CATÓLICA
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THE BIOETHICS OF GENOME EDITING IN LIVESTOCK

by

Filipa Sentieiro de Sousa Lopes

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Thesis presented to *Escola Superior de Biotecnologia of the Universidade Católica Portuguesa* to fulfil the requirements of the Master of Science degree in Applied Microbiology

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by

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Aos meus pais que sempre me deram
a mão ao longo deste percurso.

"To raise new questions, new possibilities, to regard old problems from a new angle, requires creative imagination and marks real advance in science."

Albert Einstein

Resumo

Têm vindo a surgir novas tecnologias de edição de genoma que têm a capacidade de editar o genoma com uma grande eficiência e facilidade. Estas tecnologias são baseadas com recurso de nucleases como as Nucleases de Dedo de Zinco (ZFN), Meganucleases e nucleases baseadas como Ativadores de Transcrição (TALEN). Contudo, a edição do genoma foi revolucionada, nos últimos anos, com o surgimento de Repetições Palindrômicas Curtas Agrupadas e Regularmente Interespaçadas (CRISPR) e da proteína 9 associada a CRISPR (Cas9) que com ela trouxe novas aplicações. Estas tecnologias de edição de genoma têm uma ampla gama de aplicações em animais para consumo humano como no bem-estar, na saúde, na produtividade, na resistência a doenças e ainda na melhoria da alimentação para a saúde humana e por isso, despertaram um enorme entusiasmo como também preocupação, pois levantaram novas questões éticas, debatidas em todo o mundo. Assim, o presente trabalho teve como objetivo analisar as questões éticas levantadas pela utilização da edição de genoma na pecuária assim como, perceber como poderá a bioética contribuir para a procura de recomendações em pesquisas científicas na área de edição de genoma. Para isso, foi realizada uma revisão da literatura baseada na metodologia Itens de Relatório Preferidos para Revisões Sistemáticas e Meta-Análises (PRISMA), a fim de compreender o estado da arte do tema em questão. Questões como as implicações éticas, legais e sociais foram abordadas. Contudo, como trabalho futuro parece-nos necessário uma revisão sobre a corrente regulação ou então a definição de limites para melhor esclarecer a finalidade do uso dessa tecnologia. Por outro lado, a elaboração de uma avaliação de risco e procedimento de gestão, bem como a aceitação do público, são também fatores cruciais para uma maior integração da edição do genoma na nossa sociedade. Em suma, a edição do genoma na pecuária para todos os fins deverá ser feita com prudência e cautela até que os riscos tenham sido rigorosamente caracterizados e conhecidos, bem como o público tenha sido devidamente informado e consultado.

Palavras-chave: Bioética; Edição do Genoma; Produção Animal;

Abstract

Recently, a host of genome editing technologies have emerged that can edit the genome with progressively increasing efficiency and ease of use. These technologies are based on the use of sequence-specific engineered nucleases, like zinc finger nucleases (ZFN), meganucleases and transcription activator-like effector nucleases (TALEN). Genome editing has been revolutionized, in recent years, with the emergence of clustered regularly interspaced palindromic repeats (CRISPR) and associated protein 9 (Cas9). Consequently, there have been new applications of these genome editing technologies. These genome editing technologies have a huge range of possible applications in livestock welfare, health, productivity, disease resistance, enhance food to human health. These technologies and their applications have sparked both enthusiasm and worry, raising new questions on ethics and governance and generating many debates all over the world. The present work has, therefore, aimed to analyse the ethical issues raised through genome editing applications in livestock and how bioethics could contribute to the search for recommendations in scientific research in the area of genome editing. For this, a literature review based on methodology Preferred Report Items for Systematic Reviews and Meta-Analyzes (PRISMA) was made, in order to understand the state of the art. Issues like ethics, legal and social implications were raised. However, it seems necessary to carry out a revision of the regulatory framework or a boundary definition in order to better clarify the purpose and the limits of the use of this technology. In addition, defining a risk assessment and management procedure, as well as the public acceptance, are important factors for a higher integration of genome editing in our society. In conclusion, genome editing in livestock for all the applied purposes must be considered with prudence and caution until the risks have been rigorously characterised and answered, as well as the public has been properly informed and consulted.

Keywords: Bioethics; Genome editing; Livestock;

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1. Introduction

A host of a new genome editing technologies have recently emerged that can edit the genome with progressively increasing efficiency and ease of use. These technologies are based on the use of sequence-specific engineered nucleases, like zinc finger nucleases (ZFN), meganucleases and transcription activator-like effector nucleases (TALEN). Genome editing has been revolutionized in recent years, with the emergence of clustered regularly interspaced palindromic repeats (CRISPR) and the CRISPR-associated protein 9 (Cas9). This technology is a tool that allows us to make changes in the genome of plants, animals and humans and this has raised a lot of ethical questions in the scientific community (Thurtle-Schmidt and Lo, 2018). Consequently, new applications of these genome editing technologies have appeared. These genome editing technologies have a huge range of possible applications in livestock welfare, health, productivity, disease resistance and food enhancement with human health purposes. Besides that, the fast adoption and diffusion of genome editing in the biological sciences, particularly the CRISPR-Cas9 system, offers a precise and easy molecular mechanism for editing cells, tissues and also whole organisms. Genome editing has been widely used in scientific research, in different biological fields including biomedical, animal research (disease models or xenotransplantation), gene drives, in livestock, crops, food organisms and industrial microorganisms (Graeff et al., 2019).

Gene drive has the potential of spreading genes rapidly in wild populations through an organism's progeny and the gene can increase in frequency in a population over multiple generations (EASAC, 2017). Currently, one of the applications of gene drive research is focused on modifying disease vectors like mosquitos, in order to prevent the spread of some diseases such as malaria or dengue. CRISPR could also be used in a gene drive to reverse the development of pesticide and herbicide resistance, as well as eradicate invasive species. However, this technology may present ecological and security risks, which also present some limitations (Oye et al., 2014).

Gene drive presents new possibilities for developing endonuclease-based gene drives. CRISPR mechanisms can be incorporated into a "drive allele" that cuts a specific site on a wild type homologous chromosome. In these cases, the drive allele consists of both the desired variant that will be propagated and the CRISPR guide RNA and Cas9. In order to

repair the break, the cell uses a cell repair mechanism, homology-directed repair (HDR). After this process the drive allele is copied to the wild type chromosome, replacing the wild type DNA sequence at this position of the genome. So, on one chromosome the wild type sequence is replaced with the drive allele, which containing CRISPR components the guide RNA and Cas9 genes as well as the desired change. The organism is released in nature so it can breed with wild type individuals (Enzmann, 2018). In this way, with the CRISPR-Cas9 system, the gene drive organism carries both the altered gene, the gene for Cas9 enzyme and several guide RNAs that tell it where to cut it. When passed to offspring, the guide RNAs direct Cas9 to cut the wild type mosquito of the gene inherited from the wild type parent. The cell, then copies both the altered gene and the drive when it repairs the damage. Due to organism now has two identical copies (one in each chromosome), all of its offspring will inherit the alteration as well as the gene drive. This same process will be repeated in the next generations causing the alteration and the gene drive to spread through the population (Oye et al., 2014).

Research studies in animals play a crucial role in providing an understanding of the disease and associated pathology, as well as identifying the target in the disease to which the treatment is directed and testing new treatments (Whitelaw et al., 2016). Most biological and biomedical experiments are performed on rodents and then on larger animals. NHPs (Non-Human Primates), pigs and dogs are generally used for human disease modelling in biomedical research since these animals having many similarities with humans. The large animal models could be used in research to model cardiovascular diseases, produce a model for genetically induced muscular dystrophy caused by disruption of the dystrophin gene (DMD), generate a model for certain types of cancer, and to be models for coagulation disorders (Petersen, 2017).

Xenotransplantation is also one of the areas where gene therapy can be used. Includes the “transplantation, implantation or infusion into a human recipient of either live cells, tissues, or organs from a nonhuman animal source, or human body fluids, cells, tissues or organs that have had *ex vivo* contact with live nonhuman animal cells, tissues or organs”. Pigs have been a natural focus of xenotransplantation research due to their similarity to humans (FDA, 2019). However, there are two main challenges: the immune rejection of animal tissues as well as the risk of retroviral transmission from pig to humans (Graeff et al., 2019).

Genome editing technologies could indeed be used to overcome these problems.

Genome editing currently, offers an opportunity to improve productivity and sustainability in agriculture. In this way, the introduction of genetic changes in crop plants could be one of the major keys to the future of agriculture. Used in crops could it improve some important traits to agriculture, such as herbicide tolerance, insect and virus resistance, disease resistance, grain yield, plant height and weight, as well as non-agronomic traits like sensory and nutritional properties of crops. Moreover, the CRISPR-Cas9 system can be employed in crops to increase their resistance against biotic or abiotic stress (Samanta et al., 2016).

Industrial biology plays a crucial role in the fields of medicine, health, food as well as energy (Zhang et al., 2020). Until now, applications of CRISPR systems in bacteria include genotyping, vaccinating industrial cultures against viruses, controlling uptake and dissemination of antibiotic resistance genes by bacteria as well as engineering probiotic cultures (Barrangou and Doudna, 2016). The success of native CRISPR–Cas immune systems for the vaccination of *Streptococcus thermophilus* starter cultures used in dairy fermentations (yogurt and cheese) leads to CRISPRs in food (Barrangou and Doudna 2016).

In addition, CRISPR–Cas9 systems in anti-microbial applications have been promising. Native or engineered CRISPR–Cas9 systems can be programmed to target any bacterial species. The sequence-specific antibiotics thus generated can selectively modulate bacterial populations and so eliminate pathogens. In the antiviral fields, work is ongoing to expand on their role as natural systems in order to protect bacteria from phage infection. CRISPR-based therapies are being developed that target human viruses, including HIV-1 (Hu et al., 2014; Li et al., 2015), herpes (Wang and Quake, 2014), papillomavirus (Hu et al., 2014; Kennedy et al., 2014) and also hepatitis B virus (Dong et al., 2015).

At the same time, a challenge has emerged to deal with the increase in the world population and its needs, as well as to overcome the limitations of the use of the conventional techniques. These technologies and their applications have sparked both enthusiasm and worry, raising new questions on ethics and governance and generating many debates all over the world (Graeff et al., 2019). As such, there is a need to create a better understanding of these recent gene editing techniques and their related ethical issues, as well as seek common approaches at a global level to address ethical challenges posed by them. Taking this into consideration, this work becomes quite relevant to reinforce the need for a Bioethics reflection on the specificity of these new contexts, in order to impose

boundaries - when and if necessary - and to elaborate accurate frameworks and educate the population (Carvalho, 2010).

We live in an *era* where the paradigm of the relationship between Ethics and Science is changing. The distance between scientific findings and ethical reflection is progressively fading and that's why Bioethics has found its rhythm. What is called bioethics of the emergent situations (with origin in the new technologies applied in biotechnology, health, environment) has contributed to this progress. Currently, science takes places and bioethics reflects on its possibilities reflecting before the problems happen, measuring risks, moving forward with proposals (not scientific) that are big steps contributing to the rhythm of scientific development (Carvalho, 2010).

The intervention of genome editing could change our life, contributing to a more technologically advanced society. However, ethical problems persist and cannot be forgotten and should always be a subject of participatory debate (Sales, Ferreira, and Reis, 2018). Therefore, and taking into consideration that we don't, in a work like that, analyse all the emerging ethical issues, the aim of this work is to analyse the ethical issues of the use of genome editing technologies in livestock.

For this purpose, and in order to address the ethical issues, a literature review of genome editing in livestock for human consumption was performed using the methodology Preferred Report Items for Systematic Reviews and Meta-Analyzes (PRISMA) (Moher et al., 2009).

In this context, many unanswered questions are raised during this work about the ethical issues or the ethical dilemmas associated with genome editing in livestock. Some of these questions include: (1) Which are the long-term risks associated with the use of these technologies? (2) If we lose control, is there a possibility of interrupting the gene propagation between animals? (3) What is the impact on animal welfare? (4) Do they lose their intrinsic value and/or dignity? (5) What is the impact of the presence of these animals on the environment? (6) What regulations should be applicable? (7) Is it morally acceptable to patent a living organism? Taking all the aforementioned points into consideration, this work aims to analyse the ethical issues raised through genome editing applications on livestock and how bioethics could contribute to the search for recommendations in scientific research in the area of genome editing. From my point of view, it seems that until

all the risks are listed and fully recognized, and until the public has been properly informed, genome editing in livestock must be approached with prudence and caution.

This work is structured as follows. The chapter 1 presents a brief approach regarding science and ethics, posing the question of whether ethics prevent or condition science and research. It also presents an overview of genome editing, first by introducing the concept, and its scientific aspects, as well as some current applications. In chapter 2 we try to demonstrate the trends and challenges that agriculture has to currently deal with, such as the world's population growth, food security, the increase in the number and intensity of outbreaks and, consequently, the excessive use of antibiotics, all of which could lead to the necessity for these new technologies in order to respond to these new challenges. The chapter 3 describes the methodology used to obtain and then choose the articles for the Literature Review. In chapter 4 presents results and the final articles chosen to be part of this work. Chapter 5 describes approaches the applications of genome editing in livestock and the current research in disease resistance, animal welfare, the efficiency of food production and, lastly, in human health, while Chapter 6 presents the ethical, legal, and social considerations regarding genome editing. Lastly, Chapter 7 and 8 discuss and draw conclusions respectively; providing some consideration regarding the bioethical debate of genome editing in livestock, thus, seeking to contribute, in some way, to a more in-depth bioethical reflection on this area.

1.1 Bioethics and Science

The development of science and technology is a driving force in today's societies and affects many aspects of our lifes. Scientific research and technological applications play an important role in the way societies are organized and governed, because scientific evidence could be essential for the development of public policies. The fast pace of the scientific and technological progress offers new opportunities for the future, but, at the same time, brings new and complex questions and challenges. The answer to these new challenges demands that science be developed with solid evidence and incorporate fundamental values (Moedas, 2018).

However, according to Luís Archer (1996), when science gives humans the power initially exercised only by nature, he wonders whether we are authorized to exercise such

powers, as well as whether what is technically possible is also ethically acceptable. A question, therefore, arises: Can ethics prevent or condition science and research?

It was in the early 1970s that the term “bioethics” was successfully introduced. Its author, Van Rensselaer Potter was a biologist and oncologist, through the publication of two scientific works: “Bioethics: The Science of Survival And the book “Bioethics: Bridge to the Future”. For Potter, ethical values cannot be considered separately from biological facts.

Thus, Bioethics was born by the necessity of creating a new field of knowledge in order to create interconnections among biology, humanities, and social sciences. It is exactly from this point onwards that questions begin to emerge, raised to address the new biological and medical technologies, environmental problems, and the future of human survival. Thus, this new field have had a fast development and, in a few years, has become indispensable to medicine, biology, philosophy, theology, law, sociology and even political action (Patrão Neves, M.C and Osswald, W., 2014).

Bioethics, at its core, approaches science as its primary field of study. Among other things, Bioethics questions the *bio,* promotes the need for critical approach, questions technical absolutism and the social control of science, while offering a reflective role that could help on the elaboration of the rules. Additionally, Bioethics uses an approach to answer questions on a national, international, and global level. One of the major factors that has led to the increase in the power of Bioethics is that it promotes the involvement of the public and society's goodness, while being part of the health and science policy. Another reason is that Bioethics strives to address and contribute to global and international concerns (Patrão Neves, M.C and Osswald, W, 2014).

Finally, Bioethics is transdisciplinary, pluralist, and democratic. The transdisciplinary aspect has derived from the fact that Bioethics involves different areas of the domain and goes beyond the individual perspectives. It has, therefore, become fundamental, as it involves different authors from various fields, including biologists, philosophers, nurses, and theologists. Pluralism allows Bioethics to stay away from limited points of view and restrictions that could hinder its progress (Archer, 1996).

In summary, Bioethics doesn't want to stop the progress but rather promote and redirect it. Bioethics presents a reorientation strategy, in order for science to become sensitive to the ethical questions posed, while at the same time ethics become sensitive to scientific

questions, adjusting to them. In this way, Ethics became closely related to science. Because of that, it appears today as an important chapter of any scientific investigation (Carvalho, 2010).

In the final part of the work, we will try to illustrate through some practical examples, how bioethics questions science, giving rise to the ethical issues related to genome editing and contributing to the search for recommendations in scientific research in the area of genome editing in livestock. The technical-scientific debate related to research on genome editing, as well as the ethical issues raised by these technologies, has been the target of a lot of debates around the world because there are still many questions raised about what is feasible or not and as mentioned before, if scientific possible it should or not be ethically acceptable.

