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CRISPR-CAS9, BIOSAFETY AND BIOETHICS: A JUSPHILOSOPHICAL AND ENVIRONMENTAL ANALYSIS OF GENETIC ENGINEERING

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ABSTRACT

The new genetic engineering technique CRISPR-Cas9 projects benefits and risks of genetically manipulating and altering living organisms in order to bring about characteristics that are favorable to themselves and to humans. With an interdisciplinary method, involving Philosophy, Law, Biosafety and Bioethics, this paper aims to verify the consequences that the use of this technique can bring to the genetic nature of organisms, especially from the ethical and legal points of view. As a legal and biosafety reference, we opted for Brazilian Law n. 11.105/2005 and for philosophical and bioethical reference, we approach the controversy between the German thinkers Jürgen Habermas and Peter Sloterdijk, who analyzed the subject of genetic engineering and the risk of eugenics. It is a theoretical-bibliographic research, which uses deductive reasoning on the legal-philosophical impacts of the CRISPR-Cas9 technique. The practice of genetic engineering, despite the risks, may be an inevitable procedure in the present stage of human development and confronting it with an understanding of legal and bioethical responsibilities becomes essential.

Keywords: CRISPR-Cas9; genetic manipulation; genetic engineering; biosafety; bioethics.

CRISPR-CAS9, BIOSSEGURANÇA E BIOÉTICA: UMA ANÁLISE JUSFILOSÓFICA-AMBIENTAL DA ENGENHARIA GENÉTICA

RESUMO

A nova técnica de engenharia genética CRISPR-Cas9 projeta benefícios e riscos de se manipular e alterar geneticamente organismos vivos, de forma a trazer características favoráveis a eles mesmos e aos seres humanos. Com abordagem interdisciplinar, envolvendo a Filosofia, o Direito, a Biossegurança e a Bioética, o artigo objetiva verificar quais as consequências que o uso da referida técnica pode trazer à natureza genética dos organismos, sobretudo dos pontos de vista ético e jurídico. Como referência jurídica e de Biossegurança, optou-se pela Lei brasileira n. 11.105/2005 e, como referência filosófica e bioética, abordou-se a controvérsia entre os pensadores alemães Jürgen Habermas e Peter Sloterdijk, que analisaram o tema da engenharia genética e do risco da eugenia. Trata-se de pesquisa teórico-bibliográfica, que emprega o raciocínio dedutivo sobre os impactos jurídicos-filosóficos do uso da técnica do CRISPR-Cas9. A prática da engenharia genética, apesar dos riscos, pode ser um procedimento inevitável diante do atual estágio de desenvolvimento humano e enfrentá-la com a compreensão das responsabilidades jurídica e bioética torna-se essencial.

Palavras-chave: *CRISPR-Cas9; manipulação genética; engenharia genética; biossegurança; bioética.*

INTRODUCTION

Genetic manipulation has always been shrouded in bioethical and legal polemics. However, in recent years a new genetic engineering technique has promised to revolutionize the expensive genetic alteration processes.

The CRISPR-Cas9 technique allows the replacement of fragments of the DNA chain, correcting genetic "failures" or inserting beneficial characters into a given organism.

The possibility of having control over the genome and the genetic characteristics of the organisms raises the reflection on the risks of a nature projected for human interests and also the risks of eugenic practices when these changes are turned to the human genome.

The present research presents an interdisciplinary study, involving Philosophy, Law, Biosafety and Bioethics in order to verify how the CRISPR-Cas9 technique can affect what we know as the genetic nature of living organisms, facing the raised ethical and legal problems. The Biosafety juridic reference will be Brazilian Law n.11.105/2005, which addresses the issues of genetic engineering and manipulation, both in human cells and other living organisms.

To reflect from a philosophical and bioethical perspective, the article uses the controversy between the German thinkers Jürgen Habermas and Peter Sloterdijk, who analyzed the subject of genetic engineering and the risk of eugenics.

It is, therefore, a theoretical-bibliographic research, which employs the deductive reasoning of the legal-philosophical impacts on the use of the CRISPR-Cas9 technique.

To do so, it begins with an exposition of the CRISPR-Cas9 technique, in its biological and biotechnological aspects.

Next, it addresses the legal treatment of Biosafety in Brazil. After contextualizing Biosafety and its development, some provisions of Law n.11.105/2005 are analyzed, aiming to give an overview of its legal treatment, emphasizing the rules regarding genetic engineering and manipulation.

Finally, the CRISPR-Cas9 technique is confronted with gene therapy through adenovirus or retroviruses and an exposition is made regarding the risks of these techniques compared to Bioethics.

1 CRISPR-CAS9: AN ANALYSIS

CRISPR is an acronym for the English term *Clustered Regularly Interspaced Short Palindromic Repeats*. The first time a CRISPR sequence was identified was in a study on the *Escherichia coli* bacteria in 1987, although it was not yet understood under such name (ISHINO et al., 1987). Japanese researchers noticed in certain DNA sequences pieces of genes that did not belong naturally to the genome of *Escherichia coli*. In the early 2000s, the Spanish researcher Francisco Mojica (2000) identified CRISPR in other different species, such as archaea and other unicellular microorganisms.

The role of CRISPR will then be associated with a natural defense capacity of bacteria and archaea against viruses (MAKAROVA et al., 2006), which would justify that the genes which are not part of the genome of the studied organisms would be adaptations resulting from the viruses' attacks:

> [...] bacteria and archaea would have sophisticated immune systems. After all, viruses are the most abundant biological agents on the planet, causing roughly infections every second. The selective pressures imposed by viral predation have resulted in the evolution of numerous phage defense systems, but it was only recently that sophisticated adaptive defense systems were identified in both bacteria and archaea (ERP et al., 2015, p. 85).

Such a hypothesis will be proven in 2007 by scientists of American, French and Canadian origin who worked for Danisco, a Danish food company. Researchers have started from the notion that many bacteria are used for fermentation and biotechnological processes of food, but that such bacteria are attacked by phages, which are often not neutralized by the usual processes (BARRANGOU et al., 2007).

