Comparative Medicine
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Vol 67, No 2
April 2017
Pages 101–105

Overview

The Interplay of Ethics, Animal Welfare, and IACUC Oversight on the Reproducibility of Animal Studies

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Reproducibility in animal studies has been defined as the ability of a result to be replicated through independent experiments within the same or among different laboratories. Over the past few years, much has been written and said about the lack of reproducibility of animal studies. Reasons that are commonly cited for this lack of reproducibility include inappropriate study design, errors in conducting the research, and potential fraud. In the quest to understand the basis for this lack of reproducibility, scientists have not fully considered the potential ramifications on ethical constructs for animal research, animal welfare considerations in animal research programs, the regulatory environment, and oversight by IACUCs. Here, we review how ethical theories behind animal research, policies, and practices meant to enhance animal welfare and the IACUC oversight process influence the reproducibility of animal studies, a previously undiscussed topic in the peer-reviewed literature.

Abbreviations: ILAR, Institute for Laboratory Animal Research; PI, principal investigator

Defining the Scientific Concerns about the Reproducibility of Animal Studies

Most basic research scientists agree that one of the cornerstones of the scientific endeavor is the ability to share research data and learn from the positive—and negative—results of other scientists. Not surprisingly, this process involves the replication of studies, whether needed to validate a specific animal model that can then be used in subsequent studies or to modify specific components of an experimental paradigm to test varying hypotheses. Many times, studies are repeated to confirm results when those results were obtained in a different environment. The ability to repeat studies in different environments makes studies predictable and applicable to other animal research as well as human research.

Several commentaries, letters to the editors, review papers, and metadata analyses have indicated that poor reproducibility is indeed a very real problem for both human and animal studies.[8,17,32] Some have asserted that "…a discovery is valid only if any scientist in any lab can conduct the same experiment under the same conditions and obtain the same results."[31] When studies cannot be repeated in different environments (in other words, when the findings are not reproducible) despite scientists' attempts to adhere to all components of the previously published experiments, questions are raised. The inability to replicate a study and achieve independent confirmation of data hints at poor study design and other flaws.[16]

Received: 18 Jun 2016. Revision requested: 18 Jul 2016. Accepted: 04 Oct 2016.
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In 2014, the Institute for Laboratory Animal Research (ILAR) convened the roundtable discussion "Reproducibility Issues in Research with Animals and Animal Models" to address the specific concerns with animal studies.[19] The resulting report summarized the issue by describing how recent publications and statements demonstrate the concern regarding the "…prevalence in the number of peer-reviewed studies that cannot be reproduced, particularly those containing data from experiments using animals and animal models…".[19] According to this report and other sources, although the reproducibility problem impedes the advancement of some animal research, long-term repercussions include the erosion of the integrity and public trust in science and endangerment of the entire scientific endeavor as we currently know it leading to decreased funding and support for science and most certainly less translational research.[8,19]

Causes of a Lack of Reproducibility

The current scientific literature is replete with reasons why studies cannot be reproduced. A ubiquitous reason is statistical insufficiency, primarily underpowered studies.[5,8,18,32] Additional causes include incorrect data interpretation, unforeseen technical issues, incorrectly constituted (or absent) control groups, selective data reporting, inadequate or varying software systems, and blatant fraud.[5,8,17,18,31] Some authors have remarked that the system of 'self-correction,' which has heretofore been taken for granted in the sciences, appears to be broken in that "papers with fundamental flaws often live on," because corrections, retractions, commentaries, or other mechanisms are not used to correct the scientific record.[8,18] Others have commented that the bias towards

publishing only positive results or inflation of a study's importance leads to downstream reproducibility problems.[31]

Less has been written about the variables in animal care, health, and welfare that can affect reproducibility. Laboratory animal professionals around the world are quick to recognize differences in institutional animal care programs but often fail to appreciate differences in the approach of scientists using animals in their research programs. Therefore, scientists may point to nonanimal causes for irreproducibility, as have been described in the previous paragraph, more quickly than those linked to animal care involving research animals.