1.2 Genome Editing

Genetic engineering is a part of biotechnology that has been explored in medicine and other different fields. Genetic engineering is the artificial manipulation, modification, and recombination of DNA or other nucleic acid molecules in order to modify an organism or population of organisms, including a different set of genetic manipulation techniques in order to introduce new traits, for example, to promote industrial utilization or increased resistance to adverse conditions. Initially, the term genetic engineering referred to techniques used for the modification or manipulation of organisms as the processes of heredity and reproduction. Later, in the 20th century, the term turns more specific including the methods of recombinant DNA technology (or gene cloning), in which DNA molecules from two or more sources are joint either within cells or “in vitro” and are then inserted into host organisms in which they are able to propagate. Therefore, the most recombinant DNA technology leads to the insertion of foreign genes into the plasmids of common laboratory strains of bacteria. However, they are capable of directing protein synthesis, and, like chromosomal DNA, they are reproduced and passed on to the bacterium’s progeny. Thus, by incorporating foreign DNA (for example, a mammalian gene) into a bacterium, researchers can obtain a huge number of copies of the inserted gene (Tan et al., 2012).

A subsequent generation of genetic engineering techniques that emerged in the early 21st century was the genome editing. Genome editing includes tools as the like zinc finger

nucleases (ZFN), meganucleases and transcription activator-like effector nucleases (TALEN). Genome editing has been revolutionized in recent years, with the emergence of clustered regularly interspaced palindromic repeats (CRISPR) and the CRISPR-associated protein 9 (Cas9) (Cotter and Perls, 2018). Genome editing technologies allow the addition, removal, and modification of target DNA sequences making very precise changes on DNA (Chneiweiss et al., 2017).

All genome editing technologies mentioned above are very powerful, as they can precisely alter the genome of an organism and act by creating site-specific DNA double-stranded breaks at the target site location in the genome. They can then be to utilize repair mechanisms that are present within every cell naturally, such as non-homologous end joining (NHEJ) as well as homology directed repair (HDR) (Ruan et al., 2017).

Genome editing, despite being a set of new genetic engineering techniques (Cotter and Perls 2018), differs from the old ones due to the fact that this technology allows the alterations in the genome, and it is also is commonly used to inactivate a target gene (gene knock-out), target sequence, as well as change the gene functions (EASAC, 2017). In this way, we can affirm that genome editing can insert, replace, or remove DNA from a genome with a high degree of specificity, without insert a gene from another specie (transgene) (Friedrichs et al., 2019).

The nuclease-based genome editing technologies, particularly CRISPR-Cas9 (the latest advance), have gained an enormous relevance in different fields and are helping to renovate basic research in the field of life sciences. This method of genetic engineering offers tools for innovation in biomedicine, agriculture (crops and livestock), industrial biotechnology and other sectors related to bioeconomy (Friedrichs et al., 2019). On the other hand, the final products of this technology raise ethical questions, societal acceptance, and regulatory issues (Eriksson et al., 2018).

As previously mentioned, in the past few years, older genetic engineering tools are giving way to new molecular tools. The finding of restriction enzymes that protected bacteria against phages in the late 1970s, was a highlight in the era of recombinant DNA technology. There is also the capacity of mammals to incorporate an exogenous copy of DNA into their own genome by a process called homologous recombination. Such targeted gene integration into the genome allowed the characterization of roles of many genes in model organisms. However, this approach, has several limitations; for example, the rate of

spontaneous integration of an exogenous DNA copy was very low (1 in 103–109 cells), the integration rate depended on cell types and cellular states and lastly, the approach could result in random integration of the exogenous copy into undesired genomic "loci" at a frequency similar to or, even higher, than that of the target site (Adli, 2018).

In order to overcome these limitations, the discovery of the introduction of a double-strand break (DSB) at a target site has resulted in an increase in the frequency of targeted gene integration. This technique that could be done using endonuclease enzymes, are able to cut DNA in a very specific manner by enhancing the DNA mutation rate trough the induction of DSB at a predetermined genomic site (Petersen and Niemann, 2015).

The first tool used for genome editing was mega-nucleases, endonucleases that naturally occur in bacteria and are capable of recognizing long stretches of 14–40bp DNA and then, cleaves the DNA. Although such mega-nucleases increased the genome-editing efficiency, the approach has two major drawbacks: the first one is that each of them has only one site recognition sequence, so, the likelihood of finding a mega-nuclease that targets a desired locus was still low; the second drawback is that the biggest part of induced DSBs are repaired through the cell mechanism repair - non-homologous end joining (NHEJ). Consequently, not only may the exogenously introduced DNA template not incorporate at the DSBs, but the NHEJ repair mechanism may also randomly insert or delete DNA pieces at the break site (Adli, 2018).

To overcome such challenges, zinc finger nucleases (ZFN) started a new era in genome targeting and editing. ZFN consists of a site-specific zinc-finger DNA binding domain fused to the nonspecific cleavage domain of the FokI endonuclease. ZFN are zinc ion-regulated small protein motifs that bind to DNA in a sequence-specific way. Each zinc finger module recognizes a 3-bp DNA sequence and multiple ZFNs modules could be assembled into a larger complex in order to achieve higher DNA binding specificity, contrary to mega-nucleases. ZFNs, improved the abilities to edit genomes of living cells at specifically targeted sites and opened doors for therapeutic applications of such genome-editing tools (Bhat et al., 2017).

Transcription activator-like effector (TALE) proteins were discovered from *Xanthomonas* bacteria. This protein binds to DNA in a specifically one single base instead of three bases. As ZFNs, chimeric fusion of the Fok I DNA cleavage domain to a

combination of TALE modules serves as an effective programmable nuclease, called a TALEN (Petersen and Niemann, 2015).

Therefore, mega-nucleases are engineered restriction enzymes that recognize long stretches of DNA sequence, each ZFN recognizes triple DNA code whereas each TALE recognizes an individual base, increasing in this way the genome-editing efficacy (Adli, 2018).

To sum up, it is possible to distinguish ZFNs and TALENs by differing in three main aspects: The first one, is that TALEN repeats are 3–4 times larger than ZFNs, when recognized per base pair of the targeted DNA; The second one is the fact that the spacer length (the gap between two binding sites) is variable and not restricted to a specific length, which complicates TALEN design and could lead to greater off-target activity relative to an identical nuclease with a fixed spacer length; Lastly, ZFNs assembly requires an archive of high-quality, well characterized modules to achieve specific gene targeting because cross-talk between the individual fingers can lead to imperfect DNA recognition efficiency (Tait-Burkard et al., 2018).

1.2.1 The CRISPR-Cas9 tool

CRISPR-Cas9 is a revolution in the field of gene editing due its increasing robustness and efficiency. CRISPR has been discovered when researchers studied the defense mechanism of bacteria against a virus. That brought about the discovery that prokaryotes have defense mechanisms against viral and plasmid cellular attacks, just like multicellular organisms. One of these defense mechanisms is an adaptive immune system found in many bacteria and most archaea called Clustered Regulatory Interspaced Short Palindromic Repeats or CRISPR, along with the CRISPR-associated Proteins or Cas proteins. By integrating DNA sequences that are identical to past invaders into their genome, bacteria and archaea generate a cellular memory of past invaders, passing it down to the offspring. This viral DNA acquired allow the bacteria or archaea to recognize viral or plasmid invaders during a new attack, resulting in the degradation of the invading sequence and actives an adaptive immune system for prokaryotes (Thurtle-Schmidt and Lo, 2018).

Therefore, since this complex CRISPR-Cas9 is programmable, it can be designed to recognize particular DNA sequences. Therefore, this activity could be used in genome engineering to allow cells to make a very precise change to the DNA at the site where the break was introduced (Zhang et al., 2015). In this way, CRISPR-cas9 allow scientists to alter the genes of a wide range of organisms in a very precise and ease way (Reardon, 2016).

When the double-strand break is made by the Cas9 protein, the cell activates its repair mechanisms in order to repair the damage. These can be of two types: error-prone non-homologous end joining (NHEJ) or error-free homology-directed repair (HDR). In the first case, NHEJ, it leads to the accumulation of small insertions or deletions (indels) following repeated cycles of break and repair. In the case of HDR, a repair template with homology to the target site can be delivered with Cas9, but typically at a lower efficiency than NHEJ-mediated repair. In other words, NHEJ produces gene knockouts (deletions), whereas HDR can be used to introduce a specific change in the targeted genomic site, such as a point mutation or insertion of a longer segment of DNA. Increasing the efficiency of HDR by following with nuclease-mediated DNA breakage is widely used to fully harness the power of genome editing to introduce precise genomic alterations (Pickar-Oliver and Gersbach, 2019). As a result, sequence modifications are introduced at the break site (insertion, deletion, or mutation). If the objective is to knock-down the expression of the targeted gene, it is sufficient to allow the NHEJ repair system to mend the break by inserting and/or deleting (INDELs) nucleotides randomly. As the repair is error-prone, the ‘‘repaired gene’’ will most likely be mutated. If the objective is to correct a pre-existing mutation, then the repair must restore the original sequence after the break of the mutated gene. For this to happen, the introduction of a template DNA sequence is necessary, and thus the cell repairs the break by copying the template sequence (Chneiweiss et al., 2017).

Although the potential promissies, there are some limitations. One of the biggest challenges with this technology is the potential for off-targeted effects, editing at sites in the unwanted gene (Zhang et al., 2015). Indeed, the factors controlling the precision and accuracy of DNA target recognition by Cas9 and the mechanisms that prevent undesired off-target cleavage are still not well understood. There have been efforts in order to reduce the off-target effects (Jiang and Doudna, 2017). On target effects and mosaicism have also been described as limitations. In virtue of their precision, ease of use, and universality,

these tools are more powerful than their predecessors and have revolutionized possible applications of genetic modification (Shriver and Mcconnachie, 2018).

Also, it's important to know that CRISPR technology is a tool that allows us to make changes in the genome of plants, animals and humans and this has raised a lot of ethical questions within the scientific community and society at large (Thurtle-Schmidt and Lo, 2018).

1.2.2 Genome editing and GMOs

Genetic modification is a biological technique that make alterations in the genetic machinery of all kinds of living organisms, that consequently, includes the recombinant DNA technology. This technology is the basic tool for the development of transgenics. In this technique, a gene of interest that may or may not be native is inserted into the plant/animal or endogenous genes are modified. Recombinant DNA techniques form new combinations of heritable genetic material, followed by the incorporation of that material either indirectly through a vector system or directly through micro-injection, macro-injection and micro-encapsulation techniques. So, a transgenic, or genetically modified, organism is one that has been altered through recombinant DNA technology, which involves either the combining of DNA from different genomes or the insertion of foreign DNA into a genome. There are several important applications of this technology, e.g., development of resistance to abiotic (drought, extreme temperature, or salinity) and biotic (insects and pathogens) stresses (Eriksson et al., 2018).

Therefore, organisms as plants, animals or microorganisms in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating and/or natural recombination are called genetically modified organism (GMO). GMO implies the insertion of DNA sequences from another organism (transgene) using genetic engineering tools and consequently, gives origin a transgenic organism (Eriksson et al., 2018). However, there is not yet a consensus among the society due to the lack of comprehensive understanding of current agricultural risks, the nature of GMO and the risks to human health (Eriksson et al., 2018).

On the other hand, by genome editing no foreign DNA is incorporated in the genome (Eriksson et al., 2018), it could direct only one or a few nucleotide changes (insertions, deletions or substitutions) using "molecular scissors": ZFNs, TALENs or

CRISPR-Cas9. This process is much more controlled as well as precise and is very similar to what occurs in nature (Hefferon and Herring, 2016). In this way, genome editing refers to techniques, in which enzymes that have been modified, can insert, replace, or remove DNA from a genome with a high degree of specificity, without integration of foreign DNA (Friedrichs et al., 2019) and consequently, the resultant animal is, therefore, not transgenic. Indeed, in many cases the resultant animal may be genetically indistinguishable from natural variants or variants produced through selective breeding (Schultz-bergin, 2018).

2. Trends and challenges in agriculture

Agriculture is a sector with a crucial role in the economy of most of the countries, and provides the main source of food, income, and employment to rural populations all over the world, especially in poor countries. Nowadays, the agricultural sector has to deal with many challenges and global changes. A technological revolution which includes innovations in nutrition, genetics, informatics or precision farming and low impact agriculture is taking place (Buttriss, 2011).

The growth of world population is one of the biggest challenges that agriculture and governments are facing. In general, world population growth is slowing down, but in some parts of the planet, world population is not predicted to stop growing until 2050 and even further down in the next century. At present, the population stands at around 6.8 billion and is expected to reach 8 billion by 2030 and 9 billion in 2050. On the other hand, in many developed countries, numbers are static, but growth is still happening. So, according to these trends, the world's population will increase from 7.7 billion to 9.7 billion in 2050 and could reach a peak of nearly 11 billion later in 2100 (FAO, 2019).

Along with these trends in population growth, the demand for food will increase in the next few years. The projections indicate that, in order to feed a world population of 9.1 billion people in 2050, it would be necessary to increase overall food production and in the developing countries would need to increase for the double. (FAO, 2017).

Food security, according to FAO is when: "all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life". Nowadays, there are over 800 million people who are in a constant state of hunger in the developing world. Therefore, in global

terms, we are far from reaching enough food enough security all over the world (FAO et al., 2019). In other words, food and nutrition security as well as population growth will be one of the biggest challenges for sustainable development in the 21st century.

Nowadays, livestock is facing an increase in the number and intensity of outbreaks of transboundary animal diseases. The patterns of livestock disease incidence have shifted because climate change and globalization increase the pressure in animal-health and food-safety systems, which are dealing with new additional challenges as a result of the increase in the complexity of supply chains in the livestock sector (FAO, 2009).

Animal diseases could create two types of problems for humans: socio-economic and health related. The first type may cause losses in production, productivity and profitability, brought by disease agents and the cost of their treatment. The second category, human-health threats from livestock, comes in two different types: zoonotic diseases or food-borne illnesses (FAO, 2009).

According to the World Health Organization (WHO), zoonosis is a disease or infection that is naturally transmissible from vertebrate animals to humans. There are many different potentially pandemic viruses, all causing considerable concern about the risk of a major global pandemic (WHO, 2019). For instance, in recent years, the world has experienced the emergence of severe acute respiratory syndrome (SARS), HPAI (caused by the A[H5N1] virus) and influenza caused by the A(H1N1) virus (FAO, 2009) and currently COVID19 caused by the Coronavirus (SARS-CoV-2)[1].

Zoonotic diseases represent the majority of recently emerging infectious diseases in humans. According to OIE (World Organisation For animal health), 60 percent of existing human infectious diseases are zoonotic (OIE, 2020). Over the 20th century the number of cases where new diseases have arisen in humans has increased and this high number of cases is linked to food and farming systems, brought about by changes in land use, agricultural intensification, food industry changes, and bush meat hunting and consumption (Lee-Gammage et al., 2018)

Livestock are, therefore, a critical source of many infection pathways linking humans to sources of zoonotic. A list of issues could be identified that includes: (1) an increase in

[1] This topic will not be addressed in this work once it is a recent one, happening nowadays and whose the investigation is still occurring.

the frequency of interactions between wildlife, livestock, and humans; (2) an increase in the likelihood of disease emergence and transmission events occurring; (3) a high diversity of pathogens found in different animal populations; (4) and an increase for new diseases to spread among livestock and human populations (Lee-Gammage et al., 2018).

Farmers all over the world adopt an 'intensive' livestock production system in order to increase scale and the speed of production, combined with technology (Lee-Gammage et al., 2018). In order to keep the animals in this system, farmers cluster them in large and densely populated areas that allow pathogens to be transmitted and spread to large numbers of animals. Lastly, the intensive system increases the use of antibiotics and, consequently, due to excessive usage, the development of antibiotic resistance. Therefore, intensive livestock production methods have increased the risk of infectious disease. The proportion of antibiotics used in livestock is higher when compared with humans, as more than half of all antimicrobial use is related to the production of animals that will be consumed as food (Sandoiu, 2018). All antibiotic uses are greater in an intensive agriculture, because animals are staying in confined conditions (O'Neill, 2015). In addition, besides being harmful to animals, the high levels of antibiotics are harmful to human health because livestock farms become reservoirs of antibiotic resistant bacteria and resistance genes, which can potentially spread to the wider environment and to humans (O'Neill, 2015).

