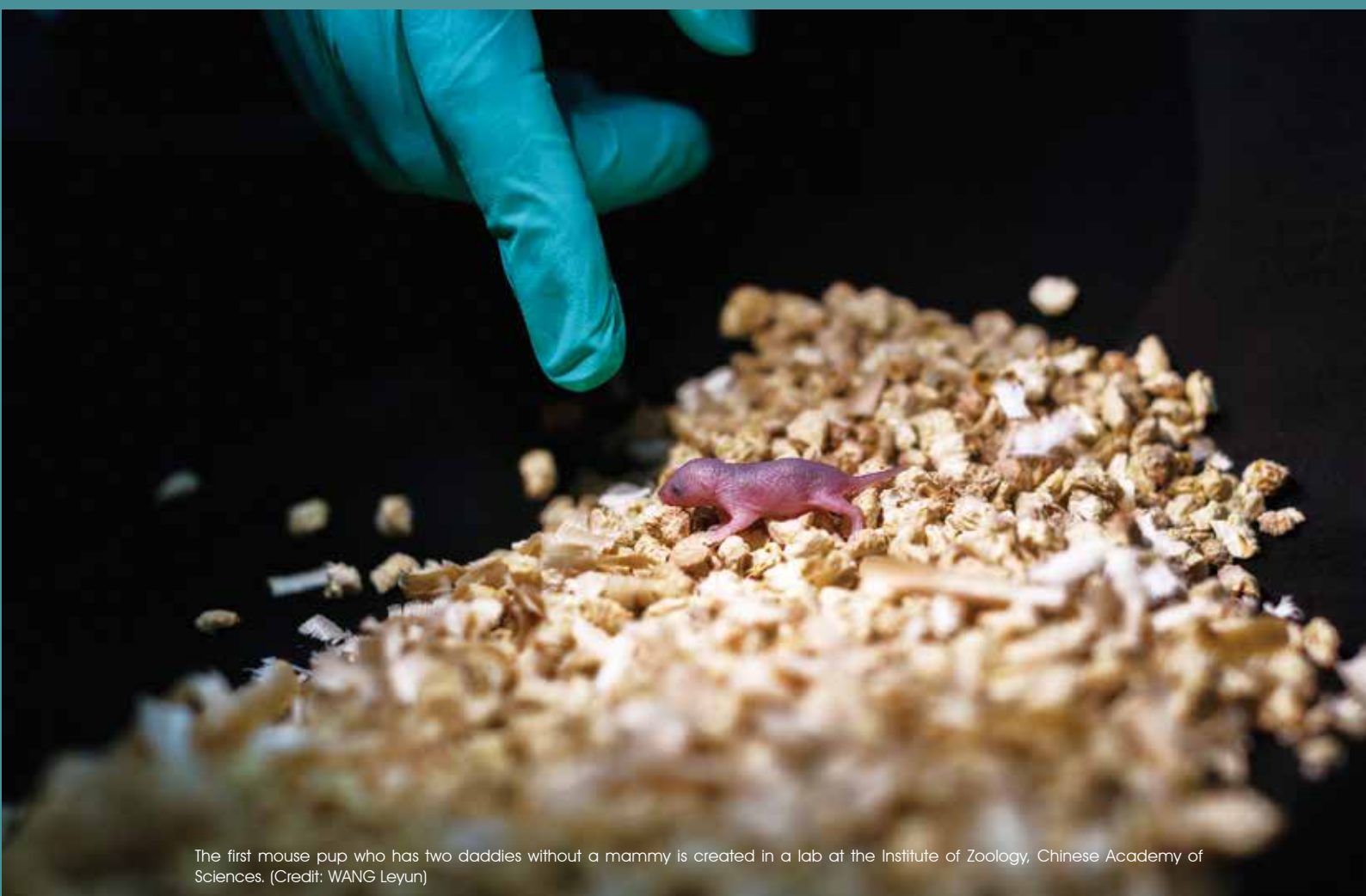
The First Creation of Mouse Pups from Two Fathers

By YAN Fusheng (Staff Reporter)

It is common sense that mammals reproduce sexually, whereby a sperm cell fertilizes an egg cell. And at some point later in reproduction, a baby with the genetic mixtures from the both parents pops out. However, this common perception was shattered by a recent study conducted by researchers from the Institute of Zoology (IOZ), Chinese Academy of Sciences (CAS). Jointly led by Prof. HU Baoyang, CAS Member Prof. ZHOU Qi and Prof. LI Wei, the team successfully created live mouse offspring descending from either two egg cells or two sperm cells. This research was published on October 11 in the journal of *Cell Stem Cell*.



