

Protective capability of nanoparticle soybean extract on Bali bull spermatozoa during cryopreservation

S. Irmawanti¹, C.D. Nugraha¹, R.N. Hayati¹, Y.N. Anggraeny¹, S.Y. Hayanti¹, H.P. Wardono¹, S. Rahayu², G. Ciptadi³, T. Susilawati³, D. Ratnawati¹

¹Research Center for Animal Husbandry, Research Organization for Agriculture and Food, National Research and Innovation Agency of the Republic of Indonesia (BRIN), ²Department of Biology, Faculty of Mathematic and Natural Science, ³Department of Animal Reproduction and Breeding, Faculty of Animal Science, Universitas Brawijaya, Malang, Indonesia

Article information

Article history:

Received February 28, 2026
Accepted May 25, 2026
Published July 5, 2026

Keywords:

Bali cattle
Freeze semen
Nanoparticle
Soybean extract

Correspondence:

D. Ratnawati
dian090@brin.go.id

Abstract

The purpose of this study was to examine formula diluents of CEP and nanoparticle soybean extract in maintaining the quality of frozen semen spermatozoa on Bali bull. The research material used one Balinese bull. The research method was experimental with a Randomized Group Design, with 12 replicates (frozen semen). Treatment of frozen semen formula: T1 (CEP + EY 10%), T2 (CEP + NSE 50%), and T3 (CEP + NSE 60%). Parameters observed included: membrane integrity, acrosome integrity, motility, viability, abnormality, SOD, MDA, DNA Fragmentation and SEM. Data on membrane integrity, acrosome integrity, motility, viability, and abnormality were analyzed using ANOVA (SPSS 16). SOD, MDA, and Fragmentation DNA data were analyzed using Microsoft excel and SEM was presented descriptively. The results of the study of post thawing frozen semen showed progressive motility, viability of spermatozoa in group T1 was higher ($P < 0.05$) than T2 and T3. The value of spermatozoa abnormalities and DNA fragmentation in the P1 group was lower ($P < 0.05$) than T2 and T3. Diluent formula of CEP and 50% and 60% nanoparticle soybean extract can support the quality of frozen semen spermatozoa (viability, membrane integrity, acrosome integrity, and spermatozoa abnormality). Nevertheless, the performance was still below CEP diluent and egg yolk. The motility characteristics are less progressive, slow movement speed, short travel distance, and low vigor. However, the swimming pattern was more linear, straighter, and does not tend to rotate. Post thawing progressive motility reached 51.35% and 45.07% and still feasible to use for artificial insemination.

DOI: [10.33899/ijvs.2026.168823.4693](https://doi.org/10.33899/ijvs.2026.168823.4693), ©Authors, 2026, College of Veterinary Medicine, University of Mosul.
This is an open access article under the CC BY 4.0 license (<http://creativecommons.org/licenses/by/4.0/>).

Introduction

The freezing process (frozen semen) causes damage and a decrease in the quality of spermatozoa so that it can reduce spermatozoa fertility. The decrease in the quality of spermatozoa due to freezing, including: a decrease in viability of 20-30%, a decrease in motility of 28.8-40%, a decrease in plasma membrane integrity, an increase in chromatin condensation errors and spermatozoa DNA

fragmentation increased by 1.84% (1). Freezing causes spermatozoa to experience cold shock and various stresses (physical, osmotic, chemical and oxidative) resulting in damage to the structure, function, permeability and fluidity of the spermatozoa plasma membrane which has an effect on reducing spermatozoa quality (viability, abnormality, membrane integrity, acrosome integrity, DNA integrity, protein profile) (2). During freezing until thawing there is an increase in ROS, which results in lipid peroxidation that

damages the structure, function and degradation of the spermatozoa membrane. One of the results of lipid peroxidation, malondialdehyde (MDA), can damage DNA and proteins resulting in changes in protein functional activity (3).

The use of an optimal diluent can minimize cryoinjury of spermatozoa during freezing. One of the diluents developed (4), is Caudal Epididymis Plasma (CEP). This diluent has an ionic composition and osmolarity similar to that of caudal epididymis seminal fluid. The components in the diluent that serve to prevent cold shock (plasma membrane stabilization) are extracellular cryoprotectants. One of the plant-based components that can be optimized as an extracellular cryoprotectant is soybean with its lecithin composition (5). The use of soy lecithin-based diluents can produce the same quality as egg yolk (6,7). The content of phosphatidylcholine and several fatty acids (linolenic, linoleic, oleic, stearic and palmitic acids) in soy lecithin plays a role in preventing damage to the plasma membrane of spermatozoa due to the process of dilution, cooling and freezing (6). The composition of soy lecithin includes phosphatidyl choline, phosphatidyl ethanolamine, glycolipid, phosphatidic acid and triglyceride. Lecithin works by forming a film to coat the spermatozoa cell membrane from damage (8). The utilization of soy lecithin as an extracellular cryoprotectant has been done before. Tris diluent with 5% soy milk can support the progressive motility of post thawing frozen semen in cows reaching $48.4 \pm 1.2\%$ (9). The progressive motility of spermatozoa in frozen semen of deer (15% soy milk) post thawing reached $32.6 \pm 1.29\%$ (10).

Nanotechnology can produce nanoparticles with physico-chemical properties that are more soluble, stable and biologically effective than their unengineered homologues. Through the touch of nanotechnology in the utilization of whole soybean extract, it is expected to increase the solubility, stability, and effectiveness of soybean extract in supporting the quality of spermatozoa during freezing.

The results of previous studies state that CEP and nanoparticle soybean extract (50% and 60%) can support the quality of liquid semen spermatozoa up to the 9th day of cold storage (11). However, studies on the use of total soybean extract as extracellular cryoprotectant in frozen semen diluents has never been done before. The purpose of this study was to examine formula diluents of CEP and nanoparticle soybean extract in maintaining the quality of frozen semen spermatozoa on Bali cattle.

