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The Effects of Different Thawing Procedures on Spermatological Parameters of Frozen Bull Sperm

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ABSTRACT

One of the most crucial elements influencing the efficacy of artificial insemination applications is semen quality. It is known that post-thaw semen quality was affected by thawing method. Errors made during the thawing process can compromise the motility, viability, morphology and DNA integrity of spermatozoa after thawing. This study was conducted to investigate the effects of four distinct thawing procedures on post-thaw semen quality in bulls. The first group was designated as 24°C (n=8); the straws in this group were thawed in a water bath at 24°C for 60 seconds. Second group was designated as 32°C (n=8); the straws in this group were thawed in a water bath at 32°C for 60 seconds. Third group was designated as 38°C (n=8); the straws in this group were thawed in a water bath at 38°C for 30 seconds. And the fourth group was designated as 70°C (n=8); the straws in this group were thawed in a water bath at 70°C for 7 seconds. Following the thawing process, motility, sperm morphology, viability, and DNA damage rate in spermatozoa were evaluated. Results showed that in 32°C group and 24°C group sperm motility significantly decreased when compared to 70°C and 38°C groups. Additionally, the 24°C group exhibited a significantly elevated DNA damage rate compared to the 70°C group, while no differences were observed in the other groups. In conclusion, the most ideal thawing procedures are at 70°C for 7 seconds and at 38°C for 30 seconds are recommended to get optimum sperm quality from frozen bull semen after thawing.

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Introduction

In bovine reproduction, the use of artificial insemination (AI) plays a pivotal role in enhancing genetic progress and reproductive efficiency. Frozen bull sperm is frequently employed in AI programs due to its preservation capabilities and ease of transportation. However, the process of thawing process frozen sperm can significantly impact its viability and fertility potential. Mistakes made during the thawing process can cause damage to the motility, viability, morphology, and DNA integrity of spermatozoa after thawing (Senger, 1980). Spermatological parameters encompass a range of factors that determine the quality and fertility potential of sperm. Key parameters include sperm motility, morphology, viability, and DNA integrity. These parameters are critical indicators of sperm health and play a vital role in successful fertilization and embryo development (Kjoestad et al., 1993). It is imperative that the recrystallization stage, occurring between -60 and 0°C, and the critical temperature stages, between 0 and 30°C, where spermatozoa are exposed to cold shock, are passed swiftly during the semen thawing process. This is to prevent the loss of motility and viability of spermatozoa (Bearden et al. 2004; Ostashko 2001; Marshall 1984).

Sperm motility is one of the most crucial determinants of fertility (Kjoestad,.et al., 1993). Studies have demonstrated that rapid thawing at higher temperatures can lead to a decrease in sperm motility compared to slower thawing methods (Rastegarnia et al., 2013; Nur et al., 2003). Rapid temperature changes during thawing can cause osmotic stress and damage to the sperm membrane, compromising motility. Sperm morphology refers to the size, shape, and structure of sperm cells. Optimal morphology is essential for successful fertilization. Thawing at higher temperatures has been associated with better preservation of sperm morphology compared to lower temperatures. Slow thawing can induce structural damage to sperm cells, leading to abnormalities in morphology (Lyashenko 2015).

Sperm viability reflects the percentage of live, functional sperm cells in a sample. Previous studies have demonstrated that thawing at moderate temperatures is more effective in maintaining higher sperm viability compared to extreme temperatures. DNA integrity is critical for the transmission of genetic information and embryo development (Zribi et al., 2012; Shoukir et al., 1998). The objective of this manuscript was to investigate the effects of various thawing temperatures on spermatological parameters, and to provide insight into the optimal methods for preserving sperm quality and fertility in bovine reproduction programs.