Specific causes of irreproducibility, from a biologic, physiologic, and animal care perspective, as described by the aforementioned ILAR Roundtable report and other sources, include variables in the following areas (although this list is not exhaustive):[3,10,26,30] 1) animal source (vendor, institution); 2) animal genetic background (inbred, outbred, or hybrid study populations as well as unique strains); 3) animal housing (food, water, bedding, sanitation frequency, air quality, caging materials, lighting, temperature, noise, and so forth); 4) animal health (disease status either active or subclinical, gut microbiota); 5) animal behavior (use of enrichment, presence of stereotypies, and so forth); and 6) animal affective or emotional states, regardless of behavior. Many of these causes could be considered normal variation within the species.

Although it is not feasible to determine every single reason for or source of variability behind a lack of reproducibility for animal studies, based on the literature the causes can be grouped into 3 main categories: 1) flaws in study design; 2) variability in study conduct; and 3) poststudy evaluation and publication bias. Ethics, animal welfare, and IACUC oversight significantly affect areas 1 and 2. Important questions that currently lack answers in the scientific and regulatory literature are raised when considering reproducibility from the viewpoints of animal welfare, ethics, regulatory mandates, and IACUC oversight. Such questions must be further examined to have a more well-rounded approach to reproducibility.

Ethical Considerations Regarding Reproducibility

The ethical construct most commonly used to justify the use of animals in research is that of the 'greater good;' this construct stems from the ethical theory of utilitarianism, which is a consequentialist theory.[27] A very basic description of this theory is that actions that produce the greatest good or happiness for the greatest number are the most moral actions.[26] The 'good' produced by animal research are the new drugs, treatments, as well as decreased suffering in humans and other animals; this 'good' justifies the use of animals in research.[27]

Animal study reproducibility directly relates to the justification of animal research based on utilitarianism. Reproducible studies contribute to the ongoing research effort and can be justified. But what happens when a study is not reproducible? Concerns about losing the justification for animal use in research due to a lack of reproducibility are seen as statements contending that animals are being "sacrificed needlessly" when reproducibility is not achieved.[17,19] Reproducibility, or replication, has been discussed within ethical texts because it may serve as a potential mechanism to determine fraud with the recognition that other variables that may prohibit replication can be in play. But now scientists themselves are asserting an ethical responsibility for reproducibility because,[17,26] with poor reproducibility, both animal lives and financial resources, time, and human energy might be wasted.[17,31] Saying that animal lives and other resources are wasted implies that they are not a part of the 'greater good' and that the ethical argument for animal research can be questioned when there is irreproducibility. Therefore, reproducibility is now an ethical topic, with the main question being "if a study was not reproducible, was it ethical?"

One can debate the usefulness of data obtained from nonreproducible studies where misconduct is not the cause of the irreproducibility.[26] Commentators and authors who state that the lack of reproducibility indicates that animal lives were wasted imply that nonreproducible results do not contribute to the greater scientific enterprise and that such studies do not meet the criteria of the greater-good argument. In reality, data from studies that are not reproducible may still be of value to other researchers or be of such a unique nature as to preclude reproducibility. Perhaps authors could provide disclosures evaluating the potential reproducibility of studies as well as a description of the intent of the study, beyond hypothesis testing, with regard to limitations on reproducibility when publishing results. Other authors have called for investigators to label their published research as 'exploratory' or 'confirmatory,' so that the emphasis on reproducibility could be placed on confirmatory studies.[15] Because of these nuances, the animal research community should be careful about making sweeping assumptions regarding the implications of reproducibility in terms of animal lives.

