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Use of experimental animals in research: Legal regulations in Türkiye

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Abstract
Experimental animals have been used in research since the beginning of medicine and still have an important place in scientific research today. Initially, there were no rules or limits regarding the treatment of animals used in experiments. When this was added to the rapid increase in the number of animals used in experiments in parallel with the increase in scientific studies, public reactions arose. To counter this reaction, governments began to make regulations on the subject, and the first legal regulations regarding experimental animals emerged. The first legal regulation covering experimental animals in Türkiye was created in 2004. The regulation issued in 2006 determined the working procedures and principles of animal experimentation ethics committees. In addition, a separate regulation was issued for aquatic vertebrates used for scientific research in 2019. Over the years, legal regulations have been revised at certain periods in order to reach the ideal in line with ethical values and emerging needs.
Keywords: *Animal experiments, animal use, ethics committees, legislation, 3Rs*

1. Introduction

Since the moment of its existence, human beings have tried to understand themselves and their surroundings and have constantly asked questions and tried to establish cause and effect relationships between events and facts. While trying to explain the reasons for the events encountered in the absence of scientific knowledge using metaphysical methods, as the level of knowledge began to increase, scientific methods began to be used and rational explanations gained importance.[1] Human beings, who are particularly trying to explain the changes in their own bodies, have noticed the physiological and anatomical similarities with some animals (especially mammals) and have started to use animals as experimental materials many years ago.[2]

In studies where the hypothesis is established in accordance with scientific rules and methods, all animals (rats, mice, rabbits, guinea pigs, fish, etc.) that are experimented on by scientists, whether vertebrates or invertebrates, to reveal the truth or falsity of the hypothesis are called experimental animals.[2,3] Experimental animals, which are the source of scientific studies, have been used in various disciplines for many years, but are mostly used in studies in the field of health sciences. Experimental animals are used in many biological experiments related to vaccines, drugs and medical supplies.[4]

This article, prepared upon invitation, provides information on the history of animal use in experiments, the first legal regulations regarding experimental animals in the world, the current legislation and current status of ethics committees in Türkiye.

2. History of animal use in experiments

The use of animals in scientific research dates back to ancient times. Because human dissection was considered taboo, physicians in Ancient Greece and Rome used animals as models of anatomy and physiology. Hippocrates (460-370 BC), Aristotle (384-322 BC), Alcmeon (6th-5th century BC), Erasistratus (310 BC- 250 BC) and Herophilus (335-280 BC) were the first researchers to experiment on animals. Galen (129-199 AD) also made important physiological studies by performing many dissections and vivisections on animals, and this information remained valid until the Renaissance.[5,6] With the advent of Christianity, people began to think that the afterlife was more important than the earthly life and spent most of their time on it. As a result, during the Middle Ages, applied sciences almost completely stopped and the use of experimental animals decreased considerably. With the Renaissance (16th century), observation and experimentation gained importance again and the use of experimental animals in scientific research began to increase again.[6,7] Especially from the 18th century onwards, animal models began to be used frequently in anatomy and physiology studies. It was understood that these studies increased the quality of life of people, and the idea that developments in medicine and biology depended on animal experiments was widely accepted.[7]

Studies in the field of medicine gained importance in the 18th and 19th centuries. There has been a significant increase in laboratory studies, both to prove previously unproven applications and to shed light on the unknown, allowing for more accurate diagnoses and increasing treatment options. In particular, the "Académie Royale de Médecine" (Royal Academy of Medicine) was founded in France in 1820 for the purpose of advancing science and medicine together. The use of animal experiments was encouraged more in the

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field of physiology and pathology to uncover unknowns and answer scientific questions. In addition, veterinarians were also included in the study in order to ensure maximum benefit from experimental animal studies at the Academy.[6] After the 1830s, many scientists in German/Prussian also contributed to the development of many fields (anatomy, physiology, histology, pathology, neurophysiology) by using experimental animals in their studies in the field of medicine. In the late 19th century, the scientific importance of animal experiments in biology and medicine began to be more widely accepted, and progress based on animal research increased to an unprecedented extent.[6,8]