Material and Methods

Semen Straws

The semen straws utilised in this study were purchased form a commercial company. Straws derived from four distinct Simmental bulls, reared in the same establish (International Center for Livestock Research and Training, Ankara, Türkiye) were evaluated for motility before purchase. Straws exhibiting motility between 65 and 70% were purchased. Eosin dye (%5), anilin blue dye, and acetic acid were sourced from a medical company. This article was supported by a project of TUBİTAK 2209 A (Project Number: 1919B012109272)

Thawing Procedures

A total of thirty-two straws were subjected to thawing for each thawing protocol. In each group eight straw from four bulls were used as material. The straws were thawed with 4 different thawing methods. The first group was designated as 24°C (n=8); the straws in this group were thawed in a water bath at 24°C for 60 seconds. The second group was designated as 32°C (n=8); the straws in this group were thawed in a water bath at 32°C for 60 seconds. The third group was designated as 38°C (n=8); the straws in this group were thawed in a water bath at 38°C for 30 seconds. And the fourth group was designated as 70°C (n=8); the straws in this group were thawed in a water bath at 70°C for 7 seconds.

Semen Examination

Immediately following thawing, semen samples were examined for motility under light microscope. The arithmetic mean of the motility rates at two different sites was recorded as the mean motility score and expressed as a percentage (%). Smears prepared with 5% eosin solution were prepared to determine the dead/viable ratios of spermatozoa and abnormal sperm ratios. Briefly, one droplet semen and one droplet of eosin dye were mixed on the glass slide and smear preparation was done. And the slides were dried on the warming table (warmed at 38°C). To determine of dead sperm rate, 200 spermatozoa cells from each preparation were examined. Spermatozoa with stained heads were recorded as dead spermatozoa, while those without stained heads were recorded as live spermatozoa and expressed as a percentage (%). In order to examine the spermatozoa morphologically, 200 spermatozoa cells from each preparation were examined. The ratio of abnormal spermatozoa was determined according to the changes occurring in the head, middle and tail parts, and expressed as a percentage (%) (Aksu et al, 2016).

The aniline blue staining method (Hatipoğlu, 2014) was employed to determine the DNA damage of semen samples. A drop of semen sample was placed on the slide, followed by the addition of a drop of 5% aniline blue dye containing 4% acetic acid to prepare a smear. A total of 200 spermatozoa cells from each preparation were examined. Spermatozoa exhibiting dark blue staining on the head were identified as DNA-damaged, while those without staining were classified as undamaged. The rates were expressed as percentages.

Statistical Analyses

The spermatological parameters were presented as mean value ± standard error of the mean (S.E.M.) value. One-way analysis of variance (ANOVA) and post-hoc Tukey test were applied for the comparison of groups in the SPSS statistical (Version 20.0, IBM SPSS Co. NY, USA) analyse programme. Differences among the groups at a significance level of $p < 0.05$ were considered statistically significant.

Results

Spermatological Parameters

The results of the spermatological parameters are presented in Table 1. The statistical analysis revealed that the motility of sperm was significantly ($p < 0.05$) lower in the 24°C and 32°C groups when compared to the 38°C and 70°C groups. Additionally, the dead sperm rate was found to be significantly ($p < 0.05$) higher in the 24°C group when compared to the other groups. Conversely, the highest DNA damage rate was observed in the 24°C group, while the lowest DNA damage was observed in the 70°C group. However, no statistical difference was found between all groups for sperm morphology.

Conclusion

Semen evaluation gives crucial insights into the prediction of fertility in the context of artificial insemination. The quality of semen following the thawing process is positively related to fertility. The decrease in sperm motility and the increase in the proportion of dead and/or abnormal spermatozoa may result in a decline in fertility success (Barros et al., 2007). The mammalian spermatozoon membrane is a bilayer lipid membrane rich in polyunsaturated fatty acids, which renders mammalian spermatozoa resistant to oxidative stress and cold shock (Aitken 1989).

Errors made during thawing can affect the quality of semen after thawing. The method of thawing has a significant impact on the motility of the spermatozoa, with losses occurring at a greater rate when a particular method is employed. This, in turn, has a detrimental effect on the fertility and return rates of the semen. The recrystallization period, which occurs between -60°C and 0°C, is characterized by the coalescence of ice crystals and the formation of larger crystals (Sönmez, 2015).

