

Article Review: The Effect of Thawing Temperature on the Quality of Bull Sperm (*Bos* sp.)

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Article Info	ABSTRACT
Article history: Received 25-12-2025 Revised 08-01-2026 Accepted 27-03-2026 Keywords: Temperature thawing Bovine sperm Artificial insemination Motility Viability	Artificial insemination (AI) is a modern reproductive technology that plays a vital role in increasing livestock productivity. One of the determining factors for successful AI is the quality of the sperm after freezing and thawing, where the thawing temperature plays a crucial role in maintaining motility, viability, morphology, and membrane and DNA integrity of spermatozoa. This study aims to examine the effect of varying thawing temperatures on bovine sperm quality (<i>Bos</i> sp.) through a literature review. Data were obtained from 11 national and international articles published in 2016–2025 that were relevant to the topic of thawing temperature and the quality of cow semen. The results of the study show that thawing at a physiological temperature of around 37°C for 30 seconds provides optimal results in maintaining motility (>70%), viability (>70%), and normal morphology of spermatozoa. Conversely, low temperatures (5°C) may increase membrane rigidity and reduce metabolic activity, while high temperatures (>39°C) trigger oxidative stress, membrane damage, and decreased DNA integrity. The conclusions of this study emphasize the importance of standardizing thawing procedures at the optimal temperature to maintain the quality of frozen semen, increase the success of AI, and support the efficiency and sustainability of livestock production.
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1. INTRODUCTION

The use of modern reproductive technology, particularly artificial insemination (AI), has become an important strategy to increase livestock productivity in the field of animal husbandry. AI technology is used to achieve efficiency and effectiveness in the use of selected males, to avoid the spread of disease through reproductive means, or to overcome obstacles in the natural mating process between males and females. The principle of artificial insemination involves the transfer of semen into the reproductive tract of a female animal during estrus with the aim of fertilizing the ovum with sperm so that the female becomes pregnant and gives birth to offspring. One of the determining factors for AI success is the quality of the sperm used, both before and after the cryopreservation and thawing processes (Purwono, 2016). The process of freezing bovine sperm is carried out to maintain its viability and fertility in the long term. However, freezing followed by thawing can cause damage to the plasma membrane, cell organelles, and even the integrity of spermatozoal DNA. This damage

often results in a decrease in motility, viability, normal morphology, and the ability of the sperm to fertilize the egg (Jauzi *et al.*, 2020).

One important factor that affects sperm quality after freezing is the conditions during thawing, particularly the temperature used in the process. The optimal combination of temperature and thawing time results in minimal damage to spermatozoa, allowing them to retain their high fertilization capacity. The thawing temperature used in cattle sperm processing can vary, but the temperature considered most optimal for artificial insemination (AI) is 37°C with a thawing time of 30 seconds (Jauzi *et al.*, 2020). Thawing temperature and duration have a significant effect on the condition of spermatozoa, particularly the integrity of spermatozoa in semen. Frozen semen to be used for AI is taken from a container containing liquid N₂ at a temperature of -196°C; therefore, it must be thawed before AI is performed. A good combination of thawing temperature and duration results in minimal damage to the spermatozoa, so that they retain a high ability to fertilize the ovum. There are many opinions on the optimal thawing temperature to achieve good sperm quality in AI (Binangkari *et al.*, 2020).

With technological developments, various studies continue to be conducted to find the most effective thawing method. Several studies have examined variations in temperature and thawing time to minimize sperm cell damage, and some have even combined this with the addition of cryoprotectants or certain additives to protect the structure of spermatozoa. These efforts are expected to improve the quality of frozen semen, so that artificial insemination technology can be optimized to support sustainable livestock productivity. Therefore, a comprehensive review of the effect of thawing temperature on bovine (*Bos* sp.) sperm quality is essential to synthesize fragmented findings and provide a standardized protocol for optimizing artificial insemination efficiency across different breeds.

