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Research article

Cryopreservation of goat spermatozoa in the Mekong Delta region of Vietnam: evaluating cryoprotectant effectiveness

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Abstract

Conserving and improving the quality of breeds is crucial for enhancing small ruminant production in Vietnam. To support this purpose, cryopreservation was offered as a promising method for semen preservation and maintaining genetic diversity. Moreover, many small-scale farmers in Mekong Delta rely on goats for both income and nutrition, making them an important part of farming activities. This study aimed to develop a sperm cryopreservation protocol and identify the optimal cryoprotectant for goat sperm in the Mekong Delta region of Vietnam. Semen samples were diluted with TCG-EggYolk freezing medium containing cryoprotectant agent (CPA) in a 1:1 ratio. The samples were then placed in 0.5 mL French straws, cooled to 15 °C and 5 °C, and subjected to nitrogen vapor before being immersed in liquid nitrogen at -196 °C. Experiment 1 utilized Glycerol as the CPA in concentrations of 0 %, 5 %, 8 %, and 11 %. Experiment 2 employed dimethyl sulfoxide (DMSO) as the CPA with the same concentrations. Thawed samples were evaluated for sperm quality. Results indicated that in Experiment 1, increasing the Glycerol concentration improved the overall motility, viability, and membrane integrity of sperm. The best outcomes were achieved with 8 % Glycerol, resulting in progressive motility of 57.5 % (P<0.05). In Experiment 2, the extender containing 8 % DMSO demonstrated significantly better performance in terms of overall motility, viability, and membrane integrity compared to 5 % or 11 % DMSO. Progressive motility was 53.5 % (P<0.05). In conclusion, the study determined that using 8 % Glycerol or 8 % DMSO is the optimal choice for freezing goat sperm in the Mekong Delta region of Vietnam.

Keywords: Cryopreservation, Dimethyl sulfoxide, Glycerol, goat, Mekong Delta, Spermatozoa

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INTRODUCTION

Goat farming is an important component of the agricultural sector in Vietnam, particularly in the Mekong Delta region. Goats are a source of income and nutrition for many small-scale farmers; therefore, they play a vital role in the livelihoods of rural communities. Natural mating is a stressful process that has a much higher tendency to result in injuries or accidents for both the animals and producers. It is well-known that male cattle often have a greater physical size than their female counterparts. Additionally, there may be instances where male cattle display aggressive behavior. Therefore, artificial insemination (AI) eliminates the potential risks associated with keeping a male on the premise. Furthermore, artificial insemination reduces the risk of transmitting diseases. Accordingly, the use of assisted reproductive technologies such as AI and cryopreservation of semen has the potential to contribute significantly to the improvement of goat breeds (Leboeuf et al., 2008; Valentina et al., 2020). However, successful cryopreservation of goat semen requires effective cryoprotectants and protocols (Benson et al., 2012; Küçük et al., 2014).

Gamete cryopreservation is an important advance in reproductive biology. Such processes are necessary to maintain endangered animals, to breed animals through artificial insemination (Tamburrino et al., 2023). In Vietnam, cryopreservation in goat sperm is still in infancy because it is still exceedingly new to goat farmers (Khuong et al., 2022). Therefore, it is crucial to assess the efficiency of cryoprotectants in preserving goat sperm through cryopreservation. This evaluation is essential for the growth of prosperous breeding initiatives and the conservation of native goat populations in the Mekong Delta area of Vietnam.

To optimize the cryopreservation of goat sperm, it is necessary to identify the optimal cryoprotectant and concentration. Several cryoprotectants, including glycerol and dimethyl sulfoxide (DMSO), have been used successfully in the cryopreservation of goat sperm (Farshad et al., 2009; Kulaksiz et al., 2013). However, to our knowledge, the optimal concentration of these cryoprotectants for goat sperm from specific regions has not been determined, and this can vary depending on breed, semen collection methods, and processing techniques.

