



Contents lists available at ScienceDirect

## Biological Conservation

journal homepage: [www.elsevier.com/locate/biocon](http://www.elsevier.com/locate/biocon)

Perspective

## The ethical assessment of Assisted Reproductive Technologies (ART) in wildlife conservation



Barbara de Mori<sup>a,b,\*</sup>, Elena Mercugliano<sup>a,b,\*</sup>, Pierfrancesco Biasetti<sup>b,c</sup>, Ilaria Pollastri<sup>a,b</sup>, Maria Michela Spiriti<sup>a,b</sup>, Daniela Florio<sup>b,d</sup>, Francesco Andreucci<sup>b</sup>, Frank Göritz<sup>c</sup>, Susanne Holtze<sup>c</sup>, Cesare Galli<sup>e,f</sup>, Jan Stejskal<sup>h</sup>, Silvia Colleoni<sup>e</sup>, Giovanna Lazzari<sup>e,f</sup>, Steven Seet<sup>g</sup>, Jan Zwillling<sup>g</sup>, David Ndeereh<sup>j</sup>, Isaac Lekolool<sup>j</sup>, Stephen Ngulu<sup>i</sup>, Dominic Mijele<sup>i</sup>, Daniel Čizmar<sup>c</sup>, Raffaella Simone<sup>c</sup>, Lisa Schrade<sup>c</sup>, Simone Basile<sup>a</sup>, Thomas B. Hildebrandt<sup>c,k,\*\*</sup>

<sup>a</sup> Department of Comparative Biomedicine and Food Science, University of Padua, Viale dell'Università 16, 35020 Legnaro, (PD), Italy

<sup>b</sup> Ethics Laboratory for Veterinary Medicine, Conservation, and Animal Welfare, University of Padua, Viale dell'Università 16, 35020 Legnaro, (PD), Italy

<sup>c</sup> Department of Reproduction Management, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany

<sup>d</sup> Department of Veterinary Medical Science, University of Bologna, Bologna, Italy

<sup>e</sup> Avantea, Laboratory of Reproductive Technologies, Via Porcellasco, 7/F, 26100 Cremona, Italy

<sup>f</sup> Fondazione Avantea, Via Porcellasco, 7/F, 26100 Cremona, Italy

<sup>g</sup> Science Communication, Science Management, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany

<sup>h</sup> ZOO Dvůr Králové, Stejskal Stefánikova 1029, 544 01 Dvůr Králové nad Labem, Czech Republic

<sup>i</sup> Ol Pejeta Wildlife Conservancy, Private Bag 10400, Nanyuki, Kenya

<sup>j</sup> Kenya Wildlife Service, P.O. Box 40241, 00100 Nairobi, Kenya

<sup>k</sup> Faculty of Veterinary Medicine, Freie Universität Berlin, Kaiserswerther Str. 16-18, 14195 Berlin, Germany

## ARTICLE INFO

## Keywords:

Conservation ethics  
Ethical assessment  
Assisted Reproductive Technologies (ART)  
Animal welfare  
Research ethics  
Biodiversity conservation

## ABSTRACT

The application of Assisted Reproductive Technologies (ART) in breeding programs to save wild animal species is a relatively new approach to conservation and their ethical implications seem still to be underestimated. However, the ethical evaluation is a decisive step for conservation projects employing ART to address crucial questions like the welfare and life of the animals involved, the survival of the targeted species, the quality and safety of the procedures and the very idea of biodiversity conservation we want to pursue. Balancing the potential benefits of ART with the risks involved requires careful ethical analysis. This paper highlights the increasing role of ART in species conservation and emphasizes the need to address their ethical implications. After discussing the lack of ethical discussion in ART application to wildlife, the contribution outlines an ethical frame to address this gap and introduces an ETHical ASsessment tool (ETHAS) for the ethical self-assessment of ART procedures in vertebrate conservation. The tool allows respondents to highlight ethical aspects to be improved and risks related to the procedure, from its design to each application. The ethical approach to the ART application, relatively novel to wildlife conservation, can contribute to assure the ethical acceptability of conservation programs while favoring discussion and communication among project partners and the integration of ethical aspects in conservation programs.

\* Corresponding authors at: Department of Comparative Biomedicine and Food Science, University of Padua, Viale dell'Università 16, 35020 Legnaro, (PD), Italy.

\*\* Correspondence to: T.B. Hildebrandt, Department of Reproduction Management, Leibniz-Institut für Zoo- und Wildtierforschung (IZW) im Forschungsvorband Berlin e.V., Alfred-Kowalke-Straße 17, 10315 Berlin, Germany.

E-mail addresses: [barbara.demori@unipd.it](mailto:barbara.demori@unipd.it) (B. de Mori), [elena.mercugliano@studenti.unipd.it](mailto:elena.mercugliano@studenti.unipd.it) (E. Mercugliano), [biasetti@izw-berlin.de](mailto:biasetti@izw-berlin.de) (P. Biasetti), [ilaria.pollastri@studenti.unipd.it](mailto:ilaria.pollastri@studenti.unipd.it) (I. Pollastri), [mariamichela.spiriti@studenti.unipd.it](mailto:mariamichela.spiriti@studenti.unipd.it) (M.M. Spiriti), [daniela.florio@unibo.it](mailto:daniela.florio@unibo.it) (D. Florio), [francesco.andreucci@aulss5.veneto.it](mailto:francesco.andreucci@aulss5.veneto.it) (F. Andreucci), [goeritz@izw-berlin.de](mailto:goeritz@izw-berlin.de) (F. Göritz), [holtze@izw-berlin.de](mailto:holtze@izw-berlin.de) (S. Holtze), [cesaregalli@avantea.it](mailto:cesaregalli@avantea.it) (C. Galli), [jan.stejskal@zoodvurkralove.cz](mailto:jan.stejskal@zoodvurkralove.cz) (J. Stejskal), [silviacolleani@avantea.it](mailto:silviacolleani@avantea.it) (S. Colleoni), [giovannalazzari@avantea.it](mailto:giovannalazzari@avantea.it) (G. Lazzari), [seet@izw-berlin.de](mailto:seet@izw-berlin.de) (S. Seet), [zwillling@izw-berlin.de](mailto:zwillling@izw-berlin.de) (J. Zwillling), [dndeereh@kws.go.ke](mailto:dndeereh@kws.go.ke) (D. Ndeereh), [stephen.ngulu@olpejetaconservancy.org](mailto:stephen.ngulu@olpejetaconservancy.org) (S. Ngulu), [dmijele@kws.go.ke](mailto:dmijele@kws.go.ke) (D. Mijele), [cizmar@izw-berlin.de](mailto:cizmar@izw-berlin.de) (D. Čizmar), [simone@izw-berlin.de](mailto:simone@izw-berlin.de) (R. Simone), [shrade@izw-berlin.de](mailto:shrade@izw-berlin.de) (L. Schrade), [simone.basile@phd.unipd.it](mailto:simone.basile@phd.unipd.it) (S. Basile), [hildebrandt@izw-berlin.de](mailto:hildebrandt@izw-berlin.de) (T.B. Hildebrandt).

<sup>1</sup> These two authors contributed equally.

<https://doi.org/10.1016/j.biocon.2023.110423>

Received 28 July 2023; Received in revised form 20 November 2023; Accepted 8 December 2023

Available online 13 January 2024

0006-3207/© 2023 Published by Elsevier Ltd.

## 1. Introduction

Breeding programs play an increasingly crucial role in biodiversity conservation, particularly in species with small, isolated populations and low reproductive success (Monfort, 2014; Herrick, 2019; Comizzoli and Holt, 2019; IUCN Action Plan, 2021). To avoid extinction, the IUCN recommends the captive maintenance and reproduction of all species whose habitat is threatened (IUCN, 2020). Assisted reproduction technologies (ART)—defined here as any procedure or technique that involves the handling of gametes or embryos with the final aim of achieving reproduction—can provide crucial support to breeding programs. They allow for overcoming infertility issues, boosting the number of individuals per generation, bridging scattered and fragmented populations without the need of translocating individuals, and providing a way for cryopreserved gene pools to be transferred back to living populations (Ebenhard, 1995; Comizzoli, 2015; Comizzoli and Holt, 2019; Herrick, 2019; Lueders and Allen, 2020; Bolton et al., 2022).