Materials and methods

Location and ethical approval

All the procedures of this research were approved by ethical clearance committee of Brawijaya University (No.059-KEP-UB-2023). This research was conducted in

Singosari National Artificial Insemination Center, East Java, Indonesia.

Materials

This study used Bali bull aged 8 years old, and fresh semen quality with progressive motility $\geq 70\%$. The design of this experimental used a Randomized Block Design (RCBD) with 3 treatments (T) and 12 replications per treatment, including: (1). CEP + Egg Yolk (EY) 10% (positive control/T1); (2). CEP + Nanoparticles Soybean Extract 50% (T2); and (3). CEP + Nanoparticles Soybean Extract 60% (T3), treatment levels of nanoparticle soybean extract added in CEP base diluent.

Nanoparticle soybean extraction procedure and preparation of based diluent (CEP)

The procedure of making nanoparticle soybean extract and preparation of based diluents (CEP) refers to (11).

Preparation of diluents A and B

Preparation of diluent A and diluent B (glycerolized) in each diluent formula. The calculation method of diluent volume was as follows:

$$\text{Total volume} = \frac{\text{fresh semen volume} \times \text{fresh semen concentration} \times 0.25 \text{ ml}}{25 \times 10^6}$$

Diluent volume = total volume - semen volume

Diluent volume A = (diluent volume/2)

Diluent volume B = diluent volume/2 (glycerol 13%)

Processing of frozen semen

Fresh semen obtained was diluted with the prepared diluents (A and B). Diluent A was added after fresh semen was obtained. The mixture of fresh semen and diluent A (glass tube), placed in becker glass with a water jacket and conditioned at 3-5 °C, maintained for 2 hours and checked the quality of spermatozoa (5 °C). The next stage was the addition of diluent B, homogenized and stored again at 3-5 °C for 30-60 minutes. The next stage was checking the quality of Before Freezing (BF) spermatozoa, and followed by filling sealing semen. Filling and sealing of semen was done using an automatic IMV machine. Pre-freezing was done for 12 minutes, including 5 minutes for adaptation at 4 °C and 7 minutes to reduce the temperature up to -140 °C, then immerse in liquid nitrogen. The frozen straw was stored in a goblet in a canister with a certain identity. Post Thawing (PT) examination was done after at least 24 hours later in a liquid nitrogen container. Thawing was done by dipping the straw into water at 37 °C for 30 seconds, and ready for spermatozoa quality analysis (post thawing).

Analysis of spermatozoa quality

The analysis of sperm quality parameters of frozen semen refers to several literatures, including: consistency, volume, pH, colour, (12); motility (11); viability and

abnormality (13); intact membrane (14); intact acrosome (15); DNA fragmentation (16); superoxide dismutase (SOD) (Universal Biological Ltd, Passhouse Farmhouse, Cambrige, United Kingdom); malondyaldehyde (MDA) (BioAssay Systems Corporate Place, California, USA); and scanning electrone microscope (SEM) (17).

Parameter and statistical analysis

Parameter of membrane integrity, viability, abnormality, and motility were analyzed using ANOVA (SPSS 16). Meanwhile, parameter of SOD, MDA, and DNA Fragmentation data were analyzed using Microsoft excel and structure of spermatozoa was presented descriptively by SEM.

Results

Membrane integrity, viability, and abnormality spermatozoa

Membrane integrity of spermatozoa in all treatments when cooling at 5°C and before freezing did not change much. The value of spermatozoa membrane integrity at various observation points (5°C, BF and PT) showed no significant difference between treatment groups. However, at post thawing there was a decrease, where the percentage of intact membrane T1 was higher than T2 and T3. The value of spermatozoa abnormality increased at post thawing, where the higher the level of soybean extract nanoparticles, the higher the level of spermatozoa abnormality. Post thawing acrosome damage in P2 and P3 spermatozoa was significantly higher ($P<0.05$) than P1 (Figure 1 and 2).

Motility and its kinematic parameter of spermatozoa

Motility of spermatozoa at observation points (5°C, BF and PT) in group P1 showed spermatozoa with higher of motility and progressive motility ($P<0.05$), and lower of static and slow sperm ($P<0.05$) than P2 and P3. Spermatozoa showed a decrease in the percentage of motility and progressive motility, while showing an increase in the percentage of static spermatozoa and slow spermatozoa (Figure 3).

SOD, MDA, and DNA fragmentation of spermatozoa

The level of SOD in P2 (3.36 ± 0.26 U/ml) and P3 (3.08 ± 0.05 U/ml) were higher than P1 (1.14 ± 0.18 U/ml), indicating that SOD antioxidant concentrations were better maintained (not depleted) in an effort to neutralize the MDA present (Figure 4).