The aim of this study is to evaluate the effectiveness of glycerol and DMSO as cryoprotectants in goat sperm cryopreservation. The study focuses on the overall motility, viability, and membrane integrity of sperm, with a specific emphasis on progressive motility which is a vital parameter for semen quality. The study also determines the optimal concentration of cryoprotectants for successful cryopreservation of goat spermatozoa in the Mekong Delta region of Vietnam. The results of this study will support the development of better protocols for goat sperm cryopreservation and provide valuable insights for the successful implementation of breeding programs.

MATERIALS AND METHODS

Ethics approval

The study received ethical approval for animal care, housing, and semen collection procedures under the Animal Welfare Assessment (BQ2022-03/VCNSHTP).

Farm, animals, and management

The study involved 2 male crossbred Boer (♂) x Bach Thao (♀) goats, aged approximately 2-3 years, with an average weight of 42-45 kg. The rations of goats were formulated to meet nutrient requirement of matured male goats (NRC, 2007). Goats were fed 3 times/day according to rations. Drinking water was fully prepared to prevent goats from getting thirsty. The barn area is built to be tall, cool, with a roof, mosquito nets, and clean. Goats were fully vaccinated against diseases and regularly monitored for health.

Semen collection

Semen was collected twice a week from each goat over a period of 4 months using an artificial vagina. First, the vagina was warmed to a temperature of 40-42 °C and lubricated with gel. Then, the female goat was placed in the male goat's cage. The semen collector held the artificial vagina with the semen collection tube between the male goat's hind legs to collect the ejaculated semen (Chemineau and Cagnie, 1991). The collected semen was evaluated for macroscopic characteristics (volume, color, and pH) and semen quality (sperm concentration, motility, viability, and membrane integrity).

Assessment of sperm concentration

The counting chamber was fixed at room temperature for 4 minutes after drawing up 9 µL of the sample. Using a microscope with a magnification of 40X, at least 200 intact spermatozoa (with full head and tail) were counted per counting chamber. To prevent duplicate counting of spermatozoa in adjacent squares, spermatozoa lying on the dividing line between 2 squares were counted once, and spermatozoa with heads lying on the dividing line above and to the left of the square were counted. The WHO guidelines were followed to calculate the sperm count (WHO, 2021).

Assessment of sperm motility

Two wet mounts with a depth of about 20 µm were prepared on a counting chamber for each sample. The motility of all spermatozoa in the same location in the field was evaluated based on three types: progressive motility (PR), non-progressive motility (NP), and immotility (IM). A random counting area was chosen, avoiding areas where only motile spermatozoa were visible. A quick assessment was performed on any field without waiting for spermatozoa to swim into the evaluation area. At least 200 spermatozoa in at least 5 fields in each wet mount were counted. The count was performed twice on two different wet mounts, and the results of the two wet mounts were compared. If the difference in the percentage of samples was within the acceptable range, the average was calculated for each motility classification (PR, NP, and IM) (Fumuso et al., 2018).

Assessment of sperm viability

The viability of sperm was measured by the Eosin-Nigrosin method (Björndahl et al., 2003). To evaluate sperm viability, 50 µL of a semen sample was mixed with 50 µL of Eosin-Nigrosin solution and allowed to stain for 30 seconds. The sample was then placed on a glass slide and air-dried. Using a microscope, 100 spermatozoa were counted and evaluated. Live spermatozoa were either white or stained red or dark pink in part of the neck region only, while the other parts of the head were unstained. Dead spermatozoa had a reddish or dark pink head region. The percentage of live spermatozoa was calculated.

Assessment of sperm membrane integrity

Sperm membrane integrity was assessed using the Hypo-Osmotic Swelling Test (HOS Test), as indicated by Jeyendran et al. (1984). A total of 20 µL of semen sample was mixed with 80 µL of HOS solution in an eppendorf tube and placed in an incubator at 37 °C. The samples were evaluated after 40 minutes of incubation. Once the samples were incubated, they were evaluated by placing 10 µL of the mixed sample onto a glass slide and observing it under a microscope. Spermatozoa with intact membranes showed swelling in the tail, while those with damaged membranes did not show swelling.

