

## Conference Paper

# Increased Integrity of Plasma Membrane and Acrosome Cap Spermatozoa Limousin Cattle at Post Thawing in Frozen Media by adding Seawater Extract

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## Abstract

This research was determined membrane integrity and acrosome cap of Limousin bull post thawing after adding seawater extract with different concentrations in extender skim milk and egg yolk to increased frozen semen quality. This research used fresh samples of Limousin bull's semen collected by using artificial vagina, then divided into 4 treatments and 6 replications. The experimental design that used was Complete Random Design. Analysis of the data using Analysis of Variant (ANOVA) one way then proceeds to the Duncan Multiple Range Test to determine significant differences between treatments. The first treatment P<sub>0</sub> no seawater extract added as a control. The second treatment P<sub>1</sub> was treated with 0.109 µL seawater extract, P<sub>2</sub> was treated with 0.426 µL seawater extract, and P<sub>3</sub> was treated with 1.09 µL seawater extract. The result showed that determine significant differences between treatments. The post thawing membrane integrity's result was P<sub>0</sub>= 22.00 ± 4.28, P<sub>1</sub>= 22.66 ± 3.61, P<sub>2</sub>= 25.00 ± 2.75, and P<sub>3</sub>= 29.00 ± 1.67. The post thawing acrosome cap's result was P<sub>0</sub>= 30.50 ± 1.37, P<sub>1</sub>= 31.50 ± 3.27, P<sub>2</sub>= 34.83 ± 2.31, and P<sub>3</sub>= 38.00 ± 1.41. The highest concentration added seawater extract to increased membrane integrity and acrosome cap spermatozoa in this research was 1.09 µL.

**Keywords:** Limousin bull; spermatozoa; seawater extract; membrane integrity; acrosome cap.

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## 1. Introduction

Artificial Insemination (AI) is a method of inserting or depositing semen into the female genital tract using a man-made device rather than a natural one. Artificial Insemination can save both cost and energy because this mating does not use direct male [1–3]. Artificial Insemination requires good semen in terms of quality and quantity both in fresh and frozen form. The quality of fresh semen tends to decrease compared to frozen semen although stored in a diluent medium or without dilution medium [4].

The process of freezing semen over the last few decades still remains at the same problem of the lifespan of spermatozoa after post-thawing is still low and limited to 50%, although it has been done through the best freezing technology [5–7]. The process of cooling, freezing, and thawing can cause physical and chemical stress on spermatozoa membrane that can decrease viability and fertility [8].

The diluent used in this study was diluent seawater extract or Nigarin. Seawater extract (cardiovit) is mineral water from sea water that is concentrated using sunlight. The process of making salt by the method of vaporization assisted by sunlight can produce nigarin [9]. According to [10], magnesium is indispensable for energy metabolism, the use of glucose, protein synthesis, synthesis and breakdown of fatty acids, all of the ATPase functions, almost all hormonal reactions and maintain the balance of cellular ionic. Magnesium will stimulate adenylyl cyclase enzyme that serves catalytic cAMP that will modulate sperm motility. The benefits of magnesium are very influential for sperm survival during the freezing process.

## 2. Materials and methods

### 2.1. Semen Collection

Semen Collection from a bull in this study using an artificial vagina performed twice a week.

### 2.2. Evaluation of Semen Before Treatment

The semen used must have good quality. Macroscopic and microscopic examinations were performed to determine the quality of semen. The macroscopic examination includes volume examination, consistency, color, odor and pH. While microscopic examination includes the motion of mass and individual motion, concentration, the percentage of live, dead and abnormal spermatozoa.

### 2.3. Seawater extract addition

Seawater extract (cardiovit) is produced by ITS, in its processing in 3 Districts: Pamekasan, Sumenep and Sampang. The seawater extract in this study was added after mixing the semen with yolk milk-egg skim diluent. Seawater extract concentrations containing Magnesium 0.109  $\mu\text{L}$ , 0.426  $\mu\text{L}$ , and 1.09  $\mu\text{L}$ .

### 2.4. Research Design

In this study there are 4 treatments: Treatment control (Po): Semen without the addition of sari seawater, Treatment 1 (P1): The addition of seawater extract on semen with a concentration of 0.109  $\mu\text{L}$ , Treatment 2 (P2): Addition of seawater extract at semen with concentration 0.426  $\mu\text{L}$ , Treatment 3 (P3): Addition of seawater extract on semen with a concentration of 1.09  $\mu\text{L}$

The determination of comparison of concentrations in this study based on previous studies [11].