The researchers studied a milk-fermenting bacteria for the production of foods such as yogurt and cheese called *Streptococcus thermophilus*. They did an experiment with bacteria and two bacteriophages that had already been isolated by the yogurt industry. Nine more phages were generated from the experiment between the previously bacteria and two phages. Subsequently, the bacterium was immune to the new phages and the authors questioned the reason. When comparing the DNAs of the bacteria and the new phages, the authors stated that the resistance of

Streptococcus thermophilus was due to the fact that CRISPR in the DNA of the bacteria had adapted to the DNA of the phages: "These results reveal that, on becoming resistant to bacteriophages, the CRISPR locus was modified by the integration of novel spacers, apparently derived from phage DNA." (BARRANGOU et al., 2007, p. 1710).

In other words:

> [...] CRISPR functioned like a molecular vaccination card: by storing memories of past phage infections in the form of spacer DNA sequences buried within the repeat-spacer arrays, bacteria could use this information to recognize and destroy those same invading phages during future infections (DOUDNA, STERNBERG, 2017, p. 56).

The bacteria, therefore, would have an ability to remember the viruses that have already infected it, from the DNA of those viruses, which would be incorporated into the CRISPRs of the bacteria. When the bacteria were attacked again by the viruses, they were resistant.

Studies on bacteria would be just the beginning of the CRISPR analysis. From then on, a number of articles were devoted to it, although not understanding how the whole procedure involved occurred. It was already known, however, that the process for bacterial resistance depended on the actuation of RNA guiding molecules. One study found that RNA would be responsible for coordinating the recognition and destruction of viral infections, and that this would involve the CRISPR defense system. RNA molecules were produced by cells, through CRISPR, to fight the DNA sequences of the invading virus (BROUNS et al., 2008). In short,

> CRISPR loci are transcribed, and the long primary transcript is processed into a library of short CRISPR-derived RNAs (crRNAs) that each contain a sequence complementary to a previously encountered invading nucleic acid. Each crRNAs is packaged into a large surveillance complex that patrols the intracellular environment and mediates the detection and destruction of foreign nucleic acid targets (WIEDENHEFT; STERNBERG; DOUDNA, 2012, p. 331).

In addition to CRISPR and the participation of RNA in its performance, the attention of researchers should also focus on *Cas* genes, which is present in the region of the genomes of the bacteria and which contains special types of proteins called enzymes, which function as

catalysts of molecular reactions in cells. Thus, by understanding the role of *Cas* protein in this process, they could understand how CRISPR actually works (DOUDNA, STERNBERG, 2017, p. 62).

Blake Wiedenheft, a researcher working with Jennifer Doudna, one of those who came to discover CRISPR's ability to "cut" any kind of gene, was able to separate numerous *Cas* proteins in one experiment (DOUDNA, STERNBERG, 2017, p. 63-64). In possession of these Cas proteins, Wiedenheft et al. (2009) found a protein enzyme Cas1, with the ability to cut DNA, which would suggest that the protein had a role along the process of constitution of the defense system and adaptation of organisms' DNAs to viruses' attacks. Other *Cas* proteins were being manipulated and discovered. Finally, it can be elucidated that bacterial defense systems had several types of *Cas* proteins, which had the function of "searching" and "cutting" ("cleaving") the viral DNAs, preventing their action. It was thus discovered the operation of the defense system that involves CRISPR. Initially, the CRISPR RNA (crRNA) molecule, which has ten or eleven different *Cas* proteins, acts in the conservation against the attacks of virus DNAs by localizing them. Next, the *Cas* proteins enzymes act by "cutting" the target DNA (DOUDNA, STERNBERG, 2017, p.63; 66). Such a process inactivates the virus genes and prevents them from acting.

Among the *Cas* proteins studied, the one that caused the greatest impact was *Cas9* (which will be known as part of the CRISPR type II system[1]). Jennifer Doudna and Emmanuelle Charpentier led a team that discovered the unique role of *Cas9*:

> They had independently been teasing out the roles of various CRISPR-associated proteins to learn how bacteria deploy the DNA spacers in their immune defenses. But the duo soon joined forces to focus on a CRISPR system that relies on a protein called Cas9, as it was simpler than other CRISPR systems.
>
> When CRISPR goes into action in response to an invading phage, bactéria transcribe

1 On a technical explanation regarding the types of CRISPR/Cas systems: "There are three types of CRISPR/Cas systems (21–23)The type I and III systems share some overarching features: specialized Cas endonucleases process the pre-crRNAs, and once mature, each crRNA assembles into a large multi-Cas protein complex capable of recognizing and cleaving nucleic acids complementary to the crRNA. In contrast, type II systems process pre-crRNAs by a different mechanism in which a trans-activating crRNA (tracrRNA) complementary to the repeat sequences in pre-crRNA triggers processing by the double-stranded (ds) RNA-specific ribonuclease RNase III in the presence of the Cas9 (formerly Csn1) protein (fig. S1) (4, 24). Cas9 is thought to be the sole protein responsible for crRNA-guided silencing of foreign DNA (25–27)." (JINEK et al., 2012, p. 16).

> the spacers and the palindromic DNA into a long RNA molecule that the cell then cuts into short spacer-derived RNAs called crRNAs. An additional stretch of RNA, called tracrRNA, works with Cas9 to produce the crRNA [...] (PENNISI, 2013, p. 834).

The discoveries so far have occurred on the natural level. The big question that emerged then is whether the researchers themselves could take advantage of *Cas9* to manipulate and manually cut out other DNA sequences: "What we wanted to do next was confirm that we could engineer Cas9 and the RNA molecules to target and cut any DNA sequence of our choice." (DOUDNA, STERNBERG, 2017, p.81).

