Assessing of Fertilization and Developmental Ability of Mouse Spermatozoa Head Separated from Tail by Freeze-drying Treatment.

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PURPOSE: Long-term preservation of mammalian sperm is needed worldwide for the strain maintenance. However, cryopreservation using liquid nitrogen is expensive to maintain and there is a risk of sperm loss due to accidents or disasters. Freeze-drying (FD) technology can easily preserve sperm at room temperature for more than one year. However, the birth rate of FD sperm is about 20%, which is significantly lower than that of fresh sperm. Selection of high-quality FD sperm is necessary to improve the offspring rate, but no such method exist yet. Recently, we identified a number of spermatozoa whose heads and tails had been separated by FD treatment (Yang, Ito et al., JRD 2023), but their fertilization and developmental ability have not yet been compared between separated and intact sperm. In this study, we examined whether the sperm head separated from the tail by FD treatment had normal fertility and developmental potential after ICSI.

METHODS: Sperm were collected from ICR and B6 strains, and FD sperm were prepared using conventional method. First, the rates of tail-separated (head-only) sperm and tail-connected (intact) sperm in fresh, freeze-thawed (FT), and FD sperm were compared. Next, ICSI was performed using head-only FD sperm or intact FD sperm. To examine the extent of DNA damage of FD sperm after fertilization, the brightness of γ-H2Ax expressed in the male pronuclei were measured by immuno-staining. To examine the quality of embryos, the frequency of abnormal chromosome segregation (ACS) was observed at the 2-cell stage, or the 2-cell stage embryos were transferred into recipient female mice and examined offspring rate. In addition, 0.5 M trehalose was added in a part of the sperm suspension to examine its protective effect against FD treatment.

RESULTS: The rates of head-only sperm in FD group (ICR:29% and B6:45%) were higher than those in fresh group (ICR:2% and B6:18%). Trehalose reduced the rate of head-only sperm in both FT and FD group. Using FD sperm, in terms of γ-H2Ax assay and ACS frequency, there was no significant difference between head-only sperm and intact sperm, and these results were similar when trehalose was added. The fertilization rate and the developmental rate to the 2-cell stage of embryos derived from head-only sperm were similar with those of intact sperm. The offspring rates of embryos fertilized with head-only sperm were comparable to those of intact sperm (ICR:21% vs. 33%; B6 31% vs. 31%, respectively).

DISCUSSION: In current study, we were unable to characterize whether head only FD sperm has high developmental ability. However, the separation phenomena of the sperm head and tail proved to be of value for a variety of uses. The head-only sperm rate increased in FD group regardless of mice strains. Trehalose reduced that rate in FT and FD groups, which seems to be due to protection of sperm

membrane at the freezing process. These results suggest that the difference of head-only sperm rate can be provided as an evaluation item that clearly reflects differences in sperm treating methods such as FT or FD. On the other hand, in the mouse ICSI, the tail of sperm must be cut off and only the sperm head are injected into oocytes. Therefore, the efficiency of ICSI can be greatly improved if sperm that are already separated from tail can be used.