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Department of Comparative Biomedicine and Food Science

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Ethical Issues of CRISPR-Based Gene Editing in De-Extinction Conservation

Relatore / Supervisor
Prof. Barbara De Mori

Laureando / Submitted by
Gabriele Maronese
Matricola n. / Student n.
2067310

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Abstract

Recent advances in genetic engineering and assisted reproductive technologies (ARTs) have renewed scientific and ethical interest in de-extinction, understood as the application of biotechnological tools to recreate extinct species or functional ecological proxies intended to support conservation outcomes by restoring ecological function. In particular, the use of CRISPR-based genome editing in combination with ARTs such as cloning, embryo manipulation, and interspecies surrogacy has expanded the technical possibilities of de-extinction projects, while at the same time raising complex ethical concerns.

This thesis presents a critical analysis of the scientific literature on CRISPR-based ART applications in de-extinction research, with the aim of identifying and examining ethical issues implied in existing studies. The methodology is based on a PRISMA-guided literature search followed by a structured ethical analysis informed by a standardized ethical assessment framework, the ETHAS tool (De Mori et al., 2024), which was developed within vertebrate wildlife conservation projects to support the ethical self-assessment of ART procedures across environmental, animal welfare, social, and research ethics domains. In this study, the ethical goals underlying ETHAS were used as an analytical guide to systematically extract and interpret ethically relevant issues emerging from the literature.

The analysis highlights recurring ethical concerns related to animal welfare during experimental procedures and throughout the lifespan of genetically engineered organisms, uncertainty regarding the efficiency and reliability of laboratory techniques, limited adherence to the principles of the 3Rs, and insufficient transparency in data management. Overall, the findings point to a fragmentation of ethical reflection and a lack of integrative ethical guidance in the current literature.

This thesis therefore argues for the need to establish an ethical framework capable of guiding research practices in a responsible and transparent manner in the context of de-extinction projects. The objective is not to hinder scientific progress, but to support its careful and ethical application in this emerging field.

This study is subject to methodological limitations, including reliance on a single database, English-language peer-reviewed literature, and predefined search queries, and should therefore be interpreted as a partial overview.

1. Introduction

1.1 Conceptual Foundations and Debates on De-Extinction

In the context of accelerating biodiversity loss and the irreversible disappearance of species worldwide, growing consideration has been given to the possibility of reversing extinction through the use of modern genomic techniques and assisted reproductive technologies (ARTs). This idea, commonly referred to as *de-extinction*, emerged as a response to historical extinctions, proposing the creation of new biological entities intended to resemble extinct species and potentially fill their former ecological roles (Novak, 2018).

Since it first appeared as a concept, de-extinction has sparked wide-ranging discussion that reaches far beyond scientific inquiry, raising ethical, social, and technological questions in both academic circles and public spheres (Novak, 2018). Part of the controversy stems from the fact that the very idea of de-extinction is conceptually unstable. The word "de-extinction" itself was only coined in 2012 and still lacks a fixed or widely agreed-upon definition. Alternative terms—such as "extinction reversal," "re-creation," "resurrection," "reviving," and "resuscitation"—are frequently used as near-synonyms, signaling persistent ambiguity about the boundaries and significance of the notion (Martinelli et al., 2014). Consequently, to engage with de-extinction is also to participate in shaping what has been termed a broader "science of resurrection," in which scientific methodologies are entangled with symbolic meanings and public expectations (Martinelli et al., 2014).

At the theoretical level, de-extinction calls into question long-held assumptions in evolutionary biology and conservation science about the definitiveness of extinction. Historically, extinction has been seen as a process that cannot be reversed. This view is reflected in the assertion that species "come into existence and pass away and, once extinct, can never come into existence again" (Hull, 1983, as cited in Slater & Clatterbuck, 2018), a claim that was intended not as a statement about technological limitations but as evidence of how species have been historically conceptualized within evolutionary biology. From this perspective, extinction represents a definitive endpoint in the historical continuity of a species.

Alongside this conceptual framing, de-extinction has also faced substantial ethical opposition, based on worries about the moral boundaries of human intervention. Some critics portray de-extinction as a kind of moral violation, as it seems to entail humans surpassing the proper limits of their sphere of action (Cohen, 2014). This opposition arises from the view that de-extinction brings together several dimensions of moral transgression: the manipulation of living beings via cloning and genome editing, the production of organisms regarded as unnatural, and the effort to restore what has already been lost through extinction. In this sense, de-extinction is viewed as intruding upon domains traditionally considered beyond human authority, including creation, the definition of naturalness, and the revival of extinct life (Cohen, 2014). While this intrinsic-value objection is philosophically significant, the present thesis does not engage with it directly. Instead, the focus is on procedural and welfare ethics: the harms arising from the technical methods used in CRISPR-based de-extinction, rather than from the metaphysical permissibility of de-extinction as such.

Despite these conceptual and ethical objections, the assumption that extinction must be absolute and irreversible has increasingly been questioned in recent decades. Advances in genetics and reproductive technologies have revived interest around de-extinction, reopening debates that were once considered resolved (Slater & Clatterbuck, 2018). These discussions unfolded alongside wider debates in ecology and conservation biology, especially those linked to rewilding projects. Ideas like "Pleistocene rewilding" promote the deliberate introduction of animals intended to stand in for extinct megafauna by fulfilling similar ecological roles, with the goal of repairing disrupted ecosystem dynamics. Such initiatives prompt significant questions concerning species substitution and ecological equivalence, challenging the idea that extinction always represents a permanent loss (Slater & Clatterbuck, 2018).

The ongoing nature of these debates points to deeper conceptual ambiguities surrounding how species and extinction are defined. Many of the contradictions in the de-extinction literature can be traced back to unresolved issues about what qualifies as a species and what it means for a species to be extinct. While conservation practice often depends on straightforward definitions, both notions have long been understood as biologically and philosophically intricate rather than strictly rigid dichotomies (Novak, 2018).

This complexity becomes clear in the graded structure of biological classification, involving multiple hierarchical levels such as species, subspecies, populations, and ecotypes. In a similar way, extinction is differentiated into various types, including functional extinction, local extinction, extinction in the wild, and global extinction. These categories challenge any straightforward view of extinction as a simple, absolute endpoint. In certain instances, species can even vanish by gradually evolving into new lineages, further blurring the line between extinction and continuity (Novak, 2018).

Within this context, de-extinction has recently increasingly been conceptualized in functional rather than strictly absolute terms. Instead of being treated as a simple yes-or-no outcome, de-extinction can be described as a continuum: at one end are organisms that very closely match extinct species at genetic, phenotypic, and ecological levels, and at the other are functional proxies that may be genetically distinct but still able to re-establish lost ecological functions (Turner et al., 2025). This perspective supports a pragmatic approach, according to which the relevance of de-extinction should be evaluated in relation to specific ecological objectives rather than abstract metaphysical criteria (Slater & Clatterbuck, 2018).

At the core of this pragmatic orientation is the recognition that different species concepts serve different theoretical aims and practical applications. Although the Biological Species Concept has historically been the most influential in evolutionary biology, it may be insufficient in de-extinction contexts that prioritize ecological function over reproductive isolation. In these contexts, alternative frameworks such as the Ecological Species Concept may be preferable, particularly when the primary concern is the role an organism plays within an ecosystem. From this angle, whether a genetically engineered mammoth-like organism qualifies as a "real" mammoth depends less on genetic continuity and more on its ability to carry out ecological functions comparable to those of extinct species (Slater & Clatterbuck, 2018).

Following from this functional interpretation, the objectives linked to de-extinction extend beyond highly publicized attempts to bring back species that have been extinct for long periods. In some contexts, de-extinction may serve a more modest yet ecologically meaningful role, for example by acting as an "ecological defibrillator" through the reintroduction of recently lost species into ecosystems where their ecological niches remain unfilled (Slater & Clatterbuck, 2018). From this perspective, the goal of de-extinction is not to create exact copies of extinct organisms, but the restoration of ecosystems and the re-establishment of lost interactions among species (Shapiro, 2015, as cited in Slater & Clatterbuck, 2018). In this sense, the term "de-extinction" can be misleading, as it suggests the complete resurrection of extinct species. Instead, current approaches aim to produce functional equivalents that can reinstate essential ecological processes.

Nevertheless, functional replacement may not fully compensate for what is lost through extinction, particularly when the value of a species is tied to its authenticity. Here, authenticity refers to the independent existence of a species within a specific historical, ecological, and evolutionary context. If extinction entails the irreversible loss of this form of value, its implications extend beyond the mere technical feasibility of de-extinction and directly influence how de-extinction should be assessed within ethical and conservation frameworks (Slater & Clatterbuck, 2018).

De-extinction can therefore be understood as a conceptual possibility, though not in a uniform or absolute sense. Its feasibility depends on the taxa involved, the underlying species concepts, and the aims being pursued. Since no single species concept categorically excludes de-extinction, the discussion nowadays is gradually moving away from purely abstract metaphysical critiques toward nuanced, context-dependent, case-specific evaluation grounded in ecological and ethical considerations (Slater & Clatterbuck, 2018).

Viewed collectively, these perspectives show that de-extinction cannot be understood as a single, clearly defined practice. Against this conceptual background, recent developments in gene-editing technologies have become progressively relevant for understanding how de-extinction is currently pursued in practice.

1.2 The Role of CRISPR in De-Extinction

Recent advances in genetic engineering have profoundly reshaped how de-extinction is discussed and, in some cases, approached in practice. Over the past two decades, a range of genome editing technologies has emerged, progressively increasing scientists' ability to modify animal genomes with greater efficiency and control (De Graeff et al., 2019). Within this broader technological trajectory, CRISPR-based systems have occupied a central position, widely described as having transformed genome editing by making targeted genetic modification faster, easier, cheaper and more accurate in a wide range of species (Greely, 2017).

Since the discovery of the structure of DNA, researchers have aimed to develop methods capable of introducing precise changes into the genome of cells and organisms. Studies on DNA repair mechanisms showed that cells possess endogenous pathways that can repair double-strand DNA breaks, suggesting that intentionally introducing such breaks at specific genomic locations could be an effective strategy for targeted genome modifications (Doudna & Charpentier, 2014).

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), was first identified in 1987 in the Escherichia coli genome. At the time, its repeated DNA sequences were considered unusual genomic elements with no known biological function (Gostimskaya, 2022). The importance of these sequences became more evident in the mid-1990s, when similar structures were discovered in archaea, suggesting that CRISPR elements were conserved across different prokaryotic organisms and likely performed an essential function (Gostimskaya, 2022).

Later research demonstrated that CRISPR systems function as an adaptive immune mechanism in bacteria and archaea. Spacer sequences within CRISPR arrays were shown to originate from viral or plasmid DNA, allowing microorganisms to store fragments of foreign genetic material and use them to recognize and defend against future infections (Gostimskaya, 2022; Doudna & Charpentier, 2014). This defense system relies on CRISPR-associated (Cas) proteins, which use RNA molecules as guides to identify and cleave invading DNA (Doudna & Charpentier, 2014).

