05$), statistical testing was performed using Duncan's test. The results of analysis showed that the P3 treatment group had the highest values ($p<0.05$) for motility and viability, at 47.16±0.40% and 58.83±0.40%, respectively, compared to the other treatment groups. The P3 treatment group showed the lowest value for abnormality at 6.00±0.00%, contributing to a decrease of abnormality in a sperm. Furthermore, observations of intact plasma membranes and intact acrosomal membranes also showed that the P3 treatment group had the highest percentages, at 59.33±0.51% and60.16±0.98%, compared to the other

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https://doi.org/10.24843/bulvet.2025.v17.i06.p10

treatment groups. The results of this study indicate that the combination of three components in the P3 treatment provides optimal protection for sperm cell structure, particularly in terms of motility, viability, abnormalities, as well as integrity of intact plasma membranes and intact acrosomal membranes. The electrolyte content in coconut water, phospholipids in egg yolk, and anthocyanins from purple sweet potato extract are thought to work synergistically to stabilize cell membranes and reduce free radical damage. Therefore, the results of this study recommend the use of local natural materials as a functional alternative diluent in pig reproductive technology.

Keywords: Landrace pig, coconut water, egg yolk, purple sweet potato, semen quality.

Abstrak

Keberhasilan inseminasi buatan pada ternak tergantung pada kualitas dan kuantitas semen yang digunakan dalam mempertahankan kualitas semen tersebut. Usaha yang dilakukan untuk meminimalisir penurunan kualitas spermatozoa selama preservasi adalah dengan pengenceran semen menggunakan pengenceran yang mengandung nutrisi yang sesuai dengan perbandingan yang tepat antara pengencer dengan semen. Penelitian ini bertujuan untuk mengetahui pengaruh penambahan ekstrak etanol ubi ungu dalam pengencer berbasis air kelapa kuning telur terhadap kualitas semen babi *Landrace*. Penelitian ini merupakan penelitian eksperimental laboratorium dengan desain penelitian berupa Rancangan Acak Lengkap (RAL) yang mengunakan semen babi *Landrace* yang diambil dari 1 ekor babi. Variabel yang diamati meliputi motilitas, daya hidup, abnormalitas, membran plasma utuh, dan membran akrosom utuh. Penelitian ini menggunakan empat perlakuan: P0 (*Beltsville Thawing Solution* BTS® sebagai kontrol), P1 (air kelapa), P2 (air kelapa kuning telur), dan P3 (air kelapa kuning telur + ekstrak etanol ubi ungu) dengan 6 pengulangan pada tiap perlakuan. Data dianalisa mengunakan *analysis of variance* (ANOVA), jika terdapat perbedaan nyata ($p<0,05$) maka dilanjutkan dengan uji statistik menggunakan Uji Duncan. Hasil analisis menunjukkan bahwa kelompok perlakuan P3 memberikan nilai tertinggi secara signifikan ($p<0,05$) pada variabel motilitas dan viabilitas yaitu masing-masing sebesar 47,16±0,40% dan 58,83 ± 0,40% dibandingkan dengan kelompok perlakuan lainnya. Pada variabel abnormalitas kelompok perlakuan P3 menunjukkan nilai terendah 6,00 ± 0,00%, yang berkontribusi dalam menurunkan tingkat abnormalitas spermatozoa. Selanjutnya pengamatan terhadap membran plasma utuh dan membran akrosom utuh juga menunjukkan bahwa kelompok perlakuan P3 memiliki presentase tertinggi, yaitu 59,33 ± 0,51% dan 60,16±0,98% dibandingkan dengan kelompok perlakuan lainnya. Hasil penelitian ini menunjukkan bahwa kombinasi tiga komponen dalam perlakuan P3 memberikan perlindungan optimal terhadap struktur sel spermatozoa, khususnya pada aspek motilitas, daya hidup, abnormalitas dan integritas membran plasma utuh maupun membran akrosom utuh. Kandungan elektrolit dalam air kelapa, fosfolipid dalam kuning telur, serta antosianin dari ekstrak ubi ungu diduga bekerja secara sinergis dalam menstabilkan membran sel dan mengurangi kerusakan akibat radikal bebas. Oleh karena itu, hasil penelitian ini merekomendasikan penggunaan bahan alami lokal sebagai alternatif pengencer fungsional dalam teknologi reproduksi babi.

