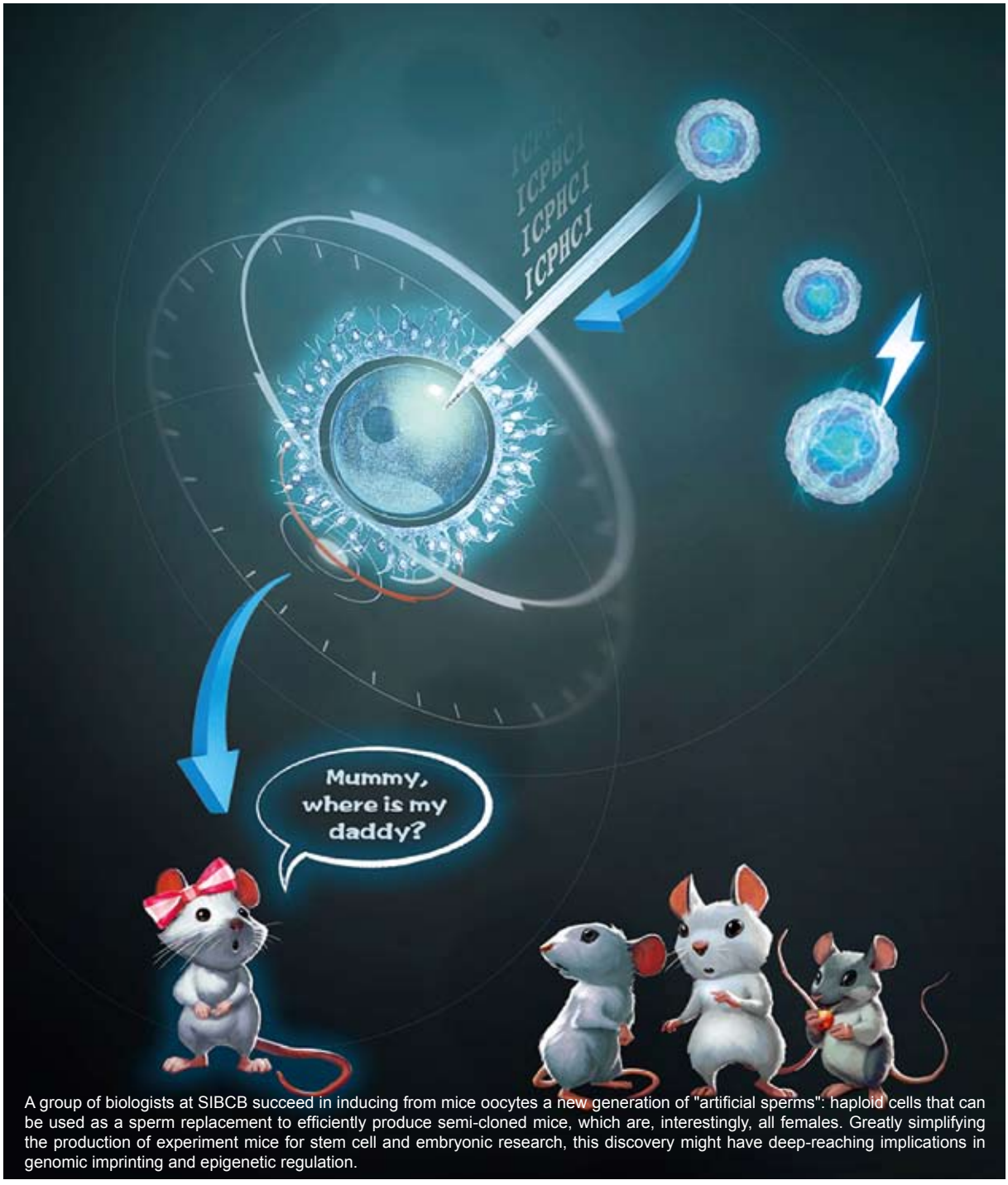
"Artificial Sperms" Induced from Mice Oocytes



A group of biologists at SIBCB succeed in inducing from mice oocytes a new generation of "artificial sperms": haploid cells that can be used as a sperm replacement to efficiently produce semi-cloned mice, which are, interestingly, all females. Greatly simplifying the production of experiment mice for stem cell and embryonic research, this discovery might have deep-reaching implications in genomic imprinting and epigenetic regulation.