The purpose of this study is to determine the effect of different thawing temperatures on the quality of bovine sperm (*Bos* sp.), including motility, viability, and normal spermatozoal morphology, so that the most optimal thawing temperature can be determined in maintaining sperm quality after freezing to support the success of artificial insemination. The benefits of this study are to provide scientific information on the quality of bovine semen after thawing at different temperatures, which is expected to serve as a reference in the application of modern reproductive technology in animal husbandry, particularly in determining the appropriate thawing procedure to improve the success of artificial insemination, as well as to serve as reference material for further research in the field of cryopreservation and animal reproduction technology.

2. RESEARCH METHOD

The method used in this study was a literature review using a systematic article selection approach adapted from the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) framework. Scientific articles were collected from several databases, including PubMed and Google Scholar, as well as journals published by MDPI, Frontiers, Springer Nature, PNAS, and Communications Biology. The keywords used in the search process included “thawing temperature”, “frozen cattle semen”, “effect of thawing temperature on cow sperm”, “heat stress sperm morphology”, and “bull sperm cryopreservation”. The publication year range was limited to 2016–2025 to obtain recent studies from both national and international sources. The inclusion criteria included articles discussing the effect of temperature on bovine (*Bos* sp.) semen quality through thawing treatment, cryopreservation, cold storage, or heat stress. The selected studies had to contain measurable sperm quality parameters such as motility, viability, morphology, membrane integrity, DNA integrity, or sperm concentration. Articles unrelated to bovine sperm quality, duplicate articles, and articles without accessible full text were excluded from the review. The article selection process consisted of identification, screening, eligibility, and inclusion stages. From the selection process, 11 relevant articles were included in the analysis.

The quality of the selected articles was evaluated based on the clarity of objectives, suitability of methods, completeness of measured parameters, and consistency of reported results. Data were analyzed descriptively by comparing the results among studies. The analysis focused on thawing temperature variables, treatment duration, measured sperm quality parameters, and the main findings reported in each study.

3. RESULTS AND DISCUSSION

Based on a literature review of 11 relevant scientific articles (2016-2025), this study successfully identified the significant effect of thawing temperature on various parameters of bovine (*Bos* sp.) sperm quality. The results of the analysis show that thawing temperature not only affects sperm motility and viability, but also has an impact on morphology, plasma membrane integrity, and sperm DNA integrity.

The discussion of the results of this literature review is divided into six main subchapters, including: (1) The Effect of Thawing Temperature on Bovine Sperm Motility; (2) The Effect of Thawing Temperature on Bovine Sperm Viability; (3) The Effect of Temperature on Bovine Sperm Morphology; (4) The Effect of Temperature on Bovine DNA and Plasma Membrane Integrity; (5) The Effect of Temperature on Bovine Sperm Quality; and (6) Practical Applications in Animal Husbandry and Cryopreservation. Each aspect is discussed in depth to provide a comprehensive understanding of the importance of controlling optimal thawing temperatures in supporting the success of artificial insemination programs.

3.1. Effect of Thawing Temperature on Cow Sperm Motility

AI is one of the efforts to maximize the utilization of superior males in order to improve the genetic quality of livestock. The principle of artificial insemination is the deposition of semen into the reproductive tract of an ovulated female animal, where it can be fertilized by sperm so that the female animal becomes pregnant and gives birth to offspring (Murtidjo, 1990). The reality in the field is different, as can be seen from observations at the Artificial Insemination Field Unit (ULIP) in Prafi SP I, which shows that the length of time taken ranges from 10–20 minutes. Thawing is done by inserting frozen semen into a bottle containing well water. Fertility is greatly influenced by temperature and duration. Meanwhile, the obstacles for inseminators include the unavailability of a minicontainer containing liquid nitrogen to carry frozen semen directly to the acceptor (Purwono, 2016).

