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# Comparison of Duroc Boar Liquid Semen Quality Diluted with Beltsville Thawing Solution and Egg Yolk Coconut Water at Different Storage Temperatures

Maria Bazelica De Nuel Mawin-Ray*, Aulia Puspita Anugra Yekti***, Yohana Figetri Sangur*, Putri Utami**, Habib Asshidiq Syah**, Achadiah Rachmawati***, Trinil Susilawati***

*Postgraduate Student, Faculty of Animal Science, Universitas Brawijaya, Malang 65145, Indonesia
Email: mariabazelica@student.ub.ac.id, yohana_figetri@student.ub.ac.id
**Doctoral Student, Faculty of Animal Science, Universitas Brawijaya, Malang 65145, Indonesia
Email: putriu73@gmail.com, habibas142000@gmail.com
***Department of Animal Reproduction, Faculty of Animal Science, Universitas Brawijaya, Malang 65145, Indonesia
Email: auliapay@ub.ac.id, achadiahr@ub.ac.id, tsusilawati@ub.ac.id
Corresponding Author Email: tsusilawati@ub.ac.id

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## Abstract:

This study compared the effectiveness of Beltsville Thawing Solution (BTS) and a coconut water-egg yolk extender in preserving the quality of Duroc boar semen under different storage methods. Fresh semen was collected from a Duroc boar and evaluated for macroscopic and microscopic parameters before being diluted with either BTS or coconut water-egg yolk extenders. The treatment used T0: BTS without a water jacket, T1: BTS with a water jacket, T2: 85% coconut water + 15% egg yolk without a water jacket, T3: 85% coconut water + 15% egg yolk with a water jacket. The diluted semen was then stored using two methods: with and without a water jacket at 5°C. Semen quality parameters, including motility, viability, abnormalities, and pH, were assessed at 0, 24, 48, 72, and 96 hours post-storage. The results showed that both extenders could maintain sperm quality for up to 96 hours. However, the coconut water-egg yolk extender with the water jacket method exhibited better motility (47.59 ± 2.68%) and viability (56.40 ± 2.62%) at 96 hours compared to BTS (motility: 45.75 ± 1.58%, viability: 56.41 ± 3.43%). The increase in sperm abnormalities over time was observed in all treatments, but values remained within the acceptable threshold for artificial insemination (<20%). The water jacket method helped reduce cold shock and provided a more stable microenvironment, thus improving semen preservation efficiency. The coconut water-egg yolk extender with the water jacket method proved a viable alternative to BTS, as it effectively preserved boar semen motility and viability during 96 hours of storage. This study suggests that natural extenders like coconut water and egg yolk could be cost-effective solutions for improving boar semen preservation in artificial insemination programs.

*Keywords* — **Artificial insemination, Beltsville Thawing Solution (BTS), boar semen, coconut water, egg yolk, Duroc**

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## I. INTRODUCTION

Pigs hold significant economic and social value, particularly in East Nusa Tenggara, Indonesia, where they play a crucial role in various traditional ceremonies and serve as a vital source of animal protein. The demand for pork in this region continues to rise in line with the expansion of the culinary industry specializing in pork-based products [1]. Consequently, efforts to enhance productivity by applying reproductive technologies

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such as artificial insemination (AI) are essential [2,3].

Artificial insemination is a reproductive technology designed to improve genetic quality, prevent reproductive diseases, and accelerate livestock population growth [4]. The success of AI is influenced by semen quality, inseminator proficiency, and semen storage methods [5]. One key approach to maintaining semen quality is the use of extenders [6,7]. Semen extenders increase semen volume while preserving spermatozoa viability and fertility during storage [8]. The Beltsville Thawing Solution (BTS) is one of the most commonly used extenders in artificial insemination for pigs. It primarily contains glucose as an energy source, EDTA as a membrane protector, and sodium bicarbonate and citrate as pH buffers. However, BTS has a limited storage capacity, typically 1 to 3 days [9].

A more cost-effective alternative is using natural extenders, such as coconut water, which is rich in glucose and fructose. Coconut water is widely available, inexpensive, and has been shown to maintain sperm motility at 52% for up to 8 hours of storage [10,11,12]. Additionally, egg yolk is frequently incorporated into semen extenders due to its lipoprotein and lecithin content, which helps reduce cold shock and protect the sperm plasma membrane during cold storage [13,14]. This study compares the quality of liquid semen from Duroc boars diluted with Beltsville Thawing Solution and a combination of coconut water and egg yolk at different storage temperatures. The findings are expected to provide an effective natural extender alternative to support the success of artificial insemination programs in pig farming.