3. The first legal regulations regarding experimental animals in the world

With the emergence of modern biomedical disciplines, the number of studies conducted, and the number of experimental animals used in these studies began to increase, and this brought with it some problems. First of all, the increasing number of animals used in experiments disturbed some groups from an ethical perspective and caused this situation to be questioned. As a result of the use of a large number of animals in experiments and the use of animals in pain and suffering without anesthesia, anti-vivisectionist movements began to become evident. As reactions and protests against experiments with live animals increased from the beginning of the 19th century, a number of legal regulations emerged for the protection of animals.[6,9] First, the *"Cruel Treatment of Cattle Act"*, one of the first animal protection laws, was enacted in England in 1822, with reference to the experiences of experimental animals. The first anti-vivisectionist association, called *"The Victoria Street Society"*, was founded in England in 1875. Following this, the *"Cruelty to Animals Act"*, the first independent law regarding the regulation of animal experiments in scientific research, came into force in 1876.[6,10,11] This law stated that live animals could only be used in experiments if they were beneficial for science, and that this could be done under the control of the state secretariat, using anesthesia, and by licensed individuals. In addition, after the experiment ended, an observer had to be present to witness that the animals used in the experiment did not experience any pain.[10] This law remained in force for 110 years until *"The 1986 Animal (Scientific Procedures) Act"* came into force in England in 1986, and it also remained the only known legislation regulating animal experiments for nearly 50 years in countries such as Germany, Sweden, Switzerland and North America.[6]

The second problem that arose in scientific research was that the animals to be used in the research were raised in random places, so standards could not be provided for each study, and therefore the results obtained in similar experiments did not support each other. In order to overcome this inconsistency in the experiment results, a standard had to be established in the breeding of animals and the same conditions had to be provided as much as possible. In order to achieve valid results in the study, it was a prerequisite that the living environment, nutrition, cleanliness and experimental conditions of the animals were kept under constant control.[3,10,12]

In 1959, William Russell (1925-2006) and Rex Burch (1926-1996) put forward the '3Rs' (Reduction, Refinement, Replacement) in their work 'The Principles of Humane Experimental Technique' regarding the use of animals in research. Reduction: Study on the smallest number of animals that will not affect the statistical calculation; Refinement: To behave in a way that causes the least discomfort to animals in experiments; Replacement; If possible, work on other materials or models that will give equally reliable results instead of animals. The 3R Theory was a solution to both of the problems mentioned above, as it aims to both increase the quality of scientific research and testing and improve the treatment of animals used in research. For these reasons, the 3R principles have become an important part of animal experimentation ethics worldwide and have become a reference for legislation.[13] In 1985, The International Foundation for Ethical Research added the principle of Responsibility to the 3R principle and the principles were developed into the 4Rs. Responsibility: The researcher should see the experimental animal as a value, be aware of their responsibilities towards it and act accordingly.[10,14] On October 15, 1978, the Universal Declaration for Animal Rights was published to protect animal rights. The eighth article of this declaration, which consists of 14 articles, was about animal experiments and stated that any experiment that would cause physical or psychological pain to animals, even if it was medical or scientific, was against animal rights.[15] The first directive for the protection of experimental animals created by the European Union was the Council Directive 86/609/EEC on the protection of animals used for experimental purposes in 1986.[4] Towards the end of the twentieth century, animal experimentation ethics committees began to be established in both European countries and Türkiye in order to protect animals and continue scientific progress.[16,17]

4. Legislation regarding experimental animals in Türkiye

In the years when there was no legal regulation regarding the use of experimental animals, a regulation (*Regulation on Drug Research*) was published on January 29, 1993. With this regulation, the ethics committee and local ethics committees were defined and ethics committees were specified as the decision-making authority.[18,19] In this context, animal experimentation ethics committees were established in universities where experimental animals were used for education or research purposes, especially in the fields of medicine, biology, veterinary medicine and pharmacy.[20] In addition, the *"Veterinary Medicine Deontology Regulation"*, which came into force on 09.07.1994, stated that veterinarians must obtain ethics committee approval before performing any procedure on experimental animals, and ethics committees were included as a monitoring body in animal experiments.[21] The first animal experimentation ethics committee was established in 1996 at Marmara University Faculty of Medicine, and in the following years, Ankara University Faculty of Veterinary Medicine Ethics Committee (1998) and Gülhane Military Medical Academy Animal Experimentation Ethics Committee (1999) were established.[7,22]