Illustrations on the CRISPR-CAS9 method: Left - DNA cut; Right - DNA Manipulation. Credits: Renato Serra

Jennifer Doudna, Emmanuelle Charpentier and the team decided to do an experiment to prove the hypotheses raised regarding CRISPR-Cas9. They decided to use genes from a jellyfish for the experiment. Researcher Martin Jinek made the process manually of CRISPR and *Cas9*. He chose five different gene sequences and "chimerically" prepared five RNA molecules to combine them. He then incubated the RNA with *Cas9* and *the jellyfish* DNA and waited for the result. He checked, then, that jellyfish DNA was cut off. The RNA molecules had acted in the exact place where the researcher had selected for the "cut", occurring through *Cas9*. A new technology capable of editing any genome in any organism was then validated and constructed! (DOUDNA; STERNBERG, 2017, p. 82-83). Without properly describing the reported research, the news regarding the CRISPR/Cas9 manipulation was mentioned in an article of the same group of scientists, published in August 2012:

> Our study further demonstrates that the Cas9 endonuclease family can be programmed with single RNA molecules to cleave specific DNA sites, thereby raising the exciting possibility of developing a simple and versatile RNA-directed system to generate dsDNA breaks for genome targeting and editing (JINEK et al., 2012, p. 816).

Such a study would trigger a series of research involving CRISPR and its potentialities. Several articles would study the genetic editing of different cell types. In addition to the dairy industry, which already benefited from the incipient applications of CRISPR, several other areas would benefit from the technique, such as agribusiness, other food areas, biotechnology and the medical field[2]. The reason for the progress of research with CRISPR involved the ability of genetic manipulation, but also the ease of manipulation and the low cost: "But the real reason that CRISPR exploded onto the biotech scene with such force and vitality was its low cost and ease of use. CRISPR finally made gene editing available to all scientists." (DOUDNA; STERNBERG, 2017, p. 111). Another factor that has contributed to the CRISPR revolution is the advancement of computational technology:

> Computers have also made gene editing easier than ever before. Using advanced algorithms that incorporate all the relevant design principles, including empirical data from the scientific literature on what kinds of targeting sequences work better than others, various software packages offer researchers an automated, one-step method to build the best version of CRISPR to edit a given gene (DOUDNA; STERNBERG, 2017, p. 112).

Progress has continued. In May 2013, Wang et al. (2013) conducted an experiment with CRISPR that would also open up many possibilities for what is perhaps one of the great dilemmas the method would have to face: the manipulation of germ cells and embryos. In addition to the first embryonic cell edition, the research was able to do several manipulations simultaneously. And although the employed technique manipulated an embryo, it opened up the possibility that CRISPR could be used on egg cells and sperm, which would allow genetic transmission for later generations: "[...] it seemed that CRISPR could be injected into any species' germ cells (eggs and sperm) or embryos, and the resulting genetic changes would be faithfully copied into all the cells and forever

2 For information regarding the entry of the CRISPR technique in the market Cf. ERP et al., 2015.

transmitted to future offspring" (DOUDNA; STERNBERG, 2017, p. 98).

Even before the CRISPR technique was officially used in the edition of human embryos in the United States, several scientists signed a manifesto called *Don't edit the human germ line*, in which they pleaded to their peers not to develop such research before a serious debate on ethics was raised. The text draws attention to the fact that until that moment, in March 2015, several researches that used the CRISPR technique were already being done with other animals, which would be a step towards research on human germ cells (ovum and sperm): "Studies using gene-editing in animals such as rats, cattle, sheep and pigs, indicate that it is possible to delete or disable genes in an embryo — a simpler process than actually correcting DNA sequences — in only some of the cells" (LAMPHIER et al., 2015, p. 411). Manipulations with human germ cells had already been done with other techniques, but with CRISPR, the changes could be passed on to later generations. Due to possible risks, many countries that have the technical capacity to make genetic manipulations in germ cells refer to legal norms to prohibit the changes:

> Many countries do not have explicit legislation in place permitting or forbidding genetic engineering in humans — considering such research experimental and not therapeutic (see go.nature.com/uvthmu). However, in nations with policies regarding inheritable genetic modification, it has been prohibited by law or by measures having the force of law. This consensus is most visible in western Europe, where 15 of 22 nations prohibit the modification of the germ line. Although the United States has not officially prohibited germline modification, the US National Institutes of Health's Recombinant DNA Advisory Committee explicitly states that it "will not at present entertain proposals for germ line alterations" (see go.nature.com/mgscb2) (LAMPHIER et al., 2015, p. 411).

The fact that a tougher legislation exists in Western European countries opens up a greater possibility of genetic manipulation at germ level with the CRISPR technique to occur in other countries, as will be seen later.

The Lamphier et al. (2015) manifest is explicit in stating that the greatest fear is regarding eugenic practice and possible harm to our own human lineage, so a public debate with specialists, scholars, and public opinion is essential to raise the discussion on whether and under what circumstances the germ-level manipulation technique in humans should

occur, which would not imply, however, in excluding all research involving genetic manipulation.

On October 28, 2016 the CRISPR-Cas9 technique was first tested on a human. A Chinese team, led by oncologist Lu You of Sichuan University in Chengdu, has modified cells using the CRISPR technique to fight lung cancer in a patient. The procedure consisted in the removal of immune cells from the patient's blood, which were manipulated with CRISPR-Cas9. Thus, a specific gene, which has the function of encoding the PD-1 protein, has been deactivated (cut). Such a protein eventually impairs the immune response of cells, causing the proliferation of cancers. The edited cells were cultured and their numbers increased. Subsequently, they were injected back into the patient. The team's hope is that edited cells without PD-1 will attack cancer (CIRANOSKI, 2016).