Species-specific optimization and increased knowledge of the reproductive biology of many taxa are still needed to unlock the full potential of ART in vertebrate conservation. However, the number of applications of these biotechnologies is steadily growing. Protocols for reproductive hormones monitoring and treatments are available for several species and are used to gather information on reproductive systems and manipulate them (Clulow and Clulow, 2016; Brown, 2018; Silla and Byrne, 2019). Artificial insemination and fertilization has been successful in several mammal and bird species, as well as in some fishes and amphibians, and even in a few reptiles (Gee et al., 2004; Comizzoli, 2016; Roth and Swanson, 2018; Comizzoli et al., 2019; Comizzoli and Holt, 2019; Silla and Byrne, 2019; Mastromonaco and Songsasen, 2020; Perry, 2021; Clulow et al., 2022; Bolton et al., 2022). Gamete collection, cryopreservation, and cryobanking are growingly recognized as important instruments in conservation, not only for their insurance value but also for facilitating genetic exchange between otherwise isolated populations (Clulow and Clulow, 2016; Comizzoli, 2018; Della Togna et al., 2020; Strand et al., 2020; Hildebrandt et al., 2021; Bolton et al., 2022). Moreover, recent developments in biotechnology have also stimulated the refinement of new laboratory techniques for assisted reproduction. Advanced ART (aART), such as inner cell mass transfer, stem-cell-associated techniques for the generation of gametes and embryos, and cloning, can offer new possibilities for expanding the gene pool of small populations and may represent in this way a new hope for critically endangered species (Hildebrandt et al., 2021; Bolton et al., 2022).

Despite the increasing role of ART in species conservation, their ethical implications are still underestimated. In this paper, after discussing the need for an ethical assessment of ART procedures, we outline a frame for such an assessment and we introduce a tool (ETHAS) to implement it when applied to vertebrate conservation, describing its functioning through customizable checklists and its main strengths and limitations. The aim of the ethical assessment is to check the ART procedures not only regarding their general protocols, but also each time they are implemented. The ethical assessment allows in this way for the continuous monitoring of ART procedures within a project, thus contributing to a responsible and ethically conscious conservation practice, while favoring discussion and communication among project partners.

## 2. The need for the ethical assessment of ART procedures in conservation

In general, the decision to use ART in a conservation project, both in situ and ex situ, can raise numerous ethical questions, concerning the welfare and life of the animals involved, the survival of the targeted species, the safety and quality of the procedures and the very idea of biodiversity conservation we want to pursue (Biasetti et al., 2022). The decision of using ART may provide the last chance for the survival of a species but does not come without risks. Pursuing the knowledge needed

to optimize a procedure can confront scientists and conservationists with the difficult choice between safeguarding the remaining individuals of a species and actively intervening to reverse population decline. Moreover, many applications of ART require the manipulation of live animals, and, in some instances, invasive procedures, sedation, or anesthesia, with possible risks to their health and welfare. In all such cases, an ethical analysis of the procedures is crucial to ensure the acceptability of the project they are part of.

Despite this, the need to analyze and assess ART application from an ethical standpoint does not seem to be sufficiently addressed (see Table 1). This can happen for several reasons. One, obviously, is that there could be no critical situations to report and analyze: if a study does not mention ethics, that does not necessarily mean it must have unaddressed ethical problems. However, in many other cases, there may be alternative explanations for this omission.

In the first place, it may be that, despite awareness of the ethical challenges their work poses (Minteer and Collins, 2005a), conservationists may lack specific training and skills to engage in ethical reasoning to fully address the ethical issues raised by their activities (Saltz et al., 2019; Brittain et al., 2020; Zemanova, 2021).

A second reason could be the underestimation of the importance of ethical standards in biodiversity conservation (Costello et al., 2016). In general, projects aiming at saving endangered species are perceived as inherently “good”, which may result in the impression that there is no need for further justification and that animal care and ethics committees

**Table 1**

Results of a search for ethical terms within original research articles on ART on wildlife.

N. articles retrieved:	500
N. repeated articles + N. articles not found/not in English:	118 + 19
N. non research articles (reviews, book chapters, conference papers, other):	149
N. articles on humans + articles on domestic animals:	34 + 31
N. articles remaining (research papers included in the analysis):	149
N. articles analyzed reporting a received formal ethical approval:	46 (31 %)
N. articles analyzed reporting following a specific animal care policy:	48 (32 %)
N. articles analyzed quoting the word “ethic*” (e.g., ethic, ethical, ethics, ethicist, ethically) in the text (not referred to the ethics committee):	15 (10 %)
N. articles analyzed reporting the application of an ethical self-assessment tool:	1 (1 %)
N. articles analyzed reporting the application of an ethical assessment tool:	1 (1 %)
N. articles analyzed reporting the application of a welfare assessment:	1 (1 %)

For this search, 28 different combinations of 2 keyword categories were run in Scopus search (<https://scopus.com/>), using the Boolean strings (“assisted reproduction technologies” OR “assisted reproduction techniques” OR “assisted reproductive techniques” OR “assisted reproductive technologies”) AND (“Wildlife” OR “wild animal” OR “Conservation” OR “rare breed” OR “animal care and use” OR “animal care” OR “animal use”). No time filter was applied to the search, with the oldest result dating back to 1993 and the latest in 2023.

Repeated results of the same article (n = 118) were not consulted again. n = 19 papers could not be retrieved or were excluded because not written in English. Reviews, books, conference papers and other non-original research papers (n = 149) were excluded because their focus is generally not on the development and optimization of assisted reproductive technologies; moreover, these types of papers do not generally require ethical approval or animal care policy, two of the here researched items. Articles regarding humans (n = 34) or domestic species (e.g. domestic cats, dogs, cattle, poultry etc.; n = 31) were excluded too, being out of scope.

n = 149 original research articles were used for the analysis, 71 % of which concerning Mammals (n = 106), 13 % Amphibia (n = 20), 5.4 % Reptiles (n = 8), 4.7 % Fish (n = 7) and 4.7 % Birds (n = 7). The content of these articles was then analyzed by searching the following terms: “Ethical Approval”, “Animal care policy”, “Ethic\*” quotes (ethic, ethical, ethics, ethicist, ethically and any other word that has as its root the term “ethic”), “Ethical self-assessment”, “Ethical tool”, “Welfare assessment” and “Welfare assessment AND ethical assessment”. Only 46 (31 %) articles mentioned obtaining formal ethical approval and only 48 (32 %) following animal care policies.

A database with references to the retrieved papers and results is provided in Appendix I.

often impose unnecessary constraints on researchers, slowing down their work.

Another reason is that, like similar cases in veterinary medicine (Clutton et al., 2017), ART fall into the category of veterinary treatments although their application may contain some typical research-related features—both translational and basic ones, as specialists proceed to optimize or test techniques by applying them. This means that they do not require an approval by an animal care and ethics committee (hence the limited number of studies reporting one), but they may nevertheless need an ethical assessment because they show a ‘boundary problem’, as the same treatment may be subjected to different standards of evaluation when performed for different purposes (Hansson, 2009, 2011).

All these reasons elicit the need for a dedicated frame to guide a proper ethical assessment of ART procedures in conservation.

### 2.1. Building a frame for the ethical assessment of ART procedures in conservation

The ethical evaluation of a biodiversity conservation project is undoubtedly complex and cannot be limited to the ethical assessment of its goals and objectives. A project may indeed have a commendable goal, and at the same time be ethically unacceptable, since its procedures are inappropriate because they fail to meet certain important values and standards or are not efficient (Costello et al., 2016; Sandler et al., 2021). Ethical assessment of procedures is even more important when a conservation project employs ART, which can be rather complex and demanding in terms of equipment and expertise, and in many cases require direct manipulation of animals. In these cases, several issues should be considered.

To start, ethical assessment of ART applications to wildlife should not be carried out exclusively by assessing protocols and the general design of the procedures. Given the field nature of biodiversity conservation efforts, and the complexity of most ART applications to wildlife, many circumstantial variables may intervene every time a procedure is performed, making it necessary to monitor each implementation. A general evaluation of the procedures may provide in this sense the backbone for the assessment, but does not remove the need to carefully check the procedures each time they are implemented (Biasetti et al., 2022).

Moreover, procedure assessment of ART should be carried out taking into consideration the larger context in which a procedure takes place. This means that even when assessing the specific implementation of an ART procedure we should always consider factors such as its overall sustainability, its scientific and technological contributions and possible consequences, its integration with the local and global social environment and so on.

Additionally, procedural evaluation should not be limited only to the specific applications of ART in the field. Given the potential risks of biomaterial collection, careful scrutiny of laboratory procedures is not only necessary for the success of a project but also for its ethical acceptability. This is true both if the material is intended to be manipulated in the laboratory (e.g., to obtain an embryo for subsequent implantation) or if it is instead banked for future use. In both cases, new risks and ethical issues arise that need to be evaluated.