Kata kunci: Babi *Landrace*, air kelapa, kuning telur, ubi ungu, kualitas semen.

INTRODUCTION

Pigs are polytocous livestock, but litter size varies between individuals, breeds, ages, and even parity (birth frequency). Pigs typically produce between 12 and 14 offspring per litter. The prolific nature of pigs makes them attractive for part-time or commercial farming, potentially increasing income (Lotu *et al*., 2017). To date, pig breeding efforts have been carried out using traditional methods, including natural mating and artificial insemination using fresh semen.

This has hampered pig development due to the lack of quality bulls and fresh semen available to pig farmers (Sungga *et al*., 2023). Tosi *et al*., (2021) stated that improving genetic quality and livestock production can be achieved by optimizing reproductive efficiency, namely by implementing an artificial insemination (AI) program with semen from superior bulls. The success of an AI program depends on the sows to be inseminated, semen quality, inseminator skills, and how to maintain fresh semen quality after ejaculation, as well as during semen preservation. Boar semen is highly sensitive to temperature changes. The lipid layer found in boar spermatozoa membranes is quite high, but its cholesterol content is low, so boar semen cannot withstand low temperatures. Boar semen can survive temperatures ranging from 15-20ºC but cannot survive long in vitro storage. Therefore, preservation efforts are needed to maintain the quality of semen over a relatively long period of time.

The quality of boar spermatozoa can be maintained for a long time if a diluent is added to the semen. The requirements for a good diluent for spermatozoa are that it is able to provide an energy source in the form of nutrients, protect spermatozoa from temperature changes, can act as a buffer, does not inhibit spermatozoa movement and is not toxic, thereby reducing the danger of lactic acid from metabolic waste. Beltsville Thawing Solution (BTS) diluent is a diluent that has a short shelf life with a defense period of 1-3 days. Beltsville Thawing Solution (BTS) is composed of Ethylene Diamine Tetraacetic Acid (EDTA) which plays a role in protecting the plasma membrane and glucose which provides nutrition for spermatozoa, sodium bicarbonate and sodium citrate act as buffers that can maintain pH stability for spermatozoa survival, antibiotics (penicillin, streptomycin) play a role in suppressing bacterial growth, and aquabidest which plays a role in diluting semen. Beltsville Thawing Solution (BTS) can be used to maintain spermatozoa motility and viability during storage at cold temperatures so that metabolic activity during the storage process can be reduced (Nahak *et al*., 2022).

Various diluents have been discovered in the development of semen dilution techniques. Several types of natural (organic) semen diluents can be used as alternative diluents. Coconut water contains carbohydrates (glucose, fructose, and sucrose), minerals, vitamins, and protein. The contents of coconut water can provide the physical and chemical needs of sperm, thus maintaining sperm quality (Mere *et al*., 2019).. Egg yolk hasSome of the contents include 49%, 16.5% protein, 0.7g carbohydrates, 32% minerals and 1.1g vitamins as well as lipoproteins derived from spermatozoa cells as a cold shock antidote. Egg yolk also contains glucose, various proteins, water-soluble vitamins and fats as well as viscosity which can benefit spermatozoa (Wawang *et al*., 2024). The addition of egg yolk containing phospholipids as a cryoprotectant is expected to help prevent damage to the membrane of boar spermatozoa (Manur *et al*., 2024).What needs to be considered to produce quality liquid semen is the right technique, such as the type and concentration of the diluent added and the antioxidant used (Sungga *et al*., 2023).

Purple sweet potato extract contains many active compounds that act as antioxidants such as vitamin C, anthocyanins, β carotene, and phenolic acids.Purple sweet potato contains natural antioxidants and, when administered in the right dosage, can minimize lipid peroxide reactions in the plasma membrane of spermatozoa, which are rich in unsaturated fatty acids, caused by free radicals.Purple sweet potato itself contains active compounds that are beneficial as a source of nutrition for spermatozoa such as glucose, minerals, and proteins that are important for spermatozoa metabolism that can bind reactive oxygen compounds in cells. Binding these oxygen compounds can prevent lipid peroxidation and membrane damage that can result in inhibited glycolysis and spermatozoa motility. The antioxidants contained in anthocyanins in purple sweet potatoes are the main variable in overcoming oxidative stress caused by free

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radicals. Antioxidants contained in purple sweet potato extract are thought to be able to inhibit free radicals that can reduce spermatozoa motility. Antioxidants are able to suppress the decline in the percentage of spermatozoa motility and viability during semen preservation by inhibiting free radicals (Shyadan *et al*., 2020).