Experimental design

Semen samples were collected from two healthy male goats at the animal experimental farm of Stem Cell Lab, Can Tho University, Vietnam. Semen samples were then evaluated for their quality. The study consisted of 2 experiments. Experiment 1 evaluated the effect of Glycerol with 4 concentrations of 0 %, 5 %, 8 %, and 11 %. Experiment 2 evaluated the effect of DMSO with 4 concentrations of 0 %, 5 %, 8 %, and 11 %.

Semen samples were diluted with Tris-Citrate-Glucose (TCG) medium and mixed with TCG-EggYolk 8 %, this freezing medium was supplemented with Glycerol or DMSO at concentrations of 5 %, 8 %, and 11 % to achieve a concentration of 2×10^9 sperms/mL. The samples were loaded into 0.5 mL French straws and stabilized through a series of steps: 15 °C for 30 minutes, 5 °C for 60 minutes, placed on nitrogen vapor for 15 minutes, and finally plunged into liquid nitrogen. After 72 hours of freezing, the samples were thawed at 37 °C for 60 seconds and evaluated for semen quality.

After cryopreservation, all samples were thawed and evaluated for overall motility, viability, and membrane integrity using standard procedures.

Statistical analysis

Data entry was performed using Microsoft Excel (ver. 2016) software. Variance analysis was conducted using Minitab (ver. 2016) software. Tukey's multiple comparison tests were used to assess the significant differences among treatment means. Statistical significance was declared at $P<0.05$. The optimal concentration of cryoprotectants for successful cryopreservation of goat spermatozoa was determined based on the highest percentage of progressive motility.

RESULTS

Fresh semen quality

The average volume, overall motility, progressive motility, viability, and membrane integrity in the ejaculates of goat spermatozoa are shown in Table 1. The results showed that the average pH was 6.9. If semen has a pH lower or higher between 6.8 and 7.5, it is abnormal semen which is not suitable for sperm vitality and fertility. The mean overall motility was 88.2 %, with progressive motility accounting for over 70 % of the total motile sperm. The mean viability was 91.7 %, indicating that the majority of the sperm cells were alive. The mean membrane integrity was 77.4 %, suggesting that a significant proportion of the sperm cells had intact membranes. Generally, the quality evaluation parameters are relatively high and meet the standards of macroscopic surveys to perform the experiment.

Table 1 Sperm quality variables (%) were recorded in freshly collected goat semen. Data are expressed as mean values ± SEM.

Color	pH	Volume (mL)	Density (*10^9 cells/mL)	Overall motility	Progressive motility	Viability	Membrane integrity
White	6.90 ± 0.10	0.80 ± 0.02	2.70 ± 0.04	88.20 ± 1.00	79.60 ± 1.30	91.70 ± 1.00	77.40 ± 0.90

Effect of different concentrations of glycerol on goat sperm quality before and after cryopreservation

Before cryopreservation, the overall motility was 88.2 %, progressive motility was 79.6 %, viability was 91.7%, and membrane integrity was 77.4 % (Table 2). After cryopreservation, compared with Glycerol 0 % (12.9 %), the overall motility significantly increased ($P<0.05$) in Glycerol 8 % (73.6 %). Progressive motility was also lowest ($P<0.05$) in Glycerol 0 % (16.9 %) and highest in Glycerol 8 % (57.5 %). The extender containing 8 % glycerol showed a significantly greater ($P<0.05$) percentage of sperm viability (82.0 %) compared with other treatments (36.2-61.0 %).

Table 2 Sperm quality variables (%) recorded in goat semen after cryopreservation with Glycerol at 5 %, 8 %, and 11 %. Data are expressed as mean values ± SEM, n = 8.