Similarly, health control procedures must also be analyzed. During a conservation project involving ART, animals may be subjected to various veterinary checks such as pregnancy checking and monitoring. Even in these cases, it is important to perform an ethical evaluation prior to the procedure in order to consider its risks and effects in the context of the conservation project.

Finally, since the ethics of biodiversity conservation intersects several dimensions of value, procedure assessment of ART should incorporate the various demands coming from environmental, social, research, and animal welfare ethics. Therefore, when evaluating ART procedures in conservation, several ethically relevant aspects should be considered (Table 2).

**Table 2**

Ethically relevant goals linked to four value dimensions: environmental, social, research, and animal welfare ethics.

Environmental ethics	Animal welfare ethics	Social ethics	Research ethics
Conservation of the biomaterial and genetic information	Health and functioning	Compliance with rules and regulations	Scientific quality and innovation
Conservation of species	Allowance of positive affective states and avoidance of negative affective states	Health, safety and empowerment of the staff and other people involved	Transparent and effective data management
Conservation of biodiversity	Expressing natural behavior and living natural lives	Positive integration with the surrounding social environment	Transparent and effective communication

A first aspect to be investigated should be the ability of the procedure to pursue conservation goals. It is necessary for instance to evaluate whether the procedure respects the conservation mission and whether it is effective in achieving the specific objectives of the projects in terms of safeguarding biodiversity at all its different levels.

The social and legal dimensions of the procedure should be also analyzed. This includes checking for compliance with the current legislation and best practices, analyzing how the safety, health, and empowerment of staff and people involved, including local ones, are ensured during all phases, and investigating integration within the surrounding social environment.

A third factor to be considered is scientific quality. It is necessary in this regard to investigate the degree of innovation and quality, the possible further technological and scientific repercussions, respect of research standards, and how communication of the results and data management is performed.

Finally, the welfare of the animals involved should be carefully scrutinized in all phases. Assessing animal welfare is highly complex since the concept itself is by its very nature multidisciplinary and difficult to define (Broom, 2008; Fraser, 2008). We can here refer to the definition adopted by the World Organization for Animal Health, as “how an animal is coping with the conditions in which it lives” (International Office of Epizootics (OIE), 2019), considering how the animal experiences its own world and life through its association with pleasant or unpleasant experiences specific for that species (Mellor et al., 2015). The aforementioned difficulties in assessing animal welfare are additionally exacerbated by the fact that, in general, wildlife welfare has received less attention than the welfare of farm and laboratory animals (Paquet and Darimont, 2010; Kirkwood, 2013; Berg et al., 2020). This is both due to a paucity of information in the literature, and to the immense diversity of wildlife species compared to the few taxa employed in agriculture and laboratory research (Field et al., 2019). Moreover, several specific issues complicate the assessment of welfare in wildlife, including the need to take into consideration the specific environment and ecosystem where the assessment takes place, and heterogeneous research conditions that impede performing the assessment (Curzer et al., 2013; Sikes and Paul, 2013; Lindsjö et al., 2016; Soulsbury et al., 2020).

Concerning ART, several elements complicate, from a welfare perspective, their application on wildlife compared to domestic animals. One complication is that many procedures in wildlife are a blueprint, and as a consequence less established than in farm animals and, often, takes place directly in the field and not in a controlled environment. Another complication is the lack in habituation of wild animals to being handled, which unlike in farm animals, may cause greater stress, or require restraint, sedation or anesthesia. Moreover, due to the need to mitigate the negative effects of restraint and captivity (Greggor et al.,

2018; Soulsbury et al., 2020), there may be limits to the applicable strategies to condition the animals for the procedures.

Good standards of animal welfare should be goals to be pursued for ethical reasons. However, there are also valid arguments related to the pursuit of conservation objectives that urge their adoption. Good animal welfare and animal care, for instance, are essential elements for the success of conservation breeding and reintroduction programs (Harrington et al., 2013; Greggor et al., 2018). Moreover, failure to respect acceptable standards of animal welfare may alienate societal support to conservation programs (McMahon et al., 2012; Beausoleil et al., 2018). In the past, conservation and animal welfare have often been considered distinct and separate goals, with the need to choose one or the other (Fraser, 2010). The need to adopt suitable animal welfare standards is, however, progressively accepted in the context of biodiversity conservation and, more generally wildlife research (Paquet and Darimont, 2010; Dubois and Fraser, 2013; Costello et al., 2016; Soulsbury et al., 2020).

To recap, ethical assessment of ART procedures should: i) monitor the specific implementations of the procedure; ii) include into the assessment the larger context into which a procedure takes place; iii) include in the assessment the aftermath of the procedure; and iv) structure the assessment starting from different ethically relevant aspects (compliance with the mission of conservation, social and legal principles, scientific and research standards, and animal welfare requirements). There are two main difficulties in building a frame capable to satisfy all these desiderata: its standardization and the possible sources from which to extrapolate its contents. ART constitute a rather heterogeneous set of procedures, further complicated by the fact that their application vastly differs between species. Currently, there are no specific guidelines for building a ready to use frame for their ethical assessment.

One strategy is to look at what has already being done in research with farm and laboratory animals. This removes the need to build from scratch a new frame of evaluation and facilitates standardization. Although farm and laboratory species represent only a tiny fraction if compared to wild species, the procedures they are subjected to are more numerous and varied than the procedures inherent to ART and the framework for their ethical assessment has been already detailed. It includes compliance with the 3Rs (i.e., Replacement of the use of animals, Reduction of the number of animals used—and of everything that can be reduced, like the number of replications and so on—and Refinement of procedures: Russell and Burch, 1959; Nuffield Council on Bioethics, 2003) and with the standards of harm–benefit analysis and research ethics.

The first point—application of the 3Rs tenet—is increasingly recognized as crucial for wildlife research (Lindsjö et al., 2016; de Mori, 2019; Soulsbury et al., 2020; Zemanova, 2020), even if it is more difficult to apply due to the heterogeneous conditions present in the field. For instance, even if complete Replacement is often not possible as the studies are mainly focused on the same species the involved individuals belong to, an incomplete replacement can be obtained by developing less invasive procedures whenever it is possible. The Reduction can be achieved by an optimized experimental design that allows for maximizing the outcome of the procedures, thereby reducing the number of replications. Refinement can be implemented with better methods of capture, anesthesia, housing, and handling, and a better definition of endpoints (Lindsjö et al., 2016; de Mori, 2019; Soulsbury et al., 2020; Zemanova, 2020).

The second point is the harm–benefit analysis. Although how it should be implemented is still under debate (Griffin et al., 2014; Bout et al., 2014; Grimm et al., 2019; Gutfreund, 2020), it is now routinely recommended in the ethical evaluation of research projects involving laboratory and farm animals (Griffin et al., 2014; Brønstad et al., 2016; Laber et al., 2016), but still underrepresented in wildlife studies (McMahon et al., 2012; Field et al., 2019; Lindsjö et al., 2019; Soulsbury et al., 2020). In particular, harm–benefit analysis has rarely been used to

evaluate the impact of veterinary procedures aimed at wildlife species conservation on individual health and welfare. Nonetheless, it has been progressively used to identify costs and benefits arising from conservation projects, in relation not only to their economic impact (Shwiff et al., 2013) but also to their positive or negative consequences for the ecosystem, local communities, and local wildlife populations (Pullin and Knight, 2009).

The third point is research ethics: ethical standards of research must be assured not only in laboratory settings but also when performing research in the field with wild animals. This can be challenging: guaranteeing standards of responsible conduct, transparency, research integrity, respect for intellectual properties, and avoidance of conflicts of interest and related biases, involves additional variables when dealing with various project partners and local communities, as is often the case when working on projects involving endangered species. Therefore, fulfillment of standards of research ethics in wildlife studies, despite being a crucial aspect, can be difficult to assess (All European Academies, 2017; TRUST, 2018; Sandler et al., 2021).