Sperm motility is one of the benchmarks for successful fertilization. The energy used in movement is influenced by energy sources in the form of fructose and electrolytes such as Na^+ , Ca^{2+} , Cl^- , and lactate. Sperm motility is one of the criteria for determining spermatozoa quality because it is very important for reaching and fertilizing the ovum (Solis *et al.*, 2024). This study aims to determine the effect of thawing temperature and time on the motility of frozen Limousin cattle semen as an indicator of semen fertility for artificial insemination. The semen used initially had an average motility of 80%, indicating that the semen quality was suitable. The results of the experiment showed that at a thawing temperature of 37°C, spermatozoa motility was relatively stable and high, namely between 71.33% and 71.67%, even though the thawing time varied from 5, 30, and 60 minutes (Purwono, 2016). However, this stability does not reflect optimal sperm function. At subphysiological temperatures, metabolic activity is suppressed and the plasma membrane undergoes a phase transition to a more rigid state, reducing the functional capacity of spermatozoa. This implies that while motility values at 5°C remain above the minimum threshold, the overall quality and fertilization potential of the sperm are not fully maintained, further supporting that 37°C remains the most optimal thawing temperature for artificial insemination purposes.

Table 1. Effect of Temperature and Thawing Time of Frozen Limousin Cow Semen on Sperm Motility (Purwono, 2016).

Temperature (°C)	Replication I (5 minutes)				Replication II (30 minutes)				Replication III (60 minutes)			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
5	75	70	69	71.33	71.5	74	69.5	71.67	68.5	61.5	55	61.67
27	71.5	72	69	70.83	51	61.5	61.5	58.00	50.5	37.5	42.5	43.50
40	52.5	62.5	47.5	54.17	57.5	42.5	40	46.67	27.5	20	25	24.17

At a temperature of 27°C, spermatozoa motility decreased significantly if the thawing time exceeded 5 minutes (down from 70.83% to 58%). shows that this intermediate temperature is less stable for thawing with long duration. Meanwhile, at a temperature of 40°C, spermatozoa motility was significantly lower with values of around 46-54% at all durations.thawing, which indicates that high temperatures are unsuitable because they cause damage to spermatozoa. Sperm motility above 50% is considered suitable for insemination, while below that level carries a risk of infertility. Low temperatures and time thawing Shorter temperatures are better for maintaining the quality of frozen semen, as high temperatures will accelerate damage to cell membranes and sperm motility. It is also important to use mini-spermcontainercontains liquid nitrogen to maintain a low temperature during semen transportation so that motility does not decrease before insemination (Purwono, 2016).

3.2. Effect of Thawing Temperature on the Viability of Cow Sperm

Sperm viability is an important indicator that determines semen quality and the chances of successful fertilization. This parameter describes the percentage of spermatozoa that are still alive with intact plasma membranes, enabling spermatozoa to move actively and maintain their reproductive function. The higher the viability rate, the greater the chances of fertilization. Fertilization can occur through natural mating or through assisted reproductive technologies such as artificial insemination (Irvanto *et al.*, 2018).

Sperm viability is greatly influenced by various factors. One factor that affects sperm viability is temperature, especially during the thawing stage of frozen semen. The freezing and thawing process often causes structural damage to sperm cells, which can reduce their viability. In this case, temperature plays an important role because the right temperature can help minimize damage caused by freezing. Meanwhile, inappropriate temperatures can worsen membrane damage and reduce spermatozoa viability (Jauzi *et al.*, 2020).

One study on the effect of thawing temperature on Madura cattle showed similar results. This study was conducted by Jauzi *et al.* (2020), who compared spermatozoa viability at three thawing temperatures: 34°C, 37°C, and 40°C. The results in Figure 1 show that the highest viability was obtained at 37°C, with a percentage of 71.56%, while at 34°C, the viability was slightly lower at 68.89%, and at 40°C, there was a significant decrease to 54.11%. These findings confirm that the physiological temperature closest to the animal's body temperature, namely 37°C, is the most optimal temperature for maintaining the viability of Madura cattle spermatozoa after thawing (Jauzi *et al.*, 2020).