Variable	Before cryopreservation	After cryopreservation				*P*-value
		Glycerol 0%	Glycerol 5%	Glycerol 8%	Glycerol 11%	
Overall motility	88.2 ± 1.0	29.0^{d} ± 0.7	46.0^{c} ± 1.8	73.6^{a} ± 0.8	52.7^{b} ± 1.2	6.63×10^{-17}
Progressive motility	79.6 ± 1.3	16.9^{c} ± 1.6	27.9^{b} ± 2.0	57.5^{a} ± 2.1	30.1^{b} ± 2.1	6.35×10^{-17}
Viability	91.7 ± 1.0	36.2^{d} ± 1.4	54.4^{c} ± 1.9	82.0^{a} ± 0.7	61.0^{b} ± 1.3	1.41×10^{-17}
Membrane integrity	77.4 ± 0.9	26.2^{c} ± 1.0	38.2^{b} ± 1.1	57.9^{a} ± 2.2	28.4^{c} ± 1.8	1.81×10^{-17}

Different lowercase letters on rows indicate a significant difference (P<0.05).

Effect of different concentrations of DMSO on goat sperm quality before and after cryopreservation

The highest (P<0.05) overall motility value was observed in the 8 % DMSO group (74.2 %), while the lowest value was recorded in the 5 % DMSO group (37.9 %; Table 3). Similarly, the highest mean progressive motility was detected in the 8 % DMSO group (53.5 %), while the lowest value was seen in the 5 % DMSO group (23.1 %; P<0.05). The trend was consistent with viability and membrane integrity, where the highest mean value was seen in the 8 % DMSO group (80.7 % and 55.3 %; P<0.05) compared with other treatments. Despite the improvement observed in the 8% DMSO group, the progressive motility was still significantly lower than that of the fresh samples.

Table 3 Sperm quality variables (%) recorded in goat semen after cryopreservation with DMSO at 5 %, 8 %, and 11 %. Data are expressed as mean values ± SEM, n = 8

Variable	Before cryopreservation	After cryopreservation				*P*-value
		DMSO 0%	DMSO 5%	DMSO 8%	DMSO 11%	
Overall motility	88.2 ± 0.5	29.8^{d} ± 1.6	37.9^{c} ± 1.1	74.2^{a} ± 1.1	44.9^{b} ± 0.7	2.33×10^{-17}
Progressive motility	79.6 ± 0.6	16.0^{c} ± 1.6	23.1^{b} ± 1.0	53.5^{a} ± 1.9	23.6^{b} ± 1.1	3.89×10^{-17}
Viability	91.7 ± 0.5	38.1^{d} ± 1.5	46.2^{c} ± 1.2	80.7^{a} ± 1.7	53.2^{b} ± 0.6	1.37×10^{-17}
Membrane integrity	77.4 ± 0.5	22.2^{c} ± 0.7	34.1^{b} ± 1.2	55.3^{a} ± 1.0	36.0^{c} ± 1.1	1.88×10^{-17}

Different lowercase letters on rows indicate a significant difference (P<0.05).

DISCUSSION

The study aimed to assess the quality parameters of goat semen in the Mekong Delta region of Vietnam and evaluate the effectiveness of two cryoprotectants, glycerol and DMSO, on goat spermatozoa cryopreservation. Results indicated that goat semen had high-quality parameters, including high overall motility, viability, and membrane integrity. However, cryopreservation without cryoprotectant agents (CPAs) resulted in low-quality goat sperm. This study identified that the optimal concentration of CPAs is crucial to improve goat sperm quality after cryopreservation. The results indicated that glycerol and DMSO are optimal cryoprotectants for goat sperm cryopreservation.

Using an 8 % concentration of glycerol or 8% concentration of DMSO was the most effective for maintaining the quality of goat semen after cryopreservation. These findings are consistent with previous studies (Kundu et al., 2000; Rini et al., 2019), which showed that glycerol concentration affects the quality and longevity of post-thaw goat semen. Nitira et al. (2013) also found a high concentration of CPA would damage goat sperm, while a low concentration failed to protect goat sperm during cryopreservation. Therefore, the current study has confirmed that an optimal concentration of glycerol or DMSO can improve goat sperm quality during cryopreservation. This finding is significant for the improvement of crossbred Boer (♂) x Bach Thao (♀) goats' breeding and conservation of genetic resources.