In addition to the 3Rs, harm and benefit analysis, and compliance with the principles of research ethics, the ethical assessment of ART procedures in conservation should include also risk assessment (Smith et al., 2018; Murray, 2004). The application of ART to animals—particularly wild animals—entails accepting a certain level of uncertainty. This is because it is often not possible to fully know the health status of the animal, and the scenario in which veterinarians have to intervene can be very heterogeneous. Even ART using biomaterial collected from animals entails some uncertainty, with the risk to waste the precious biomaterial collected. A certain level of unpredictability regarding the success of the procedures can be ethically acceptable only if a risk assessment is applied beforehand, allowing the identification of potentially harmful hazards, assessing their likelihood of occurrence, and establishing the level of their acceptability. To be applied to the ethical assessment of ART procedures, a risk assessment should be performed both in relation to the quality and safety of procedures and staff and in relation to the welfare of the animals involved. Moreover, it should be based both on traditional risk analysis (Murray, 2004) and on specific animal welfare (European Food Safety Authority (EFSA), Panel on Animal Health and Welfare (AHAW), 2012) and ethical risk analyses (Doorn, 2015; Hansson, 2018). While traditional risk assessment emphasizes the probabilities and severity of possible adverse events in general, animal welfare risk assessment specifically evaluates the probability of negative welfare consequences and the magnitude of those consequences for the animals, following exposure to a particular factor or exposure scenario (EFSA, 2012). Moreover, in order to manage situations of risk and uncertainty that cannot be assessed without a moral appraisal, the ethical risk assessment supplements the traditional risk assessment by covering specific ethical issues, such as transparency, responsible conduct, effective stakeholders involvement, inclusive decision making, etc., thus allowing for ethical considerations to be part of risk-related decisions, rather than being an afterthought to those decisions (Doorn, 2015; Hansson, 2018).

All the aforementioned points should be combined in the procedure assessment of ART to assure compliance with regulations and best practices, compliance with conservation mission and project goals, quality, communication and safety evaluation, and respect for acceptable standards of animal welfare. Moreover, given the necessity to assess the various implementations of a procedure continuously throughout the project, the assessment should take the form of a self-assessment.

Ethical self-assessment and external ethical approval share the goal of guaranteeing the ethical acceptability of a project. However, the former has a different function. It is at the same time a mean to identify any possible ethical issues arising from a project before submitting it to an external committee for approval and to continually monitor its implementation and improve its quality after the project is started. In this way, the ethical self-assessment takes place independently from an external ethical approval and can be performed both before or after the

project has received external ethical approval or when an external ethical approval is not needed, e.g. when the procedures represent standard veterinary procedures as it is the case also in livestock. An ethical self-assessment, if done routinely and consistently throughout all phases of a project, can help scientists and project partners in thinking through the ethical issues and the risks in advance and in effectively sharing information. In this way, it can contribute to the refinement of the procedures themselves and promote inclusive decision making among all involved parties. Additionally, it can assist the parties in complying with regulations and best practices (European Commission DG Research and Innovation, 2020).

### 3. ETHAS: a tool for the ethical self-assessment of ART procedures in vertebrate conservation projects

An ETHical ASessment tool (ETHAS) was built to implement the previous frame in vertebrate conservation. ETHAS has been designed in the context of BioRescue ([biorescue.org](http://biorescue.org)), an international consortium coordinated by the Leibniz Institute for Zoo and Wildlife Research in Berlin with the aim of saving the northern white rhinoceros (*Ceratotherium simum cottoni*, 1908) from extinction using ART (Hildebrandt et al., 2021; Hildebrandt et al., 2023). A first version of the tool was tested in a pilot application (de Mori et al., 2021) on ovum pick up and in vitro fertilization procedures performed on the two white rhinoceros subspecies (the northern white rhinoceros and the southern white rhinoceros, *Ceratotherium simum simum*, 1817). On the basis of the results of the pilot application, the overall frame and scoring system of ETHAS was revised, and the scope of the tool increased. In particular, ETHAS has been expanded to cover the range of ART procedures performed both in the field and in the laboratory, the associated health check procedures, and the biobanking of the different types of cells and tissues. With regard to the taxa covered, the tool was extended to assess the principal ART and associated procedures performed on placental mammals (excluding humans), as well as the procedures already in place for the other vertebrate classes (birds, amphibians, reptiles, and fish). The tool can be further expanded to cover aART procedures, including gamete production techniques from stem cell technologies and somatic cell nuclear transfer.

ETHAS is made up of checklists assessing specific procedures grouped in four categories: field procedures, laboratory procedures, health check procedures, and biobanking procedures. Table 3 contains the list of procedures covered by the tool. Some examples of checklists are provided in the appendixes to this articles, including checklists for assessing pregnancy check in mammals, semen collection in amphibians, semen collection in birds, embryo transfer in mammals, in vitro fertilization in mammals, in vitro fertilization in amphibians, and biobanking of reproductive cells and embryos for all vertebrate classes.

Each procedure is associated with two checklists (A and B). The first checklist of the pair (“Checklist A”) is designed to assess from an ethical point of view all the relevant aspects of a procedure: its protocol and its placement in the project, in the social context, and in relation to the conservation mission. The second checklist of the pair (“Checklist B”) is instead designed to assess the context-sensitive aspects of the procedure that may vary from time to time with each implementation. Together, the two checklists can be used to assess the degree of compliance of a procedure with several relevant goals of environmental, social, research, and animal welfare ethics (see Table 2). Checklist A should be completed at the beginning of the project or when defining the protocol of a procedure—in any case, before its first implementation—to obtain a full-scope evaluation. No further completions of Checklist A are needed if no major changes to the protocols or in the conditions related to the project happen. Checklist B instead should be completed before every implementation, to monitor the contextual variables that may change from time to time and affect from an ethical standpoint the procedure (see Fig. 1).

The checklists were designed by reviewing the relevant scientific

**Table 3**

List of procedures covered by ETHAS checklists.

Health check procedures	Field procedures	Laboratory procedures	Biobanking procedures
<ul style="list-style-type: none"> <li>• Health check (all vertebrate classes)</li> <li>• Pregnancy check (mammals)</li> <li>• Pregnancy monitoring (mammals)</li> <li>• Removal of extra fetus (mammals)</li> </ul>	<ul style="list-style-type: none"> <li>• Artificial insemination (mammals)</li> <li>• Artificial insemination (fishes)</li> <li>• Artificial insemination (birds)</li> <li>• Artificial insemination (reptiles)</li> <li>• Semen collection (mammals)</li> <li>• Semen collection (fishes)</li> <li>• Semen collection (amphibians)</li> <li>• Semen collection (birds)</li> <li>• Semen collection (reptiles)</li> <li>• Ovum pick up (mammals)</li> <li>• Embryo transfer (mammals)</li> <li>• Sterilization (mammals)</li> </ul>	<ul style="list-style-type: none"> <li>• In vitro fertilization (mammals)</li> <li>• In vitro fertilization (amphibians)</li> <li>• Intracytoplasmic sperm injection (mammals)</li> </ul>	<ul style="list-style-type: none"> <li>• Biobanking of tissues (all vertebrate classes)</li> <li>• Biobanking of reproductive cells and embryos (all vertebrate classes)</li> <li>• Biobanking of cell cultures (all vertebrate classes)</li> </ul>

literature, best practices, and regulations, and by analyzing the main approaches, methodologies, and relevant equipment in the field. They were further refined through an iterative consultation process between experts and stakeholders. Items in the checklists have been based on norms, regulations, and best practices, as well as the harm-benefit and research ethics evaluation, coupled with the application of the 3Rs to field research and an assessment of the significant risks that may occur during the application of ART to vertebrate species. Following the frames developed for the ethical assessment of research projects involving farm and laboratory animals (Smith et al., 2007, 2018; Hooijmans et al., 2010; Commission E., 2016), checklists are structured around sections performing documents compliance, analysis of procedure impact, and evaluation of procedure quality—including equipment, procedure area, and scientific team quality evaluation.

More specifically, the section “Documents and research ethics” checks compliance with the relevant regulation and laws (e.g., Nagoya protocol, CITES, etc.), as well as with best practices. The section “Procedure impact” analyzes the procedure in the context of the entire project, focusing on its ethically relevant consequences—including possible consequences for the involved animals, as well as for populations, species, biodiversity conservation, the environment, scientific and technological advancement, and people and communities involved. The section “Procedure evaluation” measures the degree of robustness of the scientific and technical background of the procedure, as well as compliance with the 3Rs.

Moreover, this section also assesses the degree of experience and coordination of the team performing the procedure, the satisfaction of ethical research standards, the quality of the equipment, and the organization of labs and biobanks involved. After these sections, each checklist may contain optional appendixes that collect items related to specific aspects of a procedure that may apply in some cases and not in others (e.g., anesthesia, biomaterial shipping, etc.).