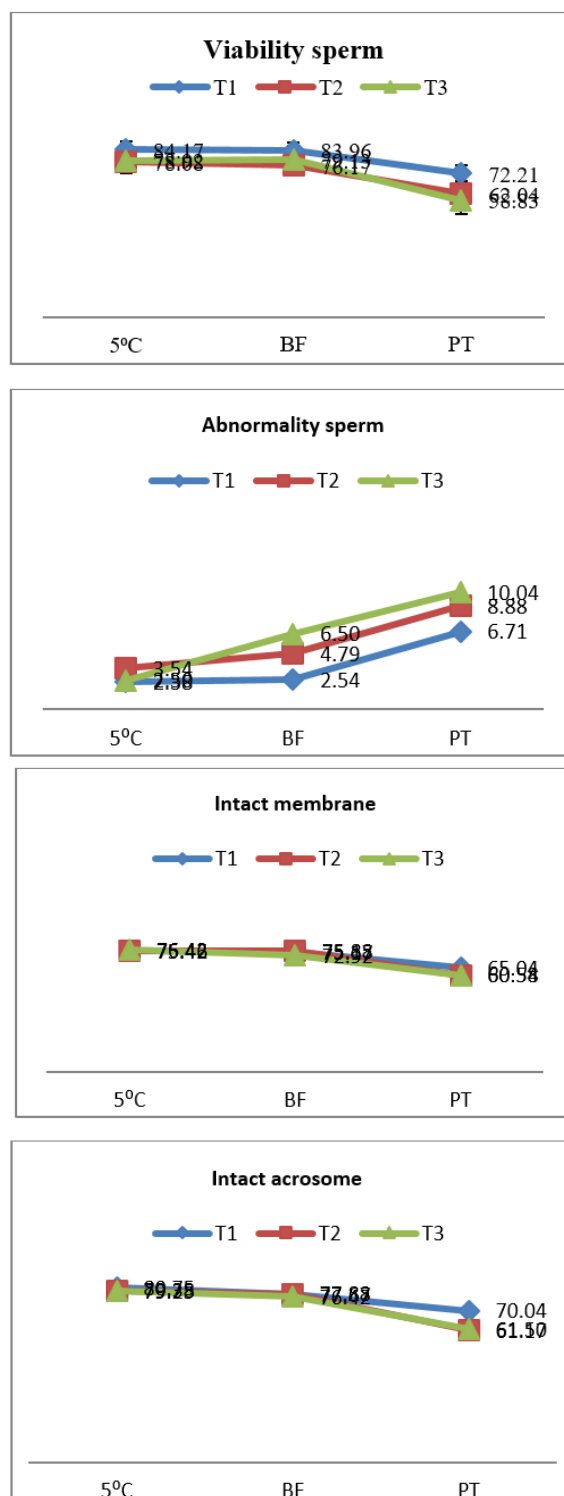


Figure 1: Membrane integrity, viability, and abnormality spermatozoa.

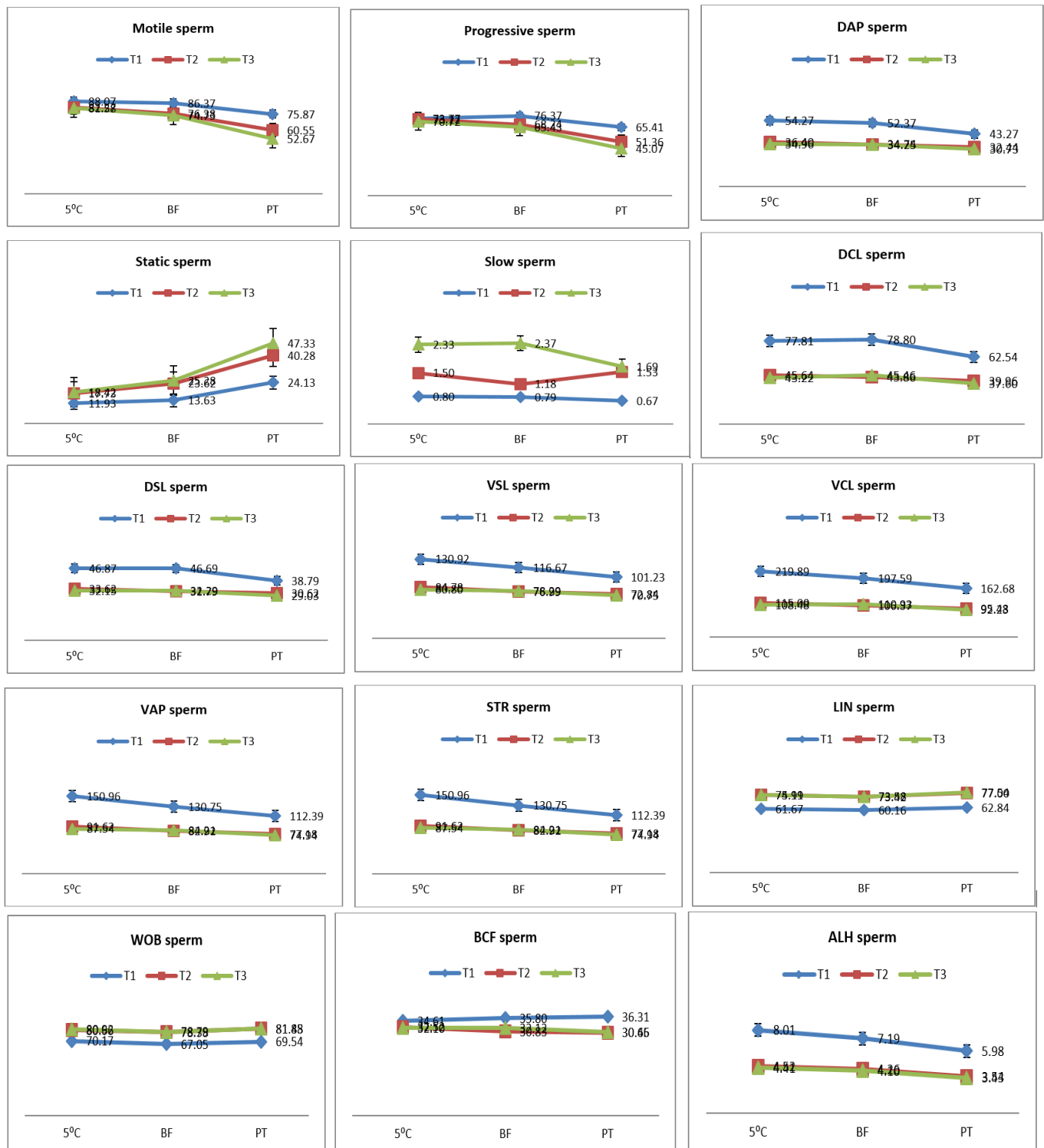


Figure 3: Motility and its kinematic parameter of spermatozoa.