Among the different identified CRISPR-Cas systems, the type II system has been particularly important for genome editing because it relies on a single protein, Cas9, to perform DNA cleavage. Cas9 contains two nuclease domains that cut the two DNA strands, generating a double-strand break at a specific location in the genome (Doudna & Charpentier, 2014). The ability of Cas9 to target a precise DNA sequence depends on the guidance provided by RNA molecules and on the presence of a short DNA sequence adjacent to the target site, known as the protospacer adjacent motif (PAM) (Doudna & Charpentier, 2014).

The CRISPR–Cas9 system uses a guide RNA to determine where the DNA cut will occur. In its natural form, this guidance is provided by two RNA molecules which were later combined into a single guide RNA (sgRNA), simplifying the system and making it easier to use in laboratory settings (Doudna & Charpentier, 2014; Gostimskaya, 2022). Guide RNA directs Cas9 to a specific genomic sequence through base pairing between RNA and target DNA. Once guide RNA binds to its complementary DNA sequence and the PAM is recognized, Cas9 introduces a double-strand break at that location (Doudna & Charpentier, 2014). This break activates the cell’s natural DNA repair mechanisms. During the repair process, targeted genetic modifications can occur, either by disrupting a gene or by introducing specific genetic changes if a repair template is provided (Doudna & Charpentier, 2014).

In 2013, several independent studies demonstrated that CRISPR–Cas9 could be used to edit the genomes of eukaryotic cells, including animal and human cells. These findings showed that RNA-guided DNA cleavage could reliably induce targeted genetic changes by exploiting endogenous DNA repair pathways (Gostimskaya, 2022; Doudna & Charpentier, 2014). The simplicity of programming Cas9 through changes in the guide RNA sequence allowed CRISPR–Cas9 to be rapidly adopted in many areas of biological research.

CRISPR–Cas9 represents a major advance in genome engineering by transforming a natural microbial defense system into a programmable tool for precise DNA modification. Its reliance on guide RNA for targeting enables flexible and efficient genome editing, making it particularly relevant for applications involving non-human animals and assisted reproductive technologies, including those proposed in de-extinction research (Doudna & Charpentier, 2014; Gostimskaya, 2022).

In the context of de-extinction, genetic engineering and CRISPR represent only one of the several possible approaches. Nonetheless, as Greely notes, gene editing has the widest range of application among these methods, specifically because it can, at least in principle, be used across different taxa and integrated with various reproductive strategies (Greely, 2017). This broad applicability has made

CRISPR increasingly prominent in de-extinction research and frequently portrayed as a key enabling technology.

Adams provides a clear description of how de-extinction through genetic engineering is generally carried out in practice. It involves reconstructing the genome of an extinct species and comparing it with that of its closest living relative, identifying relevant genetic differences, synthesizing specific genomic segments associated with extinct traits, and inserting them into the genome of living species using gene editing technologies—most notably CRISPR–Cas9. Cells carrying the modified genome can then be used in conjunction with nuclear transfer techniques to generate living organisms expressing the desired characteristics (Adams, 2017). Although conceptually straightforward, Adams emphasizes that this process is extraordinarily complex and that the technical challenges involved in accurately reconstructing extinct genomes remain immense.

The increasing technical feasibility of gene editing does not eliminate biological uncertainty. As De Graeff et al. emphasize, CRISPR-based interventions may still result in off-target mutations, unintended genomic effects, or mosaicism, all of which can have implications for animal health and development (De Graeff et al., 2019). While enhanced design and validation approaches can mitigate these risks, they can never fully eliminate them. In de-extinction settings, such uncertainties carry particular weight, because modified organisms are expected not only to survive but also to function within complex ecological networks. It should also be emphasized that these concerns should refer not only to the final engineered organisms, but likewise to the experimental animals used throughout the various laboratory procedures, who must be protected from avoidable suffering.

It now becomes evident that the same characteristics that make gene editing so powerful also amplify the ethical complexity of de-extinction tied to CRISPR interventions, reinforcing the need for careful ethical evaluation throughout the whole experimental procedure.

1.3 The Ethical Gap

Over the last several years, de-extinction has started to transition from a mainly theoretical idea to concrete research projects. Among the most notable players in this field is the biotechnology company Colossal Biosciences. To date, the company has announced projects involving extinct species such as the woolly mammoth, the dire wolf, the Tasmanian tiger, the dodo, and the moa. Although these projects are still ongoing and many technical uncertainties remain, they all share the use of CRISPR genome editing.

The literature increasingly highlights the need for ethical reflection in this context, mentioning issues such as animal welfare, ecological risk assessment, transparency, and responsible decision-making.

However, these discussions are often fragmented, and no study has yet systematically collected the ethical concerns related specifically to CRISPR-based de-extinction practices.

This gap provides the starting point for the present thesis. The aim of this work is not to oppose scientific innovation, but to carry out a structured review of the current literature in order to identify and analyze the main ethical problems in relation to CRISPR-based de-extinction.

The analysis focuses on procedural and welfare concerns—the ethical significance of technical uncertainty in CRISPR editing—rather than on intrinsic-value questions such as whether de-extinct organisms can possess the authenticity that characterises naturally evolved species (Slater & Clatterbuck, 2018; Sandler, 2014). The Colossal Biosciences projects serve throughout as reference points, illustrating how the procedural concerns identified here apply to ongoing real-world research. By organizing these issues within a standardized ethical assessment framework such as the ETHAS tool (De Mori et al., 2024), developed within vertebrate wildlife conservation projects to support ethical self-assessment of ART procedures across environmental, animal welfare, social, and research domains, the thesis seeks to contribute to future discussions on ethical regulation and responsible research in this field.

2. Materials and Methods

This thesis is based on a structured literature analysis aimed at identifying and examining ethical issues arising from the application of CRISPR-based genome editing in assisted reproductive technologies (ARTs) in non-human animals, with specific relevance to de-extinction and wildlife genetic engineering projects. The methodological approach does not consist of an experimental or quantitative study, but rather of a systematic, comprehensive and transparent analysis of existing scientific literature.

2.1 Literature Search

This study used the Web of Science (WoS) database that index peer-reviewed scientific research, due to its broad disciplinary coverage and widespread use in systematic and semi-systematic literature reviews. The use of a single database is acknowledged as a methodological limitation; studies indexed exclusively in Scopus or PubMed may not have been captured.

In Web of Science, searches were performed using the Topic Search function, which includes titles, abstracts, and keywords. This search mode was chosen to maximize the retrieval of relevant studies while maintaining a focus on ethically meaningful content.

Initial broad queries were used to map the overall research landscape related to CRISPR applications in animals and to identify recurring technical and ethical themes. This preliminary search was conducted using general keywords such as "CRISPR" AND "Ethics", "CRISPR" OR "Gene-editing" AND "Conservation" OR "Ecology", "CRISPR" AND "Bioethics", “CRISPR” AND “Behaviour” AND “Ethics”. The purpose of this phase was exploratory, aiming to assess the breadth of the literature, dominant research areas, and to evaluate whether ethical considerations related to the application of CRISPR in wildlife conservation were explicitly addressed. A total of 122 publications were read at full-text level at this stage.

Among the papers examined, several addressed ethical concerns related to genome editing in non-human animals in a broad sense. Notably, the systematic review by de Graeff et al. (2019) provides an overview of ethical arguments concerning animal welfare, risk and uncertainty, environmental impacts, and public acceptability. However, no equivalent ethical review was identified that specifically examines the application of CRISPR in wildlife conservation or de-extinction contexts. This gap in the literature highlights the need for a focused ethical analysis of CRISPR-based strategies in the context of conservation biology.

The preliminary literature search allowed for the identification of the main risks, impacts, and areas of concern associated with CRISPR-based animal gene editing, including welfare-related effects, technical uncertainty, and potential downstream consequences. This step also helped to determine whether ethical considerations related to CRISPR use in animals—and specifically in wildlife conservation contexts—were directly addressed or only indirectly implied within technical or methodological discussion. On this basis, key ethical themes and relevant terminology were identified and integrated into the following stage of query development.

In addition to the systematic search of peer-reviewed literature, relevant grey literature—including policy documents, ethical guidelines, institutional reports, and conceptual or methodological publications not indexed in Web of Science—was also referred to support the broader ethical discussion and interpretation of findings. However, grey literature was excluded from both the formal systematic search and the query strings, as the selected database did not yield relevant grey literature directly addressing CRISPR-based genome editing in non-human animals in conservation contexts. Therefore, grey literature sources were employed in a complementary manner, contributing to the development of the conceptual framework, the interpretation of ethical issues, and the thesis discussion.

2.2 Conceptual Scope

The development of search queries was guided not only by technical terminology related to CRISPR and genome editing, but also by a set of ethical and conceptual domains implicitly connected to de-extinction and animal genetic engineering. These domains included:

- Bioethical implications of genome editing in non-human animals;
- Ethical considerations in wildlife conservation and biodiversity-related genetic interventions;
- Laws, regulations, and oversight mechanisms related to CRISPR applications;
- Animal welfare implications of gene editing and reproductive manipulation.

These thematic areas shaped the construction of search queries and later guided the qualitative ethical analysis of the selected literature.

2.3 Inclusion and Exclusion Criteria

Clear inclusion and exclusion criteria were defined before the screening process.

Inclusion criteria were as follows:

1. Studies focusing on the application of CRISPR for gene editing in non-human animals;
2. Studies involving reproductive or offspring-producing purposes, including germline modification or ART-related procedures;
3. Peer-reviewed original research articles, meta-analyses, or reviews involving original statistical analysis;
4. Publications written in English;
5. Studies published from 2013 onward, corresponding to the emergence of CRISPR technologies;
6. Studies falling within relevant subject areas, including ecology, zoology, evolutionary biology, genetics and heredity, molecular biology, biodiversity conservation, environmental sciences, veterinary sciences, behavioral sciences, agriculture, and animal and dairy sciences.

Exclusion criteria included:

1. Studies not involving animals;
2. Studies not related to reproduction or offspring production;
3. Studies not involving CRISPR-based genome editing;
4. Publications focusing exclusively on plants, food products, or human clinical applications;
5. Non-peer-reviewed materials such as editorials, conference proceedings, and opinion pieces.

2.4 Query Development

As mentioned previously, the literature search strategy was developed through a stepwise and progressive process aimed at balancing sensitivity and specificity. Initial queries combined general terms related to gene editing and CRISPR with references to animals and ethics, such as "Gene editing" OR "Genome editing" OR "CRISPR" OR “Clustered Regularly Interspaced Short Palindromic Repeats” OR “CRISPR/Cas Systems” OR "CRISPR/Cas9" AND "Animals" OR "Animal" AND "moral" OR "morals" OR "ethics" OR "bioethics". These early searches returned relatively small datasets (around 60-70 papers) and mainly retrieved papers that explicitly presented their discussion in ethical or philosophical terms.

