DERLEME REVIEW

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We are Able to Edit the Genome of Human Embryos with CRISPR/Cas9 Technology, But are We Allowed to Do so?

İnsan Embriyosunun Genomunu CRISPR/Cas9 Tekniği ile Değiştirebiliriz; Ancak Bunu Yapmaya İznimiz Var mı?

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ABSTRACT In 2018, a Chinese scientist claimed the birth of the two girls whose C-C chemokine receptor type 5 (CCR5) gene(s) were deleted by CRISPR/Cas9 [clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas)] technology. The reason for this experimental procedure was the father being infected with human immunodeficiency virus (HIV). The national and international authorities heavily criticized the scientist in terms of scientific pitfalls of the technique used and ethics and showed condemnation against him. Two of the greatly criticized issues were that the implanting the genetically edited embryos to a woman with, and fabrication of the Institutional Review Board approval. Eventually, the Chinese Government sentenced him to three-year prison and fine. In this review, we aim to present the case, introduce the history of in vitro fertilization, its current implications, history of HIV, advocate reproductive rights of the individuals infected with HIV, and present CRISPR/Cas9 technique and its pitfalls. Finally, we critically evaluate the case based on the international and Turkish National Ethical Codes, including the Declaration of Helsinki, Nuremberg Code, and the Convention on Human Rights and Biomedicine. It is certain that we need stricter institutional, national, and international guidelines for gene editing of the human embryos. We also made several suggestions for updates in the curriculums of medicine and biological sciences regarding the CRISPR/Cas9 use in human subjects. We conclude that although it was an unethical human experiment conducted with inadequate scientific grounds, the CRISPR/Cas9 is a very promising technique for the cure of many human diseases. Therefore, more animal experiments with CRISPR/Cas9 should be encouraged and funded to achieve our goal of being healthier humans.

Keywords: Bioethics; CRISPR/Cas9; human genome; HIV; IVF; embryo; gene editing

ÖZET 2018 yılında Çinli bir bilim insanı, C-C kemokin reseptörü tip 5 (CCR5) genini, CRISPR/Cas9 [düzenli aralıklarla bölünmüş kısa palindromik tekrar kümeleri (CRISPR)/CRISPR ilişkili nükleaz 9 (Cas9)] teknolojisi ile sildiğini ve de bunun sonucunda ikiz kız bebek doğumu gerçekleştiğini iddia etti. Bu deneysel çalışmanın nedeni olarak ileri sürülen babanın insan bağışıklık yetmezliği virüsü [human immunodeficiency virus (HIV)] pozitif olduğu, ulusal ve uluslararası otoriteler tarafından yoğun olarak tartışılmış ve etik açıdan eleştirilmiştir. En fazla eleştirilen konulardan ikisi ise genetiği değiştirilmiş embriyoların bir kadına implante edilmesi ve de etik kurul onayının uydurma oluşudur. Çin Hükümeti'nin bu çalışmadaki araştırmacıyı, 3 yıl hapis ve aynı zamanda ağır bir tazminat ile cezalandırmasının ardından konu ile ilgili tartışmalar farklı bir boyut kazandı. Bu derlemede; olguyu sunmayı, in vitro fertilizasyon tarihini, onun mevcut sonuçlarını, HIV tarihini, HIV ile enfekte kişilerin üreme haklarını savunmayı ve CRISPR/Cas9 tekniğini ve tehlikelerini sunmayı amaçladık. Son olarak ise bu vakayı Helsinki Deklarasyonu, Nürnberg Kodu ve İnsan Hakları ve Biyotıp Sözleşmesi dâhil olmak üzere uluslararası ve Türk Ulusal Etik Kurallarına göre eleştirel olarak değerlendiriyoruz. İnsan embriyolarının gen düzenlemesi hususunda daha sıkı kurumsal, ulusal ve uluslararası yönergelere ihtiyacımız olduğu kesindir. Ayrıca tıbbi ve biyolojik bilimlerin müfredatlarında, insan araştırmalarında CRISPR/Cas9 kullanımına dair güncellemeler için çeşitli önerilerde bulunduk. Bilimsel dayanağı yetersiz olan ve etik olmayan bir insan deneyi olmasına rağmen CRISPR/Cas9'un birçok insan hastalığının tedavisi için çok umut verici bir teknik olduğu sonucuna vardık. Bu nedenle, sağlıklı insanlar olma hedefimize ulaşmak için CRISPR/ Cas9 tekniği ile daha fazla hayvan deneyi teşvik edilmeli ve finanse edilmelidir.