In the early 2000s, the Republic of Türkiye entered into a process of harmonization with the European Union,

and in this direction, the "*Turkish National Programme for the Adoption of the European Union Acquis*" and the "*Decision on the Implementation, Coordination and Monitoring of the Turkish National Programme for the Adoption of the European Union Acquis*" were accepted on 23.06.2003. With this decision, the legislation on veterinary services in force in the European Union was compared with the legislation of the Republic of Turkey. These legislative studies also include regulations on experimental animals.[23] The first legal regulation covering experimental animals in Türkiye is the regulation titled *"Regulation on the Protection of Experimental Animals Used for Experimental and Other Scientific Purposes, Establishment, Operation, Supervision, Procedures and Principles of Production Places of Experimental Animals and Laboratories to Conduct Experiments"* issued by the Ministry of Agriculture and Rural Affairs on 16.05.2004 in accordance with the Council Directive No. 86/609/EEC on the protection of animals used for experimental purposes, prepared by the European Union.[4] With this regulation, the use of experimental animals in scientific research has gained a legal basis. The regulation aims to establish a standard for the structure of the production facilities of animals used in research and to ensure animal welfare in the care and use of animals. In the section of the Regulation titled 'Issues Regarding Experimental Animals', experimental and scientific studies in which experimental animals can be used are specified, and instructions are given regarding the general care of experimental animals, housing conditions, procedures to be applied to animals in experiments, and the sale of animals and their use in scientific research. Under the title of 'Establishment Permit, Work Permit and Employee-Related Issues for Organizations Producing or Providing Experimental Animals', the establishment permit, work permit, technical and hygienic conditions and employee-related issues of organizations that will produce or provide experimental animals have been evaluated. The fourth section, titled 'Issues Related to Organizations', includes the rules that organizations using experimental animals must comply with, the points to be taken into consideration when using animals for education and training purposes, and the provisions regarding obtaining information from these organizations.[24]

In addition, Article 9 of the *"Animal Protection Law"* numbered 5199, enacted in the same year (01.07.2004), includes provisions on the use of animals in scientific experiments, the conduct of scientific experiments in a way that protects animals, the proper care and housing of animals to be used in experiments, and ethical committees under the title of "animal experiments".[25]

Based on this law, the second legal regulation on animal experiments, the *"Regulation on the Working Principles and Procedures of Animal Experimentation Ethics Committees"*, entered into force on 06.07.2006. The purpose of the regulation is to determine the minimum ethical standards regarding the methods used in scientific research to be conducted with experimental animals, to express opinions in line with ethical principles, and to determine the establishment and working principles of the Animal Experimentation Central Ethics Committee and animal experimentation local ethics committees, to monitor their practices and to determine the principles regarding the termination of relevant procedures when necessary. With this regulation, the Animal Experimentation Central Ethics Committee was established for the first time. The second part of the regulation specifies the purposes of using experimental animals, the establishment of central and local ethics committees, their terms of office, working methods, and their duties and authorities. The Animal Experimentation Center Ethics Committee consists of 18 members from the Ministry of Environment and Forestry, the Ministry of Agriculture and Rural Affairs, the Ministry of Health, Veterinary Faculties, Medical Faculties, Turkish Medical Association, Turkish Veterinary Association, the Scientific and Technological Research Council of Türkiye and non-governmental organizations for the protection of animals. At least one of the members is preferred to be from the medical deontology or veterinary deontology branch. Animal experiments cannot be conducted in institutions and organizations that do not have a local ethics committee. It is preferable for medical or veterinary ethics experts to be present on local ethics committees. The duties of the animal experimentation local ethics committee include determining the ethically acceptable limits of all procedures to be performed on experimental animals, approving or rejecting study protocols with justification, and supervising that the process of using experimental animals within the institution is carried out in accordance with ethical rules, and making the necessary arrangements for this purpose. The mouse (*Mus musculus*), rat (*Rattus norvegicus*), rabbit (*Oryctolagus cuniculus*), guinea pig (*Cavia porcellus*), golden hamster (*Mesocricetus auratus*), dog (*Canis familiaris*), cat (*Felis catus*), quail (*Coturnix caturnix*), and primate species other than humans that will be used in experiments must be purchased from registered legal laboratory animal producers and suppliers. Stray domesticated species such as cats and dogs are not used in experiments. In the third part of the regulation, evaluations were made regarding the training of personnel who will work with experimental animals and it was made mandatory for the researchers who will perform procedures on animals to obtain an animal experiment use certificate. In the fourth section, it is stated that local ethics committee approval must be obtained for any national or international scientific activity using experimental animals and that any changes made to the study after receiving ethics committee approval must be notified to the local ethics committees in writing and approval must be obtained. In the fifth section, issues related to the identification of experimental animals and registration are reported.[26]

