Genetically Modified Mishaps

Dr. T. Guaio, an established faculty member at Great Eastern University (GEU), studies animal models of hemophilia with the goal of finding effective gene therapies. Recently, Guaio completed the statistical analyses of 6 months' worth of data from his rabbit research. Much to his dismay, the results were inconclusive and rather chaotic. This was surprising, and troubling, because prior results were clear and statistically significant with a low p-value. Consequently, Guaio consulted with his colleague Dr. Altra Ipotesi.

During the conversation, Ipotesi asked Guaio about the specific animals used in the experiments; e.g., how they were housed, from where they were obtained, and were there any other health issues. Guaio responded that he bred the rabbits in-house – some rabbits were purchased from a vendor and some were obtained from another GEU faculty member, Dr. Stesso Virale. As the conversation progressed, Guaio disclosed that the animals he obtained from Virale were the progeny of transgenic (TG) rabbits, a model created by Virale for studying anemia. These TG rabbits were created by stable introduction of recombinant nucleic acid molecules.

In an attempt to uphold the principles of the 3R's (i.e., reduction), Virale donated TG offspring that were unusable for his own research (i.e., did not have the homozygous genotype) to other IACUC-approved animal users. The donated rabbits did not exhibit the anemic phenotype and were viewed as "normal" animals.

Guaio immediately realized his error and re-analyzed the data. After separating the data of the purchased, wild-type animals from that of the TG animals, it became evident that the TG animals responded differently to the treatment and could not be included in the dataset.

When Guaio submitted a request to the IACUC for more animals to replace the TG animals that he used, GEU's IACUC resulted in potentially unnecessary animal pain and distress and a poor scientific outcome. ❐

A WORD FROM OLAW AND USDA

Response from OLAW

There are several concerns arising from the Principal Investigator's decision to use rabbits from a variety of sources and consider the animals comparable. One concern is whether the research protocol that the IACUC approved described the varied genetic backgrounds of the rabbits. If animals with a transgenic background were not described in the approved protocol, then a failure to adhere to the protocol has occurred and is reportable to OLAW[1]. Another concern is the unauthorized transfer of transgenic animals to other investigators without IACUC or IBC approval. This circumstance requires investigation by both committees, corrective actions to prevent recurrence acceptable to the IACUC and IBC, and reporting to OLAW and the National Institutes of Health (NIH), Office of Science Policy[1,2]. If the findings are found to be programmatic, corrective approaches applicable to all laboratories may be required. Revisions to policies, standard operating procedures, IACUC and IBC forms, and training may be necessary. Assuming the study was NIH-funded, of concern is the lack of uniformity in the research design that compromises the rigor of the study. Since 2019, NIH has required grant applicants to describe in the Approach section of the Research Strategy how the experimental design and methods proposed will achieve robust and unbiased results[3]. To assist applicants, NIH provides guidance on how to address rigor and reproducibility in the application, explains how the application will be evaluated for scientific merit, and provides application preparation resources including links to a free online tool for designing animal experiments[4,5].

Word from USDA

The Animal Welfare Regulations require a proposal to conduct an activity involving animals to contain "a complete description of the proposed use of the animals[6]". This complete description should include information regarding the acquisition and disposition of the animals. The IACUC should have closely reviewed Dr. Virale's plan for disposition of transgenic offspring that do not carry of the gene of interest since this is an expected outcome when breeding transgenic animals. Before Dr. Virale used an alternate disposition method (e.g., transfer the animals to a different research protocol), the IACUC is required to review and approve such a significant change in ongoing activities[7]. Similarly, Dr. Guaio's protocol should have described acquisition of animals, and acquiring animals from a different source than is listed in the protocol requires IACUC review and approval. Additionally, although the regulations are silent regarding subspecies and transgenics, the regulations do require proposals to include a rationale for involving animals, and for the appropriateness of the species[8]. Dr. Guaio failed to consider the appropriateness of the animals he received from Dr. Virale, which

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6. AWR §2.31(e)(3).
7. AWR §2.31(d)(1).
8. AWR §2.31(e)(2).