Anahtar Kelimeler: Biyoetik; CRISPR/Cas9; insan genomu; HIV; IVF; embriyo; gen değiştirme

The first genetically edited humans were claimed to be born in China almost two years ago. The case has raised both ethical and scientific concerns and critics by hundreds of scientists worldwide.[1] The scientist stated that he deleted C-C chemokine receptor type 5 (*CCR5*) because the father had human immunodeficiency virus (HIV) infection but not the mother. He used a recent technique called clustered regularly interspaced short palindromic repeats (CRISPRs)/CRISPR-associated pro-

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tein nine (CRISPR/Cas9). Among all the critics, the two most problematic parts were for the first time implanting the genetically edited embryos to a woman with, and fabrication of the The Institutional Review Board (IRB) approval. The person who led this experimental procedure had sentenced to three-year prison and fine by the Chinese government.

In this review, we aim to present the case, introduce a brief history of in vitro fertilization (IVF), its current implications, history of HIV pandemic, advocate reproductive rights of the individuals infected with HIV, and present CRISPR/Cas9 technique and its pitfalls. After scientifically evaluating the techniques used for this case, we critically reviewed the experiment based on the international and Turkish National Ethical Codes, including the Declaration of Helsinki, Nuremberg Code, and the Convention on Human Rights and Biomedicine.

What happened in China showed us that we need stricter institutional, national, and international guidelines for gene editing of the human embryos, especially for those that will be transferred to the human uterus. Education has a crucial role in ethical values. For this reason, we stressed necessity updates in the curriculums of medicine and biological sciences regarding the CRISPR/Cas9 use in human subjects. We conclude that although this case was accepted as an unethical human experiment conducted with inadequate scientific grounds, the CRISPR/Cas9 is a very promising technique that we can use to cure many human diseases. Therefore, we need to encourage and fund more animal experiments using the CRISPR/Cas9 technique to achieve our goal of being healthier humans.

THE CASE

He Jiankui, is a Chinese biophysicist who used to work as a faculty member at Southern University of Science and Technology in China. On November 25, 2018, before the Second International Summit on Human Genome Editing in Hong Kong, He published a video on YouTube where he claimed that twin girls were born after their being genome editing by using the CRISPR/Cas9 technology.[2] He also presented his study during the conference the day after. Interestingly, yet, he has not published his work involving this claim of the birth of the first gene-edited human babies in the world. Nevertheless, it is highly understandable that the journals such as Nature and JAMA rejected publishing his paper due to trying to report an evidently misconducted research that did not meet the bioethical standards to be published.[3] Hence, we do not have a clear description of the methods he used for these experiments. Considering that he involved in over ten companies in multiple roles, the conflict of interest has become even further important than usual for this experiment.[1]

Even though China's National Health Commission has initiated an investigation regarding his experiments, we still do not have a full-text article. Later, he also announced that the second pregnancy with a gene-edited embryo had been going on.[4]

Not surprisingly, it was found that he made a presentation on gene editing of human embryos during another conference in 2017.[1] Although the studies of gene editing of human embryos were known thus far, it was the first time the embryos were implanted into a woman and the birth of the genetically edited babies. That was the main focus of the critics.

He deleted *CCR5*, also known as CD195, the gene from two embryos. The father of the embryos was an individual infected with HIV. It is likely that, based on the theoretical information, He offered to delete the *CCR5* gene to enable having healthy offspring for the prospective parents for this experiment. He was able to delete both copies of the *CCR5* gene from one girl.[5] However, the other girl still has one copy of the *CCR5* gene.[5] At the same time, He also announced that the second pregnancy with gene-edited embryo had been going on.[4] He also stated that he checked the DNA of the newborn girls, where there were no off-target results.[1] Later, the scientists from the audience of the Second International Summit on Human Genome Editing raised concerns about his methods by showing them unwanted off-target effects.[6] Interestingly after all of these critics, another scientist from Russia,

Denis Rebrikov, announced that he is planning to do more babies with CRISPR-edited genes.[7]

Science Magazine published a copy of the informed consent in English that used for this experimental procedure on August 1, 2019.[8] That informed consent did not include any information regarding IRB approval.[8] Later, Nature updated one of its articles based on the fact that He's IRB approval was announced to be fake; in other words, he fabricated the IRB approval.[9]

In January 2019, the university fired He.[4] Due to "illegal medical practices," the Chinese judicial system decided to a three-year prison sentence for He.[10] A fine of three-million Chinese yuan ($429,000) was also imposed on him.[10]