Table 1. Spermatological parameters of all groups

	24°C	32°C	38°C	70°C
Motility (%)	44.63 ± 1.18 [a]	46.63 ± 2.98 [a]	64.13 ± 1.53 [b]	68.13 ± 1.49 [b]
Dead Sperm (%)	14.38 ± 1.87 [a]	5.25 ± 0.37 [b]	5.25 ± 0.41 [b]	5.88 ± 0.77 [b]
Abnormal sperm (%)	11.75 ± 0.90	11.63 ± 1.84	12.5 ± 0.94	13.75 ± 1.78
DNA damage	6.75 ± 0.94 [a]	5.63 ± 0.26 [ab]	5.38 ± 0.70 [ab]	3.75 ± 0.41 [b]

(a-b): The presence of different superscript letters in the same row indicates a statistically significant difference ($p<0.05$)

If this period is prolonged, the larger crystals formed damage the filter membrane. Furthermore, ice crystals will also damage the mitochondria and axoneme of the spermatozoa (Courtens et al., 1989). During the thawing process, the period between 0-25°C is a critical phase in which spermatozoa are exposed to cold shock. Maintaining the internal temperature of the straws at these levels results in the spermatozoa undergoing cold shock, accompanied by a reduction in motility. Sperm cells are highly differentiated cells. Damage to sperm DNA, which constitutes half of the genetic material of the embryo to be formed in fertilization, reduces fertilization success. Sperm DNA damage has a 50% effect on the fertilization, embryo development, implantation and pregnancy (Baskaran et al., 2019). It has been reported that sperm DNA damage of more than 30% decreases the pregnancy rate (Santi et al., 2018). Therefore, sperm DNA damage is an important marker to prediction of potential fertilization success (Selvam et al., 2020).

In practice, the process of thawing frozen bull semen at 37°C for 30 seconds is a widely used method in the field (Nur et al., 2003; Hayashi and Isobe, 2005). However, scientists have conducted studies examining the effects of different thawing methods on post-thawing sperm quality. In a study by Lyashenko (2015), it was found that straw thawing at 65-70°C for 7 seconds significantly increased sperm motility and sperm viability after thawing compared to thawing at 35°C for 20 seconds. In this study, the effects of four different thawing methods on post-thawing sperm quality were investigated. The results demonstrated that thawing at 70°C for 7 seconds yielded the most favourable outcomes. However, the thawing protocol at 38°C for 30 seconds exhibited a comparable result. Conversely, thawing semen at 32°C for 60 seconds resulted in a statistically significant decline in sperm motility. Furthermore, the rate of dead spermatozoa as a resulting from thawing at 32°C was comparable to that observed in the 38°C and 70°C groups, and there was no statistical difference in terms of DNA damage when the results of this method were compared in the 38°C and 70°C groups. However, when the thawing method was employed for 60 seconds at 24°C, which is considered to be room temperature, it was observed that motility was significantly lower and the rate of dead spermatozoa was increased significantly higher compared to the other groups. This result can be attributed to the longer time to cold shock exposure and recrystallization period Furthermore, the use of this method was found to significantly increase the damage to spermatozoon DNA compared to the result obtained at 70°C.

The findings of this study indicate that, optimizing thawing protocols is essential for maximisation of sperm quality and fertility potential in AI programs. It is recommended that a gradual thawing at moderate temperatures, preferably around 70°C and 37°C, be employed to minimize stress on sperm cells and preserve spermatological parameters. However, the 30 at 37°C protocol is the most feasible in the field. Furthermore, the implementation of quality control measures to assess sperm quality post-thawing can help to ensure the success of AI procedures.

References

Aitken, R.J., Clarkson, J.S. & Fishel, S. (1989). Generation of reactive oxygen species, lipid peroxidation and human sperm function. *Biol. Reprod.* **41,** 183-197.

Aksu, E. H., Akman, O., Ömür, A. D., Karakuş, E., Can, I., Kandemir, F. M., Dorman, E. & Uçar, Ö. (2016). 3, 3 diindolylmethane leads to apoptosis, decreases sperm quality, affects blood estradiol 17 β and testosterone, oestrogen (α and β) and androgen receptor levels in the reproductive system in male rats. *Andrologia*, *48*(10), 1155-1165.