Research and information related to the use of purple sweet potato ethanol extract on pig spermatozoa is still very limited, so this study was conducted to determine the effect of adding natural diluents such as coconut water, egg yolk and the addition of purple sweet potato ethanol extract on spermatozoa quality, namely motility, viability, abnormalities, intact plasma membranes and intact acrosome membranes in Landrace pig semen. With the special characteristics of coconut water egg yolk diluents with the addition of purple sweet potatoes, it is necessary to study them.

RESEARCH METHOD

Ethical Clearance of Experimental Animals

This study did not require ethical clearance because it did not use laboratory animals. The sample used was fresh semen obtained from Landrace pigs.

Research Object

The object of this study was a male Landrace pig aged >8 months as a source of semen. Semen collection was carried out at the Regional Artificial Insemination Center (UPTD), the Livestock and Forage Publishing House/Bali Provincial Livestock Institute, Baturiti, Tabanan.

Research Design

This research is an experimental laboratory study with a completely randomized design (CRD). The pig semen studied was Landrace pig semen divided into 4 groups based on the treatment given, namely P0 which was pig semen diluted with BTS as a control, P1 which was pig semen diluted withcoconut water,P2 is boar semen diluted with egg yolk coconut waterAndP3 is boar semen diluted with egg yolk coconut water + purple sweet potato ethanol extract. The number of samples used is calculated based on the Federer formula, namely: (t-1) (n-1) ≥ 15. Where: t is the number of treatments; n is the number of repetitions. It is known that t = 4 so that with the formula it becomes (4-1) (n-1) ≥ 15, then getting 3n ≥ 15 with the result n = 6. So the number of samples for each treatment is 6. Thus the number of samples used in this study is 24 units.

Research Variables

The variables in this study consist of three variables, namely: 1). The independent variable is Beltsville Thawing Solution (BTS).®, natural diluents namely coconut water, egg yolk coconut water, egg yolk coconut water + purple sweet potato ethanol extract. 2). The dependent variables are spermatozoa motility, spermatozoa abnormalities, spermatozoa viability, intact plasma membrane, intact acrosomal membrane. 3). The controlled variables are age, body weight of male Landrace pigs and semen motility collected at 70%.

Data Collection Methods

The research procedures carried out in this study are:

Semen Collection

Semen collection in pigs uses the glove-hand technique or massage with the aid of a dummy show. Only the second fraction, the sperm-rich fraction, is collected. A set of semen collection tools is used for collection (Priharyanthi *et al*., 2021).

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Preparation of Diluent Material

The coconut diluent is taken and cut using a sterile machete moistened with alcohol at the blunt end of the coconut. Once the inside of the coconut is visible, insert a syringe into the coconut while the coconut water is sucked into the syringe. Pour the coconut water into a measuring cup, add 1000 IU of penicillin and 1 mg of streptomycin, and cover with aluminum foil. (Mere *et al*., 2019).

Fresh chicken eggs were cleaned with alcohol cotton, then a small hole was made in the pointed part to separate the yolk and white. The yolk was slowly poured onto filter paper to absorb the remaining egg white and to prevent the yolk membrane from breaking. The yolk was then cracked and poured into a measuring cup. The yolk was then mixed with coconut water according to the desired concentration and homogenized using a stirrer (Butta *et al*., 2021). The egg yolks used were free-range chicken egg yolks, weighing between 25 and 55 grams, with a yolk volume of 20 and 25 ml per egg (Manehat *et al*., 2021).