Another legal regulation regulating the issues related to animals used for experimental and other scientific purposes in Türkiye is the *"Veterinary Services, Plant Health, Food and Feed Law"* numbered 5996, issued by the Ministry of Agriculture and Rural Affairs on 13.06.2010. In this law, the welfare of experimental animals is also included in the scope and instructions regarding experimental animal producers and suppliers are included.[27]

In the following years, these regulations were updated within the framework of European Union standards. Firstly, the "*Regulation on the Protection of Experimental Animals Used for Experimental and Other Scientific Purposes, Establishment, Operation, Supervision, Procedures and Principles of Production Places of Experimen-*

tal Animals and Laboratories to Conduct Experiments" was repealed and replaced by the *"Regulation on the Welfare and Protection of Animals Used for Experimental and Other Scientific Purposes"* issued by the Ministry of Food, Agriculture and Livestock on 13.12.2011. This regulation has been prepared in parallel with the relevant provisions of the European Union Directive No. 2010/63/EC. The purpose of the regulation is to ensure the welfare and safety of animals by determining the procedures for raising, feeding, housing, using and killing animals to be used for experimental and other scientific purposes. The regulation states that it is mandatory to establish an animal welfare unit and states that the principles of Replacement, Reduction and Refinement (3Rs) should be applied. It also includes provisions regarding anesthesia and states that procedures involving serious injuries that may cause severe pain cannot be performed without anesthesia. Article 23 describes which animals can be reused in research and the procedures for reuse. Section 7 of the Regulation specifies the general authority and responsibilities of the personnel working in the unit.[28]

The "*Regulation on the Working Principles and Procedures of Animal Experimentation Ethics Committees*" was also repealed in accordance with European Union standards and updated by the Ministry of Forestry and Water Affairs under the same name (*Regulation on the Working Principles and Procedures of Animal Experimentation Ethics Committees*) and entered into force on 15.02.2014. This Regulation has been prepared based on Articles 9 and 17 of the Animal Protection Law No. 5199 and in parallel with the European Union Directive No. 2010/63/EU. The purpose of the regulation is to determine acceptable ethical standards regarding the methods used in scientific research to be conducted with experimental animals, to express opinions in line with ethical principles and to monitor the establishment, working principles and practices of the Animal Experimentation Central Ethics Committee and animal experiments Local Ethics Committees. It also ensures that all procedures performed on experimental animals are recorded and auditable and determines the principles regarding their termination when necessary. The regulation states that the 3Rs (Replacement, Reduction and Refinement) principles should be used. The second part of the regulation includes the purposes of using experimental animals, the establishment of ethics committees, their term of office, working methods, duties and authorities. The Animal Experiments Center Ethics Committee consists of 21 members in total, from the Ministry of Forestry and Water Affairs, the Ministry of Food, Agriculture and Livestock, the Ministry of Health, Veterinary Faculties, Medical Faculties, the Turkish Medical Association, the Turkish Veterinary Association, the Scientific and Technological Research Council of Türkiye and non-governmental organizations for the protection of animals. In this regulation, the establishment and working methods of animal experimentation local ethics committees are determined. Animal experiments cannot be performed in institutions and organizations that do not have an animal experimentation local ethics committee and animal welfare unit. It is preferable for medical or veterinary ethics experts to be present on the animal experimentation local ethics committee. It is required that the species of mouse (*Mus musculus*), rat (*Rattus norvegicus*), guinea pig (*Cavia pocellus*), Syrian (golden) hamster (*Mesocricetus auratus*), Chinese hamster (*Cricetulus griseus*), Mongolian gerbil (*Meriones unguiculatus*), rabbit (*Oryctolagus cuniculus*), dog (*Canis familiaris*), cat (*Felis catus*), all species of non-human primates, frog [*Xenopus* (laevis, tropicalis), Rana (temporaria, pipiens)], zebrafish (*Danio rerio*) and all animals to be used in the experiments must be purchased from registered legal laboratory animal producers and suppliers. Stray domesticated species such as cats and dogs are not used in experiments. However, if there is a need for studies on the health and welfare of animals, if they pose a serious threat to the environment, human and animal health, and if there is scientific justification that the purpose of the study can only be achieved using stray animals, these animals may be used in experiments. Great apes cannot be used in experiments. The regulation details the training of personnel who will handle experimental animals. It includes provisions regarding anesthesia applications, re-use of animals in experiments, and termination of experiments. It is also stated that laboratory animal use certificate programs will be opened and conducted for the training of researchers who will work with laboratory animals, and that laboratory animal use certificates will be given to those who are successful in the programs.[29]