At the opposite extreme, broader searches combining CRISPR-related terminology with animal-related keywords, such as "Gene editing" OR "Genome editing" OR "CRISPR" OR “Clustered Regularly Interspaced Short Palindromic Repeats” OR “CRISPR/Cas Systems” OR "CRISPR/Cas9"

AND "Animals" OR "Animal", generated very large result sets (around 6000 papers). These datasets included a high proportion of studies unrelated to ethical analysis, wildlife research, or reproductive applications.

Later refinements integrated ethically relevant keywords, including terms related to biosafety, biosecurity, off-target effects, conservation, risk assessment, and research guidelines, thereby allowing the identification of studies in which ethical concerns were presented within methodological discussions rather than explicitly labeled as ethical analysis. Additional refinement was achieved by incorporating reproduction-related keywords (e.g. reproduc, reproductive techniques, offspring), which are central to de-extinction strategies and advanced assisted reproductive technologies.

The final query used in Web of Science was:

("CRISPR" OR "CRISPR-Cas" OR "CRISPR-Cas9" OR "CRISPR/Cas9") AND (“animal” OR “livestock” OR "farm animal" OR “wildlife” OR “biodiversity”) AND (“reproduc*” OR “reproductive technique” OR “offspring”) AND (“ethic*” OR “bioethic*” OR "ethical issue" OR “off-target” OR "biosafety" OR "biosecurity" OR “conservation” OR "guideline*") NOT "plant" OR "fruit" OR "in silico" OR “food” OR “human”

2.5 Screening and Selection Process

The screening and selection process was conducted in line with the PRISMA 2020 framework in order to ensure transparency, consistency, and traceability of the literature selection process.

Records were retrieved from the selected bibliographic database using the predefined search queries. Duplicate entries were identified and removed before screening to avoid redundancy. No automation tools were used during this phase.

Because article titles may not consistently indicate the methodological details or ethical significance of a study, they were not screened; instead, abstracts were screened directly to assess eligibility. Abstracts that met at least one of the eligibility requirements proceeded to full-text review. During the eligibility assessment phase, full-text articles were examined in detail to confirm compliance with the inclusion and exclusion criteria and later included in the final dataset.

2.6 Data Extraction and Ethical Analysis Approach

The ethical analysis was guided by a structured framework based on the twelve ethically relevant goals articulated within the ETHAS (ETHical ASsessment) tool for Assisted Reproductive Technologies in wildlife conservation, as reported in de Mori et al., 2024. The twelve ethical goals underlying ETHAS are organized into four overarching ethical domains: environmental ethics, animal welfare ethics, social ethics, and research ethics.

Environmental Ethics	**Animal Welfare Ethics**	**Social Ethics**	**Research Ethics**
E1 - Conservation of the biomaterial and genetic information	**A4 -** Health and functioning	**S7 -** Compliance with rules and regulations	**R10 -** Scientific quality and innovation
E2 - Conservation of the species	**A5 -** Allowance of positive affective states and avoidance of negative affective states	**S8 -** Health, safety and empowerment of the staff and other people involved	**R11 -** Transparent and effective data management
E3 - Conservation of biodiversity	**A6 -** Expressing natural behaviour and living natural lives	**S9 -** Positive integration with the surrounding social environment	**R12 -** Transparent and effective communication

Table 1. The twelve ethical goals of the ETHAS framework, organized into four ethical domains (de Mori et al., 2024).

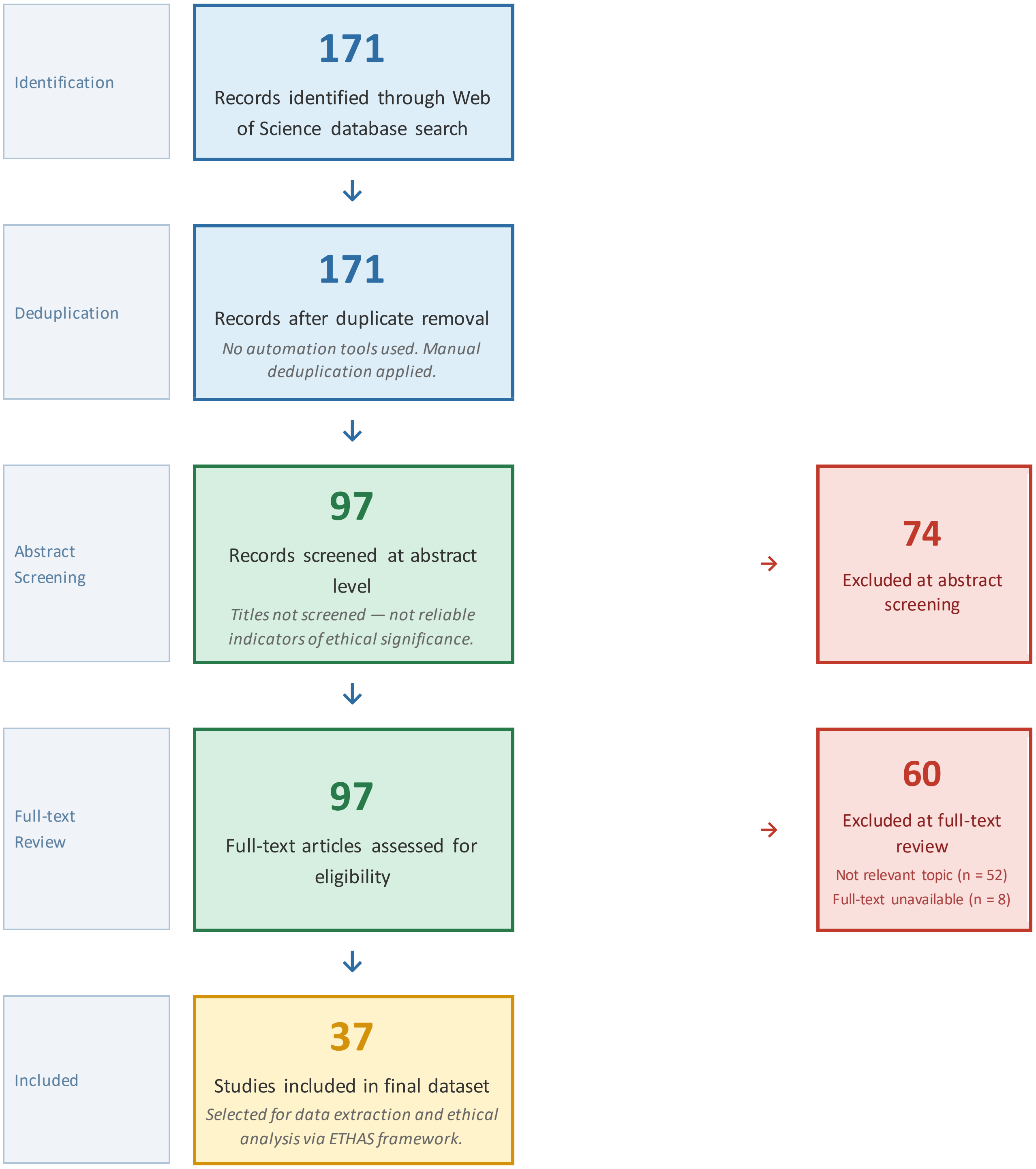
In this thesis, the ethical goals outlined above were used in two complementary ways. First, they served as analytical categories to guide the extraction of ethically relevant issues from the selected literature. Ethical concerns were captured not only when authors addressed them explicitly, but also when they could be reasonably inferred from experimental designs, methodological choices, or declared research objectives. Each identified issue was mapped to one or more of the twelve ethical goals, allowing for a structured comparison across studies. Second, the ethical goals provided a conceptual foundation for reflecting on the adequacy and limitations of current ethical discussions surrounding CRISPR-based ARTs.

3. Results

A total of 171 articles were identified through database searching. After abstract and full-text screening, 37 articles were included for data extraction and analysis in the final dataset.

PRISMA 2020 – Literature Selection Flow

Web of Science · CRISPR-based de-extinction ethics



Page, M. J. et al. (2021). The PRISMA 2020 statement. BMJ, 372, n71. https://doi.org/10.1136/bmj.n71

The studies included in the final dataset are predominantly focused on the technical development and refinement of CRISPR-based genome editing in non-human animals, with particular emphasis on applications involving reproduction and alterations of the germline. Most of the literature discusses how to improve the efficiency, precision, and reliability of CRISPR/Cas9 interventions, especially when applied at early developmental stages such as oocytes, zygotes, embryos, or germ cells. Across species—including rodents, livestock, fish, and other vertebrates—many studies investigate delivery methods such as microinjection or electroporation, strategies to reduce mosaicism, and approaches to increase successful editing rates.

A considerable portion of the dataset also examines off-target effects and unintended genetic modifications. In this context, safety is mainly framed in technical terms, focusing on mutation rates, genetic integrity, and reproducibility across generations, rather than on broader ethical or welfare-related implications.

Several papers explore genome editing as a tool for improving production traits in livestock, such as growth, fertility, or disease resistance. In these cases, CRISPR is generally presented as a promising and innovative technology, with discussion centered on feasibility and potential benefits.

Despite the extensive technical focus, explicit ethical discussion is limited across the included studies. Ethical issues are rarely (almost never) addressed as a main objective and, when mentioned, are often implicit or limited to brief considerations related to technical risks, regulatory compliance, or experimental limitations. Only a small number of papers explicitly discuss ethical aspects of genome editing in animal reproduction, and none provides a systematic ethical analysis specifically focused on wildlife conservation or de-extinction. Overall, the dataset reflects a research landscape in which technical innovation has progressed more quickly than structured ethical reflection, especially in relation to emerging conservation-oriented applications of CRISPR.

The results, presented in the below tables 1, 2, 3, and 4 were grouped (whenever possible) into thematic areas to facilitate analysis and consultation, preventing them from being fragmented into individual findings that would be less meaningful when interpreted in isolation. The grouped results were then mapped onto the ETHAS framework. They outline the ethical issues raised in the selected studies, but they do not, on their own, clarify their wider ethical significance. The discussion therefore seeks to interpret these results, examining how technical difficulties in CRISPR-based animal research generate recurring ethical concerns that deserve attention for current and future applications of this tool in de-extinction contexts.