Recent advancements in the field of sperm cryopreservation go beyond just cryobanking and other assisted reproductive technologies. The utilization of cryopreservation techniques has had significant implications for animal breeding, genetic exchange, as well as improvement and conservation initiatives. However, despite its long-standing presence, challenges such as cryoinjury and inconsistencies in cryopreservation outcomes persist, posing important considerations in the field of sperm cryobiology (Bryan Salinas et al., 2021). The quality of sperm during cryopreservation depends on various factors, such as freezing techniques, cryoprotective diluent composition, dilution methods, cooling rates, and thawing methods (Aboagla and Terada, 2004). Cryopreservation can harm cells by reducing viability, causing membrane and DNA integrity loss (Bagchi et al., 2008; Salinas et al., 2021). Goat sperm is especially susceptible to peroxidative damage during cryopreservation due to the high unsaturated fatty acid content in plasma membrane phospholipids and the relatively low antioxidant capacity of goat semen plasma (Watson, 2000). Unlike sheep, goat seminal plasma contains phospholipase, which can affect spermatozoa viability (Chunrong et al., 2019). The susceptibility of spermatozoa to damage caused during cryopreservation varies in different breeds of goats due to sperm membrane lipid composition (Holt, 2000). Sperm membrane disruption during cryopreservation can result from liquid phase transition changes and increased lipid peroxidation (Alvarez et al., 1992). Cryoprotectants are included in freezing media to minimize the physical and chemical stresses resulting from the freezing and thawing of spermatozoa (Purdy, 2006). Sperm are inevitably exposed to the toxicity and evaluation pressure of cryoprotectants. Still, single-celled spermatozoa are unable to transcribe and translate, making them incapable of recovering from trauma after vitrification.

The quality of sperm after freezing is not only determined by the male and the quality of the pre-frozen sperm but also by many other factors that play an important role in the results after freezing. These include cryoprotectants and their concentrations, freezing and defrosting rates, type of cryoprotectant, and extender used (Mocé et al., 2003). The possibility of using free-egg yolk extenders, faster and simpler application, and lower cost compared to conventional cryopreservation techniques (Sánchez et al., 2012; Pilar et al., 2015; Taiwo et al., 2019). Conventional techniques can lead to extensive cell shrinkage and sperm damage due to intra- or extra-cellular ice crystal formation and osmotic changes (Gao et al., 1995; Watson, 1995).

This research provides significant valuable information on the quality of goat semen in the Mekong Delta region of Vietnam and the effectiveness

of different cryoprotectants for its cryopreservation. The results suggest that goat semen in this region is suitable for cryopreservation, and glycerol at a concentration of 8 % is the most effective cryoprotectant for maintaining its quality after cryopreservation. This information can be used by breeders and researchers to improve goat breeding programs in the region.

This study has limitations such as a small sample size and a lack of investigation into the long-term viability and fertility of spermatozoa after cryopreservation. However, the study's strength is elucidating the effectiveness of TCG EggYolk enriched with glycerol or DMSO as cryoprotectants, which are commonly used for goat sperm cryopreservation. Despite the limitations, this study provides valuable insights into the cryopreservation of goat spermatozoa and contributes to the knowledge base of cryopreservation techniques for goat breeding and genetic resource conservation. The study's findings have significant implications for the breeding and preservation of goat in the Mekong Delta. Further research with larger sample sizes and long-term evaluation of the spermatozoa's viability and fertility is necessary to fully validate the effectiveness of the developed cryopreservation method.

CONCLUSIONS

In conclusion, this study demonstrates that adding Glycerol or DMSO to the freezing medium for goat sperm cryopreservation results in high sperm quality. Based on the results, it can be concluded that using 8 % Glycerol or 8 % DMSO is effective for goat sperm cryopreservation. The findings of this study can aid breeding programs in enhancing the quality of local goat breeds and preserving the goat population in the Mekong Delta of Vietnam. Furthermore, this study contributes to the ever-growing field of reproductive biotechnology and cryobiology.

CONFLICT OF INTEREST

Authors declare that there are no conflicts of interest.

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