With all the aforementioned risks, it becomes clear that animal welfare is crucial in this sector. According to OIE, good animal welfare management includes: (1) "freedom from hunger, malnutrition and thirst; (2) freedom from fear and distress; (3) freedom from heat stress or physical discomfort; (4) freedom from pain, injury and disease; (5) and lastly, freedom to express normal patterns of behaviour". Nowadays, the rules are being applied in large-scale intensive systems like, in poultry and pig systems. However, animal welfare also applies when animals are kept in by small-scale producers. Due to the increasing shift toward larger-scale livestock production, there is an urgent need to work with producers and governments to improve animal health and welfare, because if animals are kept in a healthy and clean space, it could reduce the number of diseases and that would later affect human health (FAO, 2009).

So, it is necessary to take action in this sector in order to able it to respond to the demand in ways that will contribute to a reduction in poverty, an increase in food security, environmental sustainability and human health, without depleting the resources available in nature. Future genome editing technologies could help to increase food production in

order to feed the growing population, improve animal welfare, contribute to food security, environmental sustainability and human health. However, genome editing does not offer the only nor the absolute response to the challenges; it could also give rise to new concerns, such as product safety, consumer choice and impact on farmed animals, environment and people. Besides that, genome editing should not be seen as the only solution because changes in people food choices are urgent and in the way people are treating the farming animals.

3. Methodology

A literature review of genome editing in livestock to human consumption was based on PRISMA: Preferred Report Items for Systematic Reviews and Meta-Analyzes (Moher et al., 2009). In this research, PubMed and Web of Science were used as databases and "genome editing" and "systematic review" as the keywords. After this research, the selected systematic review was "The ethics of genome editing in non-human animals: a systematic review of reasons reported in the academic literature" by Graeff et al. 2019.

Publications that did not contain genome editing in animals, agriculture, a new generation of genome editing or that were not a systematic review were excluded.

Based on the previous systematic review, articles were selected with the following criteria:

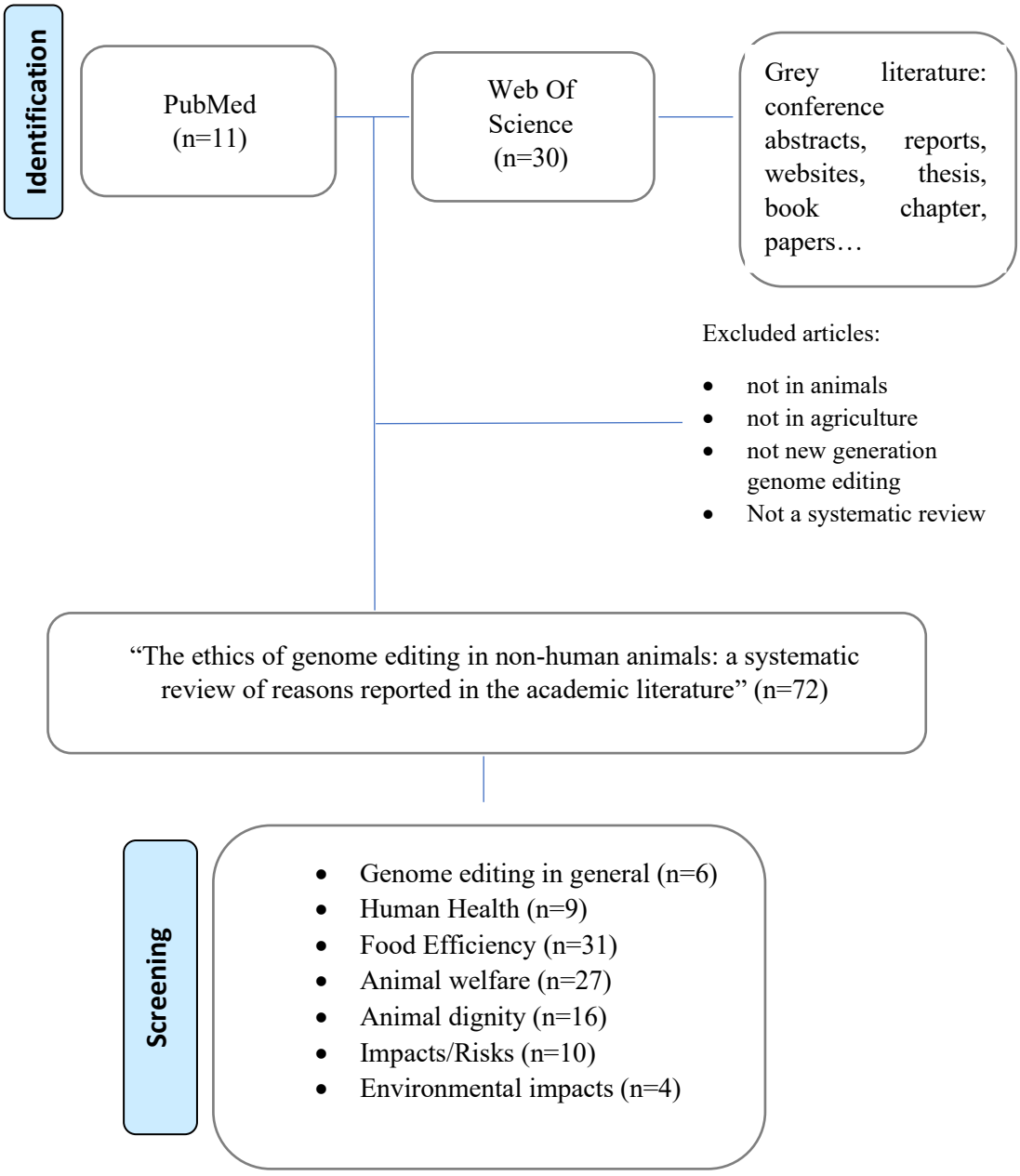
1. Genome editing in general,
2. Genome editing in human health,
3. Genome editing in food efficiency,
4. Genome editing in animal welfare,
5. Genome editing in animal health,
6. Genome editing in animal dignity,
7. Genome editing and their impacts/risks,
8. Genome editing and their environmental impacts.

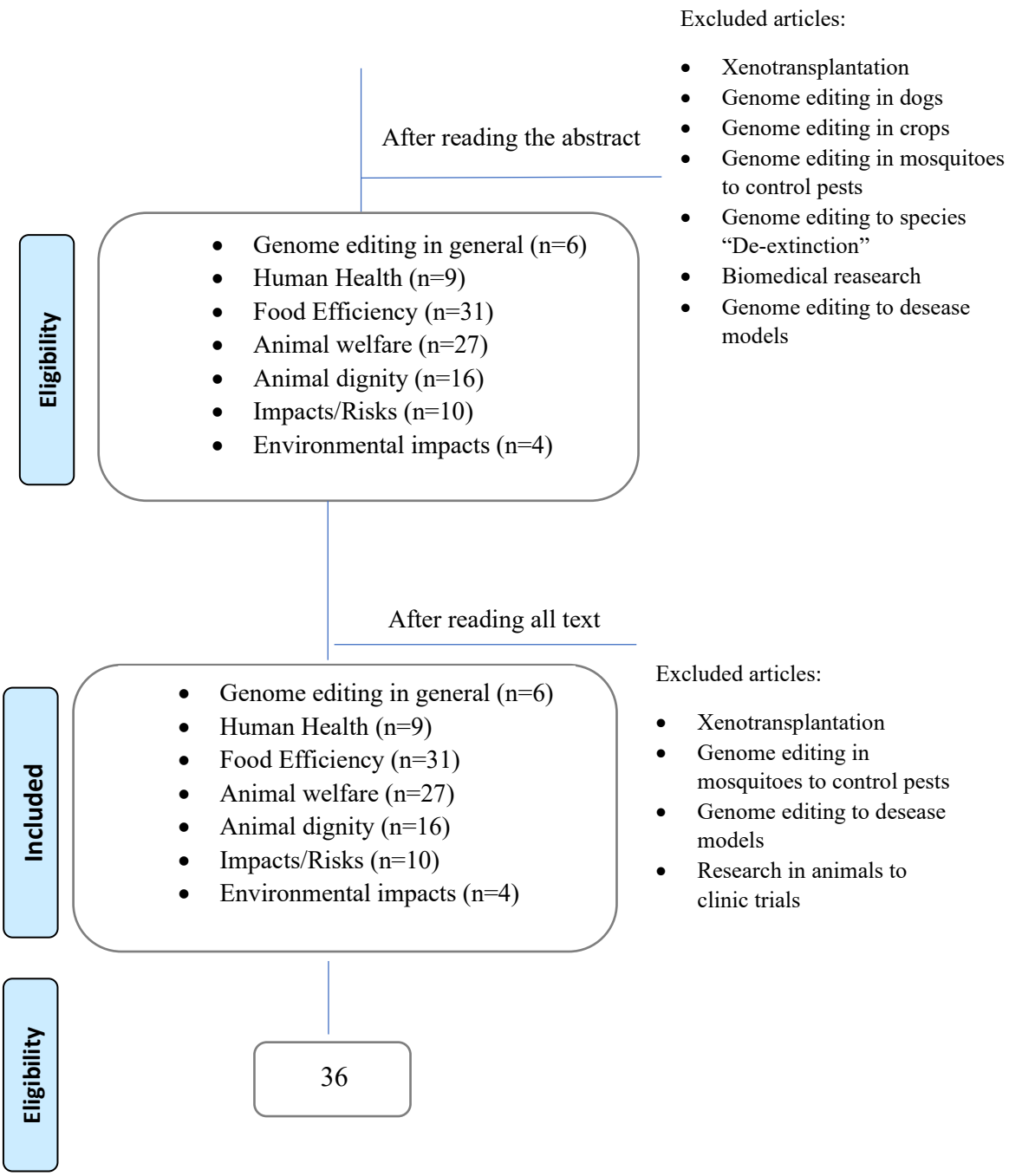
Once the articles were chosen, all the abstracts applying the inclusion criteria described above were read, and then assessed them for eligibility. Topics like xenotransplantation, genome editing in dogs, genome editing in crops, genome editing in mosquitoes to control

pests, genome editing to species “De-extinction”, biomedical research and genome editing to disease models were excluded.

After the process of abstract screening, the full text was read to obtain a final list of articles to extract the data to be included in the review.

Once the selection of the final articles to be included in this work was done, grey literature was included, with documents such as reports, government publications, theses, websites, conference abstracts, and book chapters.





4. Results

The initial potential sample included 11 articles from PubMed and 30 articles from Web of Science. The titles and abstracts were read to assess their relevance in relation to this study. According to the inclusion criteria, the systematic review selected was "The ethics of genome editing in non-human animals: a systematic review of reasons reported in the academic literature".

From 134 articles belonging to this research's bibliography, 62 documents were excluded during the first analysis, while 72 stayed, keeping only the articles that included topics such as genome editing in general, in human health, in food efficiency, in animal welfare/health or dignity, and the genome editing impacts/risks or their environmental impacts. Some articles includes more than one topic.

After reading the abstract and the full text a total of 36 articles were selected for data extraction, in order to integrate them into this study and these final articles were detailed in the following tables. These tables were grouped based on different topics: genome editing in general, in human health, in food efficiency, in animal welfare/health or dignity, and the genome editing impacts/risks or their environmental impacts.

Genome editing in general

Summary	Reference
After hatching, the male chicks are killed because they are not profitable in meat production. Explain the current practice and technological alternatives to this practice.	(Leenstra et al., 2011)
PRRS is one of the most challenging diseases in pig production. Alteration of key genes PRRS pathogenesis, like CD163 knockout has led to the production of pigs resistant to PRRSV.	(Reiner, 2016)
Genome editing to improve the welfare of agriculture animals. Despite having some comparative disadvantages, there are important reasons for an on-going pursuit of welfare improvements via genetic modification.	(Shriver and Mcconnachie, 2018)
Myostatin (MSTN) is an inhibitor of skeletal muscle development and growth. Mutations in the MSTN gene can lead to muscle hypertrophy or double-muscled (DM) phenotype. The MSTN-mutant pigs offer a genetic improvement of meat (increase skeletal muscle mass).	(Qian et al., 2015)
Specific alterations to complex genomes to enhancement farm animals for food production.	(Tan et al., 2012)
Creation of a chicken strain that produces eggs with a low allergenicity.	(Reardon, 2016)

Human Health

Summary	Reference
Genome editing livestock to benefit animals, consumers, and the environment.	(Laible, Wei, and Wagner, 2015)
Genome editing in food production as well as nutritional enhancement to feed the global population in a sustainable and equitable way.	(Buttriss, 2011)
CRISPR ethical challenges.	(Caplan et al., 2015)
Discovery and development of the CRISPR-Cas9 system in applications like genome regulation and epigenome engineering and the challenges faced.	(Lau and Davie, 2017)
The social acceptance of food products derived from genome-edited livestock.	(Ishii, 2015)
Specific alterations to complex genomes to enhance farm animals for food production.	(Tan et al., 2012)
Genome editing to improve the welfare of agriculture animals. Despite having some comparative disadvantages, there are important reasons for the on-going pursuit of welfare improvements via genetic modification.	(Shriver and Mcconnachie, 2018)
Creation of a chicken strain that produces eggs with a low allergenicity.	(Reardon, 2016)
Genome editing in farm animals and the ethical questions regarding animal welfare.	(Baltimore, Charo, and Kevles, 2016)

Food Effiency

Summary	Reference
Genome editing technologies to livestock traits improvement for production and disease resistance.	(Bhat et al., 2017)
Genes drives and environmental security challenges as well as benefits.	(Oye et al., 2014)
Genome editing in food production as well as nutritional enhancement to fed the global population in a sustainable and equitable way.	(Buttriss, 2011)
Genome editing in dairy cattle breeding. Principles of animal integrity, naturalness, risk perception as well as animal welfare.	(Eriksson et al., 2018)
Gene drive systems' that use CRISPR- cas 9.	(Graham, 2016)
Current development of CRISPR systems and its applications as well as limitations.	(Xing-Da Ju, Jing Xu, 2018)
Molecular scissors: Zinc Finger Nucleases (ZFN), Transcription-activator like endoncleases (TALENS) and meganucleases.	(Petersen and Niemann, 2015)
Potential of gene drives with genome editing to intensify genetic gain in livestock breeding programs.	(Gonen et al., 2017)
PRRS is one of the most challenging diseases in pig production. Alteration of key genes PRRS pathogenesis, like CD163 knockout has led to the production of pigs resistant to PRRSV.	(Reiner, 2016)
"Animal disenhancement": application of concepts from political theory and an inquiry into the mode of valuation that underlies industrial animal agriculture and disenhancement suggestions.	(Schultz-bergin, 2014)
Genome editing to improve the welfare of agriculture animals. Despite having some comparative disadvantages, there are important reasons for the on-going pursuit of welfare improvements via genetic modification.	(Shriver and Mcconnachie, 2018)
Myostatin (MSTN) is inhibitor of skeletal muscle development and growth. Mutations in MSTN gene can lead to muscle hypertrophy or double-muscled (DM) phenotype.	(Qian et al., 2015)

The MSTN-mutant pigs offer a genetic improvement of meat (increase skeletal muscle mass).	
CRISPR/Cas9 as a powerful tool for livestock improvement by simultaneously targeting multiple genes.	(Wang et al., 2016)
CRISPR-Cas systems: production of new artificial genes, synthetic proteins, and new transgenic organisms	(Webber, 2014)
Genome editing in large animals and their practical applications.	(West and Gill, 2016)

Animal Welfare

Summary	Reference
How genome editing could help in food production, agriculture and medicine.	(Hefferon and Herring, 2016)
The social acceptance of food products derived from genome-edited livestock.	(Ishii, 2015)
Creation of a chicken strain that produces eggs with a low allergenicity.	(Reardon, 2016)
Applications of CRISPR technologies in different areas	(Barrangou and Doudna, 2016)
Genome editing in cattle: hornless cows.	(Carroll et al., 2016)
Potential applications of genome editing and the related ethical as well as the social questions.	(Fears and Meulen, 2017)
Genome editing in dairy cattle breeding. Principles of animal integrity, naturalness, risk perception as well as animal welfare.	(Eriksson et al., 2018)
Liberal theory: stem cell research and the genetic engineering of people or animals.	(Fox, 2010)