Table1.Environmental Ethics

	Reference in the papers	Ethical relevance	Frequency
E1 - Conservation of the biomaterial and genetic information			
E2 - Conservation of the species	Escape of edited animals into wild populations	Causes irreversible genetic contamination, affecting biodiversity and natural species integrity	1/37
E3 - Conservation of biodiversity			

Table 2. Animal Welfare Ethics

	Reference in the papers	Ethical relevance	Frequency
A4 - Health and functioning	Mosaicism	Mosaicism increases uncertainty in genotype and phenotype prediction, raising the risk of unintended suffering due to undetected harmful genetic outcomes	9/37
	Unpredictability and risks of NHEJ-mediated genome editing	Imprecise NHEJ repair can create random insertions or deletions that disrupt gene function, increasing the risk of harmful or abnormal phenotypes	6/37
	Limitations and optimization challenges of HDR-based genome editing	Low HDR efficiency and unintended indel formation may introduce harmful genetic changes, increasing the risk of abnormal development or health problems	2/37
	Off-target effects and unintended genomic alterations	Off-target mutations may produce harmful or unpredictable phenotypes, increasing the risk of developmental defects and unintended suffering	11/37
	Limitations of CRISPR applications in large animal models	Technical limitations of CRISPR in large animal models, including low targeting efficiency and high mosaicism have an impact on animal health	1/37

	Variable efficiency and developmental impact of CRISPR/Cas9 procedures	Variable CRISPR efficiency may disrupt embryonic development or produce incomplete edits, increasing the risk of unintended harm and abnormal phenotypes	18/37
A5 - Allowance of positive affective states and avoidance of negative affective states	Mosaicism	Inconsistent editing outcomes can lead to variable or delayed developmental effects, requiring careful and long-term welfare monitoring	9/37
	Unpredictability and risks of NHEJ-mediated genome editing	Unpredictable NHEJ outcomes may lead to chronic discomfort, illness, or behavioral stress, limiting animals' ability to experience positive affective states	6/37
	Limitations and optimization challenges of HDR-based genome editing	Editing variability associated with HDR can lead to chronic physiological problems or reduced vitality, limiting well-being	2/37
	Off-target effects and unintended genomic alterations	Off-target effects can cause delayed or latent health problems, requiring long-term monitoring to detect hidden phenotypic consequences	11/37
	Variable efficiency and developmental impact of CRISPR/Cas9 procedures	Inconsistent editing outcomes can lead to variable or delayed developmental effects, requiring careful welfare monitoring	18/37
A6 - Expressing natural behaviour and living natural lives			

Table 3. Social Ethics

	Reference in the papers	Ethical relevance	Frequency
S7 - Compliance with rules and regulations	Need for strong legal oversight and socially responsible governance	Pioneering research in genetically edited animals is often framed as innovative and forward-looking, but it requires clear governance structures to ensure that scientific advancement proceeds responsibly. Ethical concerns arise when	1/37

		safety, regulatory compliance, and social accountability are insufficiently addressed	
S8 - Health, safety and empowerment of the staff and other people involved			
S9 - Positive integration with the surrounding social environment			

Table 4. Research Ethics

	Reference in the papers	Ethical relevance	Frequency
R10 - Scientific quality and innovation	Mosaicism	Mosaicism undermines the reliability of genotype assessment and experimental outcomes, challenging scientific quality and requiring improved editing and validation strategies	9/37
	Unpredictability and risks of NHEJ-mediated genome editing	Because NHEJ produces variable and imprecise edits, it challenges experimental consistency and highlights the need for refined editing strategies and rigorous validation	6/37
	Limitations and optimization challenges of HDR-based genome editing	HDR's technical complexity and sensitivity to donor design challenge experimental consistency, emphasizing the need for robust optimization and high-quality validation standards	2/37
	Off-target effects and unintended genomic alterations	Off-target events reduce the reliability and reproducibility of CRISPR outcomes, requiring improved detection methods and validation strategies	11/37
	Methodological limitations in mutation detection and genomic screening	Incomplete mutation detection weakens the accuracy of genome-editing workflows, highlighting the need for improved screening methods and more robust validation strategies	13/37
	Data biases in sgRNA design and library construction	Poor sgRNA design or incomplete guide libraries can reduce editing precision and increase off-target risk, highlighting the	7/37

		need for optimized design strategies and high-quality methodological standards	
	Limitations of CRISPR applications in large animal models	Technical limitations of CRISPR in large animal models, including low targeting efficiency and high mosaicism, reduce experimental reliability and require improved species-specific protocols	1/37
	Variable efficiency and developmental impact of CRISPR/Cas9 procedures	Variable CRISPR efficiency reduces experimental reliability and reproducibility, highlighting the need for optimized protocols and rigorous validation	18/37
R11 - Transparent and effective data management	Methodological limitations in mutation detection and genomic screening	Undetected deletions, mosaicism, or off-target mutations can lead to genotype misclassification, requiring careful data reporting and comprehensive record-keeping to avoid misleading conclusions	13/37
	Data biases in sgRNA design and library construction	Biases in sgRNA selection or library construction must be clearly documented, as incomplete or redundant guide sets can distort results and lead to misleading interpretation of editing outcomes	7/37
R12 - Transparent and effective communication	Limitations of CRISPR applications in large animal models	Because CRISPR performance differs markedly in large animals, researchers must clearly communicate methodological limitations and uncertainties to avoid overstating results or feasibility	1/37
	Gaps in off-target assessment and reporting	Limitations in off-target assessment must be clearly communicated, as incomplete reporting of heritable risks and long-term effects can hinder reproducibility and mislead interpretation across studies	2/37

4. Discussion

4.1 Editing errors and unintended outcomes (A4, A5, R10, R11)

4.1.1 Mosaicism

Genetic mosaicism emerged as one of the most recurrent concerns in the analyzed literature, being reported in 9 out of 37 papers. Mosaicism is defined as the presence of two or more cell lineages carrying different genotypes within the same individual and may arise naturally during early embryonic development as a result of errors introduced during DNA replication (Biesecker and Spinner, 2013, as cited in Salvesen et al, 2024). In the context of genome editing, mosaicism occurs when genome modification takes place after the first embryonic division, leading to variable genetic outcomes across different cells and tissues of the developing organism (Salvesen et al., 2024). The resulting individual will therefore have some cells that are successfully edited and some that are not.

Several studies indicate that mosaicism is not an exceptional outcome but rather a common outcome of direct zygotic genome editing. Salvesen et al. (2024) report that when conventional CRISPR-Cas9 reagents are used in livestock, mosaicism is the expected consequence. Similarly, mosaic animals with variable expression patterns and integration events have been observed following in vivo injection of Cas9–gRNA complexes or mRNA into unfertilized eggs (Ikmi et al., 2014).

The degree of mosaicism is potentially influenced by multiple variables, including the developmental stage at which editing is performed, the delivery method used, the animal species and gene editors components (Tanihara et al., 2021). Jin et al. (2021) point out that CRISPR-based genome editing, particularly when applied at suboptimal embryonic stages, inevitably generates mosaic founder animals, which are not suitable for reliable phenotypic assessment because different cell lineages within the same individual may carry distinct genotypes. The resulting phenotypes cannot, therefore, be reliably attributed to the intended genetic modification. The authors further note that mosaicism in F0 individuals is strongly influenced by the embryonic stage at which genome editing takes place. If CRISPR components act after the first embryonic division, genetic modifications may be introduced unevenly across cells, a risk that is worsened by species-specific differences in embryonic development, as guide RNAs and other CRISPR components may degrade at different rates depending on the developmental pace of the species involved (Jin et al. 2021).

Although some strategies, such as introducing Cas9 protein in a more appropriate timing, have shown partial success in reducing mosaicism in mice, these approaches have not fully resolved the issue in larger species such as pigs, cattle, or primates (Yamashita et al., 2020).

Mosaicism also complicates genetic screening and monitoring. Studies in sheep show that relying on a single tissue sample is insufficient to assess the full extent of mosaicism, which can result in misleading conclusions about an individual's genotype and its heritability (Zhang et al., 2025). Pre-implantation embryo biopsies are often proposed as a method to ensure that only edited embryos are transferred; however, their predictive value is limited in the presence of mosaicism (Vilarino et al., 2018). Vilarino et al. (2018) also demonstrate that mosaicism can significantly reduce the reliability of embryo biopsies results by causing biased amplification and inaccurate allele representation, which in turn undermines decision-making that relies on partial genetic data.

4.1.2 Ethical implications

Mosaicism introduces a special kind of unpredictable harm (A4, A5) to the animals involved that goes against the refinement principle. In fact, the animals may develop complex pathologies that are not found in nature because an edit may be present in some tissues but absent in others, resulting in genetically diverse animals that may suffer from random physiological or developmental abnormalities and be put in a state where internal genetic conflicts jeopardize their health.

Additionally, in order to isolate the desired modification, mosaicism requires extra breeding steps spread across several generations. Since founder animals serve primarily as vehicles for establishing a heritable line rather than as experimental subjects in their own right—meaning the research benefit accrues to their offspring rather than to them—the fact that more generations are required due to the presence of mosaicism implies a serious violation of the Reduction principle. This point is also emphasized by Mehravar et al. (2019), who note that to address mosaicism, one must first produce a founder animal carrying the intended genetic alterations, and then establish new mutant lines by outcrossing these mosaic founders—a procedure that requires months in rodents but can take years in other species, including non-human primates (Mehravar et al., 2019).

An ethical dilemma arises when a technology promoted for its accuracy actually needs a more wasteful, trial-and-error method of animal production because of this "clean-up" procedure, which substantially raises the total number of animals used and extends the duration of experiments, in clear contrast with the Reduction principle (Montoliu, 2024). The use of animals in research is only ethically acceptable if the benefits outweigh the risks. When mosaicism makes experimental data hard or impossible to interpret, and technical limitations make it impossible to reliably predict the welfare trajectory of an edited organism, the harm-to-knowledge ratio becomes ethically unjustifiable: the animal bears a real and unpredictable cost, while the epistemic gain is undermined by the same uncertainty that generated that cost. This compromise of scientific validity further weakens the proportionality justification for animal use.

4.1.3 Off-target effects

Another leading technical and ethical concern in the application of CRISPR/Cas9 – being mentioned in 11 out of 37 papers - is the presence of unintended genomic alterations referred to as off-target effects. They are characterized by genomic alterations arising at non-target loci which may trigger unforeseen biological consequences, including the introduction of novel mutations or the impairment of endogenous gene functions (Aboelhassan & Abozaid, 2024; Carey et al., 2019). These effects are primarily driven by the guide RNA (gRNA) annealing to non-targeted sequences that possess homology to the intended 20 base pair target; because the gRNA can tolerate several base pair mismatches, it has the potential to bind to and cleave thousands of unintended sites (Barkova et al., 2022; Hallerman, 2021). Numerous studies show that more than 50% of mutations caused by early CRISPR agents resulted in such off-target effects (Fu et al., 2013; Cho et al., 2014, as cited in Barkova et al., 2022).

These unintentional effects go beyond simple point mutations; studies report major structural damage, including “chromosomal translocations,” “aneuploidies,” “deletions,” and “gross alterations” such as inversions. (Li et al., 2018; Montoliu, 2024). Hallerman (2021) emphasizes that these mutations can lead to "unknown or unintended effects," such as the disruption of normal gene activity, gene silencing, or complete knockouts. An important complicating factor is that these mutations are “difficult to detect,” since both their precise number and their exact locations remain unknown to the staff performing the procedures (Hallerman, 2021).

To address these "potential drawbacks" (Li et al., 2018), researchers have explored technical refinements. The transition from plasmid-based systems to Ribonucleoproteins (RNPs) is highlighted as a critical strategy to prevent the "random integration of plasmid DNA," resulting in less toxicity and fewer off-target effects (Xu et al., 2022). Furthermore, the development of Cas9 Nickase systems—which only produce single-stranded breaks—has been identified as a method to reduce non-specific incisions, though it requires a more complex setup with two gRNAs (Hay et al., 2017; Shamsara et al., 2023). Despite these improvements, recent studies in primates confirm that unintended editing remains a "major concern" that can result in "unexpected phenotypes" (Ryu et al., 2024). Ultimately, the literature indicates that although certain mutations might not have an immediate effect on animal health, the possibility of triggering unexpected phenomena remains inevitable (Montoliu, 2024; Ruan et al., 2017).