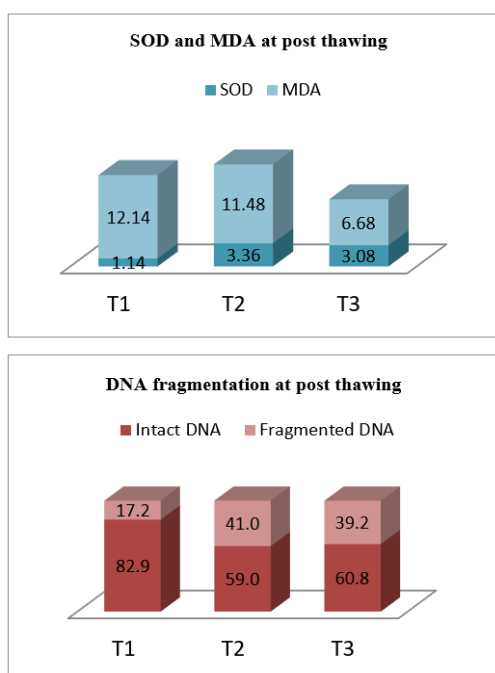


Figure 4: SOD, MDA, and DNA fragmentation of spermatozoa.

Discussions

Membrane integrity, viability, and abnormality spermatozoa

The decrease in the quality of post thawing spermatozoa in P2 and P3 is due to extreme osmotic stress. The extreme osmotic stress of spermatozoa occurred due to the high viscosity of the suspension due to the high level of nanoparticle soybean extract (50% and 60%) and 6.5% glycerolization. This causes a decrease in osmotic pressure resulting in fluid transfer from extracellular to intracellular and swelling of spermatozoa. This condition can increasingly cause lysis or destruction of the spermatozoa membrane and end in death (18). Another impact of the ballooning effect is the increase in spermatozoa abnormalities (19) and decrease of acrosome integrity. Observations of spermatozoa abnormality showed that the types of abnormality that were commonly found were in the midpiece and tail. Abnormality of the head (morphology) is closely related to the quality of DNA (genetic material) in it so that it greatly affects the fertility of spermatozoa (16). The midpiece and tail abnormalities that are commonly found include bent tail, bent midpiece, slightly bent midpiece, and coiled tail. These types of abnormalities are secondary abnormalities, where abnormalities occur due to environmental factors or spermatozoa handling. This abnormality is closely related to the osmotic pressure conditions of the spermatozoa environment, which increases

the viscosity of the suspension so that the osmotic pressure drops (19).

Abnormality in the spermatozoa tail has an impact on reducing spermatozoa motility. The freezing process affects the ability of the membrane to react to osmotic pressure. The thawing process causes 15-20% damage to the spermatozoa membrane. The spermatozoa membrane also undergoes structural changes after the spermatozoa freezing process, as much as 18% of changes occur in the spermatozoa head membrane. The reference value of spermatozoa membrane integrity in frozen semen is >50% (19). Related to this, frozen semen in all treatments has fulfilled the minimum standards for IB.

The acrosome status of spermatozoa is related to the condition of the spermatozoa membrane, because one of the functions of the spermatozoa membrane is to protect the organelles in the spermatozoa, one of which is the acrosome. This can be seen from intact membrane and acrosome values which are not much different at post thawing. Spermatozoa acrosome integrity plays an important role in measuring spermatozoa quality. The acrosome hood plays a role in protecting the acrosome, where there are enzymes that function and play a role in penetrating the zona pellucida of the egg during fertilization. These enzymes include hyaluronidase, acrosin and other hydrolytic enzymes (19). The integrity of the acrosome has a decisive influence on the penetration and fusion ability of spermatozoa in the egg, thereby affecting the fertility of frozen semen. Cold shock and various stresses, especially osmotic stress in P2 and P3 spermatozoa, have the effect of reducing acrosome integrity after freezing.

Soybean extract nanoparticles contain components: lecithin, fatty acids, glucose, amino acids, flavonoids, isoflavones, terpenoids, ubiquinone and vitamin E. The components in soybean extract nanoparticles (lecithin, fatty acids, glucose) can protect the spermatozoa cell membrane through several mechanisms. Lecithin functions to coat (coating) and fusion (bonding) the spermatozoa membrane so that the spermatozoa membrane is more stable (20). Lecithin also plays a role in the substitution of phospholipids and lipoproteins that are degraded by ROS. Fatty acids function to maintain the stability of the spermatozoa membrane. Lecithin is a phospholipid distributed in plants and plays a role in the regulation of animal cell bio-membranes. However, the limiting factor in the use of this diluent formula is the composition of soybean extract nanoparticles and glycerol which is not optimal, thus increasing viscosity and having a negative effect on the quality of spermatozoa.

Motility and its kinematic parameter of spermatozoa

The mechanism of decreased motility of frozen semen spermatozoa occurs due to cold shock (thermal stress) freezing (-196°C) and various stresses experienced by

spermatozoa during frozen storage. Stresses that occur during freezing include physical, chemical, osmotic and oxidative stresses that cause damage to the spermatozoa membrane. There is a decrease in the protective function of the membrane against spermatozoa cell organelles, one of which is mitochondria, which function to produce ATP as an energy source for spermatozoa movement. Another membrane function is as a medium for transporting metabolic materials from extracellular to intracellular. Damage to the membrane will reduce the metabolic rate of spermatozoa so that the energy for spermatozoa movement drops. Metabolic processes occur through the mechanisms of glycolysis, Krebs cycle, electron transfer, and oxidative phosphorylation. Mitochondria are key organelles in ATP synthesis and spermatozoa motility, and mitochondrial damage can cause apoptosis (21). Cryopreservation of spermatozoa is a procedure that causes the integrity of the mitochondrial membrane potential to be compromised. Oxidative phosphorylation by mitochondria is the main source of ATP production required for flagellar movement and mitochondrial changes are associated with loss of spermatozoa motility. This is consistent with the observation that there was a decrease in motility and progressive motility, as well as an increase in static spermatozoa and slow spermatozoa (21).