After hatching, the male chicks are killed because they are not profitable in meat production. Explain the current practice and technological alternatives to this practice.	(Leenstra et al., 2011)
Modifications that eliminate animal telos and what animal modifications can or cannot be justified.	(Noll, 2013)
"Disenhancing" animals in order to improve animal welfare and the human role in creating animals.	(Palmer, 2011)
"Animal *Telos":* modifying animals in order to avoid animal suffering.	(Rollin, 2014)
Genome editing and its applications in agriculture, medicine and its association with societal issues.	(Carroll and Charo, 2015)
PRRS is one of the most challenging diseases for pig production. Alteration of key genes PRRS pathogenesis, like CD163 knockout has led to the production of pigs resistant to PRRSV	(Reiner, 2016)
"Animal disenhancement": application of concepts from political theory and an inquiry into the mode of valuation that underlies industrial	(Schultz-bergin, 2014)
Dignity of Diminished Animals: enhancing animals and improving welfare.	(Schultz-bergin, 2017)
CRISPR impacts on animal welfare.	(Schultz-bergin, 2018)
Genetic engineering to welfare enhanced animals in order to reduce harm to animals without sacrificing and the associated ethical questions.	(Shriver, 2015)
Genome editing to improve the welfare of agriculture animals. Despite having some comparative disadvantages there are important reasons for the on-going pursuit of welfare improvements via genetic modification.	(Shriver and Mcconnachie, 2018)
Genome editing in farm animals and the ethical questions regarding animal welfare.	(Baltimore, Charo, and Kevles, 2016)
Specific alterations to complex genomes to enhance farm animals for food production.	(Tan et al., 2012)

CRISPR-Cas systems: production of new artificial genes, synthetic proteins and new transgenic organisms.	(Webber, 2014)
Genome editing in large animals and their practical applications.	(West and Gill, 2016)

Animal Dignity

Summary	Reference
The social acceptance of food products derived from genome-edited livestock.	(Ishii, 2015)
Dignity for non-human living beings?	(Heeger, 2015)
Genome editing in dairy cattle breeding. Principles of animal integrity, naturalness, risk perception as well as animal welfare.	(Eriksson et al., 2018)
Liberal theory: stem cell research and the genetic engineering of people or animals.	(Fox, 2010)
Modifications that eliminate animal telos and what animal modifications can or cannot be justified.	(Noll, 2013)
"Disenhancing" animals in order to improve animal welfare and the human role in creating animals.	(Palmer, 2011)
"Animal *Telos":* modifying animals in order to avoid animal suffering.	(Rollin, 2014)
"Animal disenhancement": application of concepts from political theory and an inquiry into the mode of valuation that underlies industrial.	(Schultz-bergin, 2014)
Dignity of Diminished Animals: enhancing animals and improve welfare.	(Schultz-bergin, 2017)
Genome editing to improve the welfare of agriculture animals. Despite having some comparative disadvantages there are	(Shriver and Mcconnachie, 2018)

important reasons for the on-going pursuit of welfare improvements via genetic modification.	
Genome editing in farm animals and the ethical questions regarding animal welfare.	(Baltimore, Charo, and Kevles, 2016)

Impacts/Risks

Summary	Reference
The social acceptance of food products derived from genome-edited livestock.	(Ishii, 2015)
Genome editing in cattle: hornless cows.	(Carroll et al., 2016)
Genome editing in dairy cattle breeding. Principles of animal integrity, naturalness, risk perception as well as animal welfare.	(Eriksson et al., 2018)
Molecular scissors: Zinc Finger Nucleases (ZFN), Transcription-activator like endoncleases (TALENS) and meganucleases	(Petersen and Niemann, 2015)
Myostatin (MSTN) is an inhibitor of skeletal muscle development and growth. Mutations in MSTN gene can lead to muscle hypertrophy or double-muscled (DM) phenotype. The MSTN-mutant pigs offer a genetic improvement of meat (increase skeletal muscle mass).	(Qian et al., 2015)
Specific alterations to complex genomes to enhance farm animals for food production.	(Tan et al., 2012)

Environmental impacts

Summary	Reference
Genome editing in dairy cattle breeding. Principles of animal integrity, naturalness, risk perception as well as animal welfare.	(Eriksson et al., 2018)
Genome editing to improve the welfare of agriculture animals. Despite having some comparative disadvantages, there are important reasons for the on-going pursuit of welfare improvements via genetic modification.	(Shriver and Mcconnachie, 2018)
Genome editing in farm animals and the ethical questions regarding animal welfare.	(Baltimore, Charo, and Kevles, 2016)

From the results previously presented, the discussion has been organized in different subchapters: genome editing in farmed animals and human health; genome editing in farmed animals and food efficiency; genome editing in farmed animals and animal welfare and lastly, from chapter 2 to 5 scientific approaches were described and from 6 to 8 ethical and legal issues were detailed. [2]

5. Genome editing in farmed animals

Animals have been domesticated by humans for a long time, in order to produce dairy products, clothing, meat… For a long time, selective breeding has led to the development of animals with characteristics are beneficial to humans. Those characteristics include increased muscle mass, fast growth rate, high fertility, docility and resistance to disease. These features also have implications for animal welfare, which may be positive or negative. As the majority of these features imply genetic alterations, this selective procedure has implicitly involved the animals' genome characteristics (The Nuffield Council on Bioethics, 2019).

With the development of new genome editing technologies as well as, genome sequencing, it becomes possible to understand the association between some genetic

[2] The criteria 1 ("Genome editing in general"), includes articles that have been previously addressed in the introduction. Therefore, this chapter will start with discussion of the criteria 2 – Genome editing and human health.

variants and observable phenotypes. This knowledge is essential when the objective is to choose animals with desirable genetic traits and the use of genetic techniques that allow the introduction of new traits that either do not naturally exist in the breed or could not be achieved through selective breeding (The Nuffield Council on Bioethics, 2019).

The growth and development of animals are influenced by multiple genes and different production traits are often controlled by different and/or multiple genes. It is therefore important to consider that in improving livestock production and/or quality through genome editing is the requirement of precise genome editing at multiple *loci*. For instance, different mutation sites or mutation types in the same gene may also have a large impact on production performance. Therefore, the significant enhancement of multiple livestock production traits requires precise genome editing at multiple genomic sites which could be achieved with CRISPR-Cas9 technology because it is a multi-faceted technology; in other words, it allows the simultaneous targeting of multiple genomic loci (Ruan et al., 2017).

5.1 Genome editing in farmed animals - Human Health

The world's population expanding but it is also undergoing rapid development, and with this comes an increasing demand for protein rich foods, especially meat and dairy products (Buttriss, 2011). Milk and dairy foods supply energy, protein and micronutrients, are known to be important components for infants and young children and have also important characteristics for improving undernutrition (for instance, cow's milk is rich and diverse in terms of rich nutrients as protein, fat, carbohydrate, and mineral contents). Although, milk and eggs are widely consumed and offers a beneficial source of protein, many people have allergies to certain proteins, as the β-lactoglobulin (BLG) in milk as well as ovalbumin and ovomucoid in eggs (Ishii, 2015). Genome editing technologies will open up unlimited possibilities of modifying these foods, making them more suitable for human health, improving the functional properties also improving their nutritional traits (Laible, Wei, and Wagner, 2015).

5.1.1 Milk modification: Beta-lactoglobulin (BLG) Knockout

Milk is a major source of protein for human consumption and it is produced worldwide as a commercial product. There are different ways that gene editing could help

to enhance milk, including the removal of allergens, the improvement of its nutritional value, and an increase in shelf life (Wei et al., 2018).

Beta-lactoglobulin (BLG) is a major allergen in cow milk. Genetic modification could be a more direct approach to reduce BLG levels in ruminant milk, and both ZFNs and TALENs have been used to mutate BLG in cattle. Therefore, cows without BLG gene production could provide an alternative to the other expensive techniques of creating hypoallergenic, BLG-free milk (Wei et al., 2018).

The disruption of the LGB gene, which encodes BLG, has been approached by the introduction of site-specific indels (insertion or deletion). These can be generated by NHEJ repair, triggered by a double-strand break that is introduced with a programmable nuclease. Wei et. al (2018), generated free of off-target mutations via zygotic co-injection of TALENs and an HDR-template. Theoretically, this type of mutation could also appear in nature. This strategy minimizes the potential residual risk due to the application of genome editing. Thus, the cattle edited to produce BLG-free milk should provide a valuable line of cattle for the safe production of hypoallergenic milk without the introduction of genes from other species (Wei et al., 2018).

On the other hand, Sun et. al (2018), used a different approach. The researchers created DNA-free BLG bi-allelic knockout cow by ZFNs mRNA and produced BLG-free milk without any off-target events and the trait could be transmitted through the germline by breeding. Therefore, this leads to new possibilities of adjusting milk composition to make it more suitable for human health, while also improving the properties of milk (Sun et al., 2018).

5.1.2 Chicken strain without allergenic components from eggs

Allergy to chicken eggs is a very common condition affecting up to 2.5% of children and is the second most common food allergy (Reardon, 2016). This presents a huge food safety issue because eggs are used in such a huge range of food products. Besides that, the widespread use of egg-based flu vaccines leads to additional risks (Doran et al., 2017).

Egg allergy is caused by four proteins that comprise the egg white: ovomucoid (OVM), ovalbumin (Ova), ovotransferrin, and lysozyme. It is the OVM that is the most

allergenic of the four proteins. Thus, the absence of a function for OVM together with its low abundance may allow for the successful targeted deletion of the OVM gene in layer hens (Doran et al., 2017).

Gene disruption like ovalbumin (OVA) and ovomucoid (OVM) has the potential to reduce the allergenicity in eggs, thereby reducing immune responses in individuals sensitive to items such as egg white–containing food products, as well as vaccines. To produce these genetically modified chickens, it is necessary to develop efficient technologies, because the genetic modification of chickens is harder than in other organisms due to the difficulty to access and manipulate the zygote. Nowadays, primordial germ cells (PGCs) have been used, because they can be cultured and genetically modified "in vitro", by taking advantage of their germline competency after injection into recipient embryos (Doran et al., 2017).

Oishi et. al (2016), targeted the gene of OVM in the chicken using the CRISPR-Cas9 system. CRISPR-Cas9-mediated mutagenesis in chickens was used to disrupt the OVM. Subsequently, the OVM-homozygous offspring mutants were produced by crossing the chicken mutants, thus generating a chicken strain with low allergenicity. In summary, CRISPR-Cas9 opens the possibility of producing allergen-free eggs to eliminate food safety issues associated with egg allergies and to also improve the safety of vaccines that are grown in chicken eggs (Oishi et al., 2016).

5.2 Genome editing in farmed animals - Food Efficiency

As was mentioned before, the global population is now 6.8 billion and is estimated to reach over 9 billion by 2050. The world's population is expanding also undergoing rapid development, and with this comes increasing demand for protein rich foods, especially meat and dairy products. In this way, without any change, food production will continue to be a motor problem to environment sustainability and contributing to climate change and destruction of biodiversity. Eventhough, according to FAO data the global food production needed to increase (Buttriss, 2011).

While many farmed animals have undergone the process of domestication, managed selective breeding programs have resulted in motor improvements in

productivity. Genetic improvement could result in faster, cheaper, healthier, and more-efficient animal production, with reduced impact on the environment. The current genome-editing technologies may offer new opportunities for genetic improvement in order to achieve desirable traits faster, and increase the production efficiency (Tait-Burkard et al., 2018).

5.2.1 Myostatin Knock-Out: Super-muscly Pigs

Myostatin (GDF-8) is a dominant inhibitor of skeletal muscle development and growth. Mutations in myostatin gene can lead to muscle hypertrophy or even double-muscled (DM). Thus, the myostatin gene was a target for gene editing in farmed animal species because the disruption of this single gene has significant effects on a trait of economic importance. Until now, the farmed animals in which the myostatin gene has been edited are cattle, sheep, goat, and channel catfish. However, the pig myostatin gene became the most frequently targeted one, due to pork being the biggest source of meat by weight, and/or due to the lack of natural disruptive mutations detected in this gene to date (Tait-Burkard et al., 2018).

Qian et. al, (2015), to study the influences of MSTN mutations on skeletal muscle growth in pigs, created MSTN-mutant Meishan pigs with no marker gene via zinc finger nucleases (ZFN) technology. The results were that the MSTN-mutant pigs developed and grew normally and had increased muscle mass with decreased fat accumulation when compared with wild type pigs. Besides that, in this study, it has been demonstrated that 20% MSTN-mutant pigs had one extra thoracic vertebra. In other words, MSTN-mutant pigs developed and grew normally, had increased growth performance after 6 months of age with dramatic skeletal muscle mass, producing a significant amount of lean tissue, while also exhibiting less body fat. All of these are important economic traits in agriculture that can increase meat economic value.

Although the animals were generated by genetic manipulations by somatic cell nuclear transfer, the MSTN-mutant pigs do not have any foreign DNA and associated proteins, as a result of DNA sequence deletion by ZFN. In addition, they were examined

to see if ZFN plasmid DNA had integrated into the porcine genome and no integration of ZFN plasmid was detected in the DNA from all piglets tested (Qian et al., 2015).

Aa a result, MSTN-mutant pigs offer a way of fast genetic improvement of lean meat for local fat type indigenous pig breeds. So, highly lean meat yield combined with low body fat has long been one of the ultimate goals in the breeding strategy of the livestock industry (Qian et al., 2015).

5.2.2 FGF5 knockout in sheep

The wool industry plays an important role in the global agricultural economy and the most important quality trait in sheep breeding is wool length. Fibroblast growth factor 5 (FGF5) is the main inhibitor of the length that acts during the first phase of the cycle of hair follicles, the anagen inhibits the follicle growth (Hu et al., 2017).

Hu et al. (2017), generated FGF5 knockout sheep using the CRISPR-Cas9 system. The results show that the FGF5 knockout sheep showed longer wool length when compared with wild-type sheep. Besides that, these results further confirmed that the loss-of-function mutation in FGF5 has positive effects on wool length and follicle activation. In this way, this approach could provide an efficient way for the genetic improvement of sheep breeding and, consequently, could promote the development of the wool industry (Hu et al., 2017).

5.3 Genome editing in farmed animals - Animal Welfare and Disease Resistance

Animal welfare is a complex to define due to its scientific, ethical, economic, cultural, social, religious and political dimensions. However, the World Organization for Animal Health (OIE) defines animal welfare as: “freedom from hunger, malnutrition and thirst; freedom from fear and distress; freedom from heat stress or physical discomfort; freedom from pain, injury and disease; and freedom to express normal patterns of behaviour (OIE, 2018)”. In animal agriculture, there are different welfare problems related with current practices, for instance, the intensive confinement, where the natural behaviors are severely restricted by limited space and/or high stocking densities for an extended period of time resulting in the frustration of innate desires as well as in aggressive behaviours between animals. Besides that, many routine agricultural procedures used to

mitigate some of the consequences of intensive confinement, such as dehorning of cattles or castration of pigs that are often performed without the use of painkillers, resulting in both immediate and often chronic pains. Moreover, might facilitate the spread of infectious disease due to the close proximity to other livestock, jeopardizing the human and animal health (Shriver and Mcconnachie, 2018).

Therefore, there is increasing interest in the new molecular tools that might be used to reduce the negative impacts of farming on animals, in order to improve their quality of life, focus on more-efficient farming practices and animal and human welfare. Genome editing has been used in farmed animals with the main aim of reducing the suffering or pain during some painful procedures that animals have to undergo in order to live all together in confined spaces.

5.3.1 Animal Welfare

5.3.1.1 Dehorning cattle

Genetic dehorning of cattle is a potential candidate for the application of genome editing of farm animals on a large scale. The procedure of dehorning cattle is a good example of a labor-intensive procedure that could benefit from genome editing (Carroll et al., 2016). In modern dairy and beef farms, dehorning is frequently used to prevent injuries to animals and humans, especially in overcrowded housing systems. Today, more than 80% of European dairy cattle are dehorned, in most cases without pain relief medication (Eriksson et al., 2018).

Dairy cows, which come from the Holstein breed, naturally grow horns and horns are often physically removed in farms because they can pose a threat to other cows, as well as to the farm workers handling the cattle. So, in order to prevent injury to the animals and to the workers, are removed the horns from calves using physical procedures, such as disbudding and heat cauterization, which is a very painful and invasive procedure to cows, and clearly constituted an animal welfare issue (Carroll et al., 2016). From an economic perspective, it is also a time-consuming and expensive procedure (Carlson et al., 2016).

Genetic analyses have identified variants that are associated with ‘hornlessness’ (referred to as ‘polled’) in cattle, a trait that is common in beef but rare in dairy breeds. The

dominant POLLED locus is nearly fixed in beef cattle such as Angus, so, they don't grow horns (Carlson et al., 2016).

In 2016, a Minnesota-based company, Recombinetics, reported using TALENs to genetically edit the PC allele into the genome of bovine embryo fibroblasts. Two cloned dairy bull calves with the polled phenotype were successfully born. Whole-genome sequencing to an average coverage of 20x did not identify any off-target insertions of the PC allele nor any insertion-deletions attributable to unexpected cleavage by TALENs and repair by NHEJ. They thus created a hornless strain of dairy (Holstein) cattle via HDR and somatic cloning, that could reduce the frequency of the dehorning procedure in the dairy industry, potentially enhancing the welfare of cattle since it could reduce the risk of injury, competition for feeding and space (Carlson et al., 2016).