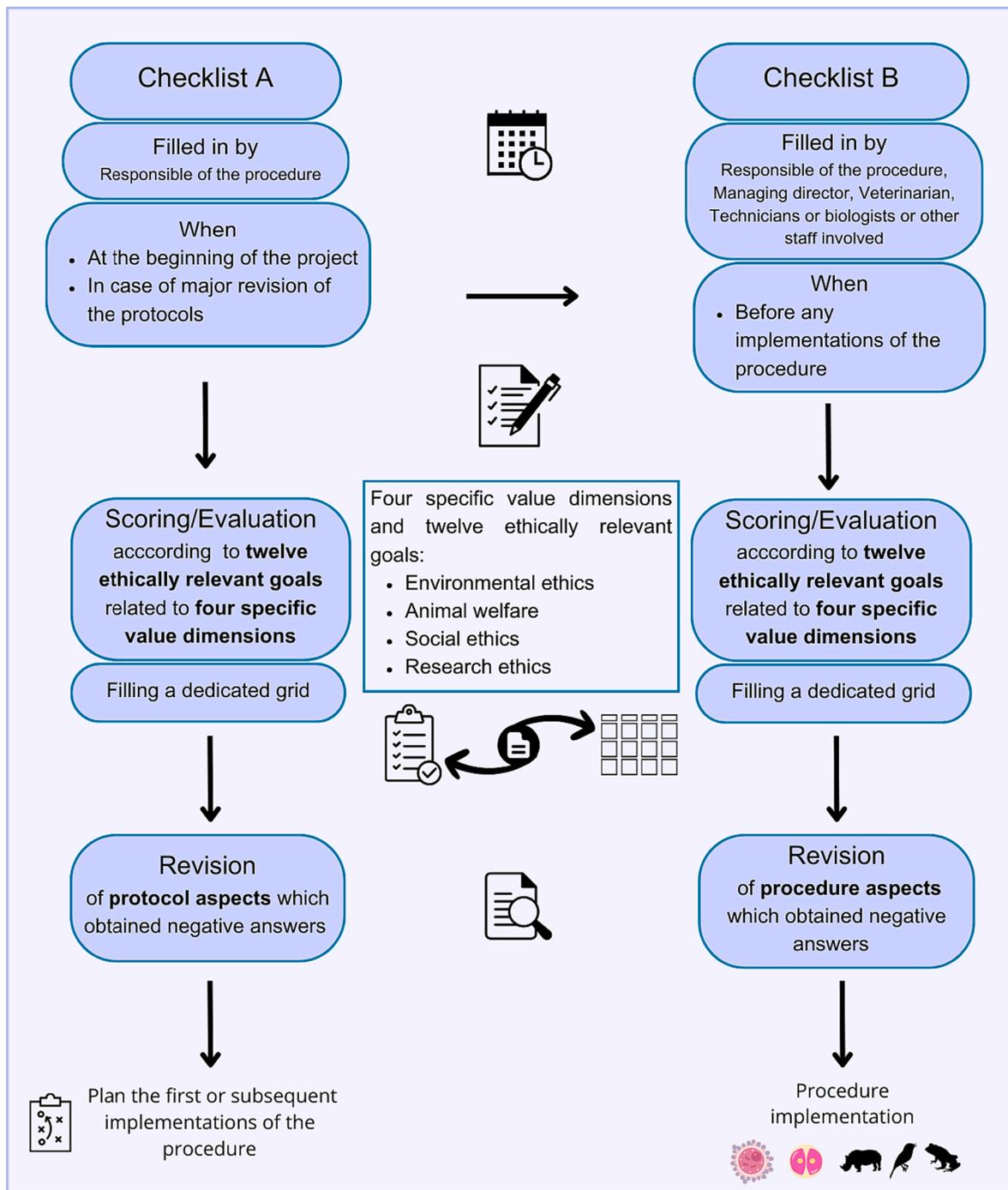


Fig. 1. ETHAS checklists application process.

Each item in the two checklists asks whether or not an ethically relevant aspect related to the procedure was complied with. It is possible to answer in three ways. A “yes” answer means that the ethically relevant element is complied with. A “no” answers means that the ethical relevant element is not complied with. A “N/A” answers means that the ethically relevant element is not pertinent to the assessed procedure.

Checklist A must be filled by the project manager, while checklist B by people with a role of responsibility in the procedure (the veterinarian performing it, the facility manager, etc.). Especially when filling checklist B, people may find it difficult to answer some items because the content is beyond their direct competence or responsibility. In this case, they should proceed to inquire with the people directly involved in the part of the procedure analyzed by the item content. This type of

crosscheck should not be brought forward only for completing the compiling of the checklist. One of the functions of ETHAS is to promote discussion and communication among the parties involved in a procedure and in a project. Widespread sharing of checklists among participants and their compilation through an informal participatory process leads to better identification and understanding of possible ethical problems, stimulates a more open and transparent discussion, and provides different points of view on their possible resolution.

Each item is associated with one or more of the twelve ethically relevant goals related to the four specific value dimensions listed in Table 2. Under environmental ethics fall the goals of: i) conservation of the biomaterial and genetic information involved; ii) conservation of the species involved; and iii) conservation of biodiversity in general. Under

animal welfare fall the goals, with respect to the animals involved, of: i) health and functioning; ii) allowance of positive affective states and avoidance of negative affective states; and iii) expression of behaviors and natural lives. Under social ethics fall the goals of: i) compliance with rules and regulations; ii) health, safety, and empowerment of staff and other people involved; and iii) positive integration of the procedure and project into the surrounding social environment. Finally, under research ethics fall the goals of: i) quality and innovation; ii) transparent and effective communication; and iii) transparent and effective data management. A negative response to an item corresponds to a failure to satisfy a specific aspect related to one of these ethically relevant goals. See Table 4 for examples of items and the ethically relevant goals associated.

By evaluating a procedure according to these twelve goals, it is possible to satisfy the desiderata identified earlier: to take into consideration the broader context in which a procedure takes place, to follow the aftermath of the procedures, and to structure the assessment from multiple ethically relevant points of view.

Scoring of the checklists is performed through dedicated grids, in which item responses are sorted according to the associated ethically relevant goals (see Appendix H for an example). The scoring process of ETHAS provides two outcomes. The first outcome is the degree of compliance of the procedure with each of the twelve ethically relevant goals. The second outcome is signaling possible ethical red flags. This is done by highlighting items (using a red circle) that contain especially important ethical requirements for the procedure. This is the case, for example, of items that ask whether certain binding laws have been complied with or whether crucial evaluations have been made regarding the possible risks of the procedure to animals or people. In these cases, a negative response indicates that the procedure is not ethically acceptable and that the issue has to be overcome before the procedure can be performed.

### 3.1. Strengths, limitations and future developments

Conceptual tools help in organizing and structuring the ethical analysis of conservation projects in an explicit, transparent, and systematic fashion (Biasetti and de Mori, 2021; Biasetti et al., 2023). The specificity of ETHAS in respect of other tools is to provide a standardized framework for evaluating the different procedures within a conservation project that employs ART—from their initial design in the planning phase of the project to their implementation during its execution. This evaluation, as noted, is not limited to ART procedures per se, but may also include associated health check and biobanking procedures. Moreover, the assessment takes in consideration the different ethical dimensions involved in conservation breeding, thus adopting as pluralistic and comprehensive a perspective as possible.

ETHAS provides support to those involved in both designing protocols and implementing procedures, ensuring immediate access to the pertinent ethical requirements. With its scoring system based on twelve ethically relevant goals, the tool allows for the prompt identification of ethical issues within a procedure. This enables users to immediately initiate targeted interventions for enhancing both quality and ethical acceptability.

However, it is important to note that the outcome provided at the end of the scoring process is not the only expected result from the application of the tool: what ETHAS does, in addition to evaluating the procedure, is to provide an opportunity for scientists and conservationists to reflect on the ethically relevant aspects of their work.

The final goal of ETHAS is to contribute to ethically responsible and conscientious practice of ART procedures within a conservation project. This can also have additional positive spin-offs on the project and the procedures. For instance, the use of ethical self-assessment for the evaluation of each procedure can provide a decisive contribution to reducing the associated risks. Additionally, this methodology can indeed be used to start an iterative process that continuously promotes the

**Table 4**

Some examples of items from ETHAS checklists and the ethical dimensions and relevant goals associated.