IN VITRO FERTILIZATION

Dr. Patrick Steptoe and Dr. Robert G. Edwards, the pioneers of IVF, reported the first successful IVF baby in 1978.[11] They refused financial support from the government and performed their experiments through private funding.[12] Dr. Edwards was awarded The Nobel Prize in Physiology or Medicine in 2010 for the development of IVF.[13] Twelve years later, in 1990, preimplantation genetic testing (PGT) was led to healthy offspring.[14] There are several clinically used purposes of PGT, such as selecting the sex of the embryos and their eye color.[15]

Eleven years after the world's first IVF baby, Louise Brown, Turkey's IVF baby, was born on April 18, 1989.[16] In 2018, The European Society of Human Reproduction and Embryology stated that the number of babies born from IVF exceeded eight million in the world.[17]

Over the years, their successful experiment of creating human embryos in a petri dish and implementing them to uterus further shaped the future of reproductive medicine. We are currently able to preserve fertility by such as oocyte and sperm and ovarian tissue cryopreservation and perform preimplantation genetic diagnosis (PGD). Moreover, a three-parent baby was born in 2016, Mexico where the mitochondrial transfer was performed from a healthy oocyte to the maternal oocyte DNA and fertilized by paternal sperm DNA in order to create a healthy offspring from a mother who had multiple miscarriages and lost young children due to Leigh's Syndrome.[18] The mutations in mitochondrial DNA have been reported to be one of the causes of Leigh's Syndrome.

IVF has its own pitfalls both for the mother and offspring, such as more risk for placenta praevia, placental abruption, and morbidly adherent placenta, and increased risk for imprinting disorders such as Beckwith-Wiedemann syndrome.[19,20]

Before initiating any IVF protocol, The Society for Assisted Reproductive Technology recommends the obligatory tests for females HIV, hepatitis B antigen, hepatitis C antibody, rapid plasma regain (RPR), Pap smear, blood group, Rh, and antibody screen, and for males HIV, hepatitis B antigen, hepatitis C antibody, RPR, complex semen analysis, anti-sperm antibodies, and strict morphology.[21]

The indications of IVF include blocked fallopian tubes, fertility preservation before gonadotoxic cancer treatment (known as oncofertility), having a genetic disorder such as cystic fibrosis that could be checked by PGD, and sperm abnormalities such as low count, poor mobility, abnormal sperm size, and shape. The case presented in this review failed to provide one of these indications. An invasive protocol such as IVF should not be considered as an option for fertile people unless indicated.

HUMAN IMMUNODEFICIENCY VIRUS

A new pandemic, acquired immune deficiency syndrome (AIDS) caused by HIV infection, emerged in the twentieth century.[22] People infected with HIV have been stigmatized by society. This, unfortunately, includes the reproduction of the individuals infected with HIV. However, we, as physicians, always act based on the best interest of our patients without any sort of discrimination and support them with their decisions with our full capacity. To raise awareness about AIDS and memorialize people died of AIDS, many stamps have been published all over the world since 1986.[22] Today, AIDS was accepted as a chronic condition thanks to the successful treatments.

HIV infects human cells by different mechanisms. One of the most commonly accepted ways is

though the *CCR5*. *CCR5* is a protein expressed by macrophages and T cells. It is well-known that *CCR5* is an important receptor for several viruses, including human immunodeficiency virus (HIV), to enter human cells. Since *CCR5* is not the sole way of HIV entering human cells, the goal of creating HIV-resistant babies by deleting *CCR5* is scientifically not acceptable. *CCR5* is also present in various human organs, including brain, and its absence might cause serious side effects.[23] The impairment of human osteoclast function was shown due to *CCR5* blockage.[23] The known roles of *CCR5* in the brain are neuroprotection, prevention of host cell damage, and T cell recruitment for some cases of encephalitis.[23]

Since transmission routes for HIV include vaginal secretions and semen fluid, reproduction was not suggested for people with HIV infection. However, reproduction options for people with HIV infection have been discussed and researched over the years. The Ethics Committee of the American Society for Reproductive Medicine does not recommend timed unprotected intercourse for HIV-serodiscordant couples to achieve pregnancy.[24] To prevent HIV transmission to females and the expected babies for male-infected HIV-serodiscordant couples, the literature showed the sperm washing technique as the first option together with either intrauterine insemination (IUI) or IVF.[25] A meta-analysis including 4184 HIV-serodiscordant couples revealed that more than half of the couples had a clinical pregnancy by sperm washing technique in IUI or IVF.[26] The same study also reported that using sperm washing in more than ten thousand IUI/IVF did not cause HIV transmission to women.[26] If couples with HIV infection are fertile, the promising data for pre-exposure chemoprophylaxis could become an option and no need for IVF to achieve pregnancy.[25] This case where the father being HIV-positive could have been achieved pregnancy without HIV transmission by these methods successfully.