Formulation of Semen Extenders and Semen Dilution Procedures

The diluents used in this study were Beltsvile Thawing Solution (BTS)® diluent as a control, coconut water, egg yolk coconut water, and egg yolk coconut water added with purple sweet potato ethanol extract. The concentration of purple sweet potato ethanol extract used in this study was 20%. The concentration of natural diluents was 80% coconut water and 20% egg yolk, so the total concentration of coconut water and egg yolk was 100% (Apriliana *et al*., 2021). The BTS® diluent was made by dissolving 50 grams of BTS in 1000 ml of aquabidestilata at 300C and then stirring until homogeneous (Bebas *et al*., 2015). The 20% concentration of purple sweet potato ethanol extract was made by adding 20 ml of purple sweet potato ethanol extract to 80 ml of egg yolk coconut water diluent. Then add 1000 IU penicillin antibiotic and 1 mg/ml streptomycin to each of the diluents and then homogenize using a magnetic stirrer and incubate at 370C.

Semen Quality Evaluation

After semen collection, macroscopic (volume, color, pH, odor, and consistency) and microscopic (mass movement, individual movement, viability, and abnormalities) tests are performed. Fresh semen quality evaluation is performed to determine its suitability for further processing. Microscopic examination of semen quality is performed after 48 hours of storage, including motility, viability, abnormalities, and intact plasma membranes and intact acrosomal membranes.

Sperm motility or individual progressive movement is assessed by looking at the comparison between progressive forward spermatozoa movement with non-progressive spermatozoa movement such as reverse, circular, vibrator and immobile or dead. The procedure at this stage is carried out by dripping one drop of semen with one drop of 0.9% physiological NaCl, both solutions are homogenized and one drop is taken then transferred to a glass object and covered with a cover glass then observed using a light microscope (Lumbantoruan *et al*., 2023).

Determination of spermatozoa viability was performed using the eosin-negrosin staining method. One drop of diluted spermatozoa was placed on a glass slide, then added with eosin-negrosin dye, and homogenized. A smear was then prepared by pressing and pushing the slide at a 45-degree angle and drying. The next step was observation under a microscope at 400x magnification. Dead spermatozoa will absorb the red dye due to the weakened permeability of their cell walls, while live spermatozoa will not absorb the dye (Apriliana *et al*., 2021).

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Evaluation of spermatozoa abnormalities was performed using differential eosin staining and observed under a microscope at 400x magnification. Abnormal spermatozoa are characterized by abnormalities in the head and tail (Marlize *et al*., 2021). Sperm abnormality testing is performed to observe any abnormalities in spermatozoa morphology. The observed abnormalities include large, double, small, bent, or broken heads in the head. Abnormalities in the tail include double, broken, and coiled tails (Bei *et al*., 2021).

The integrity of the spermatozoa plasma membrane is evaluated using the hypoosmotic swelling (HOS) test. Sperm with an intact plasma membrane are characterized by a coiled or bulging tail, while those with a damaged membrane are characterized by a long, straight tail without a tip (Marlize *et al*., 2021).

Observation of intact spermatozoa acrosome membranes (AAM) using the Saace and White method, namely 25 µl of semen was added to 100 µl of physiological NaCl solution containing 1% formalin. The solution was then homogenized and left for 5 minutes. Then, a smear preparation was made from the solution and observed under a microscope at 400x magnification on a minimum of 200 spermatozoa (Syafi'i and Rosadi, 2022). Sperm with intact acrosome membranes were characterized by a thick black head tip, while those with damaged acrosomes did not have a thick black color at the head tip (Widaringsih, 2019).

Data Analysis

Data analysis using analysis of variance (ANOVA), if there is a significant difference ($p < 0.05$) then it is continued with a statistical test using the Duncan Test.

RESULTS AND DISCUSSION

Results

From the results of macroscopic examination, the volume of semen collected was 150 ml with a thin consistency, milky white in color, distinctive aroma and a pH of 7.0. Microscopic examination revealed that fresh semen had good mass movement (++) with a motility percentage of 80%, viability of 90% and abnormalities of 4%. From the results of macroscopic and microscopic examinations, the boar semen had good quality and was suitable for further semen dilution. The results of the semen evaluation are presented in table 1. The results of the Duncan test analysis showed that P3 (coconut water egg yolk + purple sweet potato ethanol extract) provided the best results in maintaining motility, viability, abnormalities, intact plasma membranes and intact acrosome membranes of Landrace boar spermatozoa when compared to the treatment groups P0 (control = BTS), P1 (coconut water) and P2 (coconut water egg yolk). Observations of the quality of Landrace boar spermatozoa are presented in table 2.