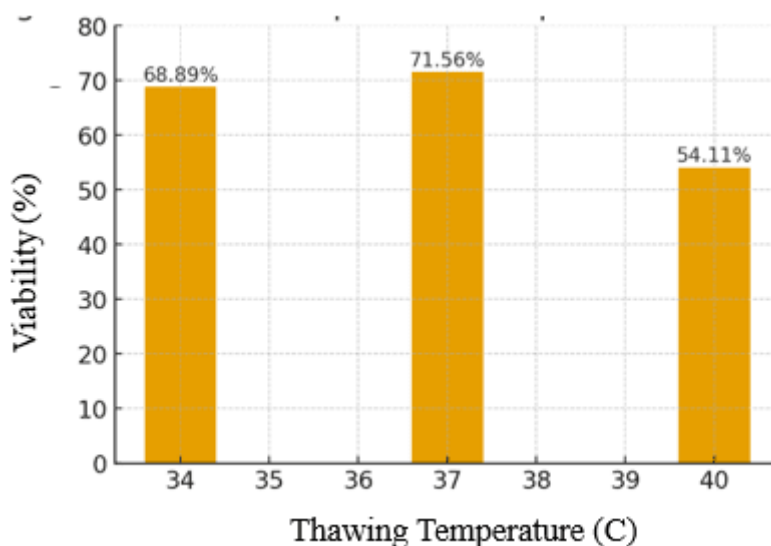


Figure 1. Effect of Temperature on the Viability of Madura Bull Spermatozoa (Jauzi *et al.*, 2020)

The decrease in viability at 40°C is caused by cell damage mechanisms due to excessive heat exposure. Excessively high temperatures accelerate the melting of ice crystals but also cause osmotic imbalance and damage the lipid structure of the plasma membrane. This condition triggers the leakage of ions, enzymes, and important molecules from within the cell and increases the production

of free radicals that damage cellular components. As a result, the integrity of the plasma membrane is disrupted, and many spermatozoa lose their ability to survive (Malinda *et al.*, 2021). Conversely, at 34°C, sperm viability can still be maintained quite well, although it is slightly lower than at 37°C. This condition is caused by the fact that melting at lower temperatures occurs more slowly. This slow process causes micro-ice crystals to not completely melt and continue to press on the plasma membrane (Malinda *et al.*, 2021). Therefore, although sperm viability is still relatively good, these conditions are less than optimal compared to a temperature of 37°C.

Physiologically, 37°C is considered the ideal temperature because it is close to the body temperature of livestock, allowing spermatozoa to be in an environment most suitable for their normal metabolism. This temperature is warm enough to quickly dissolve ice crystals but not too high, thereby minimizing thermal stress and membrane damage. These findings are also in line with research on Friesian Holstein cattle, which showed the best results at a thawing temperature of 27°C (Jauzi *et al.*, 2020). Based on the article written by Jauzi *et al.* (2020), it can be seen that the thawing temperature has a significant effect on the viability of Madura cattle spermatozoa. At the optimal temperature of 37°C, sperm viability can be maintained well because the plasma membrane remains stable and does not experience excessive damage.

Conversely, excessively high temperatures can cause a decrease in viability due to membrane damage, ion leakage, and increased oxidative stress, which causes many cells to be unable to survive. High temperatures can cause a phase transition in the lipid bilayer. This phase is a change in the structure of the membrane lipids from a liquid state to a more rigid or unstable form. This change causes the membrane to lose its flexibility, resulting in cracks or small pores forming in the membrane layer. Through these pores, important ions such as Na⁺, K⁺, and Ca²⁺ leak out, along with enzymes and metabolites that should be retained within the cell (Iskandar *et al.*, 2023).

Ion leakage causes an imbalance in the electrolyte gradient between the intracellular and extracellular environments. For example, an increase in intracellular Ca²⁺ can trigger the activation of protease and lipase enzymes, which actually exacerbates the degradation of proteins and membrane phospholipids. In addition, excessive heat exposure can increase metabolic activity, triggering the formation of excessive amounts of reactive oxygen species (ROS). These ROS can attack unsaturated fatty acids in the membrane through a process called lipid peroxidation, making the membrane more fragile and prone to rupture (Iskandar *et al.*, 2023). As a result of this combination of physical damage, spermatozoa lose their ability to maintain cellular homeostasis. Cellular energy is continuously depleted to repair the damage, motility decreases, and ultimately many cells are unable to survive (Jauzi *et al.*, 2020).