The first mouse pup who has two daddies without a mammy is created in a lab at the Institute of Zoology, Chinese Academy of Sciences. (Credit: WANG Leyun)

Cross the Reproductive Barrier

Remarkably, for the first time, the mouse pups were created from two males, with no chromosome contribution from any females to the rodents' genetic makeup. So why such a big deal? Well, in mammals, offspring generally inherit two sets of genomes, one from the father and the other from the mother. Likewise, one gene usually has two copies, one from each parent. However, for certain genes, one gene copy is shut off from either the father's or the mother's copy of genome. In this case, such a gene is imprinted, hence called an imprinted gene. For example, a paternally imprinted gene means that the gene from the father's genome is dormant (OFF), while the same gene or allele from the mother's genome is expressed (ON). The imprinting pattern between the sperm and the egg cell is distinct, however complementary in a way. Through fertilization, they add up to a complete expression of all vital genes indispensable for survival. The gene expression profile from either two sperms or two eggs alone is incomplete and thus is insufficient to yield live offspring. In other words, this genomic imprinting implies that mammals could only reproduce sexually. The scarcity of asexual reproduction in the world of higher animals, say, mammals, might make a good footnote for this. The setbacks of early research in this field also mean something: the artificially established embryos from two fathers or two mothers by nuclear transfer experiments all stopped growing at early stages of development. Now, the creation of a live mouse from two males proclaims the possibility of overcoming the restriction of sexual reproduction in mammals.

Sperm in Disguise of Egg

The key to the success lies in transforming one of the sperm cells into egg-like status, making it eligible to be "fertilized" by another sperm cell – in other words, to bypass the barrier in the mask of an egg. But how?

If one casts aside the differences in look between them, the major difference left to tell a sperm from an egg would be the way in which its genome is marked, *i.e.*, whether its genes are imprinted to be expressed from the paternal or the maternal side. To make this functional transformation possible, researchers artificially altered the expressions of certain imprinted genes using CRISPR-Cas9-mediated gene editing, which is also known as "genetic scissor." Technically, the team conducted the following workflow to fulfill this challenging task: 1) generation of stem cells with global erasure of paternal imprints from the sperm cell; 2) precise activation and screening of maternally imprinted genes to make the "transformed" sperm cell functioning like an egg cell; 3) fertilize the transformed, egg-like sperm cell by a natural sperm cell from another male; 4) implantation into a surrogate mother. Specifically, the researchers genetically switched on seven maternally imprinted clusters, which are normally only imprinted from an egg cell; whilst the paternal imprints were globally removed by using the stem cells generated from injecting a sperm cell into an empty egg cell. The use of these particular stem cells with a relative "clean background," free from the paternal imprints, was also crucial for their success, as stated by the authors. Finally, they produced 12 (~2.5%) live mice from male-only parents in 477 attempts, and two pups even survived for 48 hours after birth.

Sperm-like Egg

In addition, the team also succeeded in creating bimaternal mouse offspring from two females by transforming one of the egg cells into a more "sperm-like" status using a similar strategy. Through altering three imprinted regions, they produced 29 (~14%) live bimaternal mice from 210 embryos. As one might notice, a bit less of alterations were required to functionally transform an egg cell into sperm-like status. This is because paternally imprinted genes are generally less abundant as compared to the maternally imprinted genes. Actually, bimaternal mice had been produced previously by Prof. Kono's team from Tokyo University of Agriculture. By deleting two imprinted regions from immature eggs, Kono's lab researchers successfully produced bimaternal mice – mice with two mothers. However, the generated mice still showed defective features, and the method itself is very impractical and hard to use, as described in the literature. In their research, however, the IOZ team identified an imprinted gene in the formerly produced bimaternal mice, whose expression is different from that of wildtype mice. Based on this discovery, with aid from CRISPR-Cas9 gene-editing system, the IOZ team accurately modified this additional epigenetic imprint

together with the other two imprinted regions reported by Kono's team and eventually produced bimaternal mice that are free of defective features and able to give birth to their own progeny.