Discussion

From the results of macroscopic examination, the volume of semen collected was 150 ml with a thin consistency, milky white in color, distinctive aroma, and a pH of 7.0. The results of this examination are not much different from the research of Sungga *et al*. (2023) which has a volume ranging from 100-300 ml and Garner and Hafez (2000) which has a volume of boar semen without gelatin ranging from 150-200 ml. On microscopic examination, it was found that the collected fresh semen had good mass movement (++) with a motility percentage of 80%, viability of 90%, and abnormalities of 4%.

These results show thatThe fresh semen has good quality and is still within the normal range. Sperm mass movement is a condition in which a group of spermatozoa move together in a counterclockwise direction. Rapid sperm movement is assumed to increase the likelihood of egg fertilization. Fresh semen suitable for dilution must meet the requirements of viability

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percentage ≥70%, motility percentage ≥70%, and abnormalities ≤20%. Evaluation of fresh semen quality is essential to determine the quality of spermatozoa produced by each individual animal. Fresh semen that meets the requirements can be diluted and processed into semen suitable for insemination (Komariah *et al*., 2020).

Sperm Motility

In this study, it was found that the P3 treatment group had an average motility percentage (47.16±0.40%), which was significantly higher ($p<0.05$) when compared to P1 and P2. This indicates that the addition of 20% purple sweet potato ethanol extract can act as an antioxidant that can maintain the motility of Landrace pig spermatozoa well. The results of this study are in accordance with Syahdan *et al*. (2020) that the addition of purple sweet potato ethanol extract can maintain the motility of Etawah goat spermatozoa.

In addition, the anthocyanin and flavonoid content act as antioxidants that protect sperm cell membranes from oxidative stress. So it can be concluded that the anthocyanin, flavonoid, and vitamin C and E content in purple sweet potato has great potential in maintaining the quality of Landrace pig spermatozoa, especially by reducing the level of abnormalities, increasing motility and viability and protecting the plasma membrane from oxidative damage. Rizal & Herdis, (2010) in their research strongly emphasized that to prevent and reduce lipid peroxidation reactions, antioxidant compounds such as vitamin C, vitamin E, glutathione, and β-carotene need to be added to semen thinners, because based on the results of several studies it was reported that the addition of various antioxidants to thinners can improve the quality of frozen semen of various livestock.

The average low sperm percentage was found in group P1, at 35.00±0.00%. The low motility in group P1 was likely due to the use of high concentrations of coconut water without the addition of buffers or membrane protectors. During storage, boar spermatozoa undergo various changes, including membrane integrity, structure, function, and fertility.

Membrane dysfunction begins with instability of the phospholipid layer (Apriliana *et al*., 2021). This is in line with the findings of Sulabda and Puja (2010), who stated that coconut water has an unstable pH and low buffering capacity. One factor causing decreased motility during storage was explained by Tamoes *et al*. (2014), who stated that decreased motility is the result of cold shock and increased lactic acid concentration. The main effects of cold shock on spermatozoa include decreased motility and survival, changes in membrane permeability, and disruption of membrane lipid composition.

The accumulation of lactic acid creates acidic conditions that damage cell organelles and disrupt metabolic processes for energy production. Decreased metabolic activity results in less energy, directly impacting sperm motility and survival. Apriliana *et al*. (2021) and Nau *et al*. (2024) also emphasize that coconut water needs to be combined with antioxidants to maintain sperm quality during storage.

This is further supported by Bala *et al*. (2025), that coconut water has the potential as a diluent, but it needs to be supplemented with an energy source and membrane protector. According to Toelihere (1993) in his book Physiology of Reproduction in Livestock, individual semen motility of at least 40% is an important requirement for use in artificial insemination (AI) because spermatozoa with motility below this threshold are not capable of reaching and fertilizing the egg effectively. The percentage of motility of the P0 treatment, namely BTS®, was 47.00±0.00%.