3.3. The Effect of Temperature on the Morphology of Bovine Sperm

Sperm morphology is an important parameter in semen quality research because it is directly related to fertilization ability. Sperm with normal head, midpiece, and tail shapes have a greater chance of fertilizing an egg. Conversely, morphological abnormalities such as double heads, coiled tails, broken tails, or abnormalities in the midpiece can reduce the penetration ability of the zona pellucida and ultimately reduce the reproductive success rate (Ugur *et al.*, 2019). Therefore, sperm morphology is often used as an indicator to assess the level of damage due to temperature treatment, both during storage and during the thawing process of frozen semen.

Thawing temperature plays a crucial role in maintaining the morphological integrity of spermatozoa. Thawing too rapidly at high temperatures can cause thermal stress and increase the production of reactive oxygen species (ROS). This condition triggers membrane lipid damage, peroxidation, and rupture of the acrosome structure, which leads to an increase in the percentage of abnormal sperm (Capela *et al.*, 2022). On the other hand, thawing at temperatures that are too low causes the melting of ice crystals to occur slowly, so that the micro-ice crystals continue to press on the plasma membrane and cause mechanical damage in the form of broken or coiled tails (Nagata *et al.*, 2019). Therefore, selecting the optimal thawing temperature is crucial in maintaining spermatozoa morphology.

Research on Balinese cattle shows that a thawing temperature of 31°C provides the best results with high motility, adequate viability, and a percentage of morphological abnormalities that

is still below the threshold for artificial insemination suitability (Binangkari *et al.*, 2022). Meanwhile, research on Simmental cattle stored at 5°C for up to 72 hours showed a significant decrease in motility and viability, but morphological abnormalities were relatively stable with a low percentage, so that semen could still be used for insemination (Pasyah *et al.*, 2022). These findings confirm that although motility and viability parameters are easily affected by temperature, sperm morphology can be more stable if storage and thawing procedures are carried out properly.

In Limousin cattle, research by Purwono (2016) reported that thawing at 5°C for 5 minutes resulted in the best motility. However, this finding differs from the general recommendation that thawing should be performed at warmer temperatures (34–37°C) to minimize morphological damage. This discrepancy in results is likely influenced by technical factors such as breed variation, initial semen quality, and dilution methods. Meanwhile, molecular studies conducted in animal models such as mice have shown that exposure to high temperatures during spermatogenesis can inhibit DNA repair, increase apoptosis, and produce sperm with abnormal head and tail morphology (Trigg *et al.*, 2024).

Physiologically, morphological abnormalities resulting from temperature treatment generally include coiled, broken, or bent tails, as well as changes in the head and midpiece. This damage occurs due to changes in the structure of the plasma membrane and acrosome, ionic imbalance, and oxidative stress induced by extreme temperatures. Cryopreservation and thawing processes also increase the risk of acrosome damage and tail deformity due to osmotic pressure and cytoskeleton damage (Nagata *et al.*, 2019). Therefore, proper temperature control in each stage of frozen semen handling is a key factor in maintaining normal bovine spermatozoa morphology and supporting successful fertilization.

3.4. The Effect of Temperature on DNA Integrity and Bovine Plasma Membrane

Temperature has a major influence on the integrity of DNA and the plasma membrane of bovine spermatozoa. Storing semen at low temperatures (5°C) causes decreased motility, decreased viability, and increased abnormalities of spermatozoa, which indicates damage to the plasma membrane (The Effect of Storage at 5°C on Motility, Viability, and Abnormalities of Simmental Cattle Semen) (Pasyah *et al.*, 2022). The thawing process and post-thaw incubation can worsen DNA damage, either in the form of single-stranded or double-stranded breaks, which results in decreased in vitro fertility. This damage is exacerbated by increased production of reactive oxygen species (ROS) during storage and thawing, which can damage plasma membrane lipids and reduce the integrity of spermatozoal DNA (Jorban *et al.*, 2024).

