

## *International Journal of Scientific Research and Reviews*

### **Experimental design for animal based research**

**L. Murali Krishnan<sup>1\*</sup>, A.Varadharajan<sup>2</sup> and R.Gnanasekar<sup>3</sup>**

<sup>1,2,3</sup>Assistant Professor, Division of Animal Husbandry, Faculty of Agriculture,  
Annamalai University, Annamalai nagar 608002, Tamilnadu.

#### **ABSTRACT**

Quality and reproducibility of research is an important concern in experiments involving animals. The first and foremost step before starting a project in animal research is collection of review of literature pertaining to the topic and discussion with expertise in that area of research. This will provide a background information to the area of research and it is a prerequisite for an experimental design. A null and alternate hypothesis should be proposed to particular problem statement. Identification of suitable animal model specific to the area of research is important. Fixation of control groups, allocation of animals randomly to control/ treatment groups, calculation of animal requirement per group and suitable statistical analysis method should be considered. Permission from the ethical committee for conducting animal experiments is mandatory. All the above factors need to be carefully analyzed before designing an experiment in order to produce a scientifically valid and reproducible data and to publish the results in a reputed journal.

**KEYWORDS:** Experimental design, research, livestock

#### **\*Corresponding author**

**Dr. L. Murali Krishnan**

Assistant Professor,

Division of Animal Husbandry,

Faculty of Agriculture,

Annamalai University, Annamalai nagar 608002

Email: [drlmuralikrishnan@gmail.com](mailto:drlmuralikrishnan@gmail.com)

## INTRODUCTION

Experimental design is a systematic and scientific approach to research in which the researcher manipulates one or more variables, controls and measures any change in other variables. Animal based experiments need to be designed with utmost care due to ethical issues and to improve the quality and reproducibility<sup>1</sup>. Studies involving animals such as livestock is important for scientific development not only in animal sciences but also in human medicine. Experimental design is a vital component for the success of any research project<sup>2</sup>. It should be properly and accurately designed to produce valid reproducible results<sup>3</sup> so that the findings are easily published in standard journals. This article provides basic information on procedures that are to be considered to design an experiment using animals.

## CONDUCT OF EXPERIMENT

### *Identify, define and state the problem*

The first step is to identify and define the problem so that it can be tackled and solved. The problem statement should be precise and concise<sup>4</sup>.

### *State the objectives and propose a hypothesis*

The objectives of experiment must be clearly stated as it is basic need for conducting the experiment. Then propose and develop a hypothesis which is a statement neither considered as true or false but needs to be investigated and proven to otherwise. Collected data and the experiment carried out will provide evidence for or against the hypothesis<sup>4-7</sup>.

### *Designing and conducting the experiment*

A good design of experiment is the deciding factor in proving the hypothesis and hence achieving the stated objectives of the experiment<sup>8-10</sup>

## DESIGN OF ANIMAL EXPERIMENT

### *Research plan*

While designing an animal experiment, description of problem statement, objectives of the study and hypothesis should be properly fixed and documented<sup>11</sup>. In this stage of development the practicality of the project, time frame for data collection and evaluation be determined. Other aspects such as the life span of the animal model, time required for the progression of disease in the animal, personnel time required for the project and cost of the experiment are to be considered<sup>12-14</sup>. The space, feed requirement, mode of administration of drugs should be assessed. Standard operating

procedures should be followed for all experiments. A suitable statistical test is identified at the design stage itself. Final conclusion will be arrived from the analysis of data and based on whether the hypothesis is accepted or rejected<sup>15,16</sup>.

### ***Experimental unit***

An experimental unit consists of an individual animal or group of animals. Single goat is taken as an experimental unit if surgery is done for specific complications but flock of goat is taken as an experimental unit during drug testing. It is essential to fix appropriate experimental unit for the purpose of estimating standard error in statistical analysis<sup>15,17,18</sup>

### ***Experimental group size: N factor***

It is critical to assign appropriate number of animals to each group. Number of animals assigned in each group is determined by using statistical test on the basis of expected outcome for each group<sup>18</sup>.

### ***Controls***

Several variables will influence the outcome of the studies involving animals. To minimize the impact of external variables it is essential to use control animals for each individual experiments. Positive, negative, sham, vehicle and comparative controls are used in animal experiments.

### ***Randomization***

Animals assigned to different experimental groups must be in random. A homogeneous population of animals will have similar age, sex, weight, breed, and strain etc. whereas a heterogeneous group of animals will differ in characteristics. Randomization is done using random number tables or by computer generated sampling<sup>5,19</sup>.

## **IDENTIFICATION OF A SUITABLE ANIMAL MODEL**

For identification of a suitable animal model the following points should be considered,

- Lowest animal on the phylogenetic scale should be selected and used
- Identification of specific species or strains with specific characteristics for the study
- Cost of feeding and maintenance of animal be considered
- Consultation with colleagues in the selected field of study
- Contact with commercial vendors or government supported repositories of animal model
- Consultation with laboratory animal veterinarian
-

## EXPERIMENTAL DESIGN: FINAL CONSIDERATION

### *Experimental protocol approval*

Experiments involving small laboratory bred animals such as guinea pigs, rabbits, rats, mice, hamsters and invertebrates should be approved by Institutional Animal Ethics Committee (IAEC). For other animals such as live stocks and large animals permission must be sought from a subcommittee of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). Approval must be obtained before the animal purchase or experimentation and even before submission of grant proposal to funding agencies<sup>20-22</sup>.