5.3.1.2 Surgical castration of pigs

Surgical castration of pigs is a common practice in pork production in order to reduce the occurrence of boar taint, a smelly odor and flavor that appears when cooking and eating meat derived from some entire male pigs. The castration is a procedure that is related to animal welfare due to the fact that it avoids the expression of mounting and aggressive behaviour observed among male pigs. On the other hand, the castration is a very painful procedure for piglets, both during and after the surgery.

Sonstegard et. al (2017), as an alternative, created pigs by using TALEN, through a knock-out of the KISS1R gene, that a encodes a receptor for the onset of puberty in vertebrates which is involved in the regulation of the gonadotropin-releasing hormone. Pigs with the knock-out in KISS1R gene showed a lack of testicular development but reacted to hormone treatment, which increased testicular size. However, further experiments are necessary to see whether the animals can become fertile and whether growth properties are affected. Overall, this experience seems to show that genome editing could be used to benefit animal welfare by eliminating stressful management practices in a single generation (Sonstegard et al., 2017).

5.3.2 Disease Resistance

The potential of genome editing to increase animal health and welfare by making animals resistant to diseases is huge. Zoonotic diseases, as mentioned before, represent the majority of recently emerging infectious diseases in humans, for instance, the present COVID-19 pandemic caused by the Coronavirus (SARS-CoV-2) that the world is going through. Livestock are indeed, even before the COVID19 pandemic, an important source of many infection pathways to humans with a huge impact on public health, economic well-being as well as animal's welfare. Moreover, due to the new methods of animal production, the animal-human interface has brought about changes both in agriculture and the environment, with huge implications for zoonotic diseases and biosecurity (Graham et al., 2008). As so, we are going to discuss here some livestock zoonotic diseases and how genome editing could be a solution, to make them more resistant to these diseases.

5.3.2.1 Avian Influenza

One of the biggest outbreaks has been the avian influenza. Influenza type A viruses pose a significant risk to public health due to their capacity to cause an influenza pandemic (WHO, 2018).

The first human infections with the HPAI A(H5N1) virus were reported during an outbreak in poultry in Hong Kong in 1997. After that, the avian virus has spread since 2003 to different continents, from Asia to Europe and Africa, and it has consequently become an endemic disease in poultry populations in some countries. Outbreaks have huge consequences, such as poultry infections, several hundred human cases, and many human deaths (WHO, 2018). The virus has spread in the poultry population across the country. The huge diversity of IAVs helps them adapt to different avian and mammalian host species, including humans. In recent years, new AIVs has emerged and evolved independently of cross animal/human interface, causing mild to fatal complications in humans (Mostafa et al., 2018).

Long et al., (2019), demonstrate that the avian influenza virus cannot replicate in chicken cells without ANP32A, a cell protein. They believe that by making a few small changes to the ANP32A gene in chickens, it might be possible to generate a gene-edited chicken that is resistant to influenza. Therefore, making chickens resilient to influenza would prevent the outbreaks as well as improve the welfare of the chickens.

Long et al., (2019) in this study, used CRISPR-Cas9 to knockout the entire protein in chicken cells. The precise deletions were confirmed by Sanger sequence analysis of subcloned PCR products from genomic DNA, and both were found to be homozygous at both alleles.

The results of this research raise the possibility of producing gene-edited chickens that are resistant to the disease, which will in turn have a huge impact in the world, preventing influenza outbreaks. The genome-editing approach used in this research, the CRISPR-Cas9, is an important advancement, implying that it may be possible to use gene-editing techniques to produce chickens that are resistant to bird flu. The difference in this technology, however, is that it does not involve introducing new genetic material into the bird's DNA.

5.3.2.2 Porcine Reproductive and Respiratory Syndrome Virus

The agent that leads to porcine reproductive and respiratory syndrome (PRRS) is the PRRS virus (PRRSV), which belongs to the Arteriviridae family. This virus causes the most economically devastating disease, affecting the swine industry worldwide due to the reduction or loss of pregnancies, death in young piglets, and decreased growth rates in all PRRSV infected pigs. PRRS is, therefore, a disease that has a huge impact on welfare, production efficiency, performance as well as consumer protection (Reiner, 2016).

PRRSV has a cell tropism, limited only to cells of the monocyte/macrophage lineage. The CD163 protein is expressed with higher levels on the surface of specific macrophage. This protein has been described as a fusion receptor for PRRSV, with the scavenger receptor cysteine-rich domain 5 (SRCR5) region that have been demonstrated to be the interaction site for the virus (Burkard et al., 2018).

Burkard et. al (2018), generated pigs whose CD163 gene has been deleted, knocked out by a zygote injection of CRISPR-Cas9 editing. These pigs exhibited CD163 protein lacking SRCR5 (ΔSRCR5 CD163) but they didn’t demonstrate any adverse side effects while they were kept under standard husbandry conditions. In comparison with the wild-type control group, ΔSRCR5 pigs showed no signs of infection and no viremia or antibody response indicative of a productive infection. This shows that ΔSRCR5 pigs are fully resistant to infection by the virus.

In other words, the resulting animals were completely resistant to PRRSV infections, healthy under standard husbandry conditions and capable of keeping the biological function of the CD163 protein while being resistant to the PRRSV infection. Therefore, the creation of ΔSRCR5 pigs could have a huge impact on the pork industry worldwide, as it can improve both animal welfare and productivity. Besides that, PRRSV-resistant animals could decrease the need for antimicrobial use (Burkard et al., 2018).

5.3.2.3 African Swine Fever (ASF)

African swine fever (ASF) is endemic in sub-Saharan Africa. It later became endemic in Sardinia for several decades (OIE, 2018). This disease is caused by the African swine fever virus, a large DNA virus of the Asfarviridae family that leads to a contagious haemorrhagic viral disease of domestic and wild pigs, and, therefore, is responsible for serious economic and production losses as well as a huge impact on animal welfare (OIE, 2018).

The domestic pig is highly susceptible to infections by the African Swine Fever Virus, but in contrast, the present-day pig species found in Africa aren't, which are wildlife hosts of the virus, do not succumb to the lethal effects of infection. Differences in three amino acids between warthog (*Phacochoerus africanus*) and domestic pig (*Sus scrofa*) within the *RELA* gene, which encodes a major component of the NF-κB transcription factor that plays a key role in regulating the immune response to infections. In the later stages of an ASF infection, i.e. liberation of cytokine, an overreaction of the immune system takes place, which is thought to strongly contribute to the lethal outcome of the disease. During infection, ASFV targets the host's NF-κB transcription factor. The viral protein A238L shares homology with porcine IκBα and can substitute for this porcine protein, binding to the RELA (p65) subunit of NF-κB and reducing its ability to be activated. In this way, the differences in the warthog of this central regulator of innate and adaptive immune responses may represent a host adaptation that contributes to the lack of haemorrhagic fever in warthogs that is seen in domestic pigs (Lillico et al., 2016).

Lillico et al., (2016), by HDR of a ZFN-induced break using a plasmid repair template, producing both heterozygous and homozygous live piglets for the desired haplotype. Thus, ZFN in embryo editing of the RELA gene produced domestic pigs with the warthog RELA orthologue, which is associated with resilience to African Swine Fever.

5.3.2.4 Porcine epidemic diarrhea virus/transmissible gastroenteritis virus

Respiratory and enteric infections caused by coronaviruses have important impact on both human and animal health. The gastroenteritis virus (TGEV) or porcine epidemic diarrhea virus (PEDV) causes an infection of immunologically new-born pigs, which leads to a huge loss approaching a 100% mortality rate, caused by mal-absorptive diarrhea and dehydration caused by the destruction of infected enterocytes (Whitworth et al., 2019).

PEDV and TGEV belong to the genus *Alphacoronavirus* in the family Coronaviridae (Whitworth et al., 2019). The relevance of amino peptidase N (ANPEP) as a putative receptor for TGEV and PEDV in pigs was evaluated by using CRISPR- Cas9 in order to edit ANPEP, resulting in a premature stop codon. Cells were, therefore, created with a null ANPEP gene via CRISPR-Cas9. ANPEP null pigs did not support infection with TGEV but maintained their susceptibility to infection by PEDV. Immunohistochemistry confirmed the presence of PEDV reactivity and absence of TGEV in ANPEP null pigs. Therefore, the use of genome editing to generate pigs lacking aminopeptidase N successfully showed that pigs resistant to TGEV infection could be generated. However, the edited animals remained susceptible to PEDV infections (Whitworth et al., 2019).

5.3.2.5 Avian leukosis viruses (ALVs)

Avian leukosis viruses (ALVs) belong to a major group of oncogenic viruses that cause enormous economic losses to the poultry industry due to this virus resulting in inappetence, diarrhea, weight loss, a reduction in eggs laid, while it can also cause tumour formation in the chicken (Lee et al., 2017).

The fast spread of these viruses through both horizontal and vertical transmission infected poultry flocks makes it hard to achieve full disease control (Lee et al., 2017).

ALV-J to replicate depends on a functional cellular receptor, the chicken chNHE1. Tryptophan residue number 38 of chNHE1 (W38) in the extracellular portion of this molecule is a critical amino acid for virus entry. Lee et al., (2017), used CRISPR-Cas9 genome editing approaches to modify the critical residues of the chNHE1 receptor in

chicken cells in order to induce resistance to ALV-J infections, since the virus depends on this receptor to replicate and consequently, infect the host.

5.3.2.6 Bovine tuberculosis (bTB)

Bovine tuberculosis (bTB) is a disease of animals caused by members of the *Mycobacterium tuberculosis* complex primarily by *M. bovis*, but also by *M. caprae* and to a lesser extent by *M. tuberculosis*. It is the biggest infectious disease among cattle, which also affects other domesticated animals and certain wildlife populations, causing a general state of illness, pneumonia, weight loss, and eventual death. However, cattle are considered to be the main carrier of *M. bovis*, and are the main source of infection for humans (OIE, 2020).

The most common form of TB in humans is caused by *M. tuberculosis* and is naturally resistant to one of the antimicrobials that is commonly used to treat human tuberculosis, such as pyrazinamide, therefore representing a huge public health risk (OIE, 2020).

The protein NRAMP1 has functions as part of the innate immune response because it inhibits the proliferation of *Mycobacterium tuberculosis* through its involvement in acidification of phagosomes (Cheng, Huang, and PHD, 2015). Gao et. al (2017), introduced the *NRAMP1* gene into the genome of bovine foetal fibroblasts using the CRISPR-Cas9 technology. In the end, the genetic analysis of the calves shows that *NRAMP1* had successfully integrated into the genome at the targeted region in all of the calves. None of the calves that had the gene inserted using the new CRISPR-Cas9n technology had any detectable off target effects, whereas off-target effects have been present when the genome editing technology, other than CRISPR-CAS9 were used (Gao et al., 2017).

5.3.2.7 Mannheimia (Pasteurella) haemolytica

Mannheimia (Pasteurella) haemolytica is a commensal bacteria commonly found in the nasopharynx of cattle and other ruminants. Together with active viral infections and stress factors, it reaches the lungs, multiplies, and causes a fibrinonecrotic pleuropneumonia. The leukotoxin (Lkt) is the most critical virulence factor, secreted

by *Mannheimia* (*Pasteurella*) *haemolytica,* that is specific for ruminant leukocytes. which causes induction of respiratory disease and death in calves (Shanthalingam et al., 2016).

The leukotoxin binds to the signal peptide of ruminant CD18, which, contrary to its paradigmatic cleavage in nonruminants, remains uncleaved and intact on mature CD18 molecules expressed on the cell surface of ruminant leukocytes. So, the CD18 in ruminants is not cleaved. This happens because the signal peptide sequence of ruminants CD18 contains a cleavage-inhibiting glutamine (Q), while the nonruminants contains cleavage-inducing glycine (G). In this way, the signal peptide of ruminant CD18, the β subunit of β2 integrins, is not cleaved and hence remains intact on mature CD18 molecules expressed on the surface of ruminant leukocytes. Leukotoxin secreted by Mannheimia (Pasteurella) haemolytica binds to the intact signal peptide and causes cytolysis of ruminant leukocytes, resulting in acute inflammation and lung tissue damage (Shanthalingam et al., 2016).

Shanthalingam et. al (2016), used the genome editing approach ZFN, in order to alter the cleavage-inhibiting glutamine (Q), at amino acid position 5 upstream of the signal peptide cleavage site, with cleavage-inducing glycine (G), resulting in the cleavage of the signal peptide and annulment of leukotoxin-induced cytolysis of target cells. Thus, the gene-edited fibroblasts were used for somatic nuclear transfer and cloning to produce a bovine fetus homozygous for the Q(–5)G substitution and the results seems to show the cleavage of the signal peptide and abrogation of leukotoxin-induced cytolysis of target cells and the leukocytes were absolutely resistant to leukotoxin-induced cytolysis (Shanthalingam et al., 2016)

The development of a technology with the potential to create cattle that are naturally resistant to *M. haemolytica* leukotoxin represents a very significant improvement toward control of this economically devastating disease (Shanthalingam et al., 2016).

5.4 Conventional breeding VS Genome Editing

For thousands of years, humans have been using traditional modification methods like selective breeding and cross-breeding to breed animals with more desirable traits. Different types of breeding methods across different animal breeding programs have been used in order to improve the genetic merit of domesticated animals and, in this way, increase their productivity. Therefore, during early domestication, large livestock animals

were first genetically modified using conventional breeding and selection to produce improved livestock (Bhat et al., 2017).

However, changing animals through traditional breeding can take a long time, and it is difficult to make very specific changes so, the efficiency of such method depends on identification of high merit individuals, selection intensity, generation time, and continued genetic diversity or its conversion into short-term and long-term genetic gains (Bhat et al., 2017).

Later, in the 1970s, genetic engineering makes technology able similar changes but in a more specific way and in a shorter amount of time. Genetically modified organisms (GMOs), described as an organism that carries genetic information or "gene" that confers a desired trait, that was copied from the organism that initially carried the trait, inserting that information into the DNA of another organism (Bhat et al., 2017).

The recently developed nuclease-mediated genome editing technology improves the opportunities for making genetic modifications in livestock in an easier and cheaper way. Genome editing technologies have thus entered a new era of genome editing, through which any change in the genome can be made with precision and specificity. Besides that, it allows intensification in the frequency of desirable alleles in an animal population through gene-edited individuals, which happens faster than conventional breeding. Furthermore, precision editing in the endogenous genome, without introducing foreign DNA, could become a new breeding technology to produce genetically modified organisms for human consumption, since during the genome editing process are not introduced genes that have origin in other species (Bhat et al., 2017).

In other words, what distinguishes the genome editing and selective breeding is that selective breeding selects based the characteristics of an organism exhibited in a particular environment, i.e. the phenotype, and optimize them. On the other hand, in genome editing, specific genes are modified with the objective of obtaining organisms with a particular phenotype. Unlike the process of selective breeding, in which only genes in the initial organisms can be exchanged, in genome editing novel genes can be introduced (The Royal Society, 2016).

Eventhough in genome editing, there is the risk of causing unexpected effects, and it can suffer from its own technical complications. In gene editing, one of the main risks is that gene editing can be imprecise and thus create genetic errors by causing "off-target"

and “on-targeted” effects, meaning unintentional changes to other genes or the same, which could be harmful to animal health or welfare (Zhang et al., 2015).

Applications of gene editing in mammals depend on the use of some techniques to reproduce gene edited animals like the Somatic Cell nuclear Transfer (SCNT), Direct Editing of Zygotes, Spermatogonial Stem Cells, Primordial Germ Cells (PGCs) and Cytoplasmatic Injection (CPI). However, the SCNT has been the major technique for delivering nuclease-mediated genetic alterations in livestock and the technique involves the generation of somatic cells carrying the intended genetic modification and using these cells as donors in cloning experiments. The resulting offsprings are all clones of the original donor cell line. However, SCNT suffers from huge drawbacks, such as the impact on welfare and health; like failed pregnancies, stillbirth, deformities and early deaths in animals. On the other hand, as an alternative to SCNT, the cytoplasmic injection (CPI) has somewhat lower failure rates, though it can still raise animal welfare issues regarding failure rates, and cause additional problems, since the desired trait is not successfully expressed in all offspring or in all cells of an offspring, a phenomenon known as mosaicism (Whitelaw et al., 2016). Mosaicism refers to the presence of a genetically distinct cell population within an organism and can occur in both somatic cells and germline cells. Furthermore, mosaicism can cause changes in either nuclear or mitochondrial DNA. The phenotypes associated with mosaicism depend on the extent of the mosaic cell population, which also has important consequences with respect to animal disease (Chial, H, 2008).