## II. MATERIALS AND METHOD

This section describes the materials and methods employed in this study.

### *A. Research Period and Location*

This study was conducted at the Liquid Boar Semen Production Laboratory, Regional Artificial Insemination Center (UPTD Balai Inseminasi Buatan Daerah) in Baturiti, Tabanan Regency, Bali Province, Indonesia. The research was carried out from October to November 2024.

### *B. Research Materials*

The animal used in this study was a two-year-old Duroc boar (ID: Chapoh 02) with a body weight of 240 kg. This boar exhibited individual sperm motility more significantly than 80% and was confirmed to be in healthy condition, with a proportional body and normal reproductive organs. The board had been trained for ejaculation since the age of five months and was housed at UPTD Balai Inseminasi Buatan Daerah, Bali Province.

### *C. Research Method*

This study employed a laboratory experimental method using a Completely Randomized Block Design (CRBD) consisting of four treatments; each replicated ten times. The treatments included: T0: BTS without a water jacket, T1: BTS with a water jacket, T2: 85% coconut water + 15% egg yolk without a water jacket, T3: 85% coconut water + 15% egg yolk with a water jacket.

Semen dilution was performed after meeting the eligibility criteria, including mass movement (++ or +++), sperm concentration exceeding $150 \times 10^6$ cells/mL, and individual sperm motility above 60%. The qualified semen was then diluted using two primary extenders: Beltsville Thawing Solution (BTS) as the control and a coconut water-based extender with egg yolk variation. Each extender was placed in labeled tubes and carefully mixed with semen by pouring along the tube walls to prevent turbulence that could damage spermatozoa structures. After homogenization, the diluted semen was stored in a refrigerator at 5°C until further analysis.

### *D. Semen Collection*

Before semen collection, the boar was bathed to remove dirt around the preputium and scrotum to prevent bacterial contamination. The boar was then guided to the collection area, directed toward an artificial sow (dummy), and stimulated through massage to enhance libido. Upon erection, semen was collected using a sterile collection glass. The ejaculation process lasted between 3 and 20 min, and the collected semen was immediately transported to the laboratory for quality evaluation before dilution and storage [14].

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*E. Preparation of Beltsville Thawing Solution (BTS) Extender*

The preparation of BTS began with weighing 5 g of BTS powder, which was then placed into an Erlenmeyer flask. Subsequently, 100 mL of double-distilled water (aquabidest) was added, and the mixture was stirred for 10 min until homogeneous. After homogenization, the BTS solution was maintained in a water bath at 37°C until use.

*F. Preparation of Coconut Water Extender*

Coconut water was obtained from green coconuts at an optimal maturity stage. The coconut surface was first sanitized with alcohol-soaked cotton, and the blunt end was cut using a sterile alcohol-pretreated machete. Once the inner flesh was exposed, the coconut water was extracted using a sterile syringe, transferred into a measuring glass, and covered with sterile aluminum foil. To deactivate enzymes that may affect sperm viability, the coconut water was heated at 80°C for 25 min, then cooled to 30°C before use.

*G. Preparation of Egg Yolk Extender*

The egg yolk was obtained from commercial chicken eggs. The eggs were sanitized with alcohol, and the upper shell was carefully peeled to separate the egg whites. The yolk was slowly poured onto a filter paper to absorb residual egg white while keeping the yolk membrane intact. The yolk was then transferred into a measuring glass, placed in a centrifuge tube, and centrifuged at 2.000 rpm for 15 min. The supernatant was collected while the pellet was discarded. The purified yolk was then mixed with double-distilled water at a 1:2 ratio (15 mL egg yolk + 25 mL aquabidest) and stirred for 15 minutes until homogeneous.

*H. Preparation of of Coconut Water and Egg Yolk Extender*

A mixture of 85 mL coconut water and 15 mL egg yolk was homogenized. Antibiotics, including penicillin (1,000 IU/mL) and streptomycin (1 mg/mL), were added to inhibit bacterial growth. The solution was stirred until thoroughly mixed and maintained in a water bath at 37°C until use.