A further limitation identified in the literature, which warrants discussion despite being explicitly addressed in only two of the reviewed papers, is the systemic gap in off-target tracking and reporting. Despite the high stakes of germline modification, the frequency of genome-wide off-target mutations in edited animals and their subsequent offspring has not been sufficiently documented (Li et al., 2018). This "reporting gap" is exemplified by studies where off-target effects were not screened at all, representing a notable limitation in the validation of CRISPR outcomes (Zhang et al., 2025).

4.1.4 Ethical implications

Off-target effects create a risk of latent harm (A4, A5). Because these mutations can inadvertently disrupt stable physiological processes or silence essential genes, they may cause chronic suffering that is not immediately visible. This creates a state of biological uncertainty where the animal's physiological integrity is compromised. Ethically, this violates the principle of non-maleficence: by subjecting an animal to an imprecise tool, researchers risk inducing unique, asynchronous pathologies that serve no therapeutic purpose and cannot be mitigated through standard veterinary care.

Two further aspects that need to be considered when evaluating the consequences of off-target effects are genetic pleiotropy and epigenetics, since both can potentially harm animal welfare. A gene is considered pleiotropic when it influences several phenotypic traits, which may be distinct or seemingly unrelated. Pleiotropy can arise at different genetic levels. It may occur at the allelic level, when a single genetic variant influences multiple phenotypic traits, or at the gene or genomic region level, where different variants within the same gene or region are associated with distinct phenotypes (Solovieff et al., 2013). This implies that we lack complete control over and understanding of every individual gene. Therefore, even if we pinpoint a specific gene as responsible for a trait we aim to modify, it might also influence another unrelated trait. As a result, when we replace the unwanted gene variant with another one, we may inadvertently cause an additional, unintended phenotypic change alongside the intended modification (Schultz-Bergin, 2018). This unintended phenotypic change may translate into a decreased life quality for the animal involved.

Epigenetics studies heritable changes in gene expression that occur without altering the DNA sequence. The epigenome consists of chemical modifications that leave the DNA sequence unchanged but control how genes are expressed by modifying specific genomic regions in ways that maintain either active or silenced gene states (Jaenisch and Bird, 2003). Working together with the genome, it helps shape the phenotype. Because most epigenetic marks are not stably inherited, genome editing must work within an epigenetic landscape that is heavily shaped by random and environmental factors (Jaenisch and Bird, 2003). This implies that, even if CRISPR enables highly precise manipulation of the genome, there will still be a risk of unforeseen gene expression once that modified genome interacts with epigenetic factors. In addition, the presence or absence of epigenetic factors - despite a successful editing of the genome – may lead to no phenotypic effect (Schultz-Bergin, 2018). All this, in turn, may lead to unsuccessful research studies with a higher number of animals being employed, and again a potentially a negative impact in their physiology and behavior for which it may be needed to euthanize a "fully sentient animal" (Schultz-Bergin, 2018).

Genomic imprecision also undermines the scientific value of the research (R10). The ethical justification for using animals is predicated on the production of robust, interpretable data. When off-target mutations introduce hidden variables into the genome, they obscure the relationship between genotype and phenotype, potentially rendering the experimental results scientifically invalid. If the "noise" created by these unintended edits makes the data unreliable, the animal has been subjected to harm for a result that lacks utility. This "precision gap" therefore threatens the proportionality of the research, as the ethical cost to the animal is not balanced by a definitive gain in knowledge.

Lastly, the lack of assessment and reporting of off-target effects (R11), highlighting a tension between technical innovation and the ethical duty of accountability. When off-target effects are not screened or reported, it represents a failure in scientific accountability. If the safety profile of an animal is unknown, the researcher cannot fulfill their ethical obligation to ensure that the intervention is "precise" and "safe" for the animal and its progeny. Transparency is the cornerstone of responsible research. The lack of well-documented off-target rates across generations creates an informational gap that "creates obstacles for the future promotion of genome editing" (Ruan et al., 2017). Without transparent data, the scientific community and the public cannot make informed decisions about the technology's readiness. From the perspective of R11, a researcher's responsibility extends to the long-term monitoring of the animal lineage. By failing to document heritable off-target events, researchers pass a "burden of risk" onto future generations of animals and breeders. Responsibility implies not only achieving the desired edit but also actively identifying and disclosing the undesired ones to prevent the silent propagation of genetic errors.

Based on the literature analysis performed, mosaicism and off-target effects appear to be the most direct and tangible outcomes associated with CRISPR applications requiring an ethical consideration, especially in the light of their potential consequences on animal welfare and for the cascade of events they might trigger across the other ethical dimensions. However, the literature review revealed the involvement of specific technical factors that influence the occurrence and extent of mosaicism and off-target effects, which will be presented in the following sections.

4.2 Technical uncertainty in CRISPR genome editing (A4, A5, R10, R11)

4.2.1 Variable efficiency of CRISPR/Cas9 procedures

In the dataset, 18 out of 37 papers discuss variable efficiency and impact of how CRISPR/Cas9 procedures are performed. This matters ethically because “efficiency” here is not just about speed or convenience: it directly shapes how many embryos/animals are exposed to interventions, the likelihood of developmental disruption, and whether the resulting animals are biologically predictable and scientifically informative. Across the literature, CRISPR/Cas9 is repeatedly portrayed as a method whose outcomes depend on a precarious balance between successful genomic modification and embryo survival, with variability driven by delivery mode, timing, reagent activity, and biological context (Salvesen et al., 2024; Tröder et al., 2018; Davachi et al., 2023; Yamashita et al., 2020; Tanihara et al., 2021; Ryu et al., 2024).

<u>Delivery method</u>

Salvesen et al. (2024) explicitly link delivery choice to “editing efficiency, embryo viability, and the level of mosaicism” underlying that introduction of CRISPR components after the initial embryonic division results in inconsistent editing across cell lineages, producing offspring with a patchwork of genotypes, commonly referred to as mosaicism.

Pronuclear injection and microinjection are frequently described as reliable but invasive and technically demanding. Tröder et al. (2018) stress that pronuclear injection requires high skill and can damage embryos. Microinjection is widely described as a reliable and well-established technique for introducing CRISPR/Cas9 components into embryos, yet Nakagawa et al. (2018) emphasize that it is also time-consuming and difficult to master, requiring specialized equipment and high operator skill to avoid embryo damage. Dong et al. (2014) highlights a critical limitation of CRISPR/Cas9 microinjection approaches: only a relatively small proportion of the injected RNAs reach the primordial germ cells (PGCs). Edits that fail to reach PGCs will not be transmitted to the next generation. As a result, even when somatic editing occurs, the procedure may fail to achieve its primary objective of producing a stable, heritable genetic modification. Animals generated through microinjection may appear genetically modified but still be incapable of passing the mutation to their offspring. This disconnect between apparent success and actual heritability introduces inefficiency into the research process, as additional generations must be produced and screened to identify founders that carry edits in their germ cells. Finally, Wu et al. (2015) highlight this fundamental limitation by stating that direct injection of the CRISPR–Cas9 system into zygotes cannot produce healthy progeny with complete efficiency and may also lead to off-target genetic modifications.

Electroporation is often proposed as a faster and more scalable alternative, but multiple papers show that it introduces new welfare risks rather than eliminating them. Davachi et al. (2023) demonstrate that increasing electrical pulse duration significantly reduces embryo survival by raising the temperature of the electroporation medium. Tröder et al. (2018) further warn that some electroporation protocols rely on corrosive pre-treatments (acidic Tyrode's solution) to weaken the zona pellucida, a structure essential for embryo protection. Weakening this barrier can compromise embryo viability and is difficult to standardize, increasing the risk of unintended damage. Tröder et al. (2018) also draw attention to strain-dependent sensitivity, observing that many optimized electroporation protocols are based on robust F1 hybrid mice, whereas more sensitive inbred strains such as C57BL/6 are commonly used in research. This mismatch raises ethical concerns about generalizability: protocols that appear safe and efficient in resilient strains may cause disproportionate harm when applied to more vulnerable embryos.

Timing

A second major theme concerns the timing of CRISPR activity during early development. Salvesen et al. (2024) explain that if DNA editing occurs after the first embryonic division, variable editing across cells results in genetically mosaic offspring. Yamashita et al. (2020) further clarify that avoiding mosaicism requires that "mutations should be induced in one-cell-stage zygotes before DNA replication", a condition that is difficult to ensure consistently in practice.

Tanihara et al. (2021) generalize this issue by noting that both microinjection and electroporation often induce mosaicism due to delayed expression of mRNA-based editors or persistent activity of gene-editing components across multiple cell divisions.

Timing challenges are further compounded by reproductive context. Mukai et al. (2020) emphasize that precise knowledge of fertilization timing is critical when inducing germline changes. In settings such as natural mating or artificial insemination, where fertilization occurs over a broader time window, injections into embryos that are about to cleave can result in higher mosaicism than injections performed before or immediately after sperm entry.

Low developmental success and embryo loss

Many studies report low overall efficiency in terms of viable offspring produced. Dong et al. (2019) report that only four newborns were obtained from forty injected zygotes per strain, illustrating how a large proportion of embryos may be exposed to invasive procedures without developing into live animals. Wu et al. (2015) similarly state that direct injection of CRISPR/Cas9 into zygotes cannot produce healthy progeny at 100% efficiency, reinforcing the idea that embryo loss is an intrinsic feature of the procedure rather than an exception.

Ryu et al. (2024) provide insight into possible causes of reduced developmental success, suggesting that embryo development may be impaired by the cytotoxicity of injection materials such as Cas9 protein, sgRNA, and ssDNA. They also highlight the occurrence of chromosomal abnormalities following double-strand breaks, including large deletions, rearrangements, and ploidy issues, which can lead to chromosomal instability, cell division arrest, and reduced blastocyst formation. Even when presented as potential explanations, these findings underscore that CRISPR procedures can interfere with fundamental developmental processes.

Additional embryo handling steps can further increase vulnerability. Kaneko & Nakagawa (2020) and Nakagawa et al. (2018) note that early-stage embryos show low resistance to vitrification, indicating that developmental harm may arise not only from CRISPR activity itself, but from the cumulative stress of the entire experimental workflow.

Incomplete mechanistic understanding

Underlying many of these limitations is incomplete biological understanding. Aboelhassan & Abozaid (2024) state that the precise mechanism underlying Cas9-mediated DNA melting remains incompletely understood. Chen et al. (2023) further show that despite numerous methodological innovations, the efficiency of precise DNA integration via HDR remains suboptimal, requiring increasingly complex strategies to improve outcomes. Ryu et al. (2024) demonstrate that editing efficiency can be gene-dependent, with locus accessibility influencing double-strand break frequency, and that multiple parameters (concentration, timing, Cas9 form) must often be adjusted to improve outcomes. Additional uncertainty arises from the use of synthetic guide RNAs. Tröder et al. (2018) note that although these reagents are commercially available and increasingly used, their impact on embryo viability when delivered by electroporation had not been adequately assessed at the time of the study.