### 2.5. Inspection After Dilution

Examination after dilution is on the integrity of the plasma membrane and the integrity of the acrosome cap. Examination of the plasma membrane of spermatozoa was performed using hypoosmotic swelling test or Hypoosmotic Swelling Test (HOS). The integrity of acrosome cap spermatozoa examination was performed with 1% formalin fixation.

## 3. Results

### 3.1. Percentage Integrity of Plasma Membrane Spermatozoa Limousin Cattle

The results of the examination on the percentage integrity of plasma membrane of post-thawing Limousin cattle spermatozoa after being treated with the addition of seawater extract in the yolk milk-egg skim diluent with various concentrations: control (Po), 0.109  $\mu\text{L}$  (P1), 0.426  $\mu\text{L}$  (P2), and 1.09  $\mu\text{L}$  (P3) shows the mean and standard deviation are Po of  $22.00 \pm 4.28$ ; P1 of  $22.67 \pm 3.61$ ; P2 of  $25.00 \pm 2.75$ ; And P3  $29.00 \pm 1.67$  for which can be seen in **Table 1** and **Fig 1**.

TABLE 1: Mean and Standard Deviation Percentage of Integrity Plasma Membrane Spermatozoa Limousin Cattle Post Thawing.

Treatment	n	Integrity of Plasma Membrane % ( $\bar{X} \pm SD$ )
P0	6	22.00 <sup>a</sup> ± 4.28
P1	6	22.67 <sup>a</sup> ± 3.61
P2	6	25.00 <sup>a</sup> ± 2.75
P3	6	29.00 <sup>b</sup> ± 1.67

Superscripts in the same column with different notations indicate that there is a marked difference ( $P < 0.05$ )

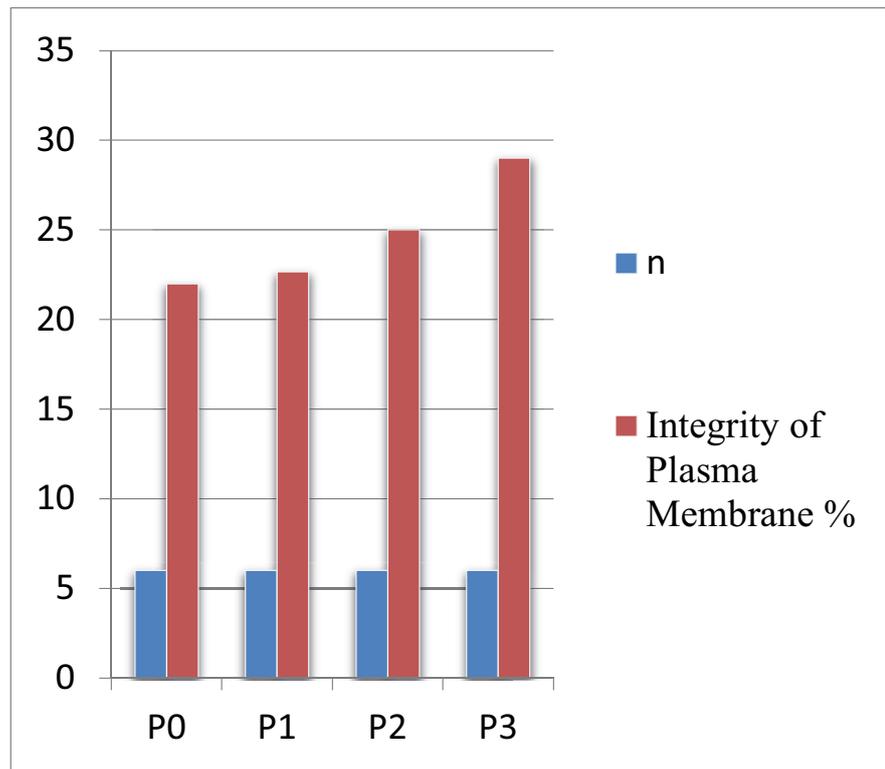
The result of ANOVA test on the percentage of plasma membrane spermatozoa of post-thawing Limousin cattle showed a significant difference between the four treatments so that it was followed by Duncan Multiple Range Test to determine the best concentration on percentage integrity of plasma membrane spermatozoa. The Duncan test results showed that P3 results (addition of 1.09  $\mu\text{L}$  seawater extract in yolk milk-egg diluent skim) were significantly different ( $p < 0.05$ ) to P0 (control without seawater addition), P1 (addition of 0.109  $\mu\text{L}$  seawater extract in yolk milk-egg skim diluent) and P2 (addition of 0.426  $\mu\text{L}$  seawater extract in yolk milk-egg skim diluent), whereas P0 was not significantly different with P1 (addition of 0.109  $\mu\text{L}$  seawater extract in diluent yolk milk-egg skim) and P2 (addition of 0.426  $\mu\text{L}$  seawater extract in yolk milk-egg skim diluent). The addition of seawater extract with a concentration of 1.09  $\mu\text{L}$  (P3) shows the highest result.