*I. Sperm Quality Parameters*

Semen quality evaluation was conducted in two stages: fresh semen analysis and post-dilution storage analysis. In the fresh semen analysis, both macroscopic and microscopic parameters were assessed. The macroscopic parameters included color, which was observed visually; ph, which was measured using a pH meter to determine the semen's acidity balance; odor, which was evaluated based on semen scent; consistency, which was assessed by examining the semen's viscosity, and volume (mL), which was measured using a sterile measuring glass [15].

The microscopic analysis involved evaluating several key sperm quality parameters. Sperm motility (%) was examined under a microscope at 400× magnification to assess the ability of spermatozoa to move effectively. Sperm viability (%) was determined using the eosin-nigrosin staining method to calculate the percentage of live spermatozoa. Sperm concentration ($\times 10^6$ cells/mL) was measured using a hemocytometer to quantify the total number of sperm cells per milliliter of semen. Sperm abnormalities (%) were analyzed by identifying the proportion of spermatozoa with morphological defects, including abnormalities in the head, midpiece, or tail. Additionally, total motile spermatozoa ($\times 10^6$ cells/mL) was calculated by multiplying the percentage of progressively motile spermatozoa by the total sperm concentration [16].

*J. Data Analysis*

The data were analyzed using Analysis of Variance (ANOVA) under a Completely Randomized Block Design (CRBD) to assess the effects of treatments on the observed parameters [17]. A post-hoc test was conducted using R Studio software if significant differences were detected.

## III. RESULTS AND DISCUSSION

### Fresh Semen Quality

The evaluation of fresh semen from Duroc boars was conducted through both macroscopic and microscopic assessments (Table I). Macroscopically, the semen appeared milky-white, had a liquid consistency, and a pH of 7.8 [18,19], who stated that the pH of fresh boar semen ranges between 7.3 and 7.8. Semen color and consistency are related to sperm concentration and quality, while pH is

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influenced by breed, environmental factors, and the buffering composition of semen [14].

The average semen volume per ejaculation was 142.0 ± 15.5 mL, which is lower than the 200–250 mL [20] but still within the 150–400 mL range [18]. Semen volume and concentration are significantly influenced by age, nutrition, environmental conditions, ejaculation frequency, and the boar's reproductive [14,20].

TABLE I
FRESH SEMEN QUALITY OF DUROC BOAR

| Semen Quality Parameters | Mean ± SD |
|---|---|
| Color | Milky-white |
| pH | 7.8 ± 0 |
| Odor | Characteristic semen odor |
| Consistency | Liquid |
| Volume (mL) | 142.0 ± 15.5 |
| Motility (%) | 78.57 ± 2.4 |
| Viability (%) | 81.0 ± 0.5 |
| Concentration ($\times10^6$ cells/mL) | 266.7 ± 15.6 |
| Abnormalities (%) | 4.7 ± 1.1 |
| Total motile spermatozoa ($\times10^6$ cells/mL) | 30,146.3 ± 4,929.1 |

Microscopically, the semen had a motility of 78.57 ± 2.4% and viability of 81.0 ± 0.5%, consistent with previous studies [21,22]. Sperm viability was higher than motility, as not all viable spermatozoa exhibit progressive movement [23]. The sperm concentration obtained was 266.7 ± 15.6 × $10^6$ cells/mL, which falls within the reported range of 200–300×$10^6$ cells/mL [18,24]. Variations in sperm concentration are influenced by genetic factors, age, nutrition, ejaculation frequency, and environmental temperature [25]. The results indicate that the Duroc boar semen meets the quality standards for dilution, with environmental factors and optimal collection techniques playing a crucial role in maintaining semen quality, ultimately impacting the success rate of artificial insemination.

### Sperm Motility After Dilution And Storage

Sperm motility is a key indicator of fertilization success, as only spermatozoa with progressive movement can navigate through the female reproductive tract and reach the oocyte. Motility was evaluated every 24 hours until sperm motility dropped below 40%, considered the viability threshold for artificial insemination.

