Abstract

Nuclear transfer techniques, including spindle chromosome complex (SC) transfer and pronuclear (PN) transfer, have been employed to mitigate mitochondrial diseases. Nevertheless, the challenge of mitochondrial DNA (mtDNA) carryover remains unresolved. Previously, we introduced a method for aggregated chromosome (AC) transfer in human subjects, offering a potential solution. However, the subsequent rates of embryonic development have remained unexplored due to legal limitations in Japan, and animal studies have been hindered by a lack of AC formation in other species. Building upon our success in generating ACs within mouse oocytes via the utilization of the phosphodiesterase inhibitor IBMX (3-Isobutyl 1-methylxanthine), this study has established a mouse model for AC transfer. Subsequently, a comparative analysis of embryo development rates and mtDNA carryover between AC transfer and SC transfer was conducted. Additionally, the mitochondrial distribution around SC and AC structures was investigated, revealing that in oocytes at the MII stage, the mitochondria exhibited a relatively concentrated arrangement around the spindle apparatus, while the distribution of mitochondria in AC-formed oocytes appeared to be independent of the AC's position. The AC transfer approach produced a marked augmentation in rates of fertilization, embryo cleavage, and blastocyst formation, especially as compared to scenarios without AC transfer in IBMX treated AC-formed oocytes. No significant disparities in fertilization and embryo development rates were observed between AC and SC transfers. However, relative real time PCR analyses revealed that the

mtDNA carryover for AC transfers was one-tenth, and therefore significantly lower than that of SC transfers. This study successfully accomplished nuclear transfers with ACs in mouse oocytes, offering an insight into the potential of AC transfers as a solution to heteroplasmy-related challenges. These findings are promising in terms of future investigation with human oocytes, thus advancing AC transfer as an innovative approach in the field of human nuclear transfer methodology.