As previously mentioned in subchapter 5.3.1.1, the University of Minnesota and Recombinetics has used genome editing and reproductive cloning to create hornless dairy cows, and Carlson et al. (2016) did not report any off-target effects in the cows. However, later in 2019, the US Food and Drug Administration (FDA) ran the available whole genome sequencing data from Carlson et al. (2016) through new DNA screening software and they found the entire plasmid, as well as a second copy of the repair template and the plasmid backbone, which were integrated into the target location of both calves. The FDA study further shows that plasmid integration including genes conferring antibiotic resistance (Regalado, 2019).

One common concern about genome editing involves the creation of disease resistant animals. These concerns are crucial in situations where varied strains exist and novel strains of the virus can arise with high mutation rates, such as the porcine

reproductive and respiratory syndrome (PRRS) in China. Animals resistant to disease could lead to pathogens evolving to become more virulent. Another risk is that even if mutated pathogen genes are introduced into an animal to make it resistant to the pathogen, those genes might recombine with wild pathogens to create a more resistant pathogen. Therefore, creating disease resistant animals could lead to the creation of a 'silent reservoir' of disease while the pathogen becomes more virulent in the environment. (Reiner, 2016).

The "Super-muscly Pigs" were created to increase muscle growth through the knockout of the myostatin gene (MSTN), which inhibits the growth of muscle cells. Several problems were observed, such as birthing difficulties due to large offspring size, enlarged tongues and severe health problems (Cotter and Perls, 2018).

On the other hand, it has been stated by Graeff et al., 2019, a genome editing offers fewer sources of risk than conventional genetic engineering, since it leaves no trace of the nuclease after it has achieved its function and it does not need to involve the introduction of bacterial and viral DNA as part of the delivery mechanism.

However, due to their novel features, as well as our incomplete knowledge and understanding of the genetic background of complex traits, the risks or consequences of genome editing technologies could be hard or even impossible to estimate (Graeff et al., 2019). However, the latest genome editing technologies have improved and quantified specificity, reducing off-target effects, and concerns. The toolkit of CRISPR-Cas reagents available, includes base editors that can edit single nucleotides in the genome without the need to induce DSBs, with a risk of off-target effect substantially lower than the frequency of spontaneous mutations naturally occurring in animal genomes (McFarlane et al., 2019).

6. Legal, Ethical and Social implications

6.1 Legal considerations

The Convention on Biological Diversity was finished in Nairobi in May 1992 and opened for signature at the United Nations Conference on Environment and Development (UNCED) in Rio de Janeiro on the 5 June 1992. One year later, it came into force on the 29th of December, 1993. One of the main questions approached by the Convention was

Biosafety, which is defined as the necessity to protect human health and the environment from the consequences that result from the products of modern biotechnology (Secretariat of the Convention on Biological Diversity, 2000).

On January 29, 2000 has been adopted the Cartagena Protocol on Biosafety. The Cartagena Protocol helps to ensure protection during the transfer, while also dealing with the use of living modified organisms resulting from modern biotechnology that may have negative effects on the conservation and sustainable use of biological diversity. Besides that, the Protocol tries to reduce the risks to human health, and mainly transboundary movements (Secretariat of the Convention on Biological Diversity, 2000).

Each member that signer the Protocol should adopt the necessary and appropriate legal, administrative, and other measures in order to implement the rules of the Protocol. Besides that, each member should ensure that the development, handling, transportation, use, transfer and release of any LMO ("living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology"), is carried out in a safe way, preventing the possible risks to biological diversity and human health (Secretariat of the Convention on Biological Diversity, 2000).

The European Union, as member of the Cartagena Protocol on Biosafety, states that the main aim of the regulatory framework for the release and placing on the market of GMOs is to guarantee the protection of human, animal and environmental health, as well as to ensure the good state of the EU internal market (Bruetschy, 2019). The EU defines a genetically modified organism (GMO) by the Directive 2001/18/EC in Article 2, as "an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination", i.e, any organism that was altered through other techniques and not natural breeding or by natural recombination, is considered to be GMO (Bruetschy, 2019).

The authorization of deliberate releases (including experimental releases) and the placing of GMOs in the market is under the Directive 2001/18/EC. On the other hand, the Regulation (EC) No 1829/ 2003 gives a specific authorization procedure in the case of GM food and feed (Bruetschy, 2019).

In 2001, when the Directive 2001/18/EC was adopted, gene editing technologies had not yet been used on agricultural organisms; there were just GMOs created by transgenesis. Gene editing techniques create specific changes at targeted locations in the genome,

leading to point mutations, targeted deletion or insertion of a gene. It could be parts of a gene, or a set of other functional DNA sequences. It is due to their precision that these gene editing techniques produce less unintended effects when compared to random mutagenesis techniques. However, this type of new technologies is not currently under a regulation (European Commission, 2018).

Thus, the increasing use of such techniques in the development of agricultural products raised questions about the applicability of the GMO legislation, and whether it is suitable for this kind of approach, and started to get the attention of stakeholders and the general public (Bruetschy, 2019). However, as these new techniques involve genome editing, there's no insertion of foreign genetic materials into cells; it only creates targeted point mutations in a gene. The question raised of whether such methods constitute a genetic modification and fall under the mutagenesis exemption has been controversial in Europe (Rehbinder, 2018). Thus, the French Conseil d'Etat asked the Court of Justice of the European Union (CJEU) to give a legal explanation on the legal status of genome edited products (Bruetschy, 2019). On 25th July, 2018, the Court of Justice of the European Union decided that all the organisms obtained via the new techniques of directed mutagenesis are considered genetically modified organisms (GMOs). Because of that, these organisms are covered by the Directive 2001/18/EC on the release of genetically modified organisms into the environment ('GMO Directive') and consequently, they are under the obligations established by the GMO Directive (SAM, 2018).

Also, the court opinion was that by Directive 2001/18 EC, all techniques and methods that artificially alter the genetic material of plants or animals are considered a genetic modification and, therefore are covered by the Directive 2001/18 EC. Besides that, the Court established that direct mutagenesis (like genome editing) might have the same consequences as well as the same irreversible effects on the environment as the insertion of foreign genetic material. As a result, this kind of technology should be subject to a robust risk assessment and must be authorized. In addition, the products obtained by genome editing may be subject to labelling, authorisation and traceability requirements, like GMOs. The Court only limited the exemption to conventional techniques and methods of random mutagenesis that had been used in a number of applications and had a significant safety record, such as chemical and radiation mutagenesis techniques (Rehbinder, 2018).

The European Commission's Scientific Advice Mechanism (SAM) is a group of chief scientific advisors that provides the EU executive with independent advice and they made

a statement about the “Regulatory Status of Products Derived from Gene Editing and the Implications for the GMO Directive”. About the safety considerations, they stated that the alterations that are introduced by random mutagenesis are more harmful than those resulting from gene editing techniques, and because unintended effects occur fewer times in gene edited products, they are potentially safer than the products by random mutagenesis (SAM, 2018).

The GMO Directive takes into account the technology used in genetic engineering and the final product. However, in scientific terms what is more important is whether the products have a long safety record, rather than the techniques used to generate them (SAM, 2018).

The capacity of gene editing to precisely introduce mutations identical to those originating in a spontaneously way or by random mutagenesis raises another concern, which is the detection of gene edited products. For instance, in the case of point mutations (mutation affecting only one nucleotide in a DNA sequence) it is hard and sometimes impossible to provide a detection method for gene edited products that will meet regulatory requirements. So, there can be no analytical approach for detecting and quantifying all possible gene edited products (SAM, 2018). As a result, it cannot be excluded that products obtained by directed mutagenesis may enter the European market undetected. It is very difficult to distinguish if the mutation has occurred in a spontaneously way or if was introduced by human intervention. It is very hard to even attribute them to a specific technique, such as random mutagenesis or directed mutagenesis, especially given the fact that in some cases the final product will be identical to that generated by other procedures. Consequently, the inability of distinguishing between spontaneously occurring mutations and different types of human interventions is a major hurdle from a regulatory point of view (SAM, 2018).

In addition, there are huge consequences for European citizens, consumers, and farmers. In legal terms, products of gene editing can be authorized in the EU according to the GMO Directive. The obligations of the GMO Directive imply cost-intensive and labour-intensive pre-market evaluations, as well as a long time for the approval process. This can be difficult and onerous to small and medium enterprises. Because of that, incentives for investment may be diminished, negatively impacting research and innovation in this field, and limiting the commercialization of gene edited products in Europe, leaving Europe at a huge disadvantage (SAM, 2018).

Besides that, the obligations imposed by the GMO Directive on traceability and labelling of GMOs entering the European market will be very difficult to implement and control due to issues related to the detection, identification and quantification of gene edited products, which will be worse when exporting countries start to market varieties that they have already decided not to regulate (SAM, 2018).

Furthermore, many consumers have reacted negatively to previous agricultural applications derived from biotechnology, particularly in the European Union (EU). The debates over GM foods focus mostly on uncertainties concerning the potential adverse effects of GM foods on human health as toxicity, allergenicity and genetic hazards in food as well as environmental safety. Although, CRISPR gene-editing is different from the genetic modification technologies, consumers may not distinguish them from the GMOs and in this way, a lack of public acceptance might stop further development of CRISPR food right before commercialization can become a reality (Shew et al., 2018).

As a result, SAM recommended revising the existing GMO directive to "reflect current knowledge and scientific evidence, particularly on gene editing and established techniques of genetic modification" (SAM, 2018). Moreover, Carlos Moedas, Commissioner for Research, Science, and Innovation, in agreement with SAM, said "Gene-editing is a critical technology with an enormous potential to improve human health and preserve the environment. I, therefore, welcome the statement from our Chief Scientific Advisors which will contribute to a well-informed debate on the regulatory framework needed to maintain high levels of protection while enabling innovations that contribute to the environment and wellbeing. Their statement also provides valuable input into our reflections on future proofing regulation so that our laws can keep up with our labs" (European Commission, 2018).

As a result, the European Group on Ethics in Science and New Technologies (EGE), held a roundtable on the ethical aspects of gene editing. The main aim was to publish an opinion on the ethics of gene editing in order to help clarify the current framework in EU and whether the regulation should be updated and in what sense (European Commission, 2018).

The regulatory framework about genome editing tends to be different in different countries all over the world. For instance, the regulatory framework is strict in New Zealand, like in the EU (Schmidt, Belisle, and Frommer, 2020). On the other hand,

countries such as Japan, Canada, Argentina, Brazil, Chile, and Australia seem to present a more permissive regulatory framework than the EU and New Zeeland. These countries are more proactive about the genome editing regulation and seem to gain a competitive advantage and higher international competitiveness (Schmidt, Belisle, and Frommer, 2020).

For instance, in Canada the organisms are not regulated as GMO unless the trait is identified as novel in Canada. According to the regulatory framework in Canada, all foods that are genetically altered, even through conventional breeding, are classified as 'novel foods' without distinction. So, the product classification is based on the final product itself, not on the process that gives origin to the trait, and thus the regulatory focus is the novel final product and not the method (Schmidt, Belisle, and Frommer, 2020).

In the case of Argentina, the biosafety authorities only make decisions on a case-by-case basis and will only regulate a new product as GMO if it contains "Novel combination of genetic material". The term "Novel combination of genetic material" comes from the definition in the Cartagena Protocol from "Living modified organism", previously described. The Argentinean regulation delegates to the biosafety commission the function to determine, for each case, if it should be considered GMO or not. This determination is carried out by evaluating the changes present in the genome intended to be introduced in the market, i.e. the final process of the breeding process. Nowadays the Argentinean regulation is being updated to apply the same criteria to gene edited animals and microorganisms. So, if no foreign DNA is detected, then the product is not regulated as GMO. Colombia, Brazil, and Chile are adopting similar regulation (Schmidt, Belisle, and Frommer, 2020).

Countries such as Switzerland, Norway, and the UK are considering new laws to facilitate the approval of products from genome editing. The discussion about the regulatory framework to genome editing is still ongoing (Schmidt, Belisle, and Frommer, 2020). In Australia if there is no foreign DNA introduced deriving from a different species, then the product is not regulated as GMO (Schmidt, Belisle, and Frommer, 2020).

In the USA, the FDA regulates animals with intentional genomic alterations developed through the use of genome editing technologies (IGAs) in animals under its FD&C Act authority. However, these animals themselves are not drugs. The agency regulates IGAs that are introduced into the animals' genomes as new animal drugs because the IGA is

intended to affect the structure or function of the altered animal. When the IGAs are approved, the FDA determines the environmental impacts and whether they are significant. (FDA, 2020).

However, currently, there are no commercialized genetically engineered farm animals (e.g: pigs, cows, chickens) anywhere in the world to human consuption. The only genetically engineered animal for human consumption is the AquAdvantage salmon, which was approved by the FDA. This genetically modified salmon grows more compared to the usual market size and in half the time that conventional Atlantic salmons need. This salmon contains a growth hormone gene from the fast-growing Pacific Chinook salmon and a promoter sequence (a fragment of DNA) from the ocean pout. When combined, the salmon is enabled to grow all the year round instead of seasonally, which is the case with wild or farmed salmon (The Nuffield Council on Bioethics, 2019).

6.2 Ethics

The fast growth of these new breeding technologies demands for an ethical reflection that keeps the higher ethical standards but, at the same time, does not hinder scientific progress and innovation in this field. Thus, different questions are raised: (1) What kind of implication CRISPR-Cas9 brings to human health, environment security and animals? (2) What happens if these technologies fall in the "wrong hands"? (Moedas, 2018); (3) How will this new entity behave in a dynamic environment and what will its interaction be with other living beings and their own ecosystem? (The National Council of Ethics for the Life Sciences, 2011)

There are currently few practical orientations about how we should address and ensure the ethical rules. Therefore, it is fundamental to delve into an investigation about ethical implications on these new technologies and, most importantly, give answers to these questions (Moedas, 2018) because it is just as essential to ask the opposite: why restrict or prohibit this type of technology if, until now, the potential risks aren't totally known (CNECV and CBE, 2011).

Therefore, it is important to know whether this type of research is morally justified, and what position should be taken regarding the products and the respective procedures, taking

into account the potential risks that may arise and the current state of these technologies is a good scenario to keep some Ethical Principles in mind (CNECV and CBE, 2011).

6.2.1 Ethical Principles:

The question arises: the manipulation of the genome of animals is morally justified? And what position should be taken regarding the products and the respective procedures, in light of the potential risks that can bring. Thus, the following ethical principles applied to genome editing tries to provide a more adequate answer to possible risks as well as to anticipate them.

- *Precautionary Principle*: Human life is full of risks. With increased environmental impacts of growing populations and industrialization, the environment was no longer able to heal itself; it had to be helped in repairing the damage caused by human activities. The Precautionary Principle (PP), appeared from the emergence of increasingly unpredictable, uncertain, and unquantifiable but possibly catastrophic risks, that has confronted societies with the necessity to develop a line of action in order to protect humans and the environment against uncertain risks of human action. The emergence of the PP shifted from post damage control (civil liability as a curative tool) to the pre-damage control (anticipatory measures) of risks. The PP is an integral principle of sustainable development, that is development that meets the needs of the present without compromising the future generations. By protecting against serious and irreversible harm to the natural resource base that might jeopardize the capacity of future generations to provide for their own needs, it builds on ethical notions of intra- and inter-generational equity. The Biosafety Protocol is part of a wider-reaching movement towards giving this principle shape in the legal framework. However, in the literature and in international treaties and declarations, there are a variety of definitions, so there is no universal definition for the precautionary principle (Bourguignon, 2015). The definition in the EU is the following ‘The precautionary principle applies where scientific evidence is insufficient, inconclusive or uncertain and preliminary scientific evaluation indicates that there are reasonable grounds for concern that the potentially dangerous

effects on the environment, human, animal or plant health may be inconsistent with the high level of protection chosen by the EU' (COMEST, 2005).

New technologies could introduce new risks and, because of that, is crucial the application of the PP since it can help to stimulate both innovation and science, achieving a better balance between the benefits of innovations and their hazards. Therefore, the PP should stimulate innovations and technological progress as well as promotes the development of innovative alternatives for potentially risky technologies. Whether one considers that the health and integrity of ecosystems and the preservation of species is important for the well-being of humanity or because they have value in their own right, any potential harm from human activities that might jeopardize these is morally unacceptable. Deliberations on the PP should explicitly consider the negative impacts that human activities may have on nature, even if these impacts do not pose direct risks for humans (COMEST, 2005).