### 3.2. Percentage Integrity of Acrosome Cap Spermatozoa Limousin Cattle

Percentage integrity of acrosome cap spermatozoa Limousin at post-thawing after being treated with addition of seawater extract in yolk milk-egg skim diluent with various concentrations: control (P0), (P1 = 0.109  $\mu\text{L}$ ), (P2 = 0.426  $\mu\text{L}$ ), and (P3 = 1.09  $\mu\text{L}$ ) Shows the average number and standard deviation are P0 (30.50 ± 1.37); P1 (31.50 ± 3.27); P2 (34.83 ± 2.31); and P3 (38.00 ± 1.41) can be seen in table 2 and Figure 2.

Superscripts in the same column with different notations indicate that there is a marked difference ( $P < 0.05$ )

The result of ANOVA test on percentage integrity of acrosome cap spermatozoa of post-thawing Limousin cattle showed a significant difference between the four treatments so that it was followed by Duncan Multiple Range Test to find out the best concentration on the percentage of whole spermatozoa acrosome hood. The Duncan



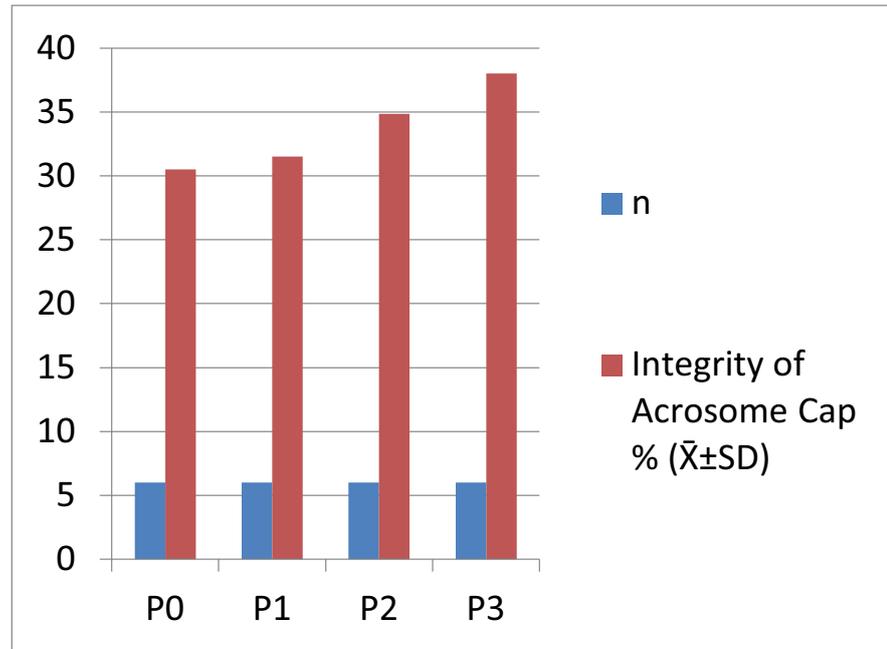
**Figure 1:** Mean and Standard Deviation Percentage Integrity of Plasma Membrane Spermatozoa Limousin Cattle Post Thawing.