TABLE II
MEAN SPERM MOTILITY AFTER DILUTION AND STORAGE

| Treatment | 0 hours | 24 hours | 48 hours | 72 hours | 96 hours |
|---|---|---|---|---|---|
| T0 | 73.14 ±5.15$^{ab}$ | 65.02 ±5.53$^{ab}$ | 57.22 ±5.64$^{b}$ | 49.61 ±2.95$^{b}$ | 41.87 ±3.72$^{b}$ |
| T1 | 73.87 ±4.49$^{a}$ | 67.42 ±3.32$^{a}$ | 61.18 ±2.32$^{a}$ | 54.98 ±1.66$^{a}$ | 45.75 ±1.58$^{a}$ |
| T2 | 72.25 ±6.11$^{b}$ | 63.27 ±3.48$^{b}$ | 56.48 ±2.21$^{b}$ | 49.65 ±2.89$^{b}$ | 39.94 ±4.63$^{b}$ |
| T3 | 73.78 ±4.38$^{a}$ | 65.83 ±3.86$^{ab}$ | 58.57 ±3.38$^{ab}$ | 53.81 ±3.20$^{b}$ | 47.59 ±2.68$^{a}$ |

Different superscript letters in the same row indicate a statistically significant difference (P<0.05)

Statistical analysis showed that sperm motility significantly differed ($P < 0.05$) between treatments. The BTS non-water jacket group (T0) maintained motility for up to 96 hours with an average of 41.87 ± 3.72%, while the BTS water jacket group (T1) exhibited higher motility (45.75 ± 1.58%) at 96 hours. Meanwhile, the coconut water-egg yolk non-water jacket group (T2) maintained motility at 49.65 ± 2.89% up to 72 hours. In contrast, the coconut water-egg yolk water jacket group (T3) sustained motility for a more extended period, up to 96 hours, with motility at 47.59 ± 2.68% (Table II).

Sperm motility in the BTS groups (T0 and T1) declined more rapidly compared to the coconut water-egg yolk groups (T2 and T3). This is because BTS is a short-term extender, which can only maintain sperm viability for 1–3 days at 5–17°C [25,26]. During storage, spermatozoa undergo metabolic processes that produce lactic acid, leading to a pH decline, which increases the risk of sperm death. Additionally, BTS lacks natural antioxidants, making spermatozoa more susceptible to oxidative stress caused by Reactive Oxygen Species (ROS), which can damage plasma membranes, degrade DNA, and reduce motility [26].

In contrast, using coconut water and egg yolk as alternative extenders demonstrated better motility preservation, especially when stored using the water jacket method. Coconut water contains glucose and fructose, which serve as primary energy sources for spermatozoa, as well as minerals and electrolytes

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that help maintain the stability of the semen environment during storage [12]. Meanwhile, egg yolk contains phospholipids, lipoproteins, and lecithin, which aid in preserving plasma membrane integrity, reducing cold shock effects, and protecting sperm from oxidative damage [14]. These findings suggest that the coconut water-egg yolk extender, particularly with water jacket storage, may serve as a viable alternative for improving semen preservation and AI success rates in boars.

The Figure 1 illustrates the comparison of sperm motility across different storage methods from 0 to 96 hours. The results indicate that motility declined more rapidly in T0 (BTS non-water jacket) and T2 (Coconut water + Egg yolk non-water jacket) compared to T1 (BTS water jacket) and T3 (Coconut water + Egg yolk water jacket). This suggests that storage methods significantly influence sperm quality, with water jacket storage providing better preservation.

The water jacket method is superior in maintaining spermatozoa quality compared to non-water jacket storage [26,27]. This method utilizes water as a medium to prevent cold shock, which results from a sudden drop in temperature, by ensuring a gradual cooling process. Storing semen in a refrigerator with water as a medium creates a more stable microenvironment, which helps spermatozoa adapt better to temperature fluctuations, reducing the risk of damage [29].

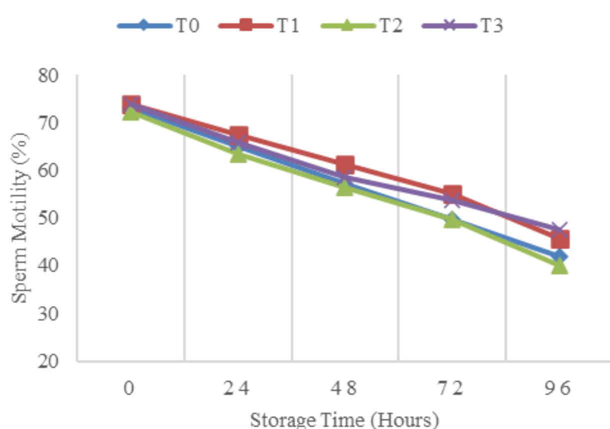


Fig. 1 Decrease in sperm motility during preservation in each treatment

## Sperm Viability After Dilution And Storage

Sperm viability, which represents the ability of spermatozoa to remain alive, was assessed using eosin-nigrosin staining, where dead spermatozoa absorbed eosin and appeared pink, whereas live spermatozoa remained transparent [30,31]. The mean sperm viability results showed that T0 (53.94 ± 2.83%) and T1 (56.41 ± 3.43%), which used BTS as the extender, exhibited significantly different results ($P < 0.05$) compared to T2 (55.09 ± 4.25%) and T3 (56.40 ± 2.62%), which used coconut water and egg yolk as the extender (Table III).