The treatment described above was against cancer, however, it should be emphasized that the CRISPR-Cas9 method has been applied to the study of several diseases, by the fact that it can revert mutations or exchange a damaged gene for healthy ones, although there are diseases that, for the time being, are not potentially treated by CRISPR-Cas9:

> Beyond cancer, HIV, and the genetic disorders discussed thus far, a quick survey of the published scientific literature reveals a growing list of diseases for which potential genetic cures have been developed with CRISPR: achondroplasia (dwarfism), chronic granulomatous disease, Alzheimer's disease, congenital hearing loss, amyotrophic lateral sclerosis (ALS), high cholesterol, diabetes, Tay-Sachs, skin disorders, fragile X syndrome, and even infertility. [...] There are all sorts of disorders — from autism to heart disease — that don't show significant genetic causation or are caused by a complex combination of genetic variants and environmental factors. In these cases, gene editing may be of more limited use (DOUDNA; STERNBERG, 2017, p. 181-182).

The gains from CRISPR-Cas9 and the real impact of his technique contributed to the US *National Academies of Sciences, Engineering, and Medicine* (2017) making a report called *Human Genome Editing: science, ethics, and governance*[3] in which they take a favorable position on genetic manipulation in embryos and germ cells, provided they follow some guidelines. The committee's conclusions can be summarized in the following principles and concluding observations:

3 The report contains 310 pages and brings many issues that could be analyzed in detail. It was decided, purposely, to emphasize certain aspects that were relevant for this *paper* .

> Genome editing holds great promise for preventing, ameliorating, or eliminating many human diseases and conditions. Along with this promise comes the need for ethically responsible research and clinical use.
> RECOMMENDATION 2-1. The following principles should undergird the oversight systems, the research on, and the clinical uses of human genome editing:
> 1. Promoting well-being
> 2. Transparency
> 3. Due care
> 4. Responsible science
> 5. Respect for persons
> 6. Fairness
> 7. Transnational cooperation (NATIONAL..., 2017, p. 182)

These principles, in turn, will result in responsibility for editing the human genome. The following explains each of the listed principles and their respective responsibilities linked.

1. Promotion of welfare: one should always seek the benefit (principle of beneficence) and prevention of harm (principle of non-maleficence) of those involved in the research ➔ The responsibilities are: a) to use the human genome edition for treatments or prevention of diseases and does not apply it in cases of great uncertainty; b) seek the benefits also bearing in mind the risks involved.

2. Transparency: Information should be given to stakeholders in a clear and comprehensible manner ➔ The responsibilities are: a) commitment to exposing the greatest amount of information in a quickly manner; b) submit information for the construction of public policies.

3. Due caution: care should be taken with those involved, acting only on the basis of strong evidence. ➔ The responsibility is: to act with caution and frequent reassessment of actions, also taking cultural opinions into account.

4. Responsible science: one should act based only on high standards of research, following the guidelines of international and professional norms.➔ The responsibilities are: a) to conduct high-level research; b) review and evaluate researches following the protocols; c) be transparent; d) correct misinformation.

5. Respect for persons: the dignity of all individuals should be acknowledged, respecting their particular decisions, and taking

all individuals with the same moral value, regardless of their genetic properties. ➔ The responsibilities are: a) to have the same commitment to all; b) respect for decisions; c) prevent eugenic practices, such as those already practiced; d) destigmatize deficiencies.

6. Equity: treat similar cases in the same way and practice distributive justice in relation to risks and benefits. The responsibilities are: a) to distribute research tasks and benefits; b) provide universal and equitable access to the benefits of the investigations.

7. Transnational cooperation: there must be international research collaboration, taking into account the different cultural contexts. Responsibilities are: a) to respect the different national policies; b) seek common standards; c) share data achieved.

The report probably encouraged embryo research to be conducted in the United States using the CRISPR-Cas9 technique. A few months later, this was confirmed by an investigation involving researchers of different nationalities and led by Shoukhrat Mitalipov, a researcher at *Oregon Health and Science University* in Portland. It was the first experiment with embryos in the United States, funded by private sectors, since the US government does not fund jobs involving human embryos (LEDFORD, 2017). The authors used the CRISPR-Cas9 technique to rectify a disease-generating mutation in embryos. The study consisted of working the mutation of a gene called MYBPC3, which generates hypertrophic cardiomyopathy, a disease that results in heart failure and is the most common cause of sudden death in young and healthy athletes. The rate of manipulated embryos that did not have the mutant gene was high (MA, 2017)[4]. The pioneering American ground-based research put the country into a silent dispute with China for embryonic research using the CRISPR-Cas9 technique.

In the middle of 2018, the CRISPR-Cas9 method received two heavy setbacks: on the one hand, two texts (HAAPANIEMI et al., 2018; IHRY et al., 2018) showed evidence that genetic editing with CRISPR-Cas9 favors the appearance of tumors, on the other, a study (KOSICKI; TOMBERG; BRADLEY, 2018) emphasized that CRISPR-Cas9 may cause more genetic destruction than the experts thought. Although they are not studies that definitely put the technique in check, they warn of possible collateral damage that should be further investigated.

In the first case, despite investigating different types of cells,

4 The results of the experiment, which would be due to the CRISPR-Cas9 technique, were later questioned by EGLI, Dieter et al. (2018). However, for the purposes of analysis, the most important is the manipulation of embryos in the United States on the experiment cited above.

human retinal pigment epithelial cells (HAAPANIEMI et al., 2018) and human pluripotent stem cells (hPSCs) (IHRY et al., 2018), researchers noticed that the DNA cut with CRISPR-Cas9 activates a gene called p53, which has the function of dealing with the damage caused by the cut: "Here, we report that genome editing by CRISPR-Cas9 induces a p53-mediated DNA damage response [...]" (HAAPANIEMI et al., 2018, p. 927). It is as if the body tried to readjust after the cut, often causing CRISPR not to have the expected effectiveness: "The toxic response to DSBs[5] was P53/TP53-dependent, such that the efficiency of precise genome engineering in hPSCs with a wild-type p53 gene was severely reduced" (IHRY et al., 2018, p. 939). That is, the effect of CRISPR would depend on the non-performance of the p53 gene. "These results suggest that p53 inhibition may improve the efficiency of genome editing of untransformed cells and that p53 function should be monitored when developing cell-based therapies utilizing CRISPR-Cas9" (HAAPANIEMI et al., 2018, p. 927). But the problem is precisely because, when p53 does not act (naturally or induced), the risk of cancer increases exponentially, which would in principle suggest a risk in the use of the technique: "P53 inhibition could alleviate toxicity but has the potential to increase off-target mutations and poses a risk for cancer." (IHRY et al., 2018, p. 945). But, also, a greater control in its application: "Controlling DNA damage signaling, such that efficient gene correction can occur but the formation and selection of potentially tumorigenic cells are suppressed, will be important in developing safer and more efficient next generation genome editing technologies." (HAAPANIEMI et al., 2018, p. 930).

