Optimization of Rehydration Process for Freeze-Dried Mouse Sperm

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Purpose: Freeze-drying is an effective method for the long-term preservation method of mouse sperm at room temperature and low and it plays a crucial role in the conservation of genetic resources. However, freeze-dried mouse sperm exhibit a significant reduction in birth rates to about one-third compared to fresh sperm, and the detailed causes of this reduction remain unclear. This study aims to elucidate the impact of the rehydration process, previously unexplored, on DNA damage in freeze-dried sperm and following development after ICSI.
Methods: Sperm and oocytes from ICR strain mice were used. Freeze-dried sperm were produced using conventional methods. For the rehydration process, we tried the following three methods that affect the soaking speed of the rehydration solution into the cytoplasm of FD sperm. 1) Type and amount of rehydration solution: Distilled water or culture medium (HTF), and the volume were altered. 2) Viscosity of rehydration solution: PVP was added to the rehydration solution at concentrations of 0 to 12% to alter its viscosity. 3) Temperature of rehydration solution: The temperature of the rehydration solution was adjusted from 0.5 to 90℃. The rate of soaking of each condition was simply measured using filter paper. DNA damage in FD sperm after rehydration was assessed using the alkaline comet assay. The quality of embryos fertilized with those sperm by ICSI was assessed using abnormal chromosome segregation (ACS)at the two-cell stage, developmental rate to the blastocysts, and birth rates after transfer of two-cell embryos into the recipient female.
Results: 1) No significant effects of different rehydration solutions or volume on DNA damage of FD sperm or birth rates were observed. 2) As the viscosity of the rehydration solution increased, DNA damage of FD sperm also increased, leading to a significant decrease in both the blastocyst rate and the birth rate. 3) As the temperature of the rehydration solution increased, a significant reduction in DNA damage of FD sperm was observed, and a significantly improved birth rate was obtained at 50℃ (room temperature 22% vs. 50℃ 37%).
Discussion: The various rehydration methods tested, from 1) to 3), suggest that the soaking rate of the rehydration solution is important to prevent/reduce the DNA damage of FD sperm during the rehydration process. While the mechanism remains unclear, a hypothesis was proposed that damage arises from the difference in expansion rates between areas of the nucleus where the soaking of solution has progressed and those where it has not. Interestingly, normal fertilization rates decreased when rehydration was performed with rehydration solutions above 70° C, but when those oocytes were artificially activated after ICSI, the birth rate of those embryos did not reduce. These results suggested that while DNA damage could be reduced with increased

temperature or soaking rate, thermal denaturation damage occurred to the FD sperm. This study shows for the first time that improving and optimizing the rate of soaking speed of rehydration solutions could lead to a much higher success rate of producing offspring from FD sperm. Future efforts should focus on minimizing DNA damage not only in the drying process but also rehydration process to achieve birth rates comparable to those of fresh sperm.