TABLE III
MEAN SPERM VIABILITY AFTER DILUTION AND STORAGE

| Treatment | 0 hours | 24 hours | 48 hours | 72 hours | 96 hours |
|---|---|---|---|---|---|
| T0 | 80.86 $\pm 0.63^{a}$ | 76.75 $\pm 2.77^{ab}$ | 68.94 $\pm 3.14^{b}$ | 62.06 $\pm 3.90^{a}$ | 53.94 $\pm 2.83^{b}$ |
| T1 | 81.18 $\pm 0.27^{a}$ | 78.83 $\pm 1.70^{a}$ | 71.95 $\pm 3.27^{a}$ | 63.04 $\pm 4.68^{a}$ | 56.41 $\pm 3.43^{a}$ |
| T2 | 80.77 $\pm 0.67^{a}$ | 76.08 $\pm 2.78^{b}$ | 70.45 $\pm 4.18^{ab}$ | 61.05 $\pm 2.43^{a}$ | 55.09 $\pm 4.25^{ab}$ |
| T3 | 81.15 $\pm 0.33^{a}$ | 77.42 $\pm 2.25^{ab}$ | 70.95 $\pm 2.27^{ab}$ | 64.60 $\pm 4.75^{a}$ | 56.40 $\pm 2.62^{a}$ |

Different superscript letters in the same row indicate a statistically significant difference ($P<0.05$)

The decrease in sperm viability was influenced by extender composition, cold shock protection, and pH changes due to lactic acid accumulation from metabolic activity [14,32,33]. The shorter viability observed in T0 and T2 is attributed to the higher coconut water content (85%) and lower egg yolk concentration (15%), leading to faster sperm metabolism.

The high fructose content in coconut water is rapidly metabolized by spermatozoa, generating higher levels of lactic acid and lowering semen pH [24]. The low egg yolk concentration was insufficient to maintain plasma membrane integrity, making spermatozoa more susceptible to membrane damage and death over time [14]. The Figure 2 illustrates the comparison of sperm viability across different storage methods from 0 to 96 hours. The results indicate that sperm viability declined more rapidly in T0 and T2 compared to T1 and T3. This suggests that water jacket storage provided better sperm viability preservation than non-water jacket storage.

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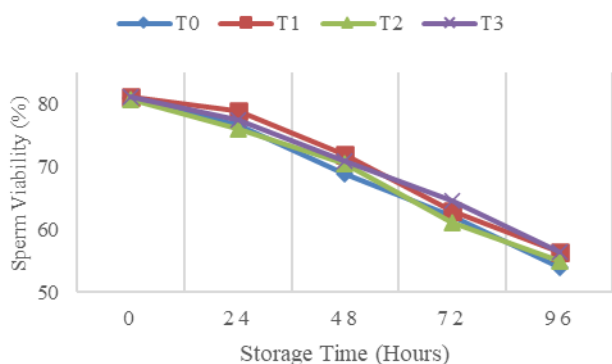


Fig. 2 Decrease in sperm viability during preservation in each treatment

Sperm viability decreases over prolonged storage as nutrient availability declines, affecting energy production, motility, and viability [33]. The water jacket method provided better preservation of sperm viability than the non-water jacket method [27]. The statistical results indicated that T1 and T3 did not show significant differences (P>0.05), while T0 and T2 showed significantly lower viability (P <0.05). The water jacket storage method provides a more stable microenvironment, allowing spermatozoa to adapt to temperature fluctuations more effectively, thereby minimizing damage and extending sperm viability [34].

### Sperm Abnormality After Dilution And Storage

The mean sperm abnormality percentages at 96 hours of storage were T0 (5.32 ± 0.22%), T1 (5.38 ± 0.78%), T2 (5.46 ± 0.43%), and T3 (5.44 ± 0.61%), all of which were within acceptable limits for AI (Table IV). These findings align with [35] who reported that longer storage duration increases sperm abnormalities due to cold stress and osmotic imbalance resulting from continued metabolic processes during storage.