Damage to the plasma membrane of spermatozoa that occurs in the midpiece, causes the release of the enzyme aspartate aminotransferase into the extracellular. The release of the enzyme AsPAT (Aspartate aminotransferase) causes the cessation of ATP production so that spermatozoa are unable to move (increased static and slow sperm) (22). This enzyme is the main enzyme involved in ATP production in the mitochondria. The loss of this enzyme causes disruption in ATP production so that motility drops (23). The use of ATP is regulated endogenously, and is influenced by the cAMP (Adenosine Monophosphate) cycle. The role of cAMP is to regulate the release of ATP and also affects the motility of spermatozoa. Another cause of decreased motility is the depletion of energy sources during the freezing process until thawing causes depletion of energy sources. Soybean phospholipids contain a low amount of carbohydrates as an energy source for spermatozoa, when compared to egg yolk (24).

Osmotic stress in frozen semen spermatozoa occurs due to changes in osmotic pressure and imbalance of extracellular and intracellular osmotic pressure due to the dilution process, addition of glycerol (intracellular cryoprotectant) or egg yolk or soybean extract nanoparticles (extracellular cryoprotectant). The high suspension viscosity in P2 and P3 is due to the high level of nanoparticle soybean extract, and glycerolization. The sugar component in the nanoparticle soybean extract reached 19.73%, contributing to increasing the suspension viscosity. This caused the spermatozoa to not be free to move so that their motility was

below P1 (control). The P1 diluent suspension has a lower viscosity because the addition of egg yolk is only 10% so that the spermatozoa are free to move. The increase in viscosity causes extracellular osmotic pressure to drop resulting in an imbalance of osmotic pressure between extracellular and intracellular (osmotic stress). Osmotic stress causes cell dehydration, and the imbalance of extracellular and intracellular solute content causes the formation of ice crystals in spermatozoa (25). All these processes increase damage to the spermatozoa membrane which can have an effect on reducing the motility of frozen semen spermatozoa.

The pattern of spermatozoa movement velocity on various trajectories (average, straight, curve) during the freezing process showed a downward trend. Velocity (VAP, VSL, and VCL) of frozen semen P1 spermatozoa at post thawing showed higher results than other treatments (P2 and P3). Velocity of P2 and P3 spermatozoa from the beginning of freezing to post thawing did not show much change. Spermatozoa in diluent P1 are more free to move quickly because the suspension is not too thick. VAP, VSL, STR, and LIN values have a positive correlation with spermatozoa progressivity. While VCL, ALH, and BCF are effective indicators of spermatozoa strength. Supplementation of 0.5% and 1.0% nanoparticle soy lecithin into the diluent showed an increase in the percentage of motility, progressive motility, VAP, VSL, VCL, LIN, and STR of post thawing spermatozoa. A lower concentration of soy lecithin can lead to higher spermatozoa movement and movement strength (26). Spermatozoa in P2 and P3 diluents showed higher LIN, STR, WOB values than P1. This indicates that spermatozoa in diluents P2 and P3 are more progressive than P1. Whereas the VCL, ALH and BCF values of P2 and P3 spermatozoa were lower than those of P1 spermatozoa, indicating a lack of vigor or effective spermatozoa strength (27).

Superoxide Dismutase (SOD), MDA, and DNA fragmentation of spermatozoa

Soy lecithin can increase enzymatic antioxidant activity (SOD) (7). Soy lecithin is an antioxidant so that it can suppress LPO which is characterized by a decrease in MDA levels (28). In contrast, egg yolk-based diluents produced higher MDA concentrations than lecithin-based ones. Egg yolk contains high unsaturated fatty acids, as well as the composition of the plasma membrane which is high in PUFAs, making it sensitive to LPO, which is characterized by high MDA (20). Supplementation of diluent with nanoparticle soy lecithin (nano-SL) can increase endogenous enzymatic activity of SOD and TAC (total antioxidant capacity). In frozen semen, soy lecithin-based diluents produced lower LPO than egg yolk lecithin-based diluents (29).

The freezing process increases the accumulation of ROS, especially during thawing. The loss of enzymatic antioxidant activity after freezing is due to changes in membrane

integrity and increased permeability due to cryopreservation, so that the intracellular activity of antioxidant enzymes drops and causes the susceptibility of spermatozoa cells to peroxidative damage. The results of this study show that higher SOD activity in P2 and P3 is not directly proportional to other spermatozoa quality parameters (viability, intact membrane, intact acrosome, abnormality). So there is a suspicion that it is not oxidative stress that dominantly plays a role in causing spermatozoa membrane damage, but other factors (osmotic stress). There was no correlation between MDA level and acrosome integrity, so that acrosome damage that may occur during the freezing process is caused by factors other than ROS and lipid peroxidation (30).

The observation of the DNA quality of frozen semen spermatozoa (post thawing) showed that spermatozoa in the control diluent showed moderate DNA damage, namely $17.15 \pm 3.04\%$ (15-30%) (31). The DFi (DNA Fragmentation Index) of 15-25% is included in the category of decreased fertility and semen production (14). Meanwhile, spermatozoa in the P2 and P3 treatment diluents showed a very high level of DNA damage or infertility category, namely $41.00 \pm 5.23\%$ and $39.20 \pm 2.12\%$ (32). However, in this study, it is known that the PTM value that has met the SNI standard does not guarantee good DNA quality, so further evaluation is needed to determine the integrity of spermatozoa DNA.

