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Production of mouse embryos with male germ cells by microinsemination and nuclear transfer techniques

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Development of germ cells is a remarkably complex process involving changes of the chromosome number and epigenetic modifications. The earliest stage at which germ cells are recognized in mouse embryos is day-7 gestation, when they exist as diploid cells, just as majority of somatic cells. The haploid state is achieved through two meiotic divisions, which are followed by gametogenesis in the female and male gonads. Diploid embryos are produced by fusion (fertilization) of these specialized germ cells (gametes) from the male and female. The definitely differentiated male germ cells, the spermatozoa, gain the fertilizing ability after maturation in the epididymis. Therefore, under in vivo and conventional in vitro fertilization conditions, only spermatozoa which have passed through the epididymis can reach the ova investment, penetrate the zona pellucida, and then fuse with the eggs. Recently developed microinsemination techniques enable us to use not only immotile spermatozoa, but also immature spermatozoa, as male gametes. In the mouse, elongated spermatids, round spermatids, and primary and secondary spermatocytes can participate in formation of diploid zygotes that develop into normal offspring by using microinsemination techniques. These findings indicate that at least a part of male germ cells complete epigenetic modifications before the first meiosis and that the male tetrad chromosomes can undergo two meiotic divisions safely in homologous oocytes. In view of the chromosomal structure, primary spermatocytes in later stages (diplotene and pachytene) are the earliest male germ cells which can produce a haploid set of chromosomes in the oocytes, thus involving in the formation of diploid zygotes together with maternal chromosomes. As the chromosomes in earlier stage spermatocytes (zygotene, leptotene) have not completed synapsis they cannot enter the first meiotic division. Spermatogonia and male primordial germ cells (PGCs) have a diploid set of chromosomes and therefore they can produce diploid embryos after introduction of their nuclei into enucleated oocytes. Some of the embryos reconstructed with the nuclei from mouse spermatogonia/PGCs develop into blastocysts and fetuses. The efficiency was very high when the cloning technique developed by Wakayama et al. was employed

In conclusion, most male germ cells from PGCs through mature spermatozoa can be used for producing diploid embryos by either microinsemination or nuclear transfer. These techniques are apparently useful to conserve genetically invaluable species/strains including genetically engineered animals. In near future, they will also become a powerful tool to study basic biological phenomena, such as meiosis, genomic imprinting, cell differentiation and dedifferentiation.

References

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