Statistical analysis (ANOVA) showed that no significant differences (P>0.05) in sperm abnormalities were observed among treatments (T0 to T3), regardless of storage method. This is likely due to the extender compositions providing sufficient energy substrates and membrane protection. BTS, in particular, supplied adequate energy for spermatozoa while reducing lipid peroxidation, contributing to sperm abnormalities [36].

TABLE IV
MEAN SPERM ABNORMALITY AFTER DILUTION AND STORAGE

| Treatment | 0 hours | 24 hours | 48 hours | 72 hours | 96 hours |
|---|---|---|---|---|---|
| T0 | 4.24 ±1.02[a] | 4.86 ±1.10[a] | 5.00 ±0.80[a] | 5.19 ±0.52[a] | 5.32 ±0.22[a] |
| T1 | 4.07 ±0.82[a] | 4.66 ±0.84[a] | 4.90 ±0.94[a] | 5.02 ±0.49[a] | 5.38 ±0.78[a] |
| T2 | 4.54 ±0.96[a] | 4.79 ±0.74[a] | 4.91 ±0.63[a] | 5.01 ±0.67[a] | 5.46 ±0.43[a] |
| T3 | 4.26 ±1.06[a] | 4.71 ±0.82[a] | 4.77 ±0.71[a] | 5.04 ±0.68[a] | 5.44 ±0.61[a] |

Different superscript letters in the same row indicate a statistically significant difference (P<0.05)

The composition of the extender plays a crucial role in preserving sperm morphology and minimizing abnormalities during storage. Coconut water contains fructose and glucose, which serve as primary energy sources for spermatozoa, thereby supporting sperm metabolism throughout the storage period [11,24]. However, excessive energy metabolism can lead to the accumulation of metabolic byproducts such as lactic acid, which may contribute to a decline in sperm quality over time. Meanwhile, egg yolk provides lipoproteins and lecithin, essential in protecting spermatozoa from cold shock during storage [37]. Phosphatidylcholine (lecithin) and low-density lipoproteins (LDL) act as cryoprotectants, maintaining plasma membrane integrity and stabilizing pH levels, especially during critical temperature transitions that occur during extended storage [38,39]. The combined properties of coconut water and egg yolk contribute to a balanced extender formulation, providing both energy substrates and membrane protection, essential for maintaining sperm viability and reducing abnormalities.

The Figure 3 compares sperm abnormalities across different storage methods over 96 hours. The results show that sperm abnormalities increased gradually over time in all treatments but remained within the acceptable threshold (<20%) for AI [40].

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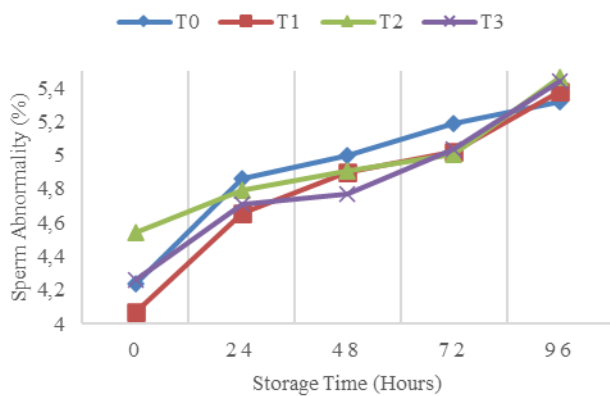


Fig. 3 Increase in sperm abnormality during preservation in each treatment

Sperm abnormalities refer to morphological deviations that can reduce fertility, as abnormal spermatozoa cannot fertilize oocytes, regardless of whether the abnormalities occur in the seminiferous tubules, male reproductive tract, or during ejaculation. The acceptable limit for sperm abnormalities in boar semen is ≤11.1% [41], abnormalities should not exceed 20% per ejaculation [25].

## IV. CONCLUSIONS

Based on the results of this study, both the Beltsville Thawing Solution (BTS) extender and the coconut water-egg yolk combination with different storage methods were able to maintain boar semen quality, including motility, viability, sperm abnormalities, and pH, for up to 96 hours of storage. The coconut water-egg yolk extender with the water jacket method demonstrated better preservation of sperm motility and viability compared to BTS. However, a gradual increase in sperm abnormalities was observed over time, indicating that extended storage duration still poses a challenge in maintaining optimal sperm quality.

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