### *Pilot studies*

Pilot study should be conducted involving small number of animals to generate preliminary data and to standardize techniques and procedures before the actual experimentation<sup>23-25</sup>.

### *Data entry and analysis*

Collection, entry and analysis of data are an important responsibility of the researcher<sup>26-28</sup>. Data entry error may occur when handling huge volume of data that may be avoided by use of quality assurance procedure. Analysis of data varies with the type of study and statistics method used to evaluate it<sup>29,30</sup>.

## CONCLUSION

For designing and conducting scientific studies using animals the investigator should conduct a complete literature search, review in consultation with an experts in the field. Specific question should be asked and an appropriate hypothesis be formulated and then design the experiments specifically to address the problem. Consultation with a biostatistician during designing phase will fetch good results. Identification of suitable animal model, choosing control group and random allocation of animals is also important. Conducting a pilot study before the actual large scale study is a prerequisite. Permission from the ethical committee for conducting animal experiments is mandatory. Ensure that utmost care is provided to the animals during the period of experimentation.

## REFERENCES

1. Smith AJ, Clutton RE, Lilley E., et al. PREPARE: guidelines for planning animal research and testing. *Laboratory Animals*.2018;52(2):135–141.
2. Merel RH, Kim W. Improving the conduct, reporting, and appraisal of animal research. *BMJ*.2018; 360 :j4935.

3. Diamond WJ. Practical Experiment Designs for Engineers and Scientists. 3rd ed. New York : Wiley;2001.
4. Kutsanedzie F, Achio S, Ameko E. Basic Concepts and Applications of Experimental Designs and Analysis.1st ed. Science Publishing Group: New York;2015.
5. Zolman JF. Biostatistics: Experimental Design and Statistical Inference . New York : Oxford University Press; 1993.
6. Ramdhani, Abdullah, Ramdhani et al. Writing a Literature Review Research Paper: A step-by-step approach. *International Journal of Basic and Applied Science*.2014;3:47-56.
7. Sproull NL. Handbook of Research Methods: A Guide for Practitioners and Students in the Social Sciences, 2nd ed. Metuchen: Scarecrow Press;1995.
8. Lawson AE.What does Galileo's discovery of Jupiter's moons tell us about the process of scientific discovery? *Sci Educ*.2002;11:1-24 .
9. Lund H, Brunnhuber K, Juhl C, et al. Towards evidence based research. *BMJ*. 2016; 355: i5440.
10. Johnson P. D and Besselsen D. G. Practical Aspects of Experimental Design in Animal Research. *ILAR Journal*.2002; 43(4):202–206.
11. O brink KJ and Rehbinder C. Animal definition: a necessity for the validity of animal experiments? *Lab Anim* 2000; 34: 121–130.
12. Keppel G. Design and Analysis: A Researcher's Handbook.3rd ed. Englewood Cliffs : Prentice Hall;1991.
13. Boer DJ, Archibald J, Downie HG. An Introduction to Experimental Surgery: A Guide to Experimenting with Laboratory Animals. New York: Elsevier;1975.
14. Davidson MK, Lindsey JR,Davis JK. Requirements and selection of an animal model. *Israel J Med Sci*. 1987;23:551-5.
15. Weber D, Skilling's JH. A First Course in the Design of Experiments: A Linear Models Approach .Boca Raton : CRC Press; 2000.
16. WuCF, Hamada M. Experiments: Planning, Analysis, and Parameter Design Optimization. New York: Wiley;2000.
17. Fitzpatrick A. Ethics and animal research. *J Lab Clin Med*. 2003;41:89-90.
18. Hoshmand, R. Design of Experiments for Agriculture and the Natural Sciences. New York: Chapman and Hall;2006.
19. Smith JA, Birke L, Sadler D. Reporting animal use in scientific papers. *Lab Anim*.1997; 31: 312–317.

20. Avey MT, Moher D, Sullivan KJ, et al. The devil is in the details: incomplete reporting in preclinical animal research. *PLoS One*. 2016; 11: e0166733.
21. Macleod MR, Michie S, Roberts I, et al. Biomedical research: increasing value, reducing waste. *Lancet*. 2014; 383:101–104.
22. DasREG. The role of ancillary in the design, analysis and interpretation of animal experiments. *ILAR J*.2002;43:214-22.
23. Festing MFW. Principles: the need for better experimental design. *Trends Pharmacol Sci*. 2003; 24:341-5.
24. Dean AM, Voss D. Design and Analysis of Experiments. New York: Springer;1999.
25. Mead, R. Statistical Methods in Agriculture and Experimental Biology. New York: Chapman and Hall;2003.
26. Chatfield C. Introduction to Multivariate Analysis. New York: Routledge;1980.
27. Cox D, Reid N. Analysis of Survival Data. New York: Routledge;1984.
28. Howard BR. The control of variability. *ILAR J*.2002;43:194-201.
29. Munafò MR, Nosek BA, Bishop DVM, et al. A manifesto for reproducible science. *Nat Hum Behav*. 2017; 1: 0021.
30. Pickvance CG. Four varieties of comparative analysis. *J Hous Built Env*.2001;16:7-28.