Thus, IVF is not the gold standard of achieving pregnancy for HIV-serodiscordant couples. Besides, not meeting any of the IVF indications, this case also put the fetuses and mother during the pregnancy into extra risks.

THE GENE EDITING TOOL: CRISPR/CAS9

CRISPR/Cas9 is a widely used molecular biology technique that enables us to insert or delete a gene of our interest to or from the human genome. CRISPR/Cas9 has several applications including genome editing, gene transcription regulation, and gene therapy.[27] Although it was discovered in 1993, it has been evolved over the years by contributions of nine countries.[28]

He deleted the *CCR5* gene from the embryos by CRISPR/Cas9. As the embryos divide zillions of times and become a fetus, there is a known risk that the deletions might not be transferred to the daughter cells due to the CRISPR/Cas9 instability.[1] This is called mosaicism. Nevertheless, it is not perfect due to off-target mutations, protospacer adjacent motif dependence, and guide RNA production.[27] The CRISPR/Cas9 technique is still evolving. By using different human cell lines, a modified CRISPR/Cas9 method showed less off-target gene editing compared to the conventional technique.[29] A recent mouse study showed that CRISPR/Cas9 could cause unwanted DNA insertions in gene-edited zygotes.[30]

CRISPR/Cas9 is a promising technique for treating various diseases, including genetic syndromes and cancer. We searched www.clinicaltrials.gov on April 11, 2020, for registered trials including CRISPR and found that currently there are 33 registries in the world where 17 recruiting, four active but not recruiting, two suspended, four withdrawn, two not yet recruiting, one unknown, one enrolling by invitation, one completed, and one terminated. Fifteen of these trials were solely taking place in China. No registry was found for the case presented, which is a clinical trial as well. Nevertheless, we need more animal studies before using this technique for human embryos' genome editing.

ETHICAL CONSIDERATIONS

Chinese government classified He's experiments as part of "high-risk biomedical technologies" and brought national approvals for such experiments in February 2019.[31]

The '*CCR5*-gene-edited twin babies' case reminds the unethical acts taken place that the doctors did not share the women who delivered the first IVF baby in England in 1978 when IVF was still considered experimental, and no live birth by IVF had been achieved yet.[32]

The four fundamental principles of biomedical ethics are autonomy, nonmaleficence, beneficence, and justice. The patient autonomy did not take place for this case due to the invalid informed consent. This case includes nonmaleficence due to knockdown of the *CCR5* gene. The beneficence is not apparent in this case based on the risks induced to the embryos due to a lack of *CCR5* gene. The knockdown of the *CCR5* gene was completely achieved on only one of the embryos, and the other still has the partial gene. This creates injustice. Overall, this case fails to fulfill any of the four fundamental principles of biomedical ethics. Additionally, the lack of proper IRB approval violated ethical values.

It was previously proposed that the medical doctor in his team should have not allowed him to conduct such an unnecessary and unethical experiment.[33] Since the peer-reviewed article has not been published, this critic stays valuable and required clarification.

Everybody has the right to reproduce. Reproduction rights are part of human rights. Ethically, it is the physicians' duty to assist their patients to have healthy offspring. This principle certainly includes people with HIV infection.

The Declaration of Helsinki is a set of ethical principles regarding human experimentation developed for the medical community by the World Medical Association.[34] It is widely regarded as the cornerstone document on human research ethics. It gives the most critical answer to the dilemma associated with research involving human subjects. Therefore, the declaration stresses the protection of the participants on the one hand and medicine's need for research, on the other hand. The goal of the Declaration of Helsinki was to prevent human subjects from being mistreated. The Declaration of Helsinki provided guidance for physicians who were conducting clinical research and focused on researchers' roles and responsibilities when it comes to protecting human subjects.

It is mandatory that the researchers, who participate in studies involving human subjects, tissues, or medical records, should be intimately familiar with the contents of the Declaration of Helsinki, as well as their local and national research standards and regulations. If any doubt or confusion arises, the local research ethics committees should be contacted for clarification and guidance. It is the responsibility of everyone involved in research to ensure that human subjects, their tissues, and their personal and medical information are protected and respected at all times, without exception. We must appreciate that it is their contributions, which has led to the present state of advanced medical science, the benefit of which we are enjoying today. This research made in China is totally out of the ethical principles of the Declaration of Helsinki; three basic ethical principles of equal importance, namely respect for persons, beneficence, and justice, permeate all other ethical principles. Principle 2: Research involving humans should be scientifically justified and described in a clear, detailed protocol. We do not see this principle fulfilled in this experiment.