Concerns about the potential effects of irreproducibility on the safety of humans participating in clinical trials built upon animal study data have also arisen but are of a different nature. Appropriate deliberation of the potential applicability of animal studies to the safety of human patients in clinical trials should be explicit within publications. If manuscript reviewers do not understand that a particular study was not undertaken in an effort to safeguard human patient safety, then false assumptions may be made, and reproducibility becomes the scapegoat when it was never the focus of the research in the first place.

Animal Welfare Considerations Regarding Reproducibility

To discuss animal welfare, one must first define it. Although multiple authoritative definitions exist, animal welfare is a multifaceted topic and even popular definitions are not uniformly accepted. In addition, the field of animal welfare science combines both scientific assessments and moral judgements.[4] Fraser[9] provides one of the best authoritative definitions of animal welfare, which is based on 3 components: biologic health, affective (emotional) states, and natural living. This definition will be used as the definition of animal welfare for the purposes of this manuscript.

As previously discussed, laboratory animal veterinarians and research scientists have already identified those variables inherent in conducting animal research in different facilities and physical environments.[19] Institutions, through their animal and veterinary care programs as well as IACUCs and comparable animal research oversight bodies, have specified those physical elements of their animal facilities that they believe provide the best animal health and welfare outcomes for laboratory animals. These ele-

ments, which directly affect both the 3 previously defined components of animal welfare as well as animal study reproducibility, include factors such as animal housing systems, animal facility macroenvironments, and source and quality of food and water. Therefore, questions regarding both animal health and animal welfare are inherent in discussions of reproducibility. These questions include "How is reproducibility ensured in different animal research environments with varying levels of animal welfare?", "Can compromises in animal welfare make an animal study or model more or less reproducible?", and "Should compromises in animal welfare be made in order to enhance study reproducibility?"

Potential answers have been offered to address reproducibility among different research environments that presumably have differing levels of animal welfare and care.[3] The previously discussed 2014 ILAR report included the concepts of publishing additional details regarding the variables and establishing detailed standards that all institutions can follow.[19] The formulation and continuing adoption of the *Animal Research: Reporting of In Vivo Experiments* (ARRIVE) guidelines along with *Guidance for the Description of Animal Research in Scientific Publications* are some of the best examples of this effort.[12,14] Although the ARRIVE guidelines are being adopted, this work has been criticized as a potential source of regulatory burden.[14,19,20]

The next 2 questions—"Can compromises in animal welfare make an animal study or model more or less reproducible?" and "Should compromises in animal welfare be made in order to enhance study reproducibility?"—are closely linked.[25] Assuming that all environmental elements and study variables are known, if one principal investigator wishes to reproduce the work of another in a different facility, will they need to ask for changes in animal management practices to facilitate potential reproducibility? Should an institution change its standards—which that institution believes fosters the desired level of animal welfare—to achieve reproducibility? Is there any guarantee that once changes are made that reproducibility will then be assured? Answers to these questions lie with an institution's veterinary staff and oversight body but pit the need to maintain institutional standards used to cultivate a preferred animal welfare status against the ability for scientists to reproduce their work in multiple environments.

Allowable tumor size limits imposed by the IACUC present a clear example of this conundrum. Many IACUCs have policies dictating that subcutaneous tumors must not exceed a certain size, usually 1.5 to 2 cm in diameter. These policies are created with significant veterinary input and represent what individual IACUCs believe to be the best way to ensure appropriate animal welfare. However, what happens if a scientist wants to replicate a research project that allowed mice to develop very large tumors so that cells from the tumor could escape and metastasize but that same tumor size is larger than that permitted by his or her home IACUC? Does this situation conflict with animal welfare or support the greater good? Should policies that represent animal welfare standards be changed or exceptions permitted in order to achieve reproducibility? Similar arguments apply to analgesia, anesthesia, postoperative care, and many other policies and institutional standards. An increasing call for animal study reproducibility will result in more of these deliberations.

From the authors' viewpoint, an obvious connection between animal welfare and reproducibility is the legitimate concern that studies performed by using animals with compromised animal welfare may not be reproducible. However, the commitment of laboratory animal professionals to the provision of high-quality animal care and to the development of animal welfare standards that promote such care can help to improve reproducibility.