**TABLE 2:** Mean and Standard Deviation Percentage Integrity of Acrosome cap Spermatozoa Limousin Cattle Post Thawing.

Treatment	n	Integrity of Acrosome Cap % ( $\bar{X} \pm SD$ )
P0	6	30.50 <sup>a</sup> ± 1.37
P1	6	31.50 <sup>a</sup> ± 3.27
P2	6	34.83 <sup>ab</sup> ± 2.31
P3	6	38.00 <sup>b</sup> ± 1.41

test results showed the results of Po (without the addition of seawater extract in yolk milk-egg skim diluent) were significantly different ( $p < 0.05$ ) against P2 (addition of 0.426 µL of sea water in the yolk skim milk thinner) and P3 (addition 1.09 µL seawater extract in diluent yolk milk-egg skim), but Po was not significantly different from P1 (addition of 0.109 µL of seawater extract in yolk milk-egg skim diluent). The addition of seawater extract with a concentration of 1.09 µL (P3) showed the highest result.

One of the causes of damage to spermatozoa during the freezing process is lipid peroxidation. The major components of cell membranes are phospholipids, glycolipids and cholesterol. The two main components contain polyunsaturated fatty acids which are highly susceptible to oxidation causing free radicals. The susceptibility of spermatozoa



**Figure 2:** Mean and Standard Deviation Percentage Integrity of Plasma Membrane Spermatozoa Limousin Cattle Post Thawing.

to increased lipid peroxidation is caused by cold stress. The process of peroxidation alters the structure of spermatozoa especially in the membrane and acrosome parts so that the membrane or acrosome will be damaged. Damage to acrosome cap affects fertilization ability of spermatozoa [12].

The addition of seawater extract with a concentration of 0.109  $\mu\text{L}$  gave a lower result compared with the application of a seawater extract of 0.426  $\mu\text{L}$  and 1.09  $\mu\text{L}$ . This is due to the addition of a seawater extract of 0.109  $\mu\text{L}$  concentration containing only a small amount of magnesium so it has not been able to produce ATP. The addition of seawater extract with a concentration of 1.09  $\mu\text{L}$  showed the highest result of plasma spermatozoa membrane at  $38.00 \pm 1.41$ . The results showed higher results than those delivered [13] that frozen semen suitable for use in the IB program should have a motility percentage of at least 40% and a minimum TAU percentage of 30%.

The integrity of the plasma membrane is a condition in which the plasma membrane remains intact to maintain the viability, motility and ability of spermatozoa fertilization. This is because the plasma membrane serves as the restriction of the continuous cell, which protects the cell organelles from mechanical damage and regulates the outflow of food substances and ions required in metabolic processes [14]. The integrity function of the spermatozoa plasma membrane is an important factor in the metabolism of spermatozoa, capacitance, acrosome reactions, and Spermatozoa bond with oocyte surface [15].

The addition of seawater extract with a concentration of 0.109  $\mu\text{L}$  gave a lower yield compared to the seawater extract with concentrations of 0.426  $\mu\text{L}$  and 1.09  $\mu\text{L}$ . This is probably due to the addition of seawater extract concentration of 0.109  $\mu\text{L}$  in the spermatozoa only in part to produce energy, therefore there is still no visible difference when compared with the control and has not been able to maintain the integrity of plasma membrane spermatozoa Limousin post thawing cattle optimally. The addition of seawater extract with a concentration of 1.09  $\mu\text{L}$  showed the highest result of spermatozoa plasma membrane at  $29.00 \pm 1.67$ . The P3 treatment generates enough energy and increases the integrity of the plasma membrane at the highest dose of 1.09  $\mu\text{L}$  resulting in ATP through metabolic processes. According to [11] also shows that the addition of Magnesium with a concentration of 5 mM can increase the motility and viability of spermatozoa. Testing of spermatozoa viability was used as an indicator of the integrity of membrane structure.

Seawater extract contains magnesium, one of the most important functions of magnesium is energy production. Spermatozoa require magnesium to activate ATP (adenosine triphosphate), which is the main energy source used by spermatozoa for movement [16]. The magnesium present in seawater extract (Nigarin) is a powerful stimulator of activity from messenger systems such as adenylyl cyclase enzymes that catalyze the formation of cAMP [17]. Cyclic AMP is a second messenger formed from ATP compounds by the action of the Adenylyl cyclase enzyme in the presence of  $\text{Mg}^{2+}$  which forms a complex with ATP to act as a substrate for reaction [18]. This increased cAMP concentration can further phosphorylate the enzyme protein kinase A (PKA) as well as the tyrosine kinase (PTK) protein. The phosphorylation of these enzymes will then activate tyrosine kinase as a biocatalyst in the process of phosphorylation of protein kinase. The phosphorylation product modulates spermatozoa movement and is followed by capacitance [19, 20].

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