The research conducted by Kosicki, Tomber and Bradley (2018), in turn, found that the use of CRISPR-Cas9 causes destructive effects at different sites where the DNA was cut, destroying other DNAs that were not involved in the process, which can cause serious pathogenic consequences, including the appearance of genes that cause cancers:

> In the clinical context of editing many billions of cells, the multitude of different mutations generated makes it likely that one or more edited cells in each protocol would be endowed with an important pathogenic lesion. Such lesions may constitute a first carcinogenic 'hit' in stem cells and progenitors, which have a long replicative lifespan and may become neoplastic with time (KOSICKI; TOMBERG; BRADLEY, 2018, p. 770).

In the view of the authors, there has been a negligence in the investigations involving the use of CRISPR-Cas9 in certain cases, since it

5 From the English "double-strand breaks" of DNA.

should be further studied:

> We speculate that current assessments may have missed a substantial proportion of potential genotypes generated by on-target Cas9 cutting and repair, some of which may have potential pathogenic consequences following somatic editing of large populations of mitotically active cells (KOSICKI; TOMBERG; BRADLEY, 2018, p. 765).

By the end of 2018, an experiment using the CRISPR-Cas9 technique would cause a riot in the scientific milieu: a Chinese researcher, He Jiankui, from Shenzhen, announced that he implanted embryos handled with the CRISPR-Cas9 technique, which resulted in the birth of two girls which would be the first genetically engineered human born. His research consisted of disabling a gene, called CCR5, which allows access to the HIV virus in a cell. His justification was to make organisms resistant to the disease, very common in China, and that the offspring did not have parental disease (he used parents with HIV and mothers without the virus). The method consisted of:

> The gene editing occurred during IVF, or lab dish fertilization. First, sperm was "washed" to separate it from semen, the fluid where HIV can lurk. A single sperm was placed into a single egg to create an embryo. Then the gene editing tool was added.
>
> When the embryos were 3 to 5 days old, a few cells were removed and checked for editing. Couples could choose whether to use edited or unedited embryos for pregnancy attempts. In all, 16 of 22 embryos were edited, and 11 embryos were used in six implant attempts before the twin pregnancy was achieved, He said.
>
> Tests suggest that one twin had both copies of the intended gene altered and the other twin had just one altered, with no evidence of harm to other genes, He said. People with one copy of the gene can still get HIV, although some very limited research suggests their health might decline more slowly once they do (MARCHIONE, 2018).

There were serious doubts of the scientific community that the research had occurred, since it did not appear in any scientific journal that could be analyzed by other researchers. There were also questions about how He Jiankui recruited the research participants, since he might not have been clear about the method used. Some scientists questioned the fact that the edition of the CCR5 gene could also make it possible for other

diseases to appear. It was also unclear whether the researcher did the right thing in the face of the competent bodies and institutions involved. There is also the questioning that there are people whose organism undergoes a natural mutation in the CCR5 gene, which makes them immune to HIV and that, therefore, the test made is implicitly justifiable by the pure and simple application of the technique (MARCHIONE, 2018). And the most fundamental question: Would He Jiankui have crossed a risky line, or would he have been the pioneer of something inevitable?

The supposed experiment generated a chain reaction. Several scientists have criticized the fact that it has been done without a consensus in the scientific world about the genetic editing on humans and its implantation. In China itself, the author's country of origin, where there is authorization for genetic editing, a group of 122 scientists wrote an open letter in which they call He Jiankui crazy and claim that such activity was a serious blow to the reputation and scientific development of China (KOLATA; WEE; BELLUCK, 2018).A few days later, the Chinese government banned He Jiankui from conducting further research. He was subsequently detained in an accommodation on the Southern University of Science and Technology, in Shenzhen, and was later fired from the same university, where he worked. The Chinese authorities, who after an investigation confirmed the researcher's achievements, are likely to take harsh measures against him and his staff, framing them on criminal charges (RAMZY; WEE, 2019).

2 BIOSECURITY AND BRAZILIAN LAW N. 11.105/2005

The incipient experiments involving the CRISPR-Cas9 technique raise safety and ethical issues in genetic manipulation procedures.

Certain risks are as yet unknown and Biosafety must act to prevent these internal (laboratory) and external (in the release of modified organisms) risks.

Biosafety is the set of techniques and procedures that works together with research on biological material, seeking the prevention, elimination or reduction of risks to human health and the environment, as well as maintaining the balance of ecosystems.

In its work with biotechnology, it can be said that Biosafety is more pragmatic than Bioethics because it aims to implement safety

procedures which should cover activities of investigation, teaching, production and distribution of inventions and biotechnological products, as well as development and the provision of services related to biotechnology.

Schramm (2010) states that Bioethics can be considered a new scope of Moral Philosophy. Its task would be to observe and discuss biotechnoscientific advances. Biosafety, in its turn, would be a new field of biotechnoscience, concerned with the safety of scientific procedures. "In sum, bioethics analyzes the morality of biotechnologies and biosafety calculates and weighs the inherent risks of biotechnology from the point of view of its safety." (SCHRAMM, 2010, p. 105).

The earliest landmark of contemporary biosafety is at meetings in Asilomar, California, where a series of meetings took place in 1975 involving leading scientists who discussed ethics in research. The most relevant issue at that time was the suggestion of a moratorium on genetic research, which occurred in the previous year by a group of scientists.

Through this meeting guidelines were established for the safety of experiments with recombinant DNA. Although the term "biosafety" was not used at the time, it was the Asilomar document that laid the foundations for biosafety.