Chair called the amendment to Full Committee Review (FCR). The IACUC was challenged with the situation and left the 4-hour meeting with even more questions than when they started! How would your institution view and handle this matter (e.g., what concerns would they have, what actions would they take)? ❐

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COMPLIANCE CONSIDERATIONS

The Protocol Review coordinators offer the following compliance considerations:

1. On donating transgenic (TG) animals
Due to the manipulation of their genome, the production and subsequent management of TG animals are governed by regulations (PHS Policy[1], NIH Guidelines[2]). Consequently, offspring of TG animals that are not used for the intended IACUC- and Institutional Biosafety Committee (IBC)-associated research would not qualify for any other research activities since they do not have the genetic background of a "normal" laboratory rabbit.

2. On the concept of "reuse"
In this case, the concept of "reusing" animals is a misapplication of the 3Rs concept of "Reduction" and is not a term used in the regulations, other than to describe a scenario that should not occur:

- "The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results[1]."
- "Reduction involves strategies for obtaining comparable levels of information from the use of fewer animals or for maximizing the information obtained from a given number of animals (without increasing pain or distress) so that upon completion of the study fewer animals were needed to achieve the same scientific goal[3]."

During the Full Committee Review (FCR) discussion of the amendment, the IACUC should identify the fact that:

- The TG rabbits did not satisfy the requirement for Guaio to select animals of an appropriate "quality"; the quality of these animals was scientifically compromised once their genetic background was altered.
- Guaio should have consulted with the IACUC and IBC prior to accepting the TG animals from Virale, which could have potentially prevented:

  a. The programmatic failure to appropriately train animal users, i.e., GEU should have had (and should now develop) a policy on the institution's expectations for the use of TG animals (thereby communicating the prohibition on donating and using TG animals for any other purpose than for what they were scientifically developed).
  b. The non-compliance (i.e., using TG animals for activities that are not the intended IACUC- and IBC-approved activities and, thus, do not qualify for research activities that are not associated with their specific phenotype).
  c. The injudicious use of animals; because the TG animals were not of an appropriate quality for Guaio's own proposed research, unnecessary procedures were conducted on animals and the outcome(s) could not contribute to Guaio's dataset.

3. Programmatic concerns of training
Virale's and Guaio's actions indicate a clear lack of training and education on the part of GEU, which speaks to a programmatic failure – for example:

a. The misinterpretation and misapplication of the 3Rs (i.e., "reusing" animals)" may have been avoided providing the required GEU training discussed the reuse of GMO animals.

The PHS Policy requires an OLAW-approved Assurance that includes "a synopsis of training or instruction in the humane practice of animal care and use, as well as training or instruction in research or testing methods that minimize the number of animals required to obtain valid results and minimize animal distress, offered to scientists, animal technicians, and other personnel involved in animal care, treatment, or use[1]."

b. The sharing of TG animals requiring IBC approval:

As a PI holding an IBC approval for the use of TG rabbits, Virale is required to manage the containment of these animals. On behalf of the institution, Virale is responsible for ensuring full compliance with the NIH Guidelines when research activities include the use of recombinant or synthetic nucleic acid molecule research. (i.e., the TG rabbits)[2]

The (potential) violation of the NIH guidelines due to inappropriate use and disposal of carcasses and other waste of TG animals would require further communication with and consultation from, for example, the GEU Biosafety Committee and the Office of Science Policy[2], NIH who oversees the use of TG animals in this case rabbits. ❐

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The problem with repurposing genetically modified organisms

Investigators are often disappointed when a gene is knocked out and animals have "no phenotype", but genetic modelers often remind them that animals may not have been appropriately challenged to reveal a phenotype and function of the gene of interest. While laudable that Dr. Guaio tried to make use of existing animals being culled by Dr. Virale, the use of "excess" genetically modified animals in unrelated experiments is not recommended, nor is it allowed at our institution without prior approval. Minimally, a pilot study would be required to demonstrate that the transgenic (TG) animals are indeed behaving as wild-type animals and appropriate to include before expanding to the larger experiment.