4.2.2 Unpredictability and risks of NHEJ and HDR-mediated genome editing

Within the reviewed literature, 6 out of 37 papers explicitly address the unpredictability and risks associated with NHEJ-mediated genome editing. Although this frequency is lower than that of efficiency-related issues, its recurrence across different species and experimental contexts indicates that NHEJ-related unpredictability represents a distinct and ethically significant concern that requires focused discussion. These papers consistently characterize NHEJ as a dominant but imprecise DNA repair pathway whose biological consequences are difficult to control, particularly in embryonic and germline editing.

A defining feature of NHEJ-mediated repair is its intrinsic lack of precision. Aboelhassan & Abozaid (2024) report that NHEJ frequently generates small, random insertions or deletions at CRISPR-induced break sites, often resulting in frameshift mutations or premature stop codons. Because these outcomes arise stochastically, the exact genetic modification introduced cannot be predicted with certainty before the intervention. Zhang et al. (2025) further clarify that while HDR can generate precise edits in the presence of donor templates, NHEJ predominates in their absence, producing variable repair outcomes. Hay et al. (2017) reinforces this limitation by emphasizing that the precision of NHEJ-mediated integrations is constrained by the unpredictability of repair junctions.

Several studies underline that NHEJ is not only unpredictable but also biologically dominant, which biases CRISPR editing outcomes toward imprecision. Hay et al. (2017) note that NHEJ occurs at a dramatically higher frequency than HDR in mammalian cells, making it the default repair pathway even when precise editing would be preferable. Zhang et al. (2025) similarly describe NHEJ as the pathway typically exploited for its high efficiency in generating insertions or deletions. This creates a structural trade-off: procedural efficiency is achieved by accepting randomness in genetic outcomes. While this may accelerate model generation, it increases uncertainty regarding genotype–phenotype relationships and reduces predictability.

Multiple sources link NHEJ directly to the formation of mosaic organisms. Yamashita et al. (2020) explain that following early embryonic cleavage, NHEJ-induced repair errors introduce random indels at successive cell divisions, leading to embryos composed of genetically heterogeneous cell populations. This makes mosaicism a predictable outcome of NHEJ activity rather than a rare complication. Hallerman (2021) adds that NHEJ-related mosaicism may include partly functional in-frame indels, resulting in mixed levels of gene function across tissues. Such heterogeneity complicates phenotypic interpretation and may lead to subtle or unexpected biological effects that are difficult to detect or anticipate. The unpredictability of NHEJ is also implicated in off-target effects. Carey et al. (2019) report that off-target mutations induced by CRISPR/Cas9 are presumably mediated through NHEJ, as the nucleotide changes observed were random. Once unintended double-strand breaks occur, NHEJ repair can introduce unpredictable genomic alterations at non-target loci, further reducing confidence in editing outcomes.

The literature describes HDR as the pathway required for precise genome editing, but also as a method that is technically complex and inefficient in practice, especially in embryos. HDR requires the delivery of substantial amounts of exogenous DNA donor templates to achieve accurate gene replacement or insertion, which increases procedural complexity and limits overall efficiency (Aboelhassan & Abozaid, 2024). In contrast, NHEJ functions effectively without requiring donor templates and thus largely determines repair outcomes, though this comes at the expense of accuracy. Optimization strategies can partially improve HDR outcomes. Zhang et al. (2025) report that single-stranded donor templates (ssODNs or ssDNA) achieve higher integration efficiency and precision than double-stranded DNA donors. However, even under optimized conditions, HDR-mediated editing frequently produces unintended indels, indicating that HDR does not fully escape the unpredictability of DNA repair processes, despite its conceptual goal of precision.

4.2.3 Data biases in sgRNA design and library construction

Within the reviewed literature, 6 out of 37 papers explicitly address issues related to biases introduced at the sgRNA design and library construction stage, showing that they can significantly affect the reliability, safety, and interpretability of CRISPR/Cas9 experiments. These biases arise from incomplete sgRNA coverage, redundancy within libraries, and insufficient control of sgRNA specificity, and they shape experimental outcomes even before genome editing is performed.

Yu et al. (2022) explicitly acknowledges limitations in their CRISPR/Cas9 knockout library, noting insufficient coverage of protein-coding genes and non-coding RNAs. Incomplete sgRNA coverage restricts the scope of genetic screening and may result in the systematic exclusion of biologically relevant targets. The same study reports the presence of redundant sgRNA sequences (Yu et al., 2022), which can skew screening results by overrepresenting certain genes while wasting sequencing and experimental resources. Redundancy threatens reproducibility and data reliability, as observed effects may reflect library structure rather than true gene function.

Several studies emphasize that sgRNA specificity is a primary determinant of off-target effects. Dong et al. (2019) highlights the use of stringent bioinformatic criteria in the selection of the right sgRNA to minimize predicted off-target potential, while also cautioning that two-sgRNA systems can significantly increase off-target risk and should be avoided unless clearly justified. Carey et al. (2019) further demonstrate that off-target frequency depends strongly on sgRNA design, reinforcing that ethical genome editing begins at the design stage. Ayabe et al. (2019) stress the importance of selecting highly specific gRNAs and Guo et al. (2016) provide experimental confirmation that careful gRNA selection can eliminate detectable off-target effects, demonstrating that high specificity is achievable when design is rigorous. Hallerman (2021) and Mukai et al. (2020) further support the role of improved algorithms, genome comparisons, and high-specificity Cas9 variants in mitigating off-target risks.

4.2.4 Methodological limitations in mutation detection and genomic screening

Issues related to mutation detection and genomic screening are discussed in 13 out of 37 papers, making this one of the most frequently recurring methodological categories in the reviewed literature. This high frequency indicates that limitations in how mutations are detected, interpreted, and validated are not peripheral technical concerns but core constraints shaping the reliability and ethical acceptability of CRISPR/Cas9 applications.

For embryo-stage analysis, pre-genetic screening (PGS) can help identify whether editing has occurred, determine zygosity, and detect mosaicism, but its reliability depends on embryo biopsy and sampling a sufficient number of cells to capture genetic heterogeneity (Salvesen et al., 2024). After birth, Salvesen et al. (2024) stress that founders must be screened across multiple tissues, including germ cells, and never assessed by phenotype alone, since genetic modifications or mosaicism may be hidden from phenotypic observation. Several studies converge on the recommendation to perform blastocyst-stage testing prior to embryo transfer, allowing early identification of mutations and reducing the propagation of uncertain genotypes (Gim et al., 2021; Dimitrieva et al., 2016; Pintér et al., 2020).

Another major limitation concerns the inability of common genotyping methods to detect certain classes of mutations. Zhang et al. (2025) report that large deletions at target sites may remain undetected by PCR when they exceed the amplicon size, potentially biasing genotyping results. Similarly, Yamashita et al. (2020) show that TIDE software analysis cannot reliably estimate indels larger than 50 bp, requiring additional electrophoretic analysis to infer mosaicism. These studies collectively show that “negative” or apparently clean genotyping results may arise from limitations of the assay itself, rather than indicating genuine genomic integrity.

The literature also highlights significant limitations in off-target detection strategies. Li et al. (2018) argue that whole-genome sequencing (WGS) provides a less biased assessment of off-target mutations than Sanger or targeted deep sequencing of pre-selected sites, which depend heavily on bioinformatic prediction. However, they also note that predicting all potential off-target sites in advance is inherently challenging, especially when mismatches distal from PAM sequences are considered. Carey et al. (2019) similarly describes off-target prediction as technically difficult, despite the availability of computational tools such as CRISPOR. At the genome-wide level, Dong et al. (2019) describes multiple highly sensitive detection methods (e.g. GUIDE-seq, CIRCLE-seq, DISCOVER-seq) but emphasize their practical limitations, including false positives, model restrictions, and experimental complexity, which limit routine application.

Additional bias arises from DNA amplification artifacts, particularly in embryo-stage analyses where DNA quantity is limited. Dimitrieva et al. (2016) report that whole-genome amplification (WGA) can introduce both false positives and false negatives, while its high cost limits sample throughput. Ryu et al. (2024) further show that WGA and PCR can generate false positive signals during ssDNA integration analysis, as homology arm sequences may unintentionally prime amplification. These findings indicate that methodological error can be introduced not only during detection, but also during sample preparation.

Finally, several authors emphasize that improving detection reliability requires broader and layered genomic approaches. Hallerman (2021) advocates for expanded use of next-generation sequencing and multi-omics to distinguish natural genetic variation from genome-editing-induced changes. Tanihara et al. (2021) similarly noted that WGS can reveal the absence of off-target cleavage and summarize a range of approaches—algorithmic design, genome-wide assays, and improved editing systems—that indirectly highlight the dependence of genome editing on robust detection strategies.

4.2.5 Ethical implications

Although discussed separately, variable efficiency, unpredictability of repair pathways, biases in sgRNA design, and limitations in mutation detection represent interconnected sources of technical uncertainty in CRISPR genome editing, which in most of the cases can somewhat indirectly lead to mosaicism and off-targets, with similar ethical patterns and implications.

In fact, across the reviewed literature, these factors converge on the same ethical concerns: increased risk of animal suffering (A4), compromised physiological integrity (A5), reduced scientific proportionality (R10), and challenges to transparency and accountability (R11). They show that ethical evaluation of CRISPR cannot focus on single technical steps but must consider the entire workflow, from experimental design to long-term monitoring.

Technical uncertainty of CRISPR creates welfare risks (A4, A5) because animals are not only exposed to interventions but may suffer specific and predictable biological harms resulting from those interventions. During embryo editing, microinjection can rupture blastomeres, damage the zona pellucida, or introduce toxic concentrations of Cas9 protein or sgRNA, leading to embryonic death or abnormal implantation. Electroporation can overheat embryos or disrupt membranes, causing developmental arrest. For donor and surrogate females, inefficiency also has direct welfare consequences. Repeated superovulation, surgical embryo collection, and embryo transfer procedures expose females to anesthesia, surgical stress, infection risk, and reproductive complications. When editing fails, the same animals may undergo additional cycles, increasing cumulative pain and physiological stress. These risks conflicting with Reduction principle, because inefficiency requires producing and manipulating many more embryos and breeding animals than would be necessary with reliable methods.

Animals that survive editing may experience long-term welfare problems linked to mosaic or unintended mutations caused by delayed CRISPR activity or editing conditions. For example, edited animals may develop immune deficiencies, metabolic disorders, neurological abnormalities, infertility, or abnormal growth patterns when gene disruption is uneven across tissues. Mosaic individuals may show inconsistent organ development, or partial loss of gene function leading to chronic illness. Off-target mutations or chromosomal rearrangements can produce subtle behavioral changes, increased cancer risk, or reduced lifespan that may not be immediately detectable. Such outcomes compromise the animal's ability to feed, reproduce, move normally, or interact socially, all core welfare indicators.