With the *"Regulation on Amendments to the Regulation on the Welfare and Protection of Animals Used for Experimental and Other Scientific Purposes"* issued by the Ministry of Agriculture and Forestry on 08.07.2019, some articles regarding establishment permits and work permits in the "*Regulation on the Welfare and Protection of Animals Used for Experimental and Other Scientific Purposes*" have been amended.[30]

The principles and practices regarding aquatic vertebrates to be used for experimental, scientific and educational purposes were specified separately in the "*Regulation on the Welfare and Protection of Aquatic Vertebrates Used for Scientific Purposes*" issued by the Ministry of Agriculture and Forestry on 20.04.2019. With the exception of dolphins and whales (Cetacea), vertebrates such as fish (Pisces), frogs (Amphibia), and aquatic reptiles (aquatic/semi-aquatic reptiles) that have to spend all or part of their life in water are classified as aquatic creatures.[31] This regulation was re-arranged on 23.12.2022 and 27.04.2024 under the name of "R*egulation on Amendments to the Regulation on the Welfare and Protection of Aquatic Vertebrates Used for Scientific Purposes*" and included instructions for the establishment of a separate central ethics committee (Aquatic Vertebrate Experiments Center Ethics Committee) and local ethics committees (Aquatic Vertebrate Experimentation Local Ethics Committee) for studies to be conducted with aquatic vertebrates.[32] In addition, one more member from the Turkish Veterinary Medical Association was added to the Aquatic Vertebrate Experiments Center Ethics Committee, increasing the number of members to 15.[33]

5. Current status of ethics committees in Türkiye

The ethics committees regulated by the *"Regulation on the Working Principles and Procedures of Animal Experimentation Ethics Committees"* are the animal ex-

perimentation local ethics committees that every institution/organization that conducts animal experiments is obliged to have within its structure and the Animal Experimentation Central Ethics Committee, which is the supreme board of these committees. The Animal Experimentation Central Ethics Committee is responsible for approving the work guidelines of animal experimentation local ethics committees for animal experiments, evaluating objections to their decisions, and supervising these institutions. The Animal Experiments Center Ethics Committee started its work with its first meeting held on 10.07.2007 and 61 meetings have been held as of February 2021. As of 2021, there are 119 Animal Experiments Local Ethics Committees in our country that have received operating permits from the Ministry of Agriculture and Forestry and whose directives have been approved by the Animal Experiments Central Ethics Committee.[34]

6. Conclusion

Since ancient times, people have used animals in their experiments to uncover the unknown. While animals were initially used in experiments without any restrictions, in later years, as society's sensitivity towards animals increased and in order to ensure certain standards in scientific research, legal regulations regarding experimental animals were enacted by governments. These regulations have been revised periodically in line with ethical values and emerging needs, and they need to continue to be revised in order to reach the ideal. In particular, in parallel with the legislative studies carried out in the process of harmonization with the European Union, it is seen that regulations and improvements have been made for experimental animals in Türkiye. As a result of these regulations, it can be said that the current legislative provisions are an important guide both in regulating the welfare of experimental animals and in determining the responsibilities of researchers towards experimental animals.

Ethical approval

This study does not require approval from the Ethics Committee.

Authors contribution

SÇA: Research, planning, article scanning, writing-original draft & review. The author approved the final version submitted.

Conflict of interest

The author has no conflicts of interest to declare.

Data availability

The data presented in this study are available on request from the author.

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