Today, biosafety procedures are mainly concerned with:

- Identify the risks of activities involving the handling of biological material;
- Characterize the risks according to the probability of their effects and the extent of their possible consequences;
- Analyze acceptable levels of exposure to hazardous materials or materials with risks as yet unknown;
- Evaluate the probability of the negative effects of the activity;
- And in case of damage, evaluate it and propose measures of containment and repair.

In Brazil, the main regulatory instrument of Biosafety is Law n. 11.105 - Lei de Biossegurança (Biosafety Law) - sanctioned by the President of the Republic on March 24, 2005.

As a general matter, the Biosafety Law establishes safety standards and inspection mechanisms for construction, cultivation, production, handling, transportation, transfer, import, export, storage, research, marketing, consumption, release into the environment and disposal of genetically modified organisms, with guidelines to stimulate scientific advances in the area of biosafety and biotechnology, protection of human,

animal and plant life and health, and observance of the precautionary principle for the protection of the environment (BRASIL, 2005, art. 1).

The Biosafety Law addresses, as central themes, embryonic stem cell research and the research and release of genetically modified organisms. However, it also creates restrictions on genetic manipulation.

The Law processing was rough, with pressure from economic groups interested in the genetic modification of soy, hitherto deregulated. It was during the procedure that the President of the Republic signed Provisional Measure n. 223, dated October 14, 2004 - later converted into Law n.11.092, of January 12, 2005 -, legalizing the planting of transgenic soybean from the 2004-2005 harvest and the commercialization of the product until January 31, 2006.

After the promulgation of the Law, another stir has been established regarding the research with embryonic stem cells. On May 30, 2005, the then Attorney General Claudio Fonteles filed a petition questioning the constitutionality of Article 5 of the Biosafety Law, which allows the use of surplus human embryos of *in vitro* fertilization techniques in research and therapies.

The Attorney General was outraged at the normative treatment given to the cryopreserved human embryo, a surplus of *in vitro* fertilization. Its use in research and therapy necessarily implied - at least at the time of enactment of the Law and proposition of Direct Action of Unconstitutionality - in the destruction of the embryo.

Under the argument that "*human life* happens *in, and from, fertilization*" Article 5 of the Biosafety Law would offend Article 1, III, and the *caput* of Article 5 of the Federal Constitution. The member of the Attorney General's Office therefore considered human embryos as being constitutionally identical to the borne human being, seeking, therefore, assistance in the opinions of doctors, geneticists and biologists.

The ADI trial n. 3510-0 began in March 2008, when the Reporting Minister Carlos Ayres de Britto and the then President of the Federal Supreme Court, Minister Ellen Gracie, voiced on the constitutionality of Article 5. The section was suspended due to the request of the Minister Carlos Alberto Menezes Direito. Resuming the trial on May 28, 2008, Ministers Menezes Direito and Ricardo Lewandowski voted for partial submission of the request for unconstitutionality of article 5 of the Biosafety Law. Minister Carmen Lúcia Rocha and Minister Joaquim Barbosa deemed it unfounded. For its dismissal, Ministers Eros Grau and Cezar Peluso also

stated, with certain reservations, in the terms of their votes. The trial was adjourned and resumed the next day, May 29, 2008. Taking the votes of the other Ministers (Min. Celso Mello, Min. Marco Aurélio and Min. Gilmar Mendes) (BRASIL, 2008).

Finally, the Federal Supreme Court, by majority vote and in accordance with the vote of the Rapporteur, dismissed the claim in the Direct Action of Unconstitutionality n.3.510-0, partially overlapping, in different extensions, the Ministers Menezes Direito, Ricardo Lewandowski, Eros Grau, Cezar Peluso and Gilmar Mendes, being allowed the research with embryonic stem cells, especially from the differentiation of the legal treatment given to the unborn child of the one spent to the embryo not gestated and frozen as a surplus of techniques of assisted human reproduction (BRASIL, 2008).

Research and therapies with human embryos are allowed by the Biosafety Law only regarding stem cells, and any technique of genetic engineering is prohibited, from which it can be inferred that the CRISPR-Cas9 technique is also prohibited. The art. 24 criminalizes and punishes with imprisonment, from 1 to 3 years, and fine, the conduct of using human embryo in disagreement with the provisions of art. 5, that is to say, the use of embryos must comply with the following requirements: a) research and therapy should have stem cells as object; b) the parents must expressly consent to the use; c) prior approval of the research ethics committees of the research institutions and health services involved; and (d) is not intended for the marketing of biological material (BRASIL, 2005).

On the other hand, the embryo genetic alteration received its own classification, being a crime punishable by imprisonment, from 1 to 4 years, and a fine (BRASIL, 2005, art.25).

Genetic engineering in human germ cell is also prohibited, which has been included in the art. 25, with the same punishments related to the embryo.

The prohibition of genetic engineering in embryos and human germ cells has been inserted with the aim of avoiding possible eugenics, or even abusive and invasive practices.

Finally, the Biosafety Law prohibits human cloning, in any of its forms, be it reproductive or therapeutic (BRASIL, 2005).

Cloning is the process of asexual reproduction, artificially produced, based on a single genetic patrimony, with or without the use of genetic engineering techniques. If it is reproductive cloning, the ultimate

goal will be to obtain a new individual, genetically the same as the previous one. If the cloning is therapeutic, its objective will be the production of genetically identical cells that can be used in medical treatment.

Cloning is prohibited through article 26, which provides for punishment of 2 to 5 years of imprisonment and a fine for the one who performed it (BRASIL, 2005).

3 BIOETHICS AND GENE THERAPY

In relation to human health, the CRISPR-Cas9 technique opens the door to the improvement and expansion of gene therapy, which consists in the treatment of diseases, inherited or acquired, in which defective genes are manipulated in order to achieve cure or stagnation of the anomaly.