These harms also contradict Refinement principle because they arise from procedures that cannot yet reliably control when and where genomic edits occur. When embryo loss, repeated breeding, or late-onset disease are foreseeable outcomes of known inefficiencies, animal suffering becomes built into the experimental design rather than an accidental side effect. Ethical genome editing therefore requires demonstrating that protocols can minimize embryo mortality, reduce repeated surgical procedures, and prevent predictable developmental pathologies, otherwise, variable efficiency represents a direct and serious welfare concern.

Another ethical concern emerging from the literature which is important to stress is biological unpredictability. Variable efficiency, stochastic NHEJ repair, incomplete HDR outcomes, sgRNA design biases, and limitations in mutation detection all reduce confidence in the genetic identity of edited organisms. From an ethical perspective, creating organisms whose development and health trajectories cannot be reliably predicted challenges the acceptability of genome editing. Ethical research requires a reasonable capacity to anticipate consequences; when the timing, location, and effects of genomic modifications cannot be controlled, it becomes difficult to assess risk–benefit ratios or to ensure that animals are not exposed to unpredictable harm.

Technical uncertainty also undermines the scientific value of CRISPR experiments (R10). Biased sgRNA design, incomplete genomic screening, inefficient editing protocols, and unpredictable repair pathways may produce ambiguous or misleading results. Hidden off-target mutations or mosaic genotypes can obscure genotype–phenotype relationships, reducing reproducibility and limiting the generalizability of findings. When data reliability is compromised, the proportional justification for animal use is weakened. Ethical animal research requires that the knowledge gained be robust enough to justify the biological cost. If additional animals must be bred or experiments repeated to compensate for unreliable methods, the ethical balance between harm and benefit becomes uncertain. In this sense, methodological rigor is not only a scientific requirement but an ethical obligation.

Because no detection method or design strategy is perfect, technical uncertainty creates a strong ethical duty for transparency and research responsibility (R11). Researchers must openly report failure rates, detection limits, sgRNA selection criteria, and uncertainty in mutation classification. Failure to disclose these limitations risks misleading other researchers, regulators, and the public, potentially leading to repetition of harmful or scientifically invalid experiments. Transparency is especially important when edited animals may be bred across generations, as undetected mutations or unreported methodological limitations can propagate genetic risks into future animal populations.

4.3 Environmental ethics (E1 – E3)

The systematic literature search yielded limited direct evidence for the environmental ethics dimensions of the ETHAS framework (E1: conservation of biomaterial and genetic information; E2: conservation of the species; E3: conservation of biodiversity). Only one paper in the dataset explicitly addressed an environmental concern—the risk of edited animals escaping into wild populations, with consequences for biodiversity and species integrity (Table 1). This absence is itself informative: it reflects the fact that the reviewed literature focuses overwhelmingly on controlled laboratory settings, where ecological consequences remain hypothetical. Nevertheless, the environmental ethics domain is arguably the most distinctive feature of de-extinction ethics relative to ordinary CRISPR research, and it deserves substantive engagement even in the absence of a strong primary literature base.

From the perspective of E2 and E3, the most direct environmental concern is the introduction of genetically novel organisms into ecosystems. Sandler (2014) argues that de-extinct organisms are not straightforward replacements for their predecessors: they are new biological entities whose ecological relationships—with prey, predators, parasites, and competitors—cannot be assumed to replicate those of the extinct species. A woolly mammoth proxy engineered from Asian elephant cells may carry the same cold-adapted traits as the extinct mammoth, yet the Siberian steppe ecosystem into which it would be released has changed substantially since the Pleistocene. The risk is not merely that the de-extinct organism will fail ecologically, but that it could succeed in unexpected ways, displacing native species or disrupting trophic cascades. These concerns are amplified by CRISPR-specific uncertainties: mosaicism and off-target mutations (Section 3.1) could produce ecological phenotypes that diverge from intended designs only after reintroduction.

With respect to E1, de-extinction raises a concern absent from standard CRISPR research: the genome of the living surrogate species may itself be altered. In projects involving Asian elephants as surrogates for woolly mammoth traits, the individuals used carry heritable germline modifications. If such individuals bred with wild conspecifics, engineered sequences could introgress into wild populations, altering the genetic integrity of a critically endangered species (IUCN/SSC, 2012). The surrogate species used in de-extinction projects are often themselves species of conservation concern, and their genomic integrity constitutes an independent conservation obligation.

Collectively, these considerations suggest that the environmental ethics domain is not merely absent from current CRISPR research but actively deferred: ecological risks are not yet encountered because de-extinct organisms have not been released. The ethical framework governing de-extinction must therefore include prospective ecological risk assessment as a mandatory step before any edited organism is introduced into a natural environment, and the ETHAS goals E1–E3 provide an appropriate structure for conducting such an assessment.

4.4 Social Ethics (S7–S9)

The literature reviewed did not provide substantial references to the domain of social ethics. This likely reflects the early stage of CRISPR-based de-extinction research, where technological development is progressing faster than legal frameworks, governance structures, and broader societal debate. Because genetically edited individuals have not yet been introduced into natural ecosystems, ecological risks remain largely hypothetical, and current discussion is focused primarily on laboratory feasibility and the scientific excitement surrounding the potential revival of charismatic extinct species. As Stephen (2024) emphasizes, the governance of CRISPR in conservation and de-extinction remains uncertain because technological progress is advancing faster than existing legal and regulatory frameworks. Regulations on genetic engineering and biodiversity protection vary widely across countries, creating a fragmented landscape that may hinder coordinated international action. Establishing clear and harmonized guidelines is therefore essential to ensure that CRISPR applications in biodiversity conservation are assessed for safety, effectiveness, and ethical acceptability. Reynolds (2021) emphasizes that since researchers, biological materials, knowledge, and potential impacts may cross national boundaries, governance frameworks will need to include a certain degree of international coordination. States have a responsibility to prevent harm resulting from activities conducted within their jurisdiction or control when these activities pose a significant transboundary risk. In this context, governments are expected to exercise due diligence by implementing measures such as requiring prior authorization, conducting environmental impact assessments, notifying and cooperating with potentially affected states in good faith, and ensuring transparency toward the public (Reynolds, 2021).

IUCN has attempted to provide some guidance for de-extinction conservation through the document *Guiding Principles on Creating Proxies of Extinct Species for Conservation Benefit*, released in 2016. A set of guiding principles was provided to evaluate the potential conservation role of creating proxies of extinct species. According to these guidelines, any de-extinction initiative must be justified by a clear expectation of conservation benefit, such as restoring ecological functions or improving ecosystem resilience. Candidate species should not be selected solely on the basis of technical feasibility; instead, projects must consider ecological relevance, potential risks, generation time, and the possibility of controlling or reversing releases if negative impacts occur. Existing alternatives, including ecological replacements using extant species, should also be evaluated before pursuing proxy creation. The IUCN recommends that projects follow established frameworks such as the Guidelines for Reintroductions and Other Conservation Translocations and conduct comprehensive risk assessments prior to any environmental release. These assessments should examine ecological, genetic, disease-related, and socio-economic risks, as well as the potential for project failure. Stakeholder involvement, including the informed consent of local communities and Indigenous peoples, is considered essential. In addition, de-extinction programs should define clear success criteria, ensure long-term monitoring of released individuals, and incorporate strategies to minimize animal suffering. Finally, projects must avoid diverting resources from the conservation of extant species and carefully evaluate opportunity costs.

The document also emphasizes and acknowledges that the creation and potential release of proxy species raise significant legal and legislative challenges. Existing conservation laws typically classify species according to measurable indicators such as population decline and geographic range, criteria that proxy species would initially lack because they have no established population or distribution. As a result, their legal status would be uncertain and could vary across jurisdictions. In addition, many legal frameworks restrict the introduction of non-native species, meaning that proxy species could only be released through specific exemptions. Different authorities may also interpret the status of proxy organisms differently, as illustrated by cases in which cloned or hybrid individuals have been classified inconsistently by regulatory bodies (IUCN/SSC, 2016). These uncertainties highlight the need for clearer regulatory frameworks capable of addressing the novel legal and governance challenges posed by de-extinction initiatives.

Another existing international regulatory framework addressing genetically modified organisms that may provide an important reference point for assessing the governance of emerging biotechnologies, including CRISPR, is the Cartagena Protocol on Biosafety under the Convention on Biological Diversity of 2000 (Reynolds, 2021). In fact, it aims to ensure the safe transboundary movement, handling, and use of living modified organisms (LMOs) that may pose risks to biodiversity and can be applied to de-extinction contexts. The Protocol establishes mechanisms for risk assessment and international oversight, including the requirement for advance informed agreement before transboundary transfers occur. In addition, the Nagoya–Kuala Lumpur Supplementary Protocol introduces obligations for response measures and liability in cases where LMOs cause significant damage to biodiversity (Reynolds, 2021). Although the definition of LMOs is provided within the Protocol, it remains uncertain whether all genetically engineered organisms produced would fall under this category.

Brodie et al. (2025) also support the idea that interventions involving engineered genes or organisms can be interpreted as extensions of existing conservation practices, such as conservation translocation or integrated pest management, which may help improve communication and policy development within the conservation community and among stakeholders. However, current regulatory frameworks often lag behind technological progress. Many international agreements regulating genetically modified organisms, such as the Cartagena Protocol on Biosafety, were established before recent advances in genetic engineering and may therefore generate regulatory uncertainty. Consequently, clearer conceptual frameworks and governance mechanisms are needed to ensure that de-extinction conservation strategies are evaluated responsibly and remain consistent with broader conservation objectives (Brodie et al., 2025). To date, the legal status and management of proxy species would need to be assessed on a case-by-case basis according to national legislation and relevant international frameworks through a coordinated approach (Reynolds, 2021).

Building public trust is another point that deserves attention. The question of public acceptability constitutes a distinct and underexplored social ethics concern in de-extinction research. De Graeff et al. (2019) demonstrate that public attitudes toward gene drive technologies in conservation are shaped not only by perceptions of risk and benefit, but by deeper concerns about naturalness, human hubris, and the appropriate scope of technological intervention in wild ecosystems. These findings are directly applicable to de-extinction: the revival of extinct megafauna through CRISPR editing is likely to elicit the same spectrum of responses, from enthusiasm driven by the charismatic appeal of species like the woolly mammoth to unease rooted in concerns about "playing God" and unpredictable ecological consequences. De Graeff et al. (2019) further argue that public engagement must go beyond information provision—it requires genuine two-way dialogue in which lay concerns can shape research priorities and governance decisions. Building public trust will therefore require transparent communication about the scientific basis of CRISPR, its potential benefits and risks, and the limits of current knowledge, as well as meaningful engagement with diverse stakeholders to support informed and socially responsible decision-making (Stephen, 2024; de Graeff et al., 2019).