Barros, P.M.H., Nichi, M., Cortada, C.N.M., Carvalho, N.A.T., Baruselli, P.S., Barnabe R.C. & Barnabe V.H. (2007). Semen evaluation of Murrah buffalo bulls using sperm functional tests. *Italian J. Anim. Sci*. **6,** 772-774.

Baskaran, S., Agarwal, A., Panner Selvam, M. K., Finelli, R., Robert, K. A., Iovine, C., Pushparaj, P.N., Samanta, L., Harlev, A. & Henkel, R. (2019). Tracking research trends and hotspots in sperm DNA fragmentation testing for the evaluation of male infertility: A scientometric analysis. *Reproductive Biology and Endocrinology*, **17**(1)

Bearden, H.J., Fuquay, F., & Willard, S.T. Applied animal reproduction. 6th ed. Pearson Prentice Hallm: New Jersey; 2004.

Courtens, J.L., Ekwall, H., Paquignon, M. & Ploen, L. (1989). Preliminary study of water and some element contents in boar spermatozoa, before, during and after freezing. *J. Reprod. Fertil*. **87,** 613-626.

Hatiboğlu, Y. (2014): Toluidin mavisi, anilin mavisi ve sperm kromatin dispersion testleri ile sperm dna hasar oranlarının kıyaslanması, Yüksek Lisans Tezi

Hayashi, Y., Isobe, N. (2005) Characteristics of cryopreserved spermatozoa from a Holstein-Friesian bull thawed at different temperature. J Inter Dev Coop 12(1): 107-110.

Kjoestad, H., Ropstad, E., & Andersen Berg, K. (1993). Evaluation of spermatological parameters used to predict the fertility of frozen bull semen. *Acta Veterinaria Scandinavica*, *34*(3), 299-303.

Lyashenko, A. (2015). Effect of different thawing procedures on the quality and fertility of the bull spermatozoa. Asian Pacific Journal of Reproduction, 4(1), 17-21.

Marshall, C. (1984) Considerations for cryopreservation of semen. Zoo Biol 3(4): 343-356.

Nur, Z., Dogan, I., Soylu, M, & Ak, K. (2003) Effect of different thawing procedures on the quality of bull semen. Rev Med Vet 154(7): 487-490.

Ostashko, F.I. Cryopreservation and long-term storage of semen producers. 2nd ed. Ukraine: Harvest; 2001.

Rastegarnia, A., Shahverdi, A., Rezaei Topraggaleh, T., Ebrahimi, B., & Shafipour V. Effect of different thawing rates on post-Thaw viability, kinematic parameters and chromatin structure of buffalo (Bubalus bubalis) Spermatozoa. Cell Journal (Yakhteh) 2013; 14(4): 306-313.

Santi, D., Spaggiari, G., & Simoni, M. (2018). Sperm DNA fragmentation index as a promising predictive tool for male infertility diagnosis and treatment management - meta-analyses. Reproductive BioMedicine Online, 37(3), 315–326.

Selvam, M.K.P., Ambar R.F., Agarwal, A. & Henkel, R. (2021) Etiologies of sperm DNA damage and its impact on male infertility. Andrologia,53(1), e12706

Senger, P.L. (1980) Handling frozen bovine semen-factors which influence viability and fertility. Theriogenology, 13, 51-62.

Shoukir, Y., Chardonnens, D., Campana, A. & Sakkas, D. (1998). Blastocyst development from supernumery embryos after intracytoplasmic sperm injection: a paternal influence? Hum. Reprod. 13, 1632-1637.

Sönmez, M. (2015) Reprodüksiyon Suni Tohumlama ve Androloji Ders Notları, Elazığ

Zribi, N., Chakroun,N. F., Abdallah F. B., Elleuch, H., Sellami, A., Gargouri, J., Rebai, T., Fakhfakh, F. & Keskes, L.A. (2012) Effect of freezing–thawing process and quercetin on human sperm survival and DNA integrity, Cryobiology, 65, 3,326-331