In theory, gene therapy can be performed on somatic cells and germ cells, although in the latter the risk is much greater, since the alteration of gametes can result in unexpected changes, such as malformations and diseases hitherto unknown. There is even the risk of generating recessive problems that may only manifest themselves in future generations.

Before the CRISPR-Cas9 technique, somatic therapy was performed by a vector, retrovirus or adenovirus, which inserted new genetic material into diseased cells. Viruses act as efficient vectors by having a genetic programming that leads them to transfer their genetic material to the infected organism.

Some retroviruses and adenoviruses have ample capacity of propagation of their genetic material without destruction of the cells of the invaded organism. In somatic therapy, part of the virus's genome is removed, maintaining its reproductive and transfer ability and inserting healthy genetic material to be transported. By infecting the patient's cells, the virus transfers the genetic material it is carrying to diseased cells of the body, modifying its structure (SÁ; NAVES, 2018).

The CRISPR-Cas9 technique does not use another organism, such as the retrovirus or the adenovirus. CRISPR is a DNA sequence that can be repeated several times, with unique sequences between the repeats, and allows to cut the DNA in specific places. Cas9 is the enzyme responsible for this cut. So, through an RNA guiding chain, one piece of the cut DNA is removed and replaced with another.

Apparently more effective, the CRISPR-Cas9 technique would be more accurate than virus therapy.

Watson and Berry (2005) report that the first successful gene therapy occurred in 1990 at the National Institutes of Health. The patients were two children suffering from adenosine deaminase deficiency (ADA), Ashanti DeSilva, aged four, and Cindy Cutshall, aged nine. ADA, which occurs by the absence of an enzyme, "deactivates" the immune system, leaving the patient vulnerable to any disease.

Immune system cells from both girls were harvested and cultured in the laboratory, and then infected with retroviruses containing the desired genetic material. The retrovirus DNA was transferred to the cells, which were reinserted into the patients. Several infusions were made for a few months. In parallel to gene therapy, the girls were submitted to enzyme replacement, as required by the National Institutes of Health.

Watson and Berry report the results:

> I can personally attest that Cutshall looked like a very healthy 11-year-old when she and her family visited Cold Spring Harbor in 1992.Eleven years later, however, the results were not as conclusive. The functioning of DeSilva's immune system is close to normal, but only about a quarter of its T cells came from gene therapy. Cutshall's blood has an even smaller proportion of T cells coming from the therapy, though her immune system is also working well. However, it is difficult to say exactly how much of this improvement is due to gene therapy and how much is a result of continuous enzyme treatment. The result, therefore, is too ambiguous to be interpreted as an unequivocal success in gene therapy (Watson, Berry, 2005: 377-378).

Some problems can be pointed out in this type of therapy, as the case of DeSilva and Cutshall shows. The cells undergoing treatment have a short life span, which means that often the healthy genetic material cannot reach the whole of the diseased cells. The difficulty in reaching only those cells needing the surrogate gene is also clear. In the case of DeSilva and Cutshall, the cells to be treated could be obtained easily because they were cells of the immune system.

Finally, somatic gene therapy has incalculable oncogenic potential. This risk can be inferred from a case that occurred in France in 2000.At Necker Hospital in Paris, under the leadership of Alain Fischer, two babies with ADA underwent therapy. The innovation was due to the use of stem cells from the bone marrow of the babies. Thus, when stem cells reproduced, they would automatically generate cells with healthy genes in a "self-regenerating genetic correction." (WATSON; BERRY,

2005, p. 380).

The results of the therapy were incredible in the early years, but in 2002, it was discovered that one of the babies had leukemia. Although the oncogenic risk is real, in the case of ADA the result obtained can still be considered advantageous, due to its characteristics and difficulties with treatments.

These risks presented by virus gene therapy, such as shorter cell lifetime and increased oncogenic potential, may also manifest in the CRISPR-Cas9 technique, as reported above.

Besides these risks, Habermas (2016) poses important ethical issues to be considered in the production of genetically programmed humans. There is, with these techniques, a change in the "ethical self-understanding of the species" that breaks with the existential notion of who we are and leads us to a possibility of constructed organic disposition. From something *given*, we become what we *give* ourselves as an organism.

Moreover, there is a great concern of the German philosopher with the self-understanding of the genetically edited person: "We cannot rule out the fact that knowledge of a eugenic programming of hereditary inheritance limits the autonomous configuration of the individual's life and undermines relations fundamentally symmetric between free and equal persons." (HABERMAS, 2016, p. 33)

Permission to interfere with the genome in search of contribution to the health of the person can be seen as beneficial, but the limit that guides what is good, preferable or bad is very tenuous. What is really therapeutic and what is just desirable?

> Genetic intervention does not open the communication space to address the planned child as a second person and to include it in a process of understanding. [...] The eugenic interventions of improvement undermine the ethical freedom as it subjects the person in question to intentions set by third parties, which the person rejects and are irreversible, preventing them from freely understanding themselves as the sole author of their own life. It may be that it is easier to identify with abilities and aptitudes than with dispositions or even qualities; but for the psychic resonance of the person in question, it only matters the intention that was linked to the purpose of the programming. Only if extreme and highly generalized evils are avoided, good reasons emerge to accept the fact that the affected individual would agree with the eugenic goal (HABERMAS, 2016, p. 86-88).

Habermas's position in *O futuro da natureza humana* (The Future of Human Nature) was a response to a writing by the German philosopher

Peter Sloterdijk[6], which would become the work *Regras para o parque humano: uma resposta à carta de Heidegger sobre o Humanismo* (Rules for the Human Zoo: A Response to the Letter on Humanism). Without referring properly to the quarrel between the two thinkers, it is important to emphasize Sloterdijk's position on the techno-scientific advance.

For Sloterdijk, the West was marked by humanism, a certain formation that would have the capacity to contain human destructive instincts: "The latent message of humanism, then, is the taming of men. And its hidden thesis is: reading the right books calms the inner beast" (SLOTERDIJK, 2009, p. 15). In this sense, there is a belief in humanism that humans are influential and that it is fundamental to offer a certain kind of control. The bestializing and domesticating tendencies would be in constant strife in the human being: "The label of humanism reminds us (with apparent innocuousness) of the constant battle for humanity that reveals itself as a contest between bestializing and taming tendencies." (SLOTERDIJK, 2009, p. 15).