IACUC and Oversight

The charge to IACUCs, and related animal research oversight bodies, does not yet include a requirement to evaluate proposed studies for reproducibility. However, even without a specific charge, the IACUC responsibilities of ensuring the humane handling, treatment, and care of animals as well as evaluating hypothesis testing, sample size, and accuracy or relevancy of controls can "contribute to enhanced reproducibility."[19] Barriers to animal study reproducibility, however, still exist in the oversight process due to the goals of various regulations and agencies, institutional policies set by internal oversight bodies, and multiple interpretations of the 3Rs (reduction, replacement, and refinement).[1,19,28]

In the United States, the USDA Animal Welfare Act and its associated regulations, in addition to the *United States Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training* and guidelines set forth by the Office of Laboratory Animal Welfare within NIH, form a core set of regulations and guidelines for animal research conduct and oversight.[2,24] The documents and accompanying interpretations, policies, and FAQs, however, do not focus on study reproducibility and in some instances can be construed as to encourage the smallest number of animals possible without regard to reproducibility.[22,23,29]

According to USDA regulations, an IACUC can only approve animal research activities after the principal investigator (PI) has provided a written assurance stating that the activities do not unnecessarily duplicate previous experiments.[29] These regulations and the USDA Animal Care Policy Manual do not address what constitutes unnecessary duplication of previous experiments, leaving that decision to the individual PI, IACUC, or institution. In addition, the term 'duplication' is not defined within the regulations and other USDA standards, which may generate confusion among the PI, IACUC, and others involved with oversight processes. Without such definitions, the PI, IACUC, and institution must decide whether the need to reproduce a study to verify reproducibility represents unnecessary duplication of previous experiments. Indeed, many institutions rely on the assurance statement that the PI signs in their description of animal research activities, whereas others use a literature search to determine unnecessary duplication—although there is no requirement or guidance for that type of literature search.

The third principle in the *United States Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training* states that animal selection should ensure that animals are of "an appropriate species and quality" and that the study uses a "minimum number required to obtain valid results."[24] Currently, many animal studies are underpowered, meaning that they do not include enough animals to provide statistically meaningful results. The word 'valid' makes a strong point but is entirely contextual. Study results may be statistically valid for a particular scientific question in a specific laboratory using certain animals with specific genetic backgrounds and re-

agents, but that validity does not guarantee that the study will be reproducible either in the same laboratory or in a different one.

In our experience, correspondence from the Office of Laboratory Animal Welfare has stated that investigators must be trained in methods that "minimize the number of animals used." No such request is made to ensure that investigators are aware of statistical justification methods to arrive at desired animal numbers, although this justification is described on the Office's website. A recommendation document released in October 2015 by the US Food and Drug Administration regarding animal medical device studies contains the statement that "A thoughtful attempt at utilizing the least number of animals that will provide meaningful interpretation is paramount and includes such measures as attention to the appropriate experimental control, consideration of potential experimental confounders, and an idea of best observation intervals…," but does not mention future reproducibility or explain whether "meaningful interpretation" equates to study reproducibility.[20] Although some of the planning committee members for the much-cited *Reproducibility Issues in Research with Animals and Animal Models: Workshop in Brief* represented government offices, none of the speakers on the agenda represented a US government office's viewpoint on the topic.[19] The *Guide for the Care and Use of Laboratory Animals* is also silent on this topic.[11] NIH's recent initiative to enhance both rigor and reproducibility does not address IACUC review of animal research.[20] Without an explicit call from regulatory agencies and guidance documents for reproducibility to be of paramount concern in animal studies, especially in the decision making process for determining animal numbers, directives regarding minimization of animal numbers will be at odds with calls for increased reproducibility as a mechanism of promoting better and safer translational research.