Item	Ethical dimension	Ethically relevant goal
Documents and research ethics Will the data collected during the procedures be available to all the participants in the project in a timely, open, transparent, and accurate manner unless otherwise agreed?	Research ethics	<ul style="list-style-type: none"> <li>• Transparent and effective data management</li> <li>• Transparent and effective communication</li> </ul>
Have protocols been established to identify whether obtaining, using, and storing biosamples falls under legally binding international regulations (e. g., CITES, Nagoya Protocol on Access and Benefit Sharing (ABS) etc.)?	Social ethics	<ul style="list-style-type: none"> <li>• Compliance with rules and regulations</li> </ul>
Procedures impact Are the procedures or specific aspects of the procedures part of a project that can positively impact the economy of indigenous people and the local communities (IPLC) involved?	Social ethics	<ul style="list-style-type: none"> <li>• Positive integration with the surrounding social environment</li> </ul>
Are the procedures or specific aspects of the procedures part of a project that can positively impact biodiversity conservation?	Environmental ethics	<ul style="list-style-type: none"> <li>• Conservation of biodiversity</li> </ul>
Procedures evaluation Has the number of people needed in each role of the procedures been assessed?	Social ethics	<ul style="list-style-type: none"> <li>• Health, safety and empowerment of the staff and other people involved</li> </ul>
Have the procedures been optimized and standardized to reduce the animal manipulation time as much as possible without impairing safety and quality?	Animal welfare ethics	<ul style="list-style-type: none"> <li>• Allowance of positive affective states and avoidance of negative affective states</li> <li>• Expressing natural behavior and living natural lives</li> </ul>
Has a plan for the mitigation of any possible adverse effect during the procedures been developed?	Research Ethics	<ul style="list-style-type: none"> <li>• Scientific quality and innovation</li> <li>• Conservation of the species</li> </ul>
	Environmental Ethics Animal welfare ethics	<ul style="list-style-type: none"> <li>• Health and functioning</li> <li>• Allowance of positive affective states and avoidance of negative affective states</li> </ul>
Anesthesia Has a cut-off time for anesthesia been established?	Animal welfare ethics	<ul style="list-style-type: none"> <li>• Health and functioning</li> </ul>
Specimens packaging and shipping Has a protocol been developed to assess the packaging condition of the specimens before the sending and after the receiving?	Environmental ethics	<ul style="list-style-type: none"> <li>• Conservation of the biomaterial and genetic information</li> </ul>
	Research ethics	<ul style="list-style-type: none"> <li>• Scientific quality and innovation</li> </ul>

exchange of knowledge and information between the participants involved and improves the transparency and quality of the procedures. In this way, continuous ethical self-assessment offers the opportunity to promote high ethical standards using detailed scrutiny of the quality of each intervention, of the process of optimization of the procedures, and of the welfare and safety of the animals and people involved.

Moreover, if the use of ETHAS is specifically included in the planning of the project, it could assist in obtaining an ethical approval by an external animal care and ethics committee. Similarly, explicitly reporting the results of the ethical self-assessment relative to the assessed procedures in research papers could help editors, reviewers, and readers to immediately identify the ethical standing of the study, and to increase the ethical acceptability of the scientific work presented. One reason a scientific journal may reject a research article is that the methodology applied in the study has obvious ethical issues that have been completely ignored or not properly addressed (Costello et al., 2016). The application of ETHAS can assist in the early identification of such ethical issues, and its inclusion among the materials and methods of the research may provide an initial assurance that the study has adopted appropriate ethical standards.

In general, the use of self-assessment tools, especially when done methodically, make it possible for project participants to critically reflect on their methodologies and goals and improve their overall ethical competence. This in turn allows both to improve strategies to avoid negative scenarios or mitigate their consequences, and to communicate in an articulate and transparent manner the reasons behind the choices made. These two characteristics could prove crucial to the success of a project, since the practice of conservation can raise numerous ethical questions (Minteer and Collins, 2005b). Failure to adequately anticipate and address these issues, in addition to creating an ethical problem in itself, can lead to conflicts (McMahon et al., 2012) that may escalate and become polarizing, creating in this way possible conditions for project failure (Crowley et al., 2017).

Alongside its strengths, also limitations of the instrument should be discussed. Ethical self-assessment of the procedures and their implementations cannot replace the need for a structured ethical assessment of the project in its entirety and of its parts (Sandler et al., 2021). However, this does not remove the need for procedural assessment, since the evaluation of a project, even if positive, does not exhaust the need to continue to monitor its implementation to ensure a transparent, accountable, and ethically conscious conservation practice.

To the best of our knowledge, ETHAS is currently the only ethical tool for analyzing conservation practice adopting the methodology of self-assessment. While some strengths of this methodology have been previously highlighted, self-assessment can also be criticized as self-referential, because it invests the same people who carry out the procedures with the task to conduct the assessment. However, it has to be noted that the primary function of ethical self-assessment is not to remove the need for external ethical evaluation but instead to help conservationists explore autonomously and in detail the ethical issues associated with their activities. Being proactive in these cases is a positive attitude even if no major ethical issues are detected because it increases awareness concerning the ethical implications of one's own work, and willingness to adapt to high standards. On a more practical level, continuous self-assessment contributes to the refinement and optimization of the procedures, by promoting communication and knowledge transfer among participants in the projects and monitoring the fluctuations in the quality of the procedures based on circumstances.

Another possible limitation of this methodology concerns the possibility for those conducting the self-assessment to compile the checklists without really taking into account the views of the various stakeholders involved. Numerous items call for evaluating the level of integration of procedures into the social reality where they take place, or for taking into account their impact on the environment, biodiversity, and animals but those compiling the checklists may not have sufficient information to answer these kinds of items or may not otherwise be able to provide

an objective answer. To avoid this kind of bias, the self-assessment process has to be constructed by seeking a real stakeholders partaking, building participatory processes to effectively involve local communities and representative associations where checklists are discussed and filled in together by the participants.

ETHAS is currently applicable to several ART and related procedures used in vertebrate conservation and will be further expanded to cover aART, starting with somatic cell nuclear transfer and induced pluripotent stem cell-based technologies for gamete production. Development of the tool will include its dissemination and application within participatory decision-making processes.

#### 4. Conclusion

The use of ART is becoming increasingly important for biodiversity conservation. In the application of ART to conservation, ethical analysis is crucial. Ethical self-assessment of each involved procedure in a conservation project provides the necessary means to assess acceptability both in relation to research and veterinary requirements, as well as in relation to the identification, evaluation, and mitigation of the potential procedure-associated risks and animal welfare issues. A ready and easy to use tool for such assessment can contribute to ensure the success of conservation projects and to protect the welfare of the concerned animals. Moreover, it contributes to raise the quality of procedures, fostering consistency, transparency and communication among the involved project partners.

#### Funding

The BioRescue project is funded by the German Federal Ministry of Education and Research (BMBF) (BMBF BioRescue: 01LC1902A).

#### CRediT authorship contribution statement

**Barbara de Mori:** Conceptualization, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Elena Mercugliano:** Investigation, Methodology, Visualization, Writing – review & editing. **Pierfrancesco Biasetti:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Ilaria Pollastri:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Maria Michela Spiriti:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Daniela Florio:** Methodology, Writing – review & editing. **Francesco Andreucci:** Methodology, Writing – review & editing. **Frank Göritz:** Writing – review & editing. **Susanne Holtze:** Writing – review & editing. **Cesare Galli:** Writing – review & editing. **Jan Stejskal:** Writing – review & editing. **Silvia Colleoni:** Writing – review & editing. **Giovanna Lazzari:** Writing – review & editing. **Steven Seet:** Writing – review & editing. **Jan Zwilling:** Writing – review & editing. **David Ndeereh:** Writing – review & editing. **Isaac Lekool:** Writing – review & editing. **Stephen Ngulu:** Writing – review & editing. **Dominic Mijele:** Writing – review & editing. **Daniel Čizmar:** Writing – review & editing. **Raffaella Simone:** Writing – review & editing. **Lisa Schrade:** Writing – review & editing. **Simone Basile:** Writing – review & editing. **Thomas B. Hildebrandt:** Funding acquisition, Supervision, Writing – review & editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

No data was used for the research described in the article.

## Acknowledgments

The authors acknowledge the supporting European zoological institutions, the staff and management of OI Pejeta Conservancy and Kenya Wildlife Service for their logistic support.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocon.2023.110423>.