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These results indicate that BTS®, a commercial diluent that is frequently used and believed to have good quality in the field, has a complete chemical composition (buffer, energy, and osmotic protection), but does not contain natural antioxidants. Therefore, the addition of antioxidant compounds is highly recommended to ward off free radicals and maintain spermatozoa viability during storage or freezing (Bebas *et al*., 2015).

Sperm Viability

The results of the Duncan test analysis showed that the P3 treatment group (coconut water egg yolk + purple sweet potato ethanol extract) had higher viability ($p<0.05$) when compared to P1 and P2. This finding indicates that the combination of the three components is able to provide optimal protection to the spermatozoa plasma membrane, so that its viability can be maintained better during storage. Ulu *et al*. (2024) explained that young coconut water functions as a natural medium rich in electrolytes and simple energy sources such as glucose and fructose. In addition, egg yolk contains phospholipids and proteins that play an important role in protecting the plasma membrane from damage due to low temperatures and osmotic stress.

Meanwhile, purple sweet potato ethanol extract is known to contain anthocyanins and antioxidant compounds that are effective in warding off free radicals, thereby preventing oxidative damage to spermatozoa (Syahdan *et al*., 2020). The combination of these three ingredients produces a synergistic effect that increases cell stability, slows the decline in viability, and prolongs spermatozoa survival. The percentage of spermatozoa survival in the P3 treatment was not significantly different from the results of the study by Na'u *et al*. (2024), which reported a viability of 59.40 ± 5.81% in the P1 treatment using a combination of young coconut water, egg yolk, and dried moringa leaf extract on Landrace pig spermatozoa.

Dried moringa leaf extract, like purple sweet potato extract, also contains antioxidant compounds that have a high ability to neutralize free radicals. Although there has not been found an identical study with the formulation of coconut water, egg yolk and purple sweet potato extract, but several studies have shown that coconut water and egg yolk based diluents supplemented with local antioxidants are indeed effective in increasing the viability of Landrace pig spermatozoa. From the results of the Duncan test it was found that there was no significant difference in viability ($p>0.05$) between the P0 group (control = BTS) 58.66±0.51% with P3. This means that the quality of viability of sperm diluted with P3 diluent has the same quality as BTS diluent which has been widely used as a commercial diluent.

Sperm Abnormalities

The results showed that treatment P3 had the lowest level of significant abnormalities ($p<0.05$) when compared to P0, P1, and P2. The abnormality of P0 was not significantly different from P2, but was significantly different from P1. P3 (coconut water egg yolk + purple sweet potato ethanol extract) produced an abnormality value of 6.00 ± 0.00%, this is the lowest abnormality value. The addition of purple sweet potato ethanol extract contributed significantly to reducing spermatozoa abnormalities.

This combination is thought to have a protective effect on sperm cell structure through several potential mechanisms. Coconut water is known to contain electrolytes that play a role in maintaining osmotic stability and reducing oxidative stress during storage. Egg yolk serves as a source of phospholipids and proteins that protect the sperm plasma membrane from damage caused by low temperatures and physical manipulation. Meanwhile, purple sweet potato ethanol extract is rich in anthocyanins, powerful antioxidant compounds that can ward off free radicals and improve cell membrane integrity.

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The value of spermatozoa abnormalities in group P3 (6.00 ± 0.00%) was slightly higher compared to the results of Na'u *et al*.'s (2024) study, which reported abnormalities of 5.83 ± 0.70% in the treatment with a combination of young coconut water, egg yolk, and dried moringa leaf extract. The addition of local antioxidants such as moringa leaves has been shown to have a protective effect on spermatozoa morphology, in line with the benefits of anthocyanins from purple sweet potatoes used in this study.

Although the abnormality value in the P3 treatment group was slightly higher than the results of Na'u *et al*. (2024), this figure is still in the good category. Meanwhile, Johnson *et al*. (2000) stated that the percentage of abnormal spermatozoa per ejaculate should not exceed 20% to remain suitable for use in artificial insemination programs. Therefore, the P3 treatment showed better potential in maintaining normal spermatozoa morphology. This is very important in supporting successful fertilization and increasing pregnancy rates in Landrace pigs.