The mechanism is that the freezing process causes various stresses on spermatozoa (physical, chemical, osmotic and oxidative), which negatively affects membrane integrity, acrosome hood, viability, and spermatozoa abnormality. Osmotic stress that occurs in P2 and P3 is higher than P1 due to higher suspension viscosity (low osmotic pressure). This affects membrane integrity so that the protective function of spermatozoa organelles decreases, one of which is DNA. This is also associated with an increase in post thawing frozen semen spermatozoa abnormalities. Head abnormality and total abnormality correlate with DNA fragmentation (16). Post thawing spermatozoa abnormality values showed an increase during the freezing process. The highest abnormality occurred in spermatozoa at P3, followed by P2 and P1. Spermatozoa membranes that are not intact or damaged increase the chance of disruption of chromatin, DNA and spermatozoa proteins. High percentage of spermatozoa viability does not guarantee good DNA quality, thus biasing spermatozoa assessment (33). This is one of the causes of the low IB success rate in the field.

Conclusion

Diluent formula of CEP and 50% and 60% nanoparticle soybean extract can support the quality of frozen semen spermatozoa (viability, membrane integrity, acrosome integrity, and spermatozoa abnormality). Nevertheless the performance was still below CEP diluent and egg yolk. The

motility characteristics are less progressive, slow movement speed, short travel distance, and low vigor. However, the swimming pattern was more linear, straighter, and does not tend to rotate. Post thawing progressive motility reached 51.35% and 45.07% and still feasible to use for artificial insemination.

Further studies are needed on the optimal combination of nanoparticle soybean extract (50% and 60%) as an extracellular cryoprotectant and glycerol as an intracellular cryoprotectant in frozen semen processing to obtain the optimal osmolarity level of the suspension to support spermatozoa quality.

Acknowledgments

Our gratitude goes to The Ministry of Agriculture of Indonesia through the Agency of Agricultural Research and Development, and National Research and Innovation Agency for funding this study.

Conflict of interest

The authors declare no real or perceived competing interests of this study.

References

1. Priyanto L, Budiyanto A, Kusumawati A, Kurniasih K. Kerusakan deoxyribonucleic acid (DNA) spermatozoa memengaruhi tingkat kebuntingan sapi Brahman. *J Vet.* 2019;20(1):119–124. DOI: [10.19087/jveteriner.2019.20.1.119](https://doi.org/10.19087/jveteriner.2019.20.1.119)
2. Peris-Frau P, Soler AJ, Iniesta-Cuerda M, Martín-Maestro A, Sánchez-Ajofrín I, Medina-Chávez DA, Fernández-Santos MR, García-Álvarez O, Maroto-Morales A, Montoro V, Garde JJ. Sperm cryodamage in ruminants: understanding the molecular changes induced by the cryopreservation process to optimize sperm quality. *Int J Mol Sci.* 2020;21:2781. DOI: [10.3390/ijms21082781](https://doi.org/10.3390/ijms21082781)
3. Priyanto L, Budiyanto A, Kusumawati A, Kurniasih K. Tingkat kerusakan DNA spermatozoa memengaruhi profil protein spermatozoa pada semen beku sapi Brahman. *J Vet.* 2018;19(4):512–520. DOI: [10.19087/jveteriner.2018.19.4.512](https://doi.org/10.19087/jveteriner.2018.19.4.512)
4. Verberckmoes S, Van Soom A, Dewulf J, De Pauw I, De Kruif A. Storage of fresh bovine semen in a diluent based on the ionic composition of cauda epididymal plasma. *Reprod Domest Anim.* 2004;39:410–416. [\[available at\]](#)
5. Salmani H, Nabi MM, Vaseghi-Dodaran H, Rahman MB, Mohammadi-Sangcheshmeh A, Shakeri M, Towhidi A, Shahneh AZ, Zhandi M. Effect of glutathione in soybean lecithin-based semen extender on goat semen quality after freeze-thawing. *Small Rumin Res.* 2013;112(1–3):123–127. DOI: [10.1016/j.smallrumres.2012.12.015](https://doi.org/10.1016/j.smallrumres.2012.12.015)
6. Layek SS, Mohanty TK, Kumaresan A, Parks JE. Cryopreservation of bull semen: evolution from egg yolk based to soybean based extenders. *Anim Reprod Sci.* 2016;172:1–9. DOI: [10.1016/j.anireprosci.2016.04.013](https://doi.org/10.1016/j.anireprosci.2016.04.013)
7. Sun L, Fan W, Wu C, Zhang S, Dai J, Zhang D. Effect of substituting different concentrations of soybean lecithin and egg yolk in tris-based extender on goat semen cryopreservation. *Cryobiology.* 2020;92:146–150. DOI: [10.1016/j.cryobiol.2019.12.004](https://doi.org/10.1016/j.cryobiol.2019.12.004)
8. Üstüner B, Alçay S, Nur Z, Sağırkaya H, Soylu MK. Effect of egg yolk and soybean lecithin on tris-based extender in post-thaw ram semen