## References

- All European Academies. 2017. The European Code of Conduct for Research Integrity. Revised edition. Berlin, Germany: All European Academies. Retrieved from <https://www.alllea.org/wp-content/uploads/2017/05/ALLEA-European-Code-of-Conduct-for-Research-Integrity-2017.pdf>.
- Beausoleil, N.J., et al., 2018. "Feelings and fitness" not "feelings or fitness"—the raison d'être of conservation welfare, which aligns conservation and animal welfare objectives. *Front. Vet. Sci.* 5, 296.
- Berg, C., Lerner, H., Butterworth, A., Walzer, C., 2020. Wildlife welfare. *Front. Vet. Sci.* 7, 576095.
- Biasetti, P., de Mori, B., 2021. The ethical matrix as a tool for decision-making process in conservation. *Front. Environ. Sci.* 9.
- Biasetti, P., Hildebrandt, T.B., Göritz, F., Hermes, R., Holtze, S., Galli, C., Lazzari, G., Colleoni, S., Pollastri, I., Spiriti, M.M., Stejskal, J., Seet, S., Zwilling, J., Ngulu, S., Mutisya, S., Kariuki, L., Lokooloo, I., Omondi, P., Ndeereh, D., de Mori, B., 2022. Ethical analysis of the application of assisted reproduction technologies in biodiversity conservation and the case of white rhinoceros (*Ceratotherium simum*) ovum pick-up procedures. *Front. Vet. Sci.* 9, 831675.
- Biasetti, P., Hildebrandt, T.B., Göritz, F., Hermes, R., Holtze, S., Stejskal, J., Galli, C., Pollastri, I., Muzzo, A., Lokooloo, I., Ndeereh, D., Omondi, P., Kariuki, L., Mijele, D., Mutisya, S., Ngulu, S., de Mori, B., 2023. Application of decision tools to ethical analysis in biodiversity conservation. *Conserv. Biol.* 37, e14029.
- Bolton, R.L., Mooney, A., Pettit, M.T., Bolton, A.E., Morgan, L., Drake, G.J., Appeltant, R., Walker, S.L., Gillis, J.D., Hvilson, C., 2022. Resurrecting biodiversity: advanced assisted reproductive technologies and biobanking. *Reprod. Fertil.* 3, 121–146.
- Bout, H.J., Van Vlissingen, J.M.F., Karssing, E.D., 2014. Evaluating the ethical acceptability of animal research. *Lab Anim.* 43 (11), 411–414.
- Brittain, S., et al., 2020. Ethical considerations when conservation research involves people. *Conserv. Biol.* 34 (4), 925–933.
- Brønstad, A., Newcomer, C.E., Decelle, T., Everitt, J.L., Guillen, J., Laber, K., 2016. Current concepts of harm–benefit analysis of animal experiments—report from the AALAS–FELASA working group on harm–benefit analysis—part 1. *Lab. Anim* 50 (1 suppl), 1–20.
- Broom, D.M., 2008. Welfare assessment and relevant ethical decisions: key concepts. *Annu. Rev. Biomed. Sci.* 10, 79–90.
- Brown, J.L., 2018. Comparative ovarian function and reproductive monitoring of endangered mammals. *Theriogenology* 109, 2–13.
- Clulow, J., Clulow, S., 2016. Cryopreservation and other assisted reproductive technologies for the conservation of threatened amphibians and reptiles: bringing the ARTs up to speed. *Reprod. Fertil. Dev.* 28, 1116–1132.
- Clulow, S., Clulow, J., Marcec-Greaves, R., Della Togna, G., Calatayud, N.E., 2022. Common goals, different stages: the state of the ARTs for reptile and amphibian conservation. *Reprod. Fertil. Dev.* 34, I–IX.
- Clutton, E., et al., 2017. Pets in clinical trials. *Vet. Rec.* 181 (8), 209–210.
- Comizzoli, P., 2015. Biotechnologies for wildlife fertility preservation. *Anim. Front.* 5 (1), 73–78.
- Comizzoli, P., 2016. Biotechnologies for wildlife fertility preservation. *Thai J. Vet. Med.* 46, 541–545.
- Comizzoli, P., 2018. Biobanking and fertility preservation for rare and endangered species. *Anim. Reprod.* 14 (1), 30–33.
- Comizzoli, P., Holt, W.V., 2019. Breakthroughs and new horizons in reproductive biology of rare and endangered animal species. *Biol. Reprod.* 101 (3), 514–525.
- Comizzoli, P., Brown, J., Holt, W. (Eds.), 2019. *Reproductive Sciences in Animal Conservation. Advances in Experimental Medicine and Biology*, vol 1200. Springer, Cham.
- Costello, M.J., et al., 2016. Field work ethics in biological research. *Biol. Conserv.* 203, 268–271.
- Crowley, S.L., Hinchliffe, S., McDonald, R.A., 2017. Conflict in invasive species management. *Front. Ecol. Environ.* 15, 133–141.
- Curzer, H.J., Wallace, M.C., Perry, G., Muhlberger, P.J., Perry, D., 2013. The ethics of wildlife research: a nine R theory. *ILAR J.* 54 (1), 52–57.
- Della Togna, G., Howell, L.G., Clulow, J., Langhorne, C.J., Marcec-Greaves, R., Calatayud, N.E., 2020. Evaluating amphibian biobanking and reproduction for captive breeding programs according to the Amphibian Conservation Action Plan objectives. *Theriogenology* 150, 412–431.
- Doorn, N., 2015. The blind spot in risk ethics: managing natural hazards. *Risk Anal.* 35 (3), 354–360.
- Dubois, S., Fraser, D., 2013. Rating harms to wildlife: a survey showing convergence between conservation and animal welfare views. *Anim. Welf.* 22 (1), 49–55.
- Ebenhard, T., 1995. Conservation breeding as a tool for saving animal species from extinction. *Trends Ecol. Evol.* 10 (11), 438–443.
- European Commission, 2016. H2020 Programme guidance how to complete your ethics self-assessment. *Eur. Comm. Dir. Res. Innov.* pp 43, version 6.1 Available: [https://ec.europa.eu/research/participants/data/ref/h2020/grants\\_manual/hi/ethics/h2020\\_hi\\_ethics-self-assess\\_en.pdf](https://ec.europa.eu/research/participants/data/ref/h2020/grants_manual/hi/ethics/h2020_hi_ethics-self-assess_en.pdf).
- European Commission DG Research & Innovation, 2020. Horizon 2020 Online Manual. Brussels. Available at: [https://ec.europa.eu/research/participants/docs/h2020-funding-guide/cross-cutting-issues/ethics\\_en.htm](https://ec.europa.eu/research/participants/docs/h2020-funding-guide/cross-cutting-issues/ethics_en.htm).
- European Food Safety Authority (EFSA), Panel on Animal Health and Welfare (AHAW), 2012. Guidance on risk assessment for animal welfare. *EFSA J.* 10, 2513.
- Field, K.A., Paquet, P.C., Artelle, K., Proulx, G., Brook, R.K., Darimont, C.T., 2019. Publication reform to safeguard wildlife from researcher harm. *PLoS Biol.* 18 (5), e3000752.
- Fraser, D., 2008. *Understanding Animal Welfare: The Science in Its Cultural Context*. Wiley-Blackwell, UFAW Animal Welfare Series, Chichester, UK.
- Fraser, D., 2010. Toward a synthesis of conservation and animal welfare science. *Anim. Welf.* 19 (2), 121–124.
- Gee, G.F., Bertschinger, H., Donoghue, A.M., Blanco, J., Soley, J., 2004. Reproduction in nondomestic birds: physiology, semen collection, artificial insemination and cryopreservation. *Avian Poult. Biol. Rev.* 15, 47–101.
- Greggor, A.L., Vicino, G.A., Swaisgood, R.R., Fidgett, A., Brenner, D., Kinney, M.E., Farabaugh, S., Masuda, B., Lamberski, N., 2018. Animal welfare in conservation breeding: applications and challenges. *Front. Vet. Sci.* 5, 323.
- Griffin, G., Clark, J.M., Zurlo, J., Ritskes-Hoitinga, M., 2014. Scientific uses of animals: harm-benefit analysis and complementary approaches to implementing the three Rs. *Rev. Sci. Tech. (Int. Off. Epizoot.)* 33 (1), 265–272.
- Grimm, H., Olsson, I.A.S., Sandøe, P., 2019. Harm–benefit analysis — what is the added value? A review of alternative strategies for weighing harms and benefits as part of the assessment of animal research. *Lab. Anim* 53 (1), 17–27.
- Gutfreund, Y., 2020. Harm-benefit analysis may not be the best approach to ensure minimal harms and maximal benefits of animal research—alternatives should be explored. *Animals* 10 (2), 291.
- Hansson, S.O., 2009. Ethics beyond application. In: Takala, T., Herisson-Kelly, P., Holm, S. (Eds.), *Cutting Through the Surface*. Leiden, The Netherlands, Brill, pp. 19–28.
- Hansson, S.O., 2011. Do we need a special ethics for research? *Sci. Eng. Ethics* 17 (1), 21–29.
- Hansson, S.O., 2018. How to perform an ethical risk analysis (eRA). *Risk Anal.* 38 (9), 1820–1829.
- Harrington, L.A., Moehrensclager, A., Gelling, M., Atkinson, R.P.D., Hughes, J., Macdonald, D.W., 2013. Conflicting and complementary ethics of animal welfare considerations in reintroductions. *Conserv. Biol.* 27 (3), 486–500.
- Herrick, J.R., 2019. Assisted reproductive technologies for endangered species conservation: developing sophisticated protocols with limited access to animals with unique reproductive mechanisms. *Biol. Reprod.* 100 (5), 1158–1170.
- Hildebrandt, T.B., et al., 2021. The ART of bringing extinction to a freeze—history and future of species conservation, exemplified by rhinos. *Theriogenology* 169, 76–88.
- Hildebrandt, T.B., Holtze, S., Colleoni, S., Hermes, R., Stejskal, J., Lokooloo, I., Ndeereh, D., Omondi, P., Kariuki, L., Mijele, D., Mutisya, S., Ngulu, S., Diecke, S., Hayashi, K., Lazzari, G., de Mori, B., Biasetti, P., Quaggio, A., 2023. In vitro fertilization program in white rhinoceros. *Reproduction* 166, 383–399.
- Hooijmans, C.R., Leenaars, M., Ritskes-Hoitinga, M., 2010. A gold standard publication checklist to improve the quality of animal studies, to fully integrate the three Rs, and to make systematic reviews more feasible. *Altern. Lab. Anim.* 38 (2), 167–182.
- International Office of Epizootics (OIE), 2019. In OIE Terrestrial Animal Health Code, 28th ed. OIE, Paris, France. <https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmlfile=sommaire.htm>.
- IUCN, 2020. The IUCN Red List of Threatened Species. Version 2020-2. <https://www.iucnredlist.org>. Accessed May 2020.
- IUCN Action Plan, 2021. <https://www.iucn.org/theme/species/our-work/influencing-policy/global-species-action-plan>.
- Kirkwood, J.K., 2013. Wild animal welfare. *Anim. Welf.* 22 (1), 147–148.
- Laber, K., Newcomer, C.E., Decelle, T., Everitt, J.L., Guillen, J., Brønstad, A., 2016. Recommendations for addressing harm–benefit analysis and implementation in ethical evaluation—report from the AALAS–FELASA working group on harm–benefit analysis—part 2. *Lab. Anim* 50 (1 suppl), 21–42.
- Lindsjö, J., Fahlman, A., Törnqvist, E., 2016. Animal welfare from mouse to moose—implementing the principles of the 3Rs in wildlife research. *J. Wildl. Dis.* 52 (2), S65–S77.
- Lindsjö, J., Cvek, K., Spangenberg, E.M.F., Olsson, J.N.G., Stéen, M., 2019. The dividing line between wildlife research and management—implications for animal welfare. *Front. Vet. Sci.* 6, 13.
- Lueders, I., Allen, W.R.T., 2020. Managed wildlife breeding. An undervalued conservation tool? *Theriogenology* 150, 48–54.
- Mastromonaco, G.F., Songsasen, N., 2020. Reproductive technologies for the conservation of wildlife and endangered species. In: Presicce, G.A. (Ed.), *Reproductive Technologies in Animals*, Chapter 7. Academic Press, pp. 99–117.
- McMahon, C.R., Harcourt, R., Bateson, P., Hindell, M.A., 2012. Animal welfare and decision making in wildlife research. *Biol. Conserv.* 153, 254–256.
- Mellor, D.J., Hunt, S., Gusset, M. (Eds.), 2015. *Caring for Wildlife: The World Zoo and Aquarium Animal Welfare Strategy*. WAZA Executive Office, Gland, 87 pp.
- Minteer, B.A., Collins, J.P., 2005a. Ecological ethics: building a new tool kit for ecologists and biodiversity managers. *Conserv. Biol.* 19 (6), 1803–1812.