IACUC forms ask several questions to determine the nature of animals to be used in experiments including the source (vendors, established colonies, external collaborators, etc.) as well as species and strain/substrain, and whether any have been genetically modified. Transfer of animals from one investigator to another requires approval by IACUC, and transport by animal care staff once an appropriate form has been completed to document the transfer. This is especially important when managing animal rooms that may have different pathogen status. Transfer without consent of IACUC would be viewed as noncompliance by both investigators and their staff participating in the transfer, with the committee subsequently determining appropriate corrective actions for all involved. We would not view the unexpected outcome as noncompliance with policies requiring the reporting of adverse events as the TG animals did not develop the anemic phenotype and presumably were otherwise healthy.

The request for additional animals to replace the TGs would be approved unless some new information challenges the rationale, justification, or value of conducting the research as originally planned (and approved by IACUC). At first glance, the total number of animals being used by Drs. Guaio and Virale would not change; however, any additional wild-type animals to be used as controls in the experimental paradigm would need additional hemophilia animals for comparison and experimental rigor. Statistical power analysis along with an updated experimental plan would be carefully reviewed by the committee, which may require an external consultant with appropriate expertise in anemia if deemed necessary.

Lastly, the researchers should not overlook the opportunity revealed by their mistake in that the transgene itself, or the gene at the integration site, or possibly the genetic makeup of animals being bred in a closed colony, affects the phenotype. To expand the studies and follow this new line of investigation, a modification of the protocol would be required. ❐

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Genetic considerations for sourcing research animals

This unfortunate mishap is an example of the reproducibility challenges we face in research. The results of their study highlight the confounding genetic variables that may be present in animal models and the need to be aware of such variables. Animal researchers are commonly aware of a range of experimental variables that require monitoring, including age, sex, pathogen status, and diet. Researchers must also be aware of the impact of various genetic elements on the experiment, including genetic background effects and the presence of any spontaneous or experimentally induced mutations or transgenes.

It is commendable that Dr. Virale donated otherwise unusable animals to fellow researchers. However, care must be taken to assure that these models have the relevant features for suitable use in other research projects. This becomes an animal welfare issue in that the study provided inconclusive results because of the use of inappropriate animals, requiring additional animals in order to complete the study.

When considering a source for research animals, relevant genetic details should be obtained, including from commercial sources or other researchers. In this case, it appears that these rabbits obtained from Dr. Virale may have genetic differences that made them unsuitable for use in Dr. Guaio's study. One possibility is that the closed breeding colony of rabbits may have a distinct genetic background from the animals used previously in Dr. Guaio's work. Some or all of these rabbits may also have harbored a transgene, perhaps in the hemizygous state, that may have influenced the results of their study. CRISPR-based genome editing tools have expanded the capacity to generate mutations and transgenes in a range of animal research models, thus consideration of these genetic-related effects will become more common. Review of the suitability of a source of research animals, and the potential for confounding genetic variables, should be assessed early in the experimental design stage.

The responsibility for confirming the suitability of an animal source must ultimately lie with Dr. Guaio. However,

the IACUC can be a valuable resource to identify potential pitfalls related to animal origin, before a study is initiated. Animal protocols should generally include the source of the animals to be used, with sufficient detail to ascertain the relevant genetic details of the animals. IACUC should be on the lookout for confounding genetic variables that could result in the unnecessary and noninformative use of research animals. Additionally, an expert in experimental animal genetics, either on the IACUC or as an ancillary subject matter expert, may be a useful resource to review well-documented protocols to identify possible confounding genetic variables in the early stages of experimental design. Complete documentation and review of the origin and nature of these animals obtained from Dr. Virale might have identified potential problems early and avoided this issue altogether. ❐

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