In interpreting Heidegger's *Letter on Humanism* , written after World War II, in 1946, Sloterdijk, based on the Heidegger's critique that humanism (and its derivations, Christianity, Marxism and existentialism) is embedded in the metaphysical tradition of the forgetfulness of being, perceives the following questioning formulated by Heidegger, which calls humanism into question: "Why should humanism and its general philosophical self-representation be seen as the solution for humanity, when the catastrophe of the present clearly shows that it is man himself, along with his systems of metaphysical self-improvement and self-clarification, that is the problem?" (SLOTERDIJK, 2009, p. 17). After two great wars, made by an educated and humanist Europe, nothing more natural than to question the formation that had been based on humanism and its millennial conception that the human being is a *rationale animal*. What interests Sloterdijk in his reading of Heidegger is the fact that humanism, as a human domestication, has been criticized. What to put, then, in place?

> What can tame man, when the role of humanism as the school for humanity has collapsed? What can tame men, when their previous attempts at self-taming have

6 The Habermas-Sloterdijk debate originated when some journalists published decontextualized sections of a speech by Sloterdijk (originally presented on June 15, 1997 in the city of Basel, at an event on humanism and then resumed in June 1999 at a symposium on Heidegger and Levinas in Elmau and that will give rise to the text *Regras para o parque humano*), implying that the author was in favor of eugenic practices.Habermas's reaction, which will initially take place in the press in a violent manner, will result in the work *O futuro da natureza humana*. In our view, Habermas never really understood Sloterdijk's text.

> led primarily to power struggles? What can tame men, when after all previous experiments to grow the species up, it remains unclear what it is to be a grown-up? Or is it simply no longer possible to pose the question of the constraint and formation of mankind by theories of civilizing and upbringing? (SLOTERDIJK, 2009, p. 20).

Following his analysis, Sloterdijk (2009) retakes Nietzsche to deduce, from this, that the human being is understood as having a domesticating force and a creative force. Socialization ended up producing men who are domesticated, but, through their creative force, man will create the superman. And it is from this creative force that Sloterdijk, despite making exceptions to Nietzsche's thought, captures the fundamental question of our age: the ability, through technique, to literally create "new human beings."

> But the discourse about difference and the control of taming and breeding – indeed, just the suggestion about the decline of awareness of how human beings are produced, and indeed of anthropotechnology – these are prospects from which we may not, in the present day, avert our eyes, lest they once again be presented as harmless (SLOTERDIJK, 2009, p. 23).

Sloterdijk, walking on dangerous terrain, which prompts a quarrel initiated perhaps by a hasty reading of Habermas, states that the history of culture is a history of selection, from literate and illiterate, and that there are two types of humans, those who created and those who are created, being that the era of the technique perpetuates such division, going towards a problematization on the biological level. Humanity will have to discuss, as the CRISPR-Cas9 technique demonstrated, the technical advances and its capacity to manipulate nature in general and human nature. Where humanism has failed to contain destructive impulses, there will be technique. And as Sloterdijk says, humanity cannot escape the questions about its own self-determination:

> But whether this process will also eventuate in a genetic reform of the characteristics of the species; whether the present anthropotechnology portends an explicit future determination of traits; whether human beings as a species can transform birth-fatalities into optimal births and prenatal selection – these are questions with which, however vague and creepy they may be, the evoluntionary horizon begins to glimmer (SLOTERDIJK, 2009, p. 24).

Finally, Sloterdijk (2009) turns to Plato and his work *O Político*, in which he finds the notion, in the early days of Western culture, that the art of politics is the art of shepherding the city. This is why all Western thought has focused on the insidious task of thinking of the human community as a zoological park, since the human being is also an animal willing voluntarily to be cared for by others, the experts, those who know how to unite the best human qualities, an idea that is more than open in this biotechnological era and that will inevitably have the CRISPR-Cas9 technique as one of the exponents.

Thus, the Bioethics space in this discussion should be expanded to allow us to understand that any decision regarding the CRISPR-Cas9 technique will imply a series of responsibilities with present and future generations, and it must not escape its interference in nature and it's consequences.

Global Bioethics is invoked as an important agent of reflection in such a thorny subject, since Genetics demands a Bioethics that projects itself "into the future leads even to a 'subject' who does not exist, does not claim and does not have his rights harmed: future generations. Moreover, it also addresses other forms of life, since ethics becomes a part of the philosophy of nature." (REIS; NAVES; RIBEIRO, 2018, p. 84)

CONCLUSION

It cannot be said with absolute certainty that the CRISPR-Cas9 technique will consolidate itself in the scientific circle. Serious science is made gradually by means of tests, counter-tests, experiments and discussions. There is, therefore, a long way to go. However, there is no denying the potential of the technique.

CRISPR-Cas9 has the capacity to enter many practical areas ranging from food production, animal manipulation, pharmacological drugs and finally biotechnology, with emphasis on manipulation in germ cells and embryos. Economic, social, political and scientific interests often cause the subject to be approached in an exalted manner.

After presenting the CRISPR-Cas9 technique, from its origin until its use in implanted embryos, the article presented elements of the Brazilian Biosafety Law. Still, using the debate between the German thinkers Habermas and Sloterdijk, the text exposed concerns about the use

of CRISPR-Cas9 in eugenic practice, but also raised the hypothesis of an unavoidable use of the technique in humans.

As the technique is usually always at the forefront of ethical-philosophical reflection and legal positioning, it is extremely necessary that both areas (Philosophy and Law) carefully reflect on the explosive potential of the CRISPR-Cas9 technique. The virtual change in all aspects of reality makes the CRISPR-Cas9 technique clear object of study for Philosophy (Environmental), Law (Environmental) and Bioethics (Environmental).

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