- Minteer, B.A., Collins, J.P., 2005b. Why we need an “ecological ethics”. *Front. Ecol. Environ.* 3, 332.
- Monfort, S.L., 2014. “Mayday mayday mayday”, the millennium ark is sinking! In: Holt, W., Brown, J., Comizzoli, P. (Eds.), *Reproductive Sciences in Animal Conservation. Advances in Experimental Medicine and Biology*, vol. 753. Springer, New York, NY, pp. 15–31.
- de Mori, B., 2019. Animal testing: the ethical principle of the 3Rs from laboratories to “field” research with wild animals. *Etica Polit./Ethics Polit.* XXI (3), 553–570.
- de Mori, B., Spiriti, M.M., Pollastri, I., Normando, S., Biasetti, P., Florio, D., Andreucci, F., Colleoni, S., Galli, C., Goeritz, F., Hermes, R., Holtze, S., Lazzari, G., Seet, S., Zwilling, J., Stejskal, J., Mutisya, S., Ndereh, D., Ngulu, S., Vigne, R., Hildebrandt, T.B., 2021. An ethical assessment tool (ETHAS) to evaluate the application of assisted reproductive technologies in mammals’ conservation: the case of the northern white rhinoceros (*Ceratotherium simum cottoni*). *Animals* 11, 1–21.
- Murray, N., 2004. *Handbook on Import Risk Analysis for Animals and Animal Products: Quantitative Risk Assessment*. Office International des Epizooties, Paris, France.
- Nuffield Council on Bioethics, 2003. *The ethics of research involving animals: a consultation paper*. Available at: [nuffieldbioethics.org/topics/animals-food-and-environment/animal-research](https://nuffieldbioethics.org/topics/animals-food-and-environment/animal-research).
- Paquet, P.C., Darimont, C.T., 2010. Wildlife conservation and animal welfare: two sides of the same coin? *Anim. Welf.* 19, 177–190.
- Perry, S., 2021. Developing assisted reproduction for reptiles, what’s next? *Clin. Theriogenol.* 13, 383–389.
- Pullin, A.S., Knight, T.M., 2009. Doing more good than harm—building an evidence-base for conservation and environmental management. *Biol. Conserv.* 142 (5), 931–934.
- Roth, T.L., Swanson, W.F., 2018. From petri dishes to politics—a multi-pronged approach is essential for saving endangered species. *Nat. Commun.* 9 (1), 1–3.
- Russell, W.M.S., Burch, R.L., 1959. *The Principles of Humane Experimental Technique*. Universities Federation for Animal Welfare, Wheathampstead (UK) (as reprinted 1992).
- Saltz, D., Justus, J., Huffaker, B., 2019. The crucial but underrepresented role of philosophy in conservation science curricula. *Conserv. Biol.* 33 (1), 217–220.
- Sandler, R.L., Moses, L., Wisely, S.M., 2021. An ethical analysis of cloning for genetic rescue: case study of the black-footed ferret. *Biol. Conserv.* 257, 109118.
- Shwiff, S.A., Anderson, A., Cullen, R., White, P.C.L., Shwiff, S.S., 2013. Assignment of measurable costs and benefits to wildlife conservation projects. *Wildl. Res.* 40 (2), 134–141.
- Sikes, R.S., Paul, E., 2013. Fundamental differences between wildlife and biomedical research. *ILAR J.* 54 (1), 5–13.
- Silla, A.J., Byrne, P.G., 2019. The role of reproductive technologies in amphibian conservation breeding programs. *Annu. Rev. Anim. Biosci.* 7, 499–519.
- Smith, J.A., Van Den Broek, F.A.R., Martorell, J.C., Hackbarth, H., Ruksenas, O., Zeller, W., 2007. Principles and practice in ethical review of animal experiments across Europe: summary of the report of a FELASA working group on ethical evaluation of animal experiments. *Lab. Anim* 41 (2), 143–160.
- Smith, J.A., Clutton, R.E., Lilley, E., Hansen, K.E.A., Brattelid, T., 2018. PREPARE: guidelines for planning animal research and testing. *Lab. Anim* 52 (2), 135–141.
- Soulsbury, C.D., Gray, H.E., Smith, L.M., Braithwaite, V., Cotter, S.C., Elwood, R.W., Wilkinson, A., Collins, L.M., 2020. The welfare and ethics of research involving wild animals: a primer. *Methods Ecol. Evol.* 11 (10), 1164–1181.
- Strand, J., Thomsen, H., Jensen, J.B., Marcussen, C., Nicolajsen, T.B., Skriver, M.B., Søgaard, I.M., Ezaz, T., Purup, S., Callesen, H., Pertoldi, C., 2020. Biobanking in amphibian and reptilian conservation and management: opportunities and challenges. *Conserv. Genet. Resour.* 12, 709–725.
- TRUST, 2018. *Global code of conduct for research in resource poor settings*. Available: <https://www.globalcodeofconduct.org/wp-content/uploads/2018/05/Global-Code-of-Conduct-Brochure.pdf>.
- Zemanova, M.A., 2020. Towards more compassionate wildlife research through the 3Rs principles: moving from invasive to non-invasive methods. *Wildl. Biol.* 2020, 1–17. <https://doi.org/10.2981/wlb.00607>.
- Zemanova, M.A., 2021. Making room for the 3Rs principles in responsible animal use in ecology: potential issues identified through a pilot survey. *Eur. J. Ecol.* 7, 18–39. <https://doi.org/10.17161/EUROJECOL.V7I2.14683>.