Protective Effects of Monosodium Glutamate on the Preparation of Freeze-Dried Sperm in Mice

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Freeze-dried (FD) preservation of sperm allows long-term storage of genetic resources at room temperature without the use of liquid nitrogen. However, compared to fresh sperm, the success rate of offspring from those FD sperm is reduced by one-third. Therefore, we focused on the use of monosodium glutamate (MSG) as a protective agent, which is a type of amino acid and enables yeast to survive after FD. In mammalian sperm, sugars and chelating agents are mainly used as FD protectants, but no examples have been found using amino acids. In this study, we investigated whether MSG could play a role as a new protective agent for FD sperm.

In this study, HTF medium was used as the basic medium. When mouse sperm were precultured in HTF medium for 1 hour and then replaced with HTF medium containing 0.5-10% MSG, almost all sperm at 5% MSG and all sperm at 10% MSG lost motility immediately after incubation. To determine the protective effect of MSG during FD treatment, those sperm were freeze-dried with MSG just after mixing with MSG, and the appearance and morphology of those sperm after FD-rehydration was observed. Then, those sperm were injected into oocytes and development rate of embryos to blastocyst and its cell number and birth rate after embryo transfer were measured. There was no difference in the appearance of the FD sperm between control and MSG treated sperm after addition of water. Blastocyst rate was 34% in controls, while the rate was significantly increased with increased the concentration of MSG, reaching a maximum of 65% at 3% MSG and did not improve further ($P< 0.05$). No significant differences were found in the number of cells in the blastocysts. When 2-cell embryos were transferred to the recipient after ICSI, the birth rate peaked at 42% (30 pups) at the 3% MSG concentration, more than twice as high in the control group (20%, 17 pups). Next, to determine whether the addition of MSG to the sperm preculture and FD treatment in succession had any adverse effects, sperm were incubated in 1% MSG-HTF for 1 hour, followed by FD treatment in the same medium. When ICSI were performed, both the developmental rate of embryos to the blastocysts (50%) and offspring (37%) derived from embryos fertilized with MSG preincubated FD sperm were increased compared to controls (38% and 20%, respectively), though the difference was not statistically significant.

This study shows for the first time that MSG, in which usually used for the yeast, also has a protective effect for mammalian sperm against FD treatment. It was also shown that the addition of MSG during preincubation before FD treatment had positive effect on sperm. Since MSG has

been suggested to be involved in membrane protection and antioxidant effects in yeast FD, it is considered to have a similar effect on sperm. Future work is needed to explore other microorganisms other than yeast to find more optimal protective agents and combinations.