PP has been introduced to help the policy makers to take prudent decisions about products or activities that could be seriously harmful to public health and the environment. For that reason, does not offer a predetermined solution to every new problem raised by scientific uncertainty. In contrast, the PP is a guiding principle that provides helpful criteria for choosing the most reasonable course of action to deal with situations of potential risk (COMEST, 2005). Although, the PP has been considered in same national, EU and international laws, it's a success, from a legal point of view, far from clear. According to diverse definitions in these legal orders and case law applications, the principle can in fact be understood in a variety of ways.

The Precautionary Principle has as its aim to analyse the risks and benefits (Bourguignon, 2015). Risk means chance or possibility of loss or bad consequence. It refers to the possibility, of damage to health, environment and goods, in combination with the nature and magnitude of the damage. Risk is the possibility that an undesirable state of reality (adverse effects) may occur as a result of natural events or human activities (COMEST, 2005).

The regulation of risky activities, such as the introduction or implementation of new technologies, always involves some form of consideration of risks

and benefits. Considering the positive and negative effects of an activity is also important in the PP. The potential harm resulting from certain activities should always be judged in view of the potential benefits they offer (compare this to the proportionality criterion in the EU approach to the PP). Therefore, negative and positive effects should be always evaluated, in order to arrive at a balanced decision on complex ethical problems even if other factors should be considered (COMEST, 2005).

Cost and benefit analysis should always be interpreted with caution and together with other methods that may be better suited to deal with political, social and ethical issues that frequently characterize situations where the PP applies. These methods can include public and transparent debate on options, particularly when phenomena are difficult to quantify and values are at stake. Nevertheless, the European Commission states that there should also be an economic cost-benefit analysis, including socio-economic impact and non-economic considerations, such as impact on health (Bourguignon, 2015).

The debate about the risks and benefits is of most importance in genome editing technologies. As a result, researchers need to be careful and aware of their own role and responsibility to look for information on potential causes of harm. Application of the precautionary principle in the EU is limited to risk management in political decisions and has been seen as a rule that stops balanced decision-making, which may lead to prohibiting scientific development and practical applications of new techniques. Therefore, a balanced interpretative of the precautionary principle is necessary. PP, it should also be seen as a process that facilitates thorough reflection and not as blocked approach that would result in a over-regulation (Eriksson et al., 2018).

In other words, the inability to foresee all potential future consequences of the alterations made to genes makes it highly uncertain to determine whether the benefits will ultimately outweigh the risks, and whether these risks, will have any consequences on the environment, human and animal health. Here, the concept of “how safe is safe enough” is of the most importance.

This is a really difficult concept that depends on the different position regarding the introduction of new technologies.

For many, PP is a process to protect human health and the environment from complex hazards, and to promote the type of progress that is better for people and their environment. To others, it is considered as a barrier that seeks to stop innovation in Europe. The EU will have to depend on the external market for its own supply. Currently, the biggest biotechnology companies applied to agriculture do not carry out research activities in this area in Europe and, for this reason, the EU is falling behind in the area of research when compared to the rest of the world, since experimentation is strongly limited. With this strict positioning of the EU, it will be hard to have research carried out in this area by European companies or even by the public sector, because it involves high levels of investment and the potential target market is very limited (CNECV, 2010).

Biotechnology companies are therefore discouraged to invest time and money in order to obtain European authorization for their latest inventions, limiting their intervention for authorization that would allow them to enter the European market with the products produced by farmers in the rest of the world (CNECV, 2010).

Consequently, there will be European technological progress in other areas, such as health and industry, as opposed to progress in the agricultural sector (CNECV, 2010).

The following guiding ethical principles applied in genome editing are based on the Precautionary Principle.

- *The "Step-by-Step" and "Case-by-Case" Principles*: These principles are related to the Precautionary Principle because they are based on precautionary measures. In other words, each decision or authorization must be justified by the requirements presented each situation, and always based on experience from other cases or similar situations that were previously authorized or inspected (CNECV and CBE, 2011).

  On the one hand, the principle of "Step-by-Step" is based on the fact that any new activity can only be carried out after an evaluation or risk assessment of the previous phases, which means that it can proceed to the next phase without any hazard (CNECV and CBE, 2011).

When it comes to the "Case-by-Case" principle, it is based on the presence of an assessment of the risks associated with any procedure or biological product, which should be evaluated individually and without generalizations (CNECV and CBE, 2011).

- *The Principle of Traceability:* This Principle helps to ensure the quality and safety of biological materials of any nature and origin. Traceability can be defined as the capacity to follow the track of the new biological identities as well as the products obtained during their production and distribution, like in the case of GMOs. Through traceability, it is easier to control the materials and products. Moreover, this principle makes it possible to remove them from the market if there's a necessity to stop their circulation – in the case of an accident, for example (Gomes et al., 2016).

  Investigators have an important role to establish mechanisms that allow tracing the origin of these products. As a result, traceability of these products should ensure that relevant information concerning any genetic modification is available at each stage of the production and market process, which should thereby facilitate accurate labelling, providing all the necessary information and making it readily available for operators and consumers, thus ensuring the protection of the consumer (CNECV and CBE, 2011). As underlined before, the compliance with this principle in genome edited products seems scientifically complex (SAM, 2018).

- *The Principle of Autonomy:* Autonomy has since been extended to individuals and has acquired meanings as diverse as self-governance, liberty rights, privacy, individual choice, freedom of the will, causing one's behaviour, and being one's own person.

  In order for someone to be able to act and decide autonomously, there are three conditions: acting intentionally; understanding the meaning of their action and decision; having no external influences that determine or control one’s decision and action (Gomes et al., 2016).

  The Autonomy Principle, therefore, emphasizes the respect for the person, for his/her choices, options, and beliefs, which must be protected (Gomes et

al., 2016). In this way, applying this Principle to genome editing is giving the right to the consumer to be informed about the genome editing products. This, in turn, gives the consumers the right to choose if they want to consume a product or not, respecting the individual as well as their capacity of decision-making. Because of that, it is essential for each country to state with clarity on the product labels the nature of these products in order for the consumer to truly have the option to make an informed choice (Gomes et al., 2016). As stated before, this represents a huge difficulty in genome editing procedures.

- *The Principle of "Beneficence" and "Non-Maleficence":*

  The principle of Non-Maleficence asserts an obligation not to inflict harm intentionally.

  On the other hand, the principle of beneficence requires that damage should always be avoided, always promoting the good by assessing the risks and benefits (Gomes et al., 2016). Therefore, these principles require taking action by helping to prevent harm, removing harm, and promoting good, whereas non-maleficence only requires intentionally refraining from actions that cause harm.

  Consequently, it is important to apply these ethical principles to genome editing, as in this situation it is important to ask "What will happen if this technology cause direct and indirect actions harmful to human, animals and environment?"

  CRISPR-Cas9 is a powerful tool to generate organisms for different purposes. This technique could be used to harm human health. The easiness and efficiency of CRISPR has raised concern because the option of selling CRISPR-Cas9 kits to a huge number of users beyond traditional biotechnological companies and research institutions challenges how this situation will be governed and regulated effectively (Rodriguez, 2017). The potential use of these technologies in bioterrorism should be considered regarding the risks.

  It is the obligation of the investigator to prove, on a scientific basis, that genome editing does not provoke any damage to animals, the environment,

biodiversity, nor, of course, to human health. Therefore, it is the researchers' obligation to promote beneficence and never maleficence, showing that their intention is always to create something good for humanity (Gomes et al., 2016).

- *The Principle of Accountability:* The German philosopher Hans Jonas published a work in which he reflected on the Principle of Responsibility for future generations, both generations of human beings and other living beings. Human beings have the responsibility to provide to future environmental biodiversity and sustainability that is, at least comparable with the present. Humans with genome editing techniques obtain great power over nature and, because of that, they are responsible to take into account the risks. Ethically, it is necessary to have limits because living beings are not mere objects and we, as human beings should only develop something that guarantees the best interest of animal beings (intra-inter-generational responsibility) (Rodriguez, 2017).

- *The Principle of Justice*: In the context of Bioethics, justice refers to the requirements of distributive justice and equity in the distribution of resources, giving to each one what is due to them, regarding their rights, duties, benefits, and responsibilities.

  The potential for the implementation of genome editing techniques in certain contexts, particularly in agriculture, has an impact on social, intergenerational or global justice (i.e. fair distribution of advantages or opportunities among different groups in a society, between one generation and the next or between nations). Such concerns require to attend to the need to ensure that measures (such as the introduction of new biotechnology) that affect welfare can only be introduced if it does not discriminate unfairly among people (The Nuffield Council on Bioethics, 2019). A number of issues related to justice and fairness are raised in terms of a global perspective. For instance, will genome editing contribute to increasing the gap between developing and developed countries that already exist? Although would be of huge importance for public health in developing

countries, these possibilities will be explored in these countries? (CNECV and CBE, 2011)

6.2.2 Ethical aspects of Patents

Patents are defined as exclusive rights conceived during a limited period of time and given by states to inventors in order to prevent others from exploiting the patent holder's invention. For a patent to be granted, the invention must be considered a novelty, and be eligible and inventive or non-obvious, as well as have an industrial application (The Nuffield Council on Bioethics, 2002).

Since biotechnologies are a dynamic sector, the European legislator considered it necessary that this development be followed by the elaboration of a safe juridical instrument. This instrument should allow European companies to develop and commercialize new products and products that result from genetic engineering. In this context, the Directive 98/44 /EC has been published, (EU Biotech Directive). The Directive 98/44/EC, ensures the legal protection of biotechnological inventions. The directive's aims is to define what kind of biotechnological inventions might or not be patentable, giving stability to entities that want to invest in this type of innovation. However, there is still uncertainty when it comes to what can be patented or not. Nevertheless, the situation is not easy, because there is an attempt of patenting new complex products which raise ethical and legal issues (Martinho da Silva, 2010).

The process of patenting technologies raises several ethical debates, as the patent holders are able to control who uses the invention as well as the purposes of use. Because of that, private interests of biotechnological companies may clash with the public's interests, preserving intellectual property and creating a culture of secrecy within the scientific community. As a result, patents on technologies, if exercised in a restrictive manner, could prevent the access to technologies for the public and the wider research community, by refusing licenses or driving up costs (Martin et al. 2020). The introduction of the right of private property in a sector so strongly connected to the survival of humanity raises the question of food rights. The aim of some companies in the production of genetically modified animals (Recombinetics being an example, in the aforementioned case of cows without horns) shows an intention to suppress the conventional varieties,

leading to the end of the way agriculture was practiced since ancient times. When these organisms are patented by companies, CEOs can decide who eats and what, and at what price. The resulting competition among companies will exacerbate this trend. In other words, the first ethical issue that arises is the privatization of information as a right of those who carry the patent, stopping the spread of information to the scientific community, as well as, the CEO's having the power to make such decisions (Martinho da Silva, 2010).

Another question that arises is the following: how is it possible to achieve compatibility between the aim of the patent system, which deals with problems related to ensuring the protection of knowledge, and the dissemination of information (benefit sharing)? Also, we can ask whether it is possible to find a balance between the protection of knowledge and the incentive for innovation and sharing of knowledge (Martinho da Silva, 2010).

The EPO has reconsidered the patentability of plants and animals derived from conventional breeding. The discussion about patenting these organisms started in the mid-1980s, and in 1998 the European Parliament adopted the Directive 98/44 /EC (Martinho da Silva, 2010). The first transgenic animal to be patented, the OncoMouse, raises a range of ethical issues and raised related highly controversial debate because of the genetic manipulation of animals, particularly mammals. Two other key issues were raised regarding the patent system: The first one is whether patents should be granted at all for animals or animal varieties, particularly for mammals, even if they meet patentability criteria (novelty, industrial applicability/usefulness, inventive step etc.). For instance, a GM plant is considered an invention. However, in the case of transgenic soy, the change in made in soy represents a small portion of the plant's genome, since this change is only one gene a the entire genome. Thus, it can be argued that only the laboratory techniques are effectively an invention. It would be difficult to draw a similar conclusion in the case of genome editing, where there is no gene introduction. The second key issue involves moral implications and the way they should be addressed in relation to the suffering caused to the transgenic animals (Idris and Arai, 2006).

The European Patent Office (EPO) considered the OncoMouse case at several levels. It was only solved in 2004 (Idris and Arai, 2006). The utilitarian balancing test was created in order to address the issue of public or morality exception. This test assesses the benefits of a claimed invention against the risks, so in this case it weighed the suffering of the animal against the medical benefits that it could bring to humanity. In addition, the

environmental risks or public acceptance were aspects that were also considered into account. Therefore, the EPO concluded that the OncoMouse utility to the humanity was greater when compared to the moral concerns about the suffering caused to the animal. It was thus decided that the exclusion on patenting animal varieties did not constitute a ban on patenting animals as such considered to be, and concluded that the OncoMouse did not fall within that exclusion because it was not animal variety (Idris and Arai, 2006). In the light of genome editing in livestock, the question remains the same: is morally acceptable that animals edited by genome editing are considered an invention and, as such, patentable? (Idris and Arai, 2006).

It is, therefore, important to find a balance between the ethical issues and the necessity of protecting investments in new technologies (Martinho da Silva, 2010).

6.2.3 Risks and Benefits: The uncertainty

As stated before, one of the fundamental questions addressed by risk-benefit analysis is "How safe is safe enough?" (Fischhoff et al., 1978).

Currently, it is possible to point out many benefits that come from genome editing in livestock. (1) Improvements regarding human health; (2) decrease the transmission of animal diseases, such as Avian Influenza (Long et al., 2019) or African Swine Fever (Lillico et al., 2016); (3) food improvement, such as the creation of chicken strains without allergenic components from eggs (Oishi et al., 2016) or beta-lactoglobulin (BLG) knockout in milk (Wei et al., 2018). Genome editing could also reduces the overuse of antibiotics in farm animals by providing these animals with disease resistance (Graeff et al., 2019). In addition, these new technologies have the capacity to improve productivity by achieving a higher meat yield. Furthermore, genome editing could be quicker or more effective when it comes to trait improvement than classic breeding since it could be more efficient, versatile, precise, easier to use or more accurate than previous editing technologies. It could thus accelerate and/or enhance the trait improvement currently accomplished via classic breeding (Graeff et al., 2019). Besides that, there are advantages in animal welfare, such as the creation of hornless cattle without requiring them to go through the painful dehorning process, which is commonly performed in the farming industry to protect both cows and farmers from injuries (Carlson et al., 2016).

On the other hand, the uncertainties in assessing the potential consequences of genome editing technologies in human health, environmental security, or even animal health and welfare, keep an intense debate all over the world. The risks and consequences of genome editing technologies are still difficult or even impossible to characterize beforehand because of their novelty, as well as due to our incomplete knowledge and understanding about the genetic background of complex traits. Because of that, it becomes difficult to calculate the magnitude and the likelihood of the risks. However, it is essential to anticipate the balance between the potential risks and the potential benefits (Graeff et al., 2019).

As previous mentioned, genome editing could result in off-target effects, on-targeted effects and mosaicism, which could be introduced when the CRISPR/Cas9 system repeatedly targets genes at different stages of embryonic development. In gene-edited animals, the off-target effects, changes to other genes that were not intended, pose a major challenge and concern because the detection of off-target effects can be identified as a genetic variation, and, because of that, some off-target effects may be undetected (Cotter and Perls, 2018). Off-target effects could unintentionally change important genes, that can lead to changes in chemistry or protein production, and there may even be a loss of function of a gene, leading to harmful events like fetal abnormalities (Rodriguez, 2017). On the other hand, the on-targeted effects, may lead to deletions and complex re-arrangements of DNA. These deletions and re-arrangements of DNA by CRISPR may cause parts of the gene (exons) to be "missed" when the DNA is read, altering the alternative splicing process, when parts (exons) of a gene are read to produce a protein. In this way, the on-targeted effect (effects on the same gene) causes an unintended exon skipping, leading to an unintended protein. This can reflect in animal health as aborted pregnancies, stillbirths and infant deaths (Cotter and Perls, 2018). Lastly, the mosaicism as was mentioned before, describes the situation in which not all cells of an individual are genetically identical but, instead, cells harbouring distinct mutations co-exist in the same organism, when the injection of CRISPR-Cas9 reagents into single-cell mouse zygotes. This implies such individuals may transmit several distinct mutations to the next generation. Mosaicism is more of a technical difficulty than a food safety, animal health/welfare or environmental concern (The Nuffield Council on Bioethics, 2019).

In this way, methods are being developed to reduce the off-target effect and mosaic mutations and the efficiency is being improved. The use of a Cas9 protein could be conducted so efficiently that no off-target cutting is detectable across the entire genome in

some organisms. However, every organism must be tested. The investigation of off-target mutations is, therefore, crucial in order to ensure the correct use of genome editing in livestock breeding from the viewpoint of animal welfare and health (Rodriguez, 2017).