IACUCs are charged with oversight according to these aforementioned regulations and guidelines, and the regulations and guidelines do not request that reproducibility factor into the IACUC's decision-making. In fact, many feel that IACUCs can hinder reproducibility by focusing on the minimization of the number of animals used, whereas others may feel that IACUCs should assume some of the responsibility to help ensure reproducibility.[1] IACUCs should carefully consider which stance is appropriate given that many factors influencing reproducibility are beyond the scope of the IACUC, it may not be the intention of the PI that the study be reproduced, or the IACUC may be aware of only one small component of an entire experiment. An IACUC deciding that their reviews must safeguard reproducibility might also be construed as an example of regulatory drift.

IACUC reviews, decisions, policies, and other actions can, however, influence the ability of studies to be reproduced. Emphasizing the minimization of the number of animals to be used has been considered an over-interpretation of one of Russell and Burch's '3Rs' in that the original publication identifies the 'R' of 'reduction' not as the minimization of the number of animal used, but rather determining the correct number of animals (based on statistical analysis) prior to conducting the study rather than afterward.[28] Ultimately, determining the appropriate number of animals prior to study initiation could reduce the number of animals "…progressively as statistical and experimental techniques are improved."[28] Striving for an "absolute minimization of animals used would be inconsistent with this aim" and can deter scientists from asking for the appropriate number of animals, resulting in underpowered studies and nonreproducible studies.[1,28]

Because many IACUCs are constituted with knowledgeable scientists and statisticians, an IACUC protocol review can reveal that an inadequate number of animals has been requested for the proposed hypothesis testing and sample sizes. Scenarios include requesting too few animals to derive statistically relevant conclusions, having inadequate or nonexistent positive and negative control groups, and not accounting for sex- and strain-associated differences or animal attrition.[17,18] What would not be obvious in IACUC review is requesting inadequate numbers due to budgetary constraints. The directive to minimize animal use does not include a companion obligation for the IACUC to request that animal numbers be increased to improve the chances for better statistical outcomes or reproducibility. In our experience, some IACUCs believe it is inappropriate to ask a PI to increase animal numbers during protocol review. However, such a request would be very much in line with the previously depicted ethical construct demanding the need for reproducibility in animal studies so that animal lives are not wasted.[17,19] On further extrapolation, one might argue that if more studies are designed with reproducibility in mind, then fewer animal lives are 'wasted' in unsuccessful attempts at study replication after results have been made public, increasing the contribution to the greater good. Concentrating on the selection of the appropriate animal numbers rather than adhering to a preconceived notion of minimizing the number of animals to be used without factoring in a need for reproducibility should be an area of training and debate for IACUC members.

Conclusions

The reproducibility of animal studies has become a highly discussed topic in the scientific community during the past few years. Peer-reviewed manuscripts, retrospective reviews, metadata analyses, webinars, workshops, journal clubs, symposia, NIH policies, and projects have all been instigated to determine the causes of this lack of reproducibility, to propose solutions to the problem, and to reproduce pivotal studies.[5,7,13,16-18,19,25] Animal research is particularly vulnerable to concerns about reproducibility because preclinical results are used to support efficacy and safety determinations for clinical studies and direct but independent oversight in human trials addresses many of the scientific concerns raised with animal studies that lack such oversight.[13] What has been largely absent from this discussion has been ethical and animal welfare considerations (questions), applicable regulatory mandates, and IACUC oversight. We have discussed the ethical construct for supporting the reproducibility in animal studies, but the call for reproducibility may potentially be undermined by animal welfare standards at individual research facilities, lack of regulatory or other guidance for IACUCs and other oversight bodies to stress reproducibility, and an emphasis on minimizing the number of animals used in proposed animal studies. With the NIH, research scientists, professional associations, pharmaceutical companies, and veterinarians asking that reproducibility receive serious deliberation, now is the time to evaluate fully all ethical, animal welfare, regulatory, and institutional influences that could, in the end, make reproducibility a reality.

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