In addition, inappropriate thawing temperatures can affect plasma membrane stability and acrosome status. Research on Bali cattle shows that varying thawing temperatures result in different outcomes for spermatozoa quality, with optimal temperatures better able to maintain plasma membranes and suppress chromatin damage (Salim *et al.*, 2012). Other studies also found that thawing at temperatures close to physiological (36–37°C) for a short duration is more effective in maintaining viability and motility, while temperatures that are too high ($\geq 39^\circ\text{C}$) actually accelerate damage to the plasma membrane and reduce fertilization ability (Jauzi *et al.*, 2020).

The effect of temperature on sperm quality is not only seen from the technical aspects of storage and thawing, but is also related to the biological function of the cells themselves. Sperm are highly sensitive to environmental changes because their membrane structure is composed of unsaturated lipids that are easily damaged by temperature changes. This membrane instability can cause disruptions in ion exchange, enzyme activity, and signal transmission, ultimately reducing the sperm's ability to survive and fertilize eggs (Leite *et al.*, 2022).

Furthermore, inappropriate temperatures can also affect sperm energy metabolism. Under optimal conditions, sperm cells utilize energy reserves in the form of ATP to maintain progressive motility. However, when exposed to temperature stress, these energy reserves are depleted more rapidly, resulting in a drastic decrease in sperm motility. This condition directly impacts the success of the fertilization process, as good motility is a key requirement for sperm to reach the ovum in the female reproductive tract (Pasyah *et al.*, 2022).

Proper temperature management during storage and thawing of frozen semen also significantly determines the effectiveness of an artificial insemination program. Inaccurate temperature settings

can result in low pregnancy rates, ultimately costing farmers both money and time (Jauzi *et al.*, 2020). Therefore, understanding the effect of temperature on the integrity of DNA and the plasma membrane of spermatozoa is not only academically important, but also has a major practical impact in supporting the sustainable improvement of livestock productivity.

3.5. The Effect of Temperature on Sperm Quality in Cows

Poor quality of frozen semen and the inseminator's skill in carrying out the thawing process are two obstacles to the success of artificial insemination in the field. Inseminators tend to pay less attention to temperature during the thawing process, which results in decreased sperm motility after thawing and re-culture. A study on the effect of different thawing methods on the quality of frozen semen and the success of artificial insemination (AI) in cattle reported that spermatozoa motility using warm water at 37°C for 30 seconds was 71.67%. Meanwhile, the observation using ice water at 5°C for 30 minutes showed a value of 63.94%. These results indicate a very significant difference ($P \leq 0.01$) between the two thawing methods. The thawing process using warm water at a temperature of 37°C for 30 seconds resulted in higher motility than the use of ice water at 5°C for 30 minutes, indicating that sperm quality is better maintained at a warm temperature (Kristiawan *et al.*, 2024).

At 5°C, sperm quality decreases because low temperatures cause the phospholipid structure of the plasma membrane to change from a liquid phase to a gel phase, or a thicker phase. Furthermore, at low temperatures, cryoprotectant release is complete, but metabolism is suboptimal, resulting in decreased sperm quality and motility. When spermatozoa are thawed at temperatures that are too low or too high, the lipid structure of the membrane protein becomes unbalanced and does not meet the physiological parameters required for sperm movement and quality due to osmotic pressure. Cryoprotectant release is ideal at low temperatures, but metabolism is suboptimal, which reduces the quality and motility of the resulting spermatozoa. The results showed that P2, which involved thawing at 5°C for 30 minutes, was lower than the P1 treatment because melting at low temperatures causes the phospholipid structure of the plasma membrane to transition from a liquid phase to a gel or thicker phase (Kristiawan *et al.*, 2024).

The metabolic process of spermatozoa will increase at high temperatures in the thawing medium, resulting in higher energy requirements. Spermatozoa rapidly lose energy under such circumstances, leading to their death (Zelpina *et al.*, 2012). Based on the research that has been conducted, the thawing method using warm water at 37°C for 30 seconds is better than using ice water at 5°C for 30 minutes. The results of microscopic examination and observation of frozen semen showed that the motility and percentage of live spermatozoa using the thawing method with water at 37°C for 30 seconds were very good and high (Kristiawan *et al.*, 2024).