The aforementioned SCNT is the most used technique to deliver nuclease-mediated genetic alterations in livestock. Until now, a lot of animal species that are important within the food market, including sheep, goats, bovines and pigs, have been cloned via SCNT. Moreover, the mechanical stress of the potentially donor egg cell and "in vitro" conditions for the embryonic culture are critical, leading to consequences to animal welfare, such as short-term abnormalities. In other words, the decreased rate of growth and chromosomal abnormalities can lead to early embryonic death; the large offspring syndrome (LOS) which is normally manifested as increased birth weight of the offspring, placental abnormalities, stillbirth or even malformations of several organs; and post-natal mortality: an early neonatal mortality, within one week after birth (European Commission, 2008).

In addition, using genome editing to increase the productivity of livestock could be undesirable, given the negative impact of farming on the environment. The intensification of farming is a consequence of generating polled or disease resistant animals that could be kept in smaller spaces and therefore at higher density (Eriksson et al., 2018). On the other hand, one of the greatest consequences is the risk of them escaping into the wider environment, as this could result in them joining existing escaped (feral) or wild populations or forming new populations. The genetic trait could spread through these populations, and it could potentially act as a gene pool — moving the genetic trait back to farm animals through reproduction. These organisms could then end up in peoples' food, or even exchange genes with the wild populations, which would bring about unknown consequences for biodiversity and the environment (Cotter and Perls, 2018). Besides that, natural selection could select these organisms instead of their wild counterparts, due to the former having a competitive advantage because of their more favourable traits (Rodriguez, 2017).

However, the environmental risks are not well defined and very few studies on what the potential risks might be have been performed. This is due to the fact that, after the introduction of organisms into the environment, it is hard to find out whether the organism will negatively impact the rest. Moreover, due to the dynamic and ever-changing nature of the environment, it is difficult to pinpoint cause–effect relationships of genome edited animals, hence the huge importance of the Precautionary Principle (Cotter and Perls, 2018).

The potential of this technology has been recognized and detailed before. However, it is crucial for the scientific community to gain a deeper understanding and knowledge about the potential risks. Thus, it is crucial to involve the general population and create an inclusive public sphere to engage society in the discussion regarding genome editing. Once the majority of population is not capable of reflect about this, the scientists together with the governmental, should provide information about the benefits, uncertainties, and risks of these technologies, while always presenting all the concepts clearly, in order to reinforce the population's interest and confidence in science. Knowledge is therefore a key part, so that each person can contribute to the future, in a sustainable and appropriate way that unites the population. In this way, the outcomes of public engagement may be crucial in to order to determine the path that we want to follow in the future (Sales, Ferreira, and Reis, 2018).

6.2.4 The Anthropocentrism and Animal Instrumentalization

With the emergence of the ecological crisis and environmental concerns, after the second world war, ecology acquired huge importance among people, and for that reason it has since then started to be seen as a social movement, under the name "Environmentalism", which aims to fight against harmful to the environment actions (Araújo, 2011).

A new type of Ethics, Environmental Ethics, has arisen through the necessity and concern about the environment, with the purpose of posing a demand for a new concept of humanism. Thus, the need for new ethics was born in the 1970s in the United States, where an unprecedented ecological crisis took place. Humans ran the risk of becoming a hostage of themselves, since they have always thought that they are capable of controlling all the consequences of their technological developments. However, the emergence of new Ecological Ethics started to make sense, based on the assumption of the inherent threats in human capacities to change the world (Araújo, 2011).

There is a distinction between Anthropocentric Environmental Ethics and Non-Anthropocentric Environmental ethics. Although both converge to the same practices and policies, their principles are very distinctive. From the perspective of Anthropocentric Ethics, the ethical purpose of acting is concerned with human beings and their relationships and interest. On the other hand, Non-Anthropocentric Ethics suggest the expansion of the

moral community so that it encompasses other beings rather than only human beings (Araújo, 2011).[3]

The anthropocentric view is based on the assumption that nature only has value when useful to humans, and everything in the world has as its primary function to serve our species. This view also attributes intrinsic value only to human beings, while centered ethical considerations human beings satisfaction and interests. It confers a merely instrumental value to the nature, since nature is only considered when possess value in terms of human needs (Araújo, 2011).

From this perspective, any environmental issue can be solved through scientific and technological progress. This view is thus considered as inherent to capitalist societies, where principles such as free market and private property prevail. Other points that characterize this vision of an anthropocentric world are the characteristic of self-determination and the centralized control of the social, political and economic elites (Araújo, 2011).

Since the premise of this vision is the satisfaction of human needs, this theory the best interest of human beings should always take priority to other beings and resources the environment could be neglected (Araújo, 2011). As a result, the notion of animals as instruments is not accepted in Animal Ethics theories. When it comes to Animal Ethics, the two main principles that are present in contemporary regarding the on the relationship between man and animals are "*Animal Liberation*" by the philosopher Peter Singer and "*The Animal Rights*" by Thomas Regan (Neves and Araújo, 2018).

In 1975 Peter Singer published a book "*Animal Liberation"*, which was critical for the beginning of the animal liberation movement. The philosopher defends that all beings are endowed with sensitivity, all the animals experience suffering as well as feelings of happiness and they therefore deserve the same consideration as humans and their interests should be considered equal (Principle of Equality). Thus, Peter Singer advocates that all animals are "sentient" in their capacity of feeling pain and satisfaction, and they have interests that should be considered; for that reason, he advocated for their protection. *Animal Libertation* encourages the disappearance of people's prejudice in relation to animals, as well as the abolition of boundaries between human animals and non-human

[3] In the Non-Anthropocentric Ethics there are different models such as: Kantian Ethics, Animal Ethics, Biocentrism, Ecocentrism and Ecofeminism (Araújo 2011).

animals. And, therefore defends and promotes total zoo-centric equality and total protection of animals, and the eradication of their exploitation by humans (Neves and Araújo, 2018).

On the other hand, Thomas Regan, author of "*The Case for Animal Rights"* (1984), considers that animal liberation only happens when we respect and recognize animal rights. He, claims the animals have rights, as they are "subjects-of-life", endowed with sensitivity, expressing preferences and wishes, and perception of world, memories and are capable of intentional actions. Above all, they own a sense of individual welfare. Therefore, Regan is completely against the use of animals in science, agriculture, commerce, hunting for sport and trapping (Neves and Araújo, 2018).

However, in our opinion, the current world tendency is still mainly anthropocentric. As previously mentioned, in this theory, human beings only deal with natural things as an instrument that will bring them benefits and satisfaction. Because of that, Anthropocentric Ethics often lead to the animals being regarded as instruments. In the case of animals edited by gene editing to increase food production (Qian et al., 2015), to improve the nutritional characteristics of food (Wei et al., 2018; Oishi et al., 2016).), breeding disease-resistant animals (Long et al., 2019; Burkard et al., 2018; Lillico et al., 2016; Whitworth et al., 2019; Lee et al., 2017; Gao et al., 2017; Shanthalingam et al., 2016), all these are examples of treating animals as means to serve human interests. In other words, subjecting them to genetic engineering for human purposes is to make them serve us better, like objects for human use. Therefore, gene editing that has focused on production traits such as the ones described above may be less acceptable, since enhanced production is a human advantage, driven by the economics of large-scale factory farming, with no potential to benefit the animal itself. Besides that, such traits often result in phenotypes that cause animals distress and may encourage poor animal welfare standards, like high-intensity production (GeneWatch UK, 2019). In conclusion, genome editing technology can exacerbate this ethical problem when the aim is industrial farming and mass production.

Gene editing may, indeed, exacerbate anthropocentrism and reduce, even more, the moral status of farm animals. Gene-edited animals like super-muscly pigs are designed to meet human desires, for example, via an increase in body mass to match their instrumental purpose—but what about their suffering? What about their needs and interests individual welfare as sentience beings? In fact, their moral status is independent from their

instrumental value as objects for our consumption. As such, the application of gene editing to animals, which to some is a step forward for technology, may be a step backwards for human moral development (Baltimore, Charo, and Kevles, 2016).

In addition, the instrumentalization of animals species integrity should also be considered. When we talk about species integrity, it is important to clarify the concept of 'telos'. This concept was coined by Aristotle, who contended that every creature had a goal in life, which he designated as 'telos'. It has since they be described as the 'dignity and integrity' or 'inherent worth' of a being. Genetically edited animals could affect the 'telos' of animals if they are genetically altered to the point where they lose the behavior that makes them that particular animal. The cost could outweigh the benefits as, by carrying out genetic engineering, their rights are violated. On the other hand, the genetic modification of animals changes our relationship with the natural world and contributes to the commoditization of animals (GeneWatch UK, 2019).

7. General Conclusions

7.1 Conclusions

Genome Editing is the practice of making targeted interventions at the molecular level of DNA or RNA function, in order to deliberately change the structural or functional characteristics of biological entities**.** The rapid uptake and diffusion of genome editing technologies across many fields of biological research is undeniable. This spread is overwhelmingly attributable to the CRISPR-Cas9 system, although this technique is still undergoing refinement. Indeed, new technologies may emerge that can affect genome editing with even greater precision and speed. Genome editing in animals has made previously infeasible research now possible. Limitations to achieve the desired modifications are compounded by the low efficiency of the procedures used to produce genetically modified livestock, although genome editing has potential advantages over other approaches in terms of safety and controllability. Because of that, research in the field is currently taking place in order to develop new features in a wide range of farmed animals, such as producing animals that are resistant to disease, with higher proportions of muscle mass and also better adapted to the environmental conditions (The Nuffield Council on

Bioethics, 2019). Consequently, these new applications raise many ethical, social and legal implications.

Indeed, a careful analysis of genome editing in livestock allows us to understand that maybe the most debate-provoking concept is whether genome editing is the same as genetically modified organism (GMO). The argument is raised particularly from the scientific research community, arguing that genome-edited organisms should not be classified as GMOs, because no transgene is involved. In this case, the resulting organism is the same as the one that would have potentially been created through conventional breeding techniques, without the inclusion of foreign DNA (from the use of a vector). In such cases, the products from genome editing and 'traditional' breeding would be indistinguishable. On the other hand, other authors assert that genome-edited organisms should be regulated as GMOs due to the method of production (The Nuffield Council on Bioethics, 2019).

This raises questions about how organisms altered by any means should be regulated, despite the ECJ having decided to regulate all the organisms obtained via the new techniques of directed mutagenesis as GMOs. However, regulation based on the characteristics of novel organisms, i.e, a product-based regulation independent of the technology used on its production, would provide more effective, robust, and futureproof regulation when compared to considerations based on the method used to generate them.

From the analysis of genome editing in livestock, a group of Ethical and Regulatory Principles arise. Principles as responsibility, beneficence or non-maleficence, precaution or autonomy do not aim to be an obstacle to develop this kind of technology or products, but a way of guaranteeing the fulfilment of requirements such as the respect for the environment, animals, humans, and science based on precautionary approach.

As previously discussed, the patenting of biotechnologies raises several ethical debates. Firstly, it raises questions regarding whether it is morally acceptable to consider animals as an invention and, consequently, whether it is ethical to consider them available to be patentable (Idris and Arai, 2006). Animals are entities with rights and intrinsic value and so it does not seem right that they are being patented, which implies that someone has a right over them. Moreover, if exercised in a restrictive manner, the patenting could stop the access to technologies for the public and the research community by refusing licenses or driving up costs (Martinho da Silva, 2010). If the patent holders do not share their findings,

this utilization will contradict the principle of benefit sharing and will consequently violate the principle of justice (CNECV and CBE, 2011).

The uncertainty has been hindering the progress of genome editing. It is possible to point out many benefits that come from genome editing in livestock. However, the uncertainties in assessing the potential consequences of this technology in human health, environmental security, as well as animal health and welfare, keep the debate open and, because of that, it becomes difficult to calculate the magnitude and the likelihood of these risks and to define "how safe is safe enough" (Graeff et al., 2019). In this way, risk management should be essential, control and follow-up, including the assessment of its potential dual-use. Just as, authorizations for all activities that assume the production of these products as well as control and inspections. In this way, these investigations should be realized step by step, in order to allow the assessment of its impact, in terms of protection of human health, environment, and animal welfare, allowing to proceed to the next step with security.

Consequently, in Europe, the Precautionary Principle is part of EU guidelines, according to which substantial uncertainties cannot be ignored, as they might include serious and undesirable consequences that may not be apparent in the short term and, in long term perspective difficult or impossible to reverse (Bourguignon, 2015). In this way, Europe presents a regulatory framework that is stricter than that of other countries in the worldwide, which could make Europe less competitive. From the point of view of the economic sustainability of European agriculture and European farmers, this conservative and restricted position of European policy may have consequences such as a huge difference between the European agriculture products and higher market prices than the international market prices (CNECV 2010).

Animals have been used for years, but with genome editing there is an increasing tendency to disregard their rights, which has to be considered because it can lead to pushing animals to their biological limits. This technology may therefore lead to exacerbating ethically problematic practices. For instance, cattle genetically modified to lack horns, might potentially be kept in even smaller spaces, which may affect further their welfare (GeneWatch UK, 2019).

However, many questions remain unanswered. As previously discussed, the risks or consequences of genome editing technologies are still hard or even impossible to

characterize. But why restrict or even forbid this kind of technology if until today their potential risks have not been fully recognized? When the benefits are believed to outweigh the risks and dangers can be avoided, should science consider moving forward with genome editing? If the answer is yes, how can researchers do so responsibly?

It is possible that denying these technologies could exacerbate their potential risks? In other words, can the future consequences be greater if these technologies are not used? If these technologies bring about some benefits in different fields, is it morally acceptable to avoid them? For instance, disease resistance is a very important topic not only for livestock, but also for human health, as these animals are more exposed to the wildlife populations and to the spread of diseases. So, the question raised is whether it is ethical to avoid this kind of technologies for these animals in order to prevent diseases.

All of these questions have one thing in common: a proactive posture in respect to genome editing technologies. However, it is necessary to reflect its balance in a precautionary way.

First of all, is it ethical to keep using this technology without understanding all of its effects in the environment, animal welfare and human health? The case of hornless dairy cows, in which Carlson et al. (2016) did not report any off-target effects in the gene edited cows is a great example. The FDA later did indeed find the entire plasmid as well as a second copy of the repair template and the plasmid backbone, which were integrated into the target location of both calves.

Besides that, are all the purposes of the genome editing justifiable? For instance, when it comes to food production, are these animals the solution to the problem of hunger in the world? The issue is more complex and is linked to multiple factors, such as the current inequality in the distribution of existing resources (FAO, 2017). So, why should animals be mutated and altered in order to address what is a human problem? In addition, genome editing seems, in this situation, maladjusted, as animals are already pushed beyond their biological limits by living in the conditions of intensive farming.

It is impossible to ignore the huge potential that biotechnology represents for current and future agriculture. However, this kind of emerging questions, such as genome editing are met with high uncertainty, and it is necessary that all the problems are presented without ambiguities and that they can be explained with clarity to promote open reflection regarding the ethical questions.

Transparency and societal debate are other important aspects of new and emerging technologies. As previously mentioned, trust and the promotion of scientific evidence are the basis for our society (Moedas, 2018) and, due to this, the discussion needs the public's involvement, and therefore since this issue could have an enormous societal impact, it is important to identify and bridge potential gaps between the public and the scientists. It is, therefore, crucial to encourage this kind of debate includes and inform the public with transparency about the benefits and risks, the uncertainties or even their rights of consumers to right information, thus not allowing the propagation of wrong information. In this way, they will know what they are buying and they will be allowed to be involved in shaping technological developments together with other stakeholders, in order to protect the consumer (Sales, Ferreira, and Reis, 2018).

Besides that, more research is really needed about what is safe and what is unsafe. Due to the absence of clear research on this field, a precautionary position should be adopted.

Therefore, Bioethics allows the reflection and discussion regarding the uncertainties and objections that genome editing currently brings. However, these ethical debates must not be seen as a barrier to investigation moving forward, but as a starting point to make good and responsible decisions in science. Taking all of this into consideration, genome editing in livestock for all applied purposes must be approached with prudence and caution until the risks have been rigorously characterised and answered, and until the public has been properly informed and consulted.

7.2 Future work

To better understand the genome editing implications, more robust research is needed in order to encounter and address all the risks. Besides that, a regulatory framework revision seems necessary. Moreover, it is essential to define a risk assessment and management procedure, while the scope of analysis should also be expanded to include social, cultural and ethical considerations and extensive public discussion to determine the future of genome editing in agriculture. Although a methodology by PRISMA was followed during this work to obtain the literature review of genome editing in livestock to human consumption, deeper research will be necessary and in order to follow the huge quantity of literature (scientific methodologies and non-scientific) to further improve the amount of data include

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