The governance challenge is compounded by the involvement of private commercial actors. Colossal Biosciences, whose mammoth, thylacine, and dire wolf projects were identified in Section 1.3 as reference points for this thesis, operates outside the regulatory frameworks typically applicable to publicly funded research. The company has announced milestones—including the claimed revival of the dire wolf—through press releases and media channels rather than peer-reviewed publication, and its projects are not subject to the institutional ethics review processes that govern academic research involving endangered species or novel organisms (Hiller, 2025). This creates a governance gap that existing frameworks do not adequately address: neither wildlife law, nor biosafety regulation, nor research ethics guidelines were designed with commercial de-extinction in mind. Meijer et al. (2023) identify this as a structural problem across emerging biotechnologies in conservation, noting that governance tends to lag behind innovation and that the burden of demonstrating safety falls disproportionately on regulators rather than developers. A precautionary approach that requires affirmative demonstration of safety and ethical acceptability before any release—rather than reactive prohibition after harm has occurred—is therefore essential for de-extinction projects proceeding in commercial contexts.

The social ethics domain of ETHAS also encompasses S9 (positive integration with the surrounding social environment). In a de-extinction context this goes beyond institutional governance: decisions about which species to resurrect and where to reintroduce them affect communities, land managers, and indigenous peoples whose relationships with affected ecosystems may predate the species' extinction. The woolly mammoth's potential reintroduction to Siberian steppe and Arctic tundra occurs in territories where indigenous communities hold distinct land rights and ecological knowledge. Programme legitimacy may therefore depend not only on scientific and regulatory approval, but on genuine participatory consultation with those most directly affected — a requirement that current de-extinction discourse has only begun to address.

4.5 Interpretation of ethical issues in de-extinction contexts

While the risk of mosaicism, off-target mutations, and other CRISPR-related unwanted consequences - widely discussed in genome editing research - are not fundamentally different in de-extinction projects, their ethical significance is amplified due to specific biological and technological conditions. In fact, these projects usually rely on editing the genome of the closest living relative of an extinct species, often through multiple CRISPR modifications and in the context of incomplete genomic knowledge. As a result, uncertainty, unpredictability, and the potential for unintended harm are here strongly increased.

4.5.1 Limited species-specific knowledge

Most CRISPR optimization experiments are initially performed in model organisms such as mice or rodents, where genetic pathways, reproductive biology, and long-term outcomes are relatively well studied. In de-extinction projects, however, the editing phase may involve often large, non-model species, for which genomic data, developmental biology knowledge, and long-term health studies are far more limited. This lack of species-specific knowledge increases the potential consequences associated with mosaicism and off-target mutations in such species, whose short and long-term effects remain poorly understood and studied in such animals, consider for example Asian elephants (the closest living relative of the woolly mammoth) or fat-tailed dunnarts (closest living relative of the Tasmanian tiger). Consequently, predicting the welfare impact of genome editing becomes more difficult, challenging risk assessment and undermining animals' life quality. Moreover, it is important to note that in de-extinction projects, where genetically edited individuals may eventually be introduced into the wild or in the unlikely scenario they may accidentally escape, uncertainties caused by mosaicism and off-targets (especially if it remains undetected), raise concerns about ecological risk assessment, the genetic stability of populations, and long-term welfare of both edited animals and the species that interact with them.

4.5.2 Longer generation time

Large mammals used in de-extinction projects have longer generation intervals compared with rodents. If CRISPR editing introduces unintended mutations that are not immediately detected, correcting these errors through breeding programs may require years if not decades. This temporal scale increases the ethical weight of initial editing, because animals may live with harmful phenotypes for extended periods before problems are identified or corrected. It also complicates welfare monitoring and increases uncertainty regarding the heritability of mosaic or off-target mutations.

4.5.3 Multi-target editing and increased genomic risk

De-extinction requires the simultaneous editing of multiple genes to reproduce polygenic traits in large animals such as thermoregulation or metabolism. Multiplex CRISPR editing strongly increases the probability of off-target mutations, large genomic rearrangements, and mosaic genotypes. Even if individual edits are accurate, the cumulative effect of many edits can create unexpected interactions, producing phenotypes that are difficult to predict or detect. This raises ethical concerns about knowingly introducing a high level of genomic uncertainty into sentient animals.

4.5.4 Unprecedented genomic combinations and long-term consequences

Editing the genome of a living species to include traits from an extinct relative creates genetic combinations that have never existed in evolutionary history. Gene interactions may therefore differ substantially from expectations based on either species alone and can affect development, immunity, behavior, or metabolism in ways that cannot be predicted through standard assays, increasing the risk of chronic or latent health problems.

In fact, CRISPR-based de-extinction produces genomes that, as an example, are neither fully mammoth nor elephant. The long-term physiological and ecological consequences of such genomes are unknown, and the effects of unintended mutations may only emerge over time or across generations. This highlights the ethical responsibility associated with germline editing and reinforces the need for conservative validation thresholds before edited animals are produced.

4.5.5 Incomplete ancient genomes

It is possible that ancient DNA may be fragmented or not fully reconstructed. As a result, some genomic regions of extinct species may be missing or inaccurately inferred. Editing based on incomplete genomic templates increases the likelihood of functional errors or unintended phenotypic outcomes. From an ethical perspective, proceeding with genome editing despite incomplete biological information challenges the adequacy of risk assessment and raises concerns about the reliability of scientific justification. The degree of genomic completeness varies considerably across de-extinction targets. The woolly mammoth genome has been sequenced to high coverage—approximately 3.7 billion base pairs at around 65x depth (Lynch et al., 2015)—making it one of the better-characterised ancient genomes. By contrast, the dodo (*Raphus cucullatus*) and the Tasmanian tiger (*Thylacinus cynocephalus*) are represented by more fragmented assemblies, and the dire wolf (*Aenocyon dirus*) genome revealed a deep evolutionary divergence from living canids that complicates functional inference (Perri et al., 2021). The ethical weight of this limitation is proportional to the degree of incompleteness: editing a genome that is 90% characterised poses qualitatively different risks than editing one that is 50% inferred. Ethical oversight bodies should therefore require explicit disclosure of reference genome completeness as a precondition for approving de-extinction editing programmes.

4.5.6 Epigenetic disruption and phenotype uncertainty

CRISPR editing procedures can alter epigenetic regulation, while pleiotropic genes may influence multiple traits simultaneously for which the connection may not be previously studied. Some edited traits may express only under specific environmental conditions, making phenotype prediction difficult in laboratory or captive settings. This uncertainty complicates welfare assessment, because animals may appear healthy in early stages while developing problems later in life or under different environmental conditions.

4.5.7 Trial-and-error editing and the 3Rs

Achieving accurate simultaneous editing often requires multiple experimental attempts. Each failed editing attempt represents additional embryos or animals subjected to genetic modification procedures. Although some level of experimentation is inherent to scientific progress, de-extinction projects may involve a higher degree of trial-and-error due to genomic uncertainty, challenging the application of the 3Rs principle.

4.5.8 Indirect welfare costs of CRISPR editing

In de-extinction workflows, the closest living relative is typically required to carry edited embryos to term. Although pregnancy itself is part of assisted reproduction, the frequency of embryo transfer attempts is strongly influenced by CRISPR-specific failure rates, which include mosaicism, off-target mutations, and undetected large genomic alterations level. When edited cell lines or embryos fail genetic validation, display unexpected phenotypes, or result in non-viable development, additional editing cycles and embryo transfers may be required.

From an ethical standpoint, this creates a downstream welfare impact that is causally attributable to CRISPR uncertainty: the less predictable the editing outcome (especially under multiplex editing), the more likely it becomes that repeated attempts will be necessary to obtain an acceptable genotype/phenotype combination. In large mammals with usually longer reproductive cycles than rodents, this implies that CRISPR imprecision can translate into repeat exposure of surrogate individuals to invasive reproductive procedures and prolonged physiological stress, even when researchers aim to minimize harm. This concern extends to the de-extinct organisms themselves. Browning (2018) argues that animals created through de-extinction face welfare challenges beyond standard captive breeding, including the absence of conspecific social learning, exposure to evolutionarily mismatched environments, and the psychological costs of being the first individuals of a kind. These challenges are compounded when CRISPR-induced mosaicism or off-target mutations add unpredictable physiological burdens on top of the inherent difficulties of captive life. More recently, Hiller (2025) raises equivalent concerns in the context of Colossal Biosciences’ dire wolf project, noting that de-extinction companies may underestimate welfare obligations to the animals they create. Together, these analyses reinforce the argument that the ethical costs of CRISPR imprecision are not limited to the laboratory phase but extend across the entire life trajectory of the animals produced.

5. Conclusion

The absence of publications specifically addressing CRISPR in de-extinction reflects the novelty of the field rather than the absence of ethical relevance. Although direct evidence remains scarce, many of the technical and ethical issues identified in genome editing research in other contexts are likely to be transferable to de-extinction applications, given the shared biological mechanisms and experimental approaches involved, but might be considerably amplified. This suggests that ethical reflection does not need to wait for large-scale implementation but can build on existing knowledge to anticipate risks and avoid dramatic consequences.

Overall, mosaicism, off-target effects, and procedural inefficiencies illustrate how technical uncertainty in CRISPR editing is ethically relevant in itself. These issues simultaneously affect animal welfare, organisms' integrity, cost-benefit evaluation, and research responsibility. Ethical governance of CRISPR therefore requires not only technological improvement and refinement but also careful justification of experimental goals, transparent reporting, and rigorous welfare standards before such approaches can be considered fully ethically acceptable in de-extinction research.

In fact, ethical evaluation must consider whether the technological uncertainty inherent in CRISPR is compatible with conservation goals, or whether it risks introducing new welfare problems into already fragile ecological systems.

The ethical analysis presented in this thesis also points to the need for a stronger and more explicit commitment to the principles of Reduction and Refinement in CRISPR-based de-extinction research. Many of the ethical concerns identified are closely linked to technical uncertainty, which can increase animal use and limit the ability to minimize harm. This indicates that improving methodological reliability, welfare assessment, and experimental design is not only a scientific objective but an ethical requirement. Future developments in genome editing should therefore be guided by a proactive integration and balance of the 3Rs, ensuring that innovation proceeds alongside concrete efforts to reduce animal use and refine experimental procedures.

These considerations also highlight the need for a standardized ethical protocol specifically tailored to CRISPR applications in de-extinction research and guided by the ETHAS framework, which would help define welfare standards, criteria for proportionality, requirements for genomic validation and monitoring, and standards for transparency and public accountability in this specific context.

It is important to acknowledge that this study is subject to several methodological limitations. The literature review was restricted to a single database (Web of Science) and to English-language peer-reviewed publications. In addition, the search strategy relied on predefined query terms, which may have influenced the scope of the retrieved literature. As a result, relevant studies indexed in other databases or present in grey literature may not have been captured. Therefore, the findings should be interpreted as a partial overview rather than an exhaustive account of the ethical issues related to CRISPR-based de-extinction.

The future of de-extinction will depend not only on whether extinct species can be recreated, but on whether the methods used to create them are ethically justifiable.

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