Intact Plasma Membrane

The evaluation results of intact plasma membranes of Landrace pig spermatozoa showed that the P3 treatment group (coconut water egg yolk + purple sweet potato ethanol extract) had the highest percentage of intact plasma membranes (MPU), namely 59.33 ± 0.51%. Statistically, this value was not significantly different ($p>0.05$) from the control group P0 (BTS®) which had an MPU of 59.00 ± 0.63%, but was significantly different ($p<0.05$) from groups P1 (45.83 ± 0.98%) and P2 (53.66 ± 1.03%). These results indicate that the combination of the three treatment components in P3 provides optimal protection for the spermatozoa plasma membrane, which is very important in maintaining cell viability and function during storage. The electrolyte content in coconut water, phospholipids in egg yolk, and anthocyanins from purple sweet potato extract are thought to work synergistically in stabilizing cell membranes and preventing damage from free radicals.

Treatment group P1, which only used coconut water as a diluent, showed the lowest MPU value and was significantly different from all other groups. This indicates that coconut water alone is not effective enough in maintaining the integrity of the spermatozoa plasma membrane. Treatment group P2, which combines coconut water and egg yolk, showed an increase in MPU value compared to P1, but was still lower than P0 and P3. This is supported by Ulu *et al*. (2024) who stated that the use of egg yolks from various birds in young coconut water diluents has been proven to prevent spermatozoa from cold stress or cold shock. This indicates that the addition of egg yolk provides an additional protective effect, although not as optimal as when combined with purple sweet potato extract. Interestingly, group P3 was able to match the effectiveness of BTS® in maintaining the integrity of the plasma membrane, despite using natural ingredients.

This reinforces the potential of locally based diluents as an efficient alternative in Landrace pig reproductive technology. Based on a recent literature search, no studies have been found that specifically use a diluent formulation of coconut water, egg yolk, and purple sweet potato ethanol extract in Landrace pigs to measure intact plasma membranes (IPM) of spermatozoa. However, several studies have used a similar approach and conceptually support the findings. A study by Putri (2019) showed similar results, where the semen treatment group was diluted using egg yolk citrate with the addition of purple sweet potato extract, resulting in an IPM percentage of 50.72.±5.16%. The antioxidant content in purple sweet potato extract, such as flavonoids and polyphenols, is known to have a protective effect on sperm plasma membranes. Furthermore, the anthocyanins contained in purple sweet potatoes act as powerful antioxidants in scavenging free radicals and maintaining cell integrity.

Other studies by Banamtuan *et al*. (2021) and Djawapatty *et al*. (2018) reported that the

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percentage of MPU of Landrace pig spermatozoa was 52.92 ± 0.61% and 49.14 ± 11.60%, respectively, using palmyra fruit juice and fructose as diluents. Palmyra fruit juice and fructose play an important role as a source of energy and nutrition for spermatozoa, as well as helping maintain plasma membrane stability during storage. This reinforces the fact that the use of natural antioxidant compounds in diluent formulations has great potential in maintaining the structural quality of spermatozoa across species.

Intact Acrosome Membrane

From the observation of the integrity of the acrosome membrane, it is known that the highest percentage of plasma membrane integrity was obtained in the P3 treatment group, which showed the highest MAU percentage (60.16%), and was statistically significantly different from the P1 and P2 groups, but not significantly different from the P0 control. This shows that the combination of egg yolk coconut water + purple sweet potato ethanol extract is able to match the effectiveness of BTS® in maintaining the integrity of the acrosome membrane.

Research specifically using a combination of coconut water, egg yolk, and purple sweet potato as diluents to test acrosome integrity is still relatively limited. However, several studies have evaluated intact acrosome membranes with different diluents, despite having similar nutritional components. One such study is by Parera *et al*. (2024), which used a tris-based diluent, fructose citrate, palmyra fruit mesocarp extract, and egg yolk (TCF+EM+KT). The results showed a level of acrosome integrity of 88.50%, with the lowest reduction in acrosome integrity compared to other treatments.

These findings indicate that the combination of nutrients in the diluent is effective in maintaining spermatozoa quality during storage. Egg yolk is known to contain lipids and phospholipids that play an important role in protecting the acrosome membrane from damage caused by cold shock and oxidative stress, as described by Sariozkan *et al*. (2010) and Hu *et al*. (2010).

