

Ultrasonic Treatment of Chicken Yolk: A Promising Strategy to Optimize Boar Semen Cryopreservation

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ABOUT THE STUDY

Boar semen cryopreservation is a fundamental technology for swine reproduction, facilitating genetic resource conservation, overcoming geographical barriers in breeding, and enhancing the utilization efficiency of elite boars. However, its commercial application remains limited due to the inherent cold sensitivity of boar sperm, which suffers significant cryoinjury during freeze-thaw cycles including ice crystal formation, osmotic stress and oxidative damage that compromises sperm motility, membrane integrity and fertilizing capacity.

Chicken egg yolk has long been a staple cryoprotective additive in boar semen extenders, primarily due to its rich content of Low-Density Lipoproteins (LDL) that shield sperm membranes from cold shock and oxidative stress. Nevertheless, traditional egg yolk preparation methods result in large, unevenly distributed particles that reduce extender stability, interfere with sperm quality assessment, and limit the full exertion of its cryoprotective potential. Our original study addressed this limitation by introducing ultrasonic treatment as a simple, cost-effective strategy to modify chicken egg yolk properties, with the goal of improving boar semen cryopreservation outcomes.

In our research, we compared the cryopreservation efficacy of boar semen using conventional chicken egg yolk (Con; control extender) and Ultrasonically Treated Chicken Egg Yolk (UT-CEY) extenders, evaluating key sperm quality parameters post-thaw. The results demonstrated that ultrasonic treatment significantly reduced yolk particle size, improved emulsion stability, and decreased the creaming index changes that enhanced the homogeneity and physical stability of the extender. Critically, sperm cryopreserved with UT-CEY exhibited significantly higher total and progressive motility, straight-line velocity, plasma membrane integrity, and acrosome integrity compared to the Con group ($p < 0.01$ or $p < 0.05$). We also observed elevated activities of Catalase (CAT) and Superoxide Dismutase (SOD) key antioxidant enzymes along with

upregulated expression of anti-apoptotic (Bcl-2) and antioxidant (CAT, SOD₂) genes in the UT-CEY group, indicating that ultrasonic treatment mitigates oxidative stress and apoptosis in cryopreserved sperm.

These findings align with and extend recent advances in boar semen cryopreservation research, which has increasingly focused on optimizing extender components and processing methods to reduce cryoinjury. For instance, our team's previous research on centrifugal treatment of chicken egg yolk has also demonstrated its effectiveness in improving post-thaw sperm quality by removing large particles, and ultrasonic treatment provides an alternative approach with its own advantages in simplicity and efficiency. Similarly, studies on ultrasonic treatment of egg yolk plasma for canine semen cryopreservation have demonstrated that ultrasonication can form LDL nanoparticles with enhanced cryoprotective efficacy, supporting the generalizability of our approach across species. Notably, our study is among the first to systematically investigate the effects of ultrasonic treatment on chicken egg yolk's physical properties and its subsequent impact on boar sperm cryosurvival, filling a gap in current knowledge.

Despite the promising results, several questions remain to be addressed in future research. First, the optimal ultrasonic parameters (e.g., frequency, duration, power) may vary depending on egg yolk source and semen quality, requiring further optimization to maximize cryoprotective efficacy. Second, while we observed improvements in in vitro sperm quality, in vivo fertility trials are needed to confirm that UT-CEY can enhance farrowing rates and litter sizes key metrics for commercial swine production. Third, the molecular mechanisms underlying the enhanced cryoprotection of UT-CEY, particularly the interaction between modified yolk components and sperm membranes, merit deeper investigation.

In conclusion, our original study highlights ultrasonic treatment of chicken egg yolk as a practical and effective strategy to improve boar semen cryopreservation. By addressing the limitations of traditional egg yolk extenders, this method has the

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potential to advance the commercial application of cryopreserved boar semen, benefiting the swine industry worldwide.

We believe this commentary will provide valuable insights to researchers and practitioners in the field of andrology and

animal reproduction, while also increasing the visibility and citation of our original work. We thank the editors of Andrology for the opportunity to share our perspectives and look forward to further advancing this research area.