- quality and in vitro fertility. Kafkas Univ Vet Fak Derg. 2014;20(3):393–398. DOI: [10.9775/kvfd.2013.10248](https://doi.org/10.9775/kvfd.2013.10248)
9. El-Keraby FE, Osman K, Ganah HB, El-Siefy EM. Soymilk-based extender for cryopreservation of bovine semen. J Anim Poult Prod. 2010;1(2):61–69. DOI: [10.21608/jappmu.2010.86093](https://doi.org/10.21608/jappmu.2010.86093)
 10. Soltan W, El-Siefy ES, Abe El-Razek I, El-Sharawy M, Kubota K, Yamauchi N, El-Shamaa I. Soybean milk-based extender for cryopreservation of buck spermatozoa. J Fac Agric. 2017;62(2):361–366. DOI: [10.5109/1854007](https://doi.org/10.5109/1854007)
 11. Ratnawati D, Ciptadi G, Rahayu S, Retnowulan D, Nafis FAD, Ridwan T, Susilawati, T. Utilization of nanoparticle soybean extract as an extracellular cryoprotectant in cooling semen of Bali cattle (Bos indicus). J Appl Animal Res. 2025;53(1):2516528 DOI: [10.1080/09712119.2025.2516528](https://doi.org/10.1080/09712119.2025.2516528)
 12. Susilawati T. Spermatology. 1st ed. Malang: Universitas Brawijaya Press; 2011. 202 p.
 13. Ax RL, Dally M, Didion BA, Lenz RW, Love CC, Varner DD, Hafez B, Bellin ME. Semen evaluation. In: Hafez B, Hafez EE, editors. Reproduction in Farm Animals. 8th ed. USA: Lea and Febiger; 2000.
 14. Pardede BP, Agil M, Yudi Y, Supriatna I. Relationship of frozen-thawed semen quality with the fertility rate after being distributed in the Brahman cross breeding program. Vet World. 2020;13(12):2649–2657. DOI: [10.14202/vetworld.2020.2649-2657](https://doi.org/10.14202/vetworld.2020.2649-2657)
 15. Prihantoko KD, Yulastuti F, Haniarti H, Kusumawati A, Widayati DT, Budiyo A. The acrosome integrity examination of post-thawed spermatozoa of several ongole grade bull in indonesia using giemsa staining method. IOP Conference Series: Earth and Environmental Science, 478. IOP Conf. Ser.: Earth Environ. Sci. 478 012042. DOI: [10.1088/1755-1315/478/1/012042](https://doi.org/10.1088/1755-1315/478/1/012042)
 16. García-Macias V, De Paz P, Martínez-Pastor F, Alvarez M, Gomes-Alves S, Bernardo J, Anel E, Anel L. DNA fragmentation assessment by flow cytometry and Sperm-Bos-Halamax (bright-field microscopy and fluorescence microscopy) in bull sperm. Int J Androl. 2007;30:88–98. DOI: [10.1111/j.1365-2605.2006.00723.x](https://doi.org/10.1111/j.1365-2605.2006.00723.x)
 17. Chemes E. Ultrastructural analysis of testicular tissue and sperm by transmission and scanning electron microscopy. In: Carrell DT, Aston KI, editors. Spermatogenesis. New York: Springer; 2013.
 18. Katepogu K, Kurumala D, Kedam T. Effect of cryopreservation on rat sperm DNA and involvement of elevated oxidative stress, apoptosis: a review. Int J Pharm Res Technol. 2012;2(4):1–9. DOI: [10.31838/ijpr/02.01.07](https://doi.org/10.31838/ijpr/02.01.07)
 19. Shukla MK. Applied Veterinary Andrology and Frozen Semen Technology. New Delhi: New India Publishing Agency; 2020.
 20. Chelucci S, Pasciu V, Succu S, Addis D, Leoni GG, Manca ME, Naitana S, Berlinguer F. Soybean lecithin-based extender preserves spermatozoa membrane integrity and fertilizing potential during goat semen cryopreservation. Theriogenology. 2015;83:1064–1074. DOI: [10.1016/j.theriogenology.2014.12.012](https://doi.org/10.1016/j.theriogenology.2014.12.012)
 21. Jurado-Campos A, Soria-Meneses PJ, Arenas-Moreira M, Alonso-Moreno C, Rodríguez-Robledo V, Soler AJ, Garde JJ, del Rocio Fernández-Santos M. Minimizing sperm oxidative stress using nanotechnology for breeding programs in rams. J Anim Sci Biotechnol. 2023;14:1–26. DOI: [10.1186/s40104-023-00907-3](https://doi.org/10.1186/s40104-023-00907-3)
 22. Sahara Y, Horie S, Fukami H, Goto-Matsumoto N, Nakanishi-Matsui M. Functional roles of V-ATPase in the salivary gland. J Oral Biosci. 2015;57:102–105. DOI: [10.1016/j.job.2014.11.002](https://doi.org/10.1016/j.job.2014.11.002)
 23. Arifiantini RI, Yusuf TL. Developing of tris soy milk diluent for Frisian Holstein bull frozen semen. HAYATI J Biosci. 2010;17(2):91–94. DOI: [10.4308/hjb.17.2.91](https://doi.org/10.4308/hjb.17.2.91)
 24. Gunawan M, Kaiin EM, Mudita GS, Chaidir RR. Soybean phospholipids-based extender as an alternative for bull sperm cryopreservation. IOP Conf Ser Earth Environ Sci. 2020;478:012014. DOI: [10.1088/1755-1315/478/1/012014](https://doi.org/10.1088/1755-1315/478/1/012014)
 25. Akhtar MF, Ma Q, Li Y, Chai W, Zhang Z, Li L, Wang C. Effect of sperm cryopreservation in farm animals using nanotechnology. Animals. 2022;12:2277. DOI: [10.3390/ani12172277](https://doi.org/10.3390/ani12172277)
 26. Sun L, He M, Wu C, Zhang S, Dai J, Zhang D. Beneficial influence of soybean lecithin nanoparticles on rooster frozen-thawed semen quality and fertility. Animals. 2021;11:1769. DOI: [10.3390/ani11061769](https://doi.org/10.3390/ani11061769)
 27. Ratnawati D, Kuswati K, Yekti AP, Ciptadi G, Rahayu S, Susilawati T. Effect of modified CEP-3 diluents with aqueous soybean extract on liquid semen quality in Ongole crossbred bull. Vet World. 2023;16(5):1075–1083. DOI: [10.14202/vetworld.2023.1075-1083](https://doi.org/10.14202/vetworld.2023.1075-1083)
 28. Dalmazzo A, Losano JD, Rocha CC, Tsunoda RH, Angrimani DD, Mendes CM, Assumpção ME, Nichi M, Barnabe VH. Effects of soy lecithin extender on dog sperm cryopreservation. Anim Biotechnol. 2017;1–9. DOI: [10.1080/10495398.2017.1334662](https://doi.org/10.1080/10495398.2017.1334662)
 29. Pipan M, Casal ML, Šterbenc N, Virant Klun I, Mrkun J. Vittrification using soy lecithin and sucrose: a new way to store the sperm for the preservation of canine reproductive function. Animals. 2020;10:653. DOI: [10.3390/ani10040653](https://doi.org/10.3390/ani10040653)
 30. Rezaei N, Mohammadi M, Mohammadi H, Khalatbari A, Zare Z. Acrosome and chromatin integrity, oxidative stress, and expression of apoptosis-related genes in cryopreserved mouse epididymal spermatozoa treated with L-carnitine. Cryobiology. 2020;95:171–176. DOI: [10.1016/j.cryobiol.2020.03.006](https://doi.org/10.1016/j.cryobiol.2020.03.006)
 31. Agatri NL, Rujito, Arjadi F. Morphology and DNA fragmentation spermatozoa in animal models with sleep deprivation-induced stress. J Kedokt Kesehatan Indones. 2020;11(3):232–240. DOI: <https://doi.org/10.20885/JKKI.Vol11.Iss3.art4>
 32. Gliozzi TM, Turri F, Manes S, Cassinelli C, Pizzi F. The combination of kinetic and flow cytometric semen parameters as a tool to predict fertility in cryopreserved bull semen. Animal. 2017;11(11):1–8. DOI: [10.1017/S1751731117000684](https://doi.org/10.1017/S1751731117000684)
 33. López-Fernández C, Johnston SD, Fernández JL, Wilson RJ, Gosálvez J. Fragmentation dynamics of frozen-thawed ram sperm DNA is modulated by sperm concentration. Theriogenology. 2010;74(8):1362–1370. DOI: [10.1016/j.theriogenology.2010.06.006](https://doi.org/10.1016/j.theriogenology.2010.06.006)