Summed up, the IOZ team successfully went across the unisexual reproduction barrier using a strategy of "sperm-egg transformation" via alteration of certain imprinted genes. The most remarkable and anti-intuitive part of this study might be the generation of live mice descending from two sperm cells.

Why This Effort?

It is well known that compared with asexual reproduction by producing "clones", sexual reproduction embraces genetic diversity through recombination, which makes combinations of good genes across two or more sites and helps get rid of bad mutations. Therefore, sexual reproduction offers more evolutionary benefits by reducing the inheritance of harmful traits and accelerating environmental adaptation. But, why should scientists study the male-only or female-only reproduction in mammals at all?

"This is an important basic research," stated ZHOU Qi, director of IOZ and Member of CAS. "Exploring the unknown is the nature of science, and scientific research is the process of exploring the unknown to increase the understanding of humans themselves and the universe. Exploring the mystery of reproduction helps us understand how we have evolved until now, the pros and cons of sexual reproduction, and how to solve the coming challenges as we face in the future. This is the reason why we did the research," he explained.

From the perspective of application, this research will have a potentially huge impact on human health research, especially in preventing potential genetic diseases beforehand. According to ZHOU, previous studies have found that these imprinted genes have a high correlation with some genetic diseases. Dr. WANG Leyun, a postdoc researcher with the team explained: "The success of mammalian homosexual reproduction provides us with a new means of animal breeding that can be used to breed animals of pure bloodline with high scientific and medical value. The genetic alteration of different imprinted genes can be seen as a way of 'genetic repairing', which provides valuable early exploration for future clinical treatment of these diseases."

So far, the molecular mechanisms behind the genomic imprinting are still elusive, said the researchers, and many open issues remain to be explored.

Further Explorations Needed

One may ask if this research gives hope for the human homosexual couples who dream of having a baby of their own biological origin. However, the authors are not intended to apply this technique in humans, considering the potential risks. As a matter of fact, they explained, the CRISPR-Cas9-mediated genetic alteration of imprinted genes is intrinsically irreversible; and studies have demonstrated that the altered genes would inevitably cause developmental abnormalities or other unknown defects in some of the offspring. Moreover, these irreversible mutations could be inherited to later generations, if applied to human beings. All this amounts to a big concern.

Possible future development in the precise manipulation of DNA methylation, however, might help ease this concern. DNA methylation, methyl tags on DNA that function as chemical switch to control gene expression, could be turned on/off reversibly, hence their accurate manipulations might circumvent the permanent changes and potential harms on the genomes. At that point, an accurate, reversible artificial regulation of epigenetic switch between maternal or paternal imprinting might be available. By that time, however, humanity will need to reach an agreement on the long-debated ethnic issue: Should we really alter our genes out of reproductive purpose?

Terminology for Fun

Epigenetic: it means that certain heritable phenotype changes that do not involve alterations in the DNA sequence. The Greek prefix epi- implies features that are "on top of" or "in addition to" the traditionally recognized genetic basis for inheritance written in the DNA sequence. The study on such topic is called epigenetics. The following is something that may put these terminologies into perspective:

Think of the human lifespan as a very long movie. The cells would be the actors and actresses, essential

units that make up the movie. DNA, in turn, would be the script – instructions for all the players of the movie to perform their roles. Subsequently, the DNA sequence would be the words on the script, and certain blocks of these words that instruct key actions or events to take place would be the genes. The concept of genetics would be like screenwriting. Follow the analogy so far? Good. The concept of epigenetics, then, would be like directing. The script can be the same, but the director can choose to eliminate certain scenes or dialogues, altering the movie for better or worse. After all, Steven Spielberg's finished product would be drastically different than Woody Allen's for the same movie script, wouldn't it?

References

Z.-K. Li *et al*. Generation of uniparental mice from hypomethylated haploid ESCs with imprinting region deletions. *Cell Stem Cell*. Published online October 11, 2018. doi: 10.1016/j.stem.2018.09.004.

Lila Abassi, Epigenetics: Lamarck's Revenge? *American Council on Science and Health*, Published online June 10, 2016. https://www.acsh.org/news/2016/06/10/epigenetics-lamarcks-revenge.