Nuremberg Code was published in 1947 after Nazi doctors' unethical experiments on humans during World War II.[35] The Nuremberg Code 3 states that "The experiment should be so designed and based on the results of animal experimentation and a knowledge of the natural history of the disease or other problem under study that the anticipated results will justify the performance of the experiment."[35] Even before evaluating the experiments of human embryo gene editing, we should carefully look at the technique used for this purpose. We aforementioned that CRISPR/Cas9 has its pitfalls and needs more animal research. The experiment presented at this paper also has the unique challenge that once one edited a gene in the human embryo, it becomes irreversible. Moreover, since no scientific full-text paper was published about this case ant its preliminary animal data, it might be considered that this case lacks providing a reliable animal experiment data. Hence, to be able to conduct such an experiment in humans, we need more and more robust data from animal experiments,

including various species, and follow-up them during their lifespan to know short- and long-term complications.

In 2017, "Human Genome Editing Science, Ethics, and Governance" was published by National Academies of Sciences, Engineering, and Medicine in the USA.[36] Nuffield Council on Bioethics, a UK-based independent group, also published a report on a gene-editing "Genome editing and human reproduction: social and ethical issues short guide" before the case presented.[37] These two documents stressed the significance of the human genome editing research before any implications used clinically.[15] The concept of 'designer baby' arose and also challenged the social norms.[15]

The United Nations Educational, Scientific, and Cultural Organization was published Universal Declaration on the Human Genome and Human Rights in 1997.[38] Research with human embryonic stem cells has been highly regulated by legal rules. One of the highly regarded ones is the Convention on Human Rights and Biomedicine within the Council of Europe signed by Turkey but not China.[39] Article 13 on interventions on the human genome stated that "An intervention seeking to modify the human genome may only be undertaken for preventive, diagnostic or therapeutic purposes and only if its aim is not to introduce any modification in the genome of any descendants."

Genetic manipulations of embryos have different levels. For instance, the embryo's sex and eye color selections can be performed in some countries. However, there are still subject to debate in many countries and societies. This has led to so-called IVF tourism from the banned countries to the allowed countries. Both procedures are not allowed to take place in Turkey.

EDUCATION IN MEDICAL SCHOOLS AND BIOLOGICAL SCIENCES

Ethical lectures about the research involving human subjects have been taught in medical curriculums for many years, including the Turkish National Core Curriculum. Here, the students learn the fundamentals of bioethics and the codes for human research. One of the crucial elements of scientific studies involving humans is the approval of IRB. This is also true for animal experiments. It seems that the CRISPR/Cas9 technique will be used more and more for human research. Thus, the ethical and scientific considerations while using CRISPR/Cas9 for the human subjects should be implemented in the medical curriculum.

The curriculum for biological sciences such as biophysics, biomedical, and biochemical sciences must include the basics of ethical conduct for the research involving humans. This should be applied to both undergraduate and postgraduate education.

CONCLUSION

The Declaration of Helsinki, Nuremberg Code, and the four fundamental principles of biomedical ethics have been taught in medical schools for many years as part of the medical curriculum. However, it is not very well known that whether they are part of the curriculum for biological sciences. We highly recommend that the biological sciences shall include the basic ethical principles to their curriculums not to allow their graduates to conduct experiments, including humans, without following the ethical rules.

Although there are several ethical codes about human genome editing, there is still an urgent need for specific legal regulations in international, national, and institutional levels and law enforcement to control human germline gene editing based on the currently available techniques such as CRISPR/Cas9. However, the prospective regulations should not slow down the research on animals that we need to excel in human research to find cures and treatments for many diseases. Declaration of Helsinki has been fully adapted in Turkey, where also based on the Convention on Human Rights and Biomedicine strictly forbids the human genome editing.

We still need the full text of this experiment, and also the report prepared, preferably by the independent organizations.[1] The principles of research must be followed for any novel experiments in both animals and humans. If the animal experiments met the required criteria, human clinical trials by following Phase 1, 2, and 3 should be conducted. However, if the research involves germline gene editing, the ani-

mal experiments should cover the lifespan of the animals before even considering any human experiments. If a germline gene editing animal experiment passes the safety rules and many other related regulations at national and international levels, the gene-edited human embryos must be followed regularly during their entire lives.

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Authorship Contributions

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