On Nov 17th, a team of researchers from the Shanghai Institute of Biochemistry and Cell Biology (SIBCB), Shanghai Institutes for Biological Sciences, CAS led by Prof. LI Jinsong reports online in *Cell Research* a novel technique to induce from mice oocytes haploid embryonic stems cells (haESCs) that can fully replace the reproductive functions of sperms, greatly simplifying the otherwise complicated techniques to produce such stem cells and semi-cloned (SC) mice. It is anticipated that this will further facilitate research in the field of stem cells and embryonic development; in addition the study might also suggest some implications in genomic imprinting and epigenetic regulation.

In their work entitled "Parthenogenetic haploid embryonic stem cells efficiently support mouse generation by oocyte injection", the team demonstrated that oocyte-originated haESCs carrying deletions in the DMRs controlling *H19* and *Gtl2* (referred to as DKO-PG-haESCs) can efficiently support the generation of SC pups at a rate of 15.5%, and hence can overcome a former defect in a line of *in vitro* regenerative cells, which can be used as "artificial sperms" and has enormously improved the efficiency of biological research in lab.

Back in 2012, in cooperation with Prof. XU Guoliang's lab at SIBCB, the team succeeded in creating the first generation of "artificial sperms", a line of haploid embryonic stem cells (AG-haESCs) from mice sperms, and published a paper in *Cell*. These cells can support full-term embryonic development upon injection into MII oocytes, leading to the successful generation of SC mice (*Cell*, 2012, 149, 605). However one major drawback of this line is, after generations of *in vitro* self-reproduction, the "artificial sperms" gradually lose their ability to produce SC mice. As a result aberrant development of AG-haESC-derived embryos and very low birth rates of healthy SC mice (around 2% of total SC embryos) were frequently observed. In a recent work, the group revealed that AG-haESCs that carry deletions in the DMRs (differentially DNA methylated regions) controlling *H19* and *Gtl2*, two paternally repressed imprinted genes (designated as DKO-AG-haESCs), can efficiently support the generation of SC pups at a rate of 20% (*Cell Stem Cell*, 2015, 17, 221).

Based on this discovery the team improved the ability of AG-haESCs in generation of SC mice; however due to the complicated nuclear-transfer technique involved in the construction of haploid embryos, its application stayed very demanding for many labs. To bypass this setback, the team made efforts to induce oocyte-originated haESCs (PG-haESCs), namely "artificial sperms" produced from oocytes, but it remained an ambitious concept until the latest work.

To test whether oocyte-originated haESCs can be used as a sperm replacement, ZHONG Cuiqing, XIE Zhenfei, YIN Qi, and their colleagues generated 6 PG-haESC lines from parthenogenetic embryos derived via chemical activation of mature oocytes. Injection of these cells into mature oocytes, as they found, failed to produce SC mice, however. To find out the reason, they attempted to distinguish the differences between PG-haESCs and AG-haESCs by performing RNA-seq analysis. Surprisingly, the results showed that the two exhibited highly similar expression profiles based on all genes and all imprinted genes. Bisulfite-sequencing analysis of several typical paternal and maternal imprinted genes indicated that DMRs of paternal genes were free of methylation as expected. Interestingly, DMRs of two maternal imprinted genes, *Snrpn* and *Peg1*, which were expected to largely retain methylation, quickly lost methylation imprints during the process of haESC derivation and cell passage, thus exhibiting an imprinting pattern similar to that of AG-haESCs. Given what they demonstrated previously, *H19* and *IG* DMRs are two barriers to the high-efficiency generation of SC mice in AG-haESCs, they tested whether the removal of both DMRs would induce high-efficiency generation of SC mice in PG-haESCs. For this sake a total of 44 DKO-PG-haESC lines were derived and tested. All efforts count. Eventually, the researchers found that over 15% of SC embryos developed into healthy pups after injection of these DKO-PG-haESC cells; moreover, they all grew into fertile adults.

This study demonstrates that removal of *H19* and *IG* DMRs can functionally convert the imprinting status of parthenogenetic haploid cells, thus can provide the equivalent of a paternally inherited genome. Drawing on previous studies, it further verifies that the same deletions can also dramatically enhance mouse generation from AG-haESCs via semi-cloning.

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