3.6. Practical Applications in Animal Husbandry and Cryopreservation

Various studies on the effect of temperature on sperm quality, including motility, viability, morphology, and several other aspects, have important implications for the application of artificial insemination in the field. The right thawing temperature has been proven to maintain various aspects of spermatozoa quality. This condition is crucial to successful fertilization because good-quality sperm have a greater chance of reaching the ovum and fertilizing it (Jauzi *et al.*, 2020). Therefore, standardizing the thawing temperature is an important step that must be considered by farmers and inseminators to maintain optimal frozen semen quality. Thawing is the process of re-liquefying frozen semen before it is used for insemination, so this stage is crucial in determining whether the spermatozoa are still viable (Malinda *et al.*, 2021).

Based on a number of references, to achieve good sperm quality, the thawing process is recommended to be carried out at a temperature of around 37°C (Syamlan *et al.*, 2021). This temperature is sufficient to quickly dissolve ice crystals without causing membrane damage due to excessive heat exposure (Malinda *et al.*, 2021). Therefore, inseminators must use a water bath with a controlled temperature, usually around 37°C, for approximately 30 seconds (Novanda *et al.*, 2025). This thawing process is used to return frozen semen to a liquid state and make it ready for artificial insemination (Khoirunnisa *et al.*, 2024). Without thawing at the appropriate temperature, the sperm cannot be used effectively because the cells have been damaged or killed. With the correct procedure,

sperm viability and motility are better preserved, making them more suitable for use in the fertilization process (Syamlan *et al.*, 2021).

In the field of animal husbandry, the use of standard thawing procedures will have a direct impact on increasing reproductive efficiency. Sperm with high viability and motility after thawing will increase the conception rate, shorten the calving interval, and reduce the costs incurred for repeat insemination (Malinda *et al.*, 2021). This certainly provides economic benefits for farmers while supporting increased livestock productivity. In addition, research on the effect of temperature also has strategic value in the conservation of local germplasm such as Madura cattle (Jauzi *et al.*, 2020). With proper cryopreservation, frozen semen from superior bulls can be stored long-term without losing quality, then distributed to various regions without the need to move animals. This not only maintains the genetic sustainability of local cattle but also expands their utilization at the national level.

The findings across the reviewed studies exhibited methodological heterogeneity. For instance, while Jauzi *et al.* (2020) focused on high-throughput experimental designs with specific cryoprotectants, the study by Purwono (2016) primarily evaluated field-scale applications. Despite these differences in scale and breeds, both studies consistently demonstrate that precise thawing temperature control is crucial in maintaining the motility and viability of spermatozoa. Therefore, understanding the importance of thawing temperature and applying proper procedures in the field can maintain consistent frozen semen quality. The application of this technology is expected to increase the success of artificial insemination, production efficiency, and the resilience of Indonesia's livestock genetic resources.

4. CONCLUSION

Based on a review of various scientific articles, the thawing process of frozen semen is a crucial step that significantly determines sperm quality after thawing. This quality is reflected in various parameters, including motility, viability, morphology, plasma membrane integrity, and genetic material (DNA) quality, all of which are greatly influenced by the thawing conditions applied. The results indicate that a thawing temperature of 37°C for approximately 30 seconds is the optimal condition because it is close to the physiological body temperature of cattle, thereby maintaining the structural and functional stability of spermatozoa while minimizing thermal stress and disruptions to osmotic balance. Under these conditions, cellular metabolic activity can proceed normally, thereby maintaining sperm motility and viability, while damage to the plasma membrane and genetic material is minimized. Furthermore, the application of the appropriate thawing temperature also plays a role in maintaining the morphological integrity of spermatozoa, which is directly related to fertilization capacity. Thus, the use of a thawing temperature of 37°C for 30 seconds can be recommended as a standard procedure in artificial insemination (AI) to improve fertilization success, reproductive efficiency, and support sustainable livestock productivity.

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