Furthermore, the presence of beta-carotene as an antioxidant in the mesocarp extract of lontar fruit also contributes to suppressing lipid peroxidation and preventing membrane damage due to exposure to reactive oxygen species (ROS) during storage. This further confirms that the use of antioxidants in diluent formulations is crucial for increasing viability and maintaining the structural integrity of spermatozoa, especially during storage at low temperatures (Parera *et al*., 2019).

From the Duncan test analysis, it was found that the P1 treatment group had the lowest MAU value, namely 46.50 ± 1.3%, significantly different from all other treatment groups. This indicates that coconut water alone is not effective enough in maintaining the structure of spermatozoa acrosomes. The P2 treatment group showed an increase of 54.16 ± 1.47% compared to P1, but still lower than P0 (60.00 ± 1.09%) and P3 (60.16 ± 0.98%). This indicates that the addition of egg yolk provides an additional protective effect, although not as optimal as when combined with antioxidants from purple sweet potatoes.

The acrosomal membrane is an essential component of the sperm head, storing hydrolytic enzymes such as hyaluronidase and acrosin, which are necessary for penetrating the zona pellucida during fertilization. The integrity of this membrane significantly determines the sperm's ability to reach and fertilize the egg. The high percentage of intact acrosomal membranes (AAMs) in group P3 indicates that the diluent formulation used provided optimal protection for the acrosome structure during storage.

Statistically and biologically, P3 diluent was shown to be the most effective in maintaining the acrosome membrane integrity of Landrace boar spermatozoa. This formulation has great

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potential as an efficient alternative to natural diluents and supports successful fertilization by maximizing cell structure protection.

CONCLUSIONS AND SUGGESTIONS

Conclusion

From the research results it can be concluded that P3 Diluent(coconut water, egg yolk + purple sweet potato ethanol extract) gave the best results in maintaining the quality of Landrace pig semen stored at 15°C for 48 hours, with 47.16% motility, 58.83% viability, 6.00% abnormality, 59.33% plasma membrane and 60.26% intact acrosome membrane.

Suggestion

It is recommended to conduct further tests with in vivo tests on the use of P3 diluent (coconut water egg yolk + purple sweet potato ethanol extract) in artificial insemination programs to determine the fertilization level of diluted semen.

ACKNOWLEDGEMENTS

The author would like to thank the Head of the Veterinary Reproduction Laboratory of the Faculty of Veterinary Medicine, Udayana University, the Regional Artificial Insemination Center (UPTD Balai Inseminasi Buatan Daerah), the Livestock Breeding and Forage/Livestock Institute (UPTD Perbibitan Ternak dan Hijauan Pakan/Lembaga Ternak) of Bali Province, Baturiti, Tabanan and all parties who have helped ensure the smooth running of this research until its completion.

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Table

Table 1. Results of macroscopic and microscopic examination of fresh semen from Landrace pigs.

Types of examination	Characteristics of semen	Observation result
Macroscopic	Volume	150ml
	Color	Milky white
	Consistency	Thin
	pH	7.0
	Aroma	Typical
Microscopic	Mass movement	++
	Motility	80%
	Viability	90%
	Abnormality	4%

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Table 2. Mean ± SD results of Landrace pig spermatozoa quality diluted with BTS® diluent, coconut water, egg yolk coconut water, egg yolk coconut water + purple sweet potato ethanol extract.

Component	Observation result (%)			
	P0	P1	P2	P3
Motility	47.00±0.00c	35.00±0.00[a]	42.33±0.51b	47.16±0.40c
Life force	58.66±0.51c	45.50±0.54a	53.33±0.51b	58.83±0.40c
Abnormality	6.66±0.51b	7.33±0.51c	7.16±0.40b,c	6.00±0.00 a
Intact Plasma Membrane	59.00±0.63c	45.83±0.98a	53.66±1.03b	59.33±0.51c
Intact Acrosome Membrane	60.00±1.09c	46.50±1.37a	54.16±1.47b	60.16±0.98c

Information: P0 (control= BTS); P1 (coconut water); P2 (coconut water egg yolk); P3 (coconut water egg yolk + purple sweet potato ethanol extract). Different letters towards the row indicate a significant difference ($p<0.05$), while the same letter does not indicate a significant difference ($p>0.05$).

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