قدرة مستخلص فول الصويا النانوي الوقائية لنطف ثور بالي أثناء الحفظ بالتجميد

سولستيو نيفتاس إيرماواني^١، الرئيس دوي نوغراها^١، ريني نور حباتي^١، يني نور أنغريني^١، ساري يانتي هايانتي^١، هيريو بونكو واردونو^٢، سري راهايو^٢، غاتوت سيبتادي^٢، ترينيل سوسيلواتي^٢، ديان رانتاواتي^٢

^١ مركز أبحاث تربية الحيوانات، منظمة أبحاث الزراعة والأغذية، الوكالة الوطنية للبحث والابتكار في جمهورية إندونيسيا (برين)، ^٢ قسم علم الأحياء، كلية الرياضيات والعلوم الطبيعية، ^٣ قسم تكاثر وتربية الحيوانات، كلية علوم الحيوان، جامعة براويجايا، مالانج، إندونيسيا

الخلاصة

كان الغرض من هذه الدراسة هو فحص مخففات الصيغة من CEP ومستخلص فول الصويا النانوية في الحفاظ على جودة النطف المجمدة لثور بالي. استخدم في البحث ثورا بالي واحدا. كانت طريقة البحث تجريبية بتصميم مجموعة عشوائية، مع ١٢ نسخة مكررة (السائل المنوي المجمد). علاج تركيبة السائل المنوي المجمد: T1 (CEP + EY 10%)، T2 (CEP + NSE 50%)، and T3 (CEP + NSE 60%). وشملت المعايير التي لوحظت: سلامة الغشاء، سلامة الأكرسوم، الحركة، الحيوية، التشوهات، DNA Fragmentation، SOD، MDA، SEM. تم تحليل البيانات التي شملت سلامة الغشاء، سلامة

الصويا النانوية يمكن أن تدعم نوعية النطف المجمدة (الحيوية، سلامة الغشاء، سلامة أكروسوم، وتشوهات النطف). ومع ذلك، كان الأداء لا يزال أقل من مخفف CEP وصفار البيض. خصائص الحركة كانت أقل تقدمية، وسرعة حركة النطف بطيئة، ومسافة المسير قصيرة، وقوة منخفضة. ومع ذلك، كان نمط الحركة أكثر خطية واستقامة ولا يميل إلى الدوران. وصلت الحركة التقدمية بعد التذويب إلى ٥١,٣٥ ٪ و ٤٥,٠٧ ٪ ولا يزال من الممكن استخدامها للتلقيح الاصطناعي.

أكروسوم، والحركة، والحيوية والتشوهات باستخدام التحليل الاحصائي ANOVA (SPSS 16). بيانات كل من SOD و MDA Fragmentation DNA تم تحليلها باستخدام تطبيق إكسل وقدم وصف لـ SEM. أظهرت نتائج دراسة السائل المنوي المجمد بعد الذوبان بأن الحركة التقدمية، وحيوية النطف في مجموعة T1 كانت أعلى ($P<0.05$) من مجموعة T2 و T3. وكانت نسبة تشوهات النطف و Fragmentation DNA في مجموعة P1 أقل ($P<0.05$) من مجموعة T2 و T3. تركيبة مخفف CEP و ٥٠ ٪ و ٦٠ ٪ من مستخلص فول