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Use of Animal models in Experimental Research in Dentistry in Brazil

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ABSTRACT

Objective: To identify the types of animals commonly used in experimental research in dentistry in Brazil. **Material and Methods:** 2721 studies were evaluated, of which 379 (13.9 %) involved animals. The variables analyzed were: area of knowledge, type of study, type of animal, number of animals, occurrence of sacrifice, body part used and financing. Descriptive and inferential statistical analyses were performed using the chi-square test ($p < 0.05$) with the SPSS 18.0 software. **Results:** Research in Dental Materials (38.8 %), Surgery, Dental Anesthesiology and Implantology (25.6 %) were those that most used animals. Most studies were *in vivo* (60.2 %), using rats (82.7 %) and average of 44.3 animals per study. Most animals were sacrificed during or at the end of the experiment (61.8 %) and the most used body part was the teeth (44.2%), especially bovine. **Conclusion:** Areas of Dental Materials and Surgery, Anesthesiology and Implantology are those that most used animals in studies, with predominance of rodents, being usually sacrificed during or at the end of the experiment.

Key-words: Animal experimentation; Models, animal; Research.

INTRODUCTION

Historically, animals have been target of investigations and observations to humans. The earliest records date from about 500 BC, when Alcmaeon performed dissections on animals in order to know more about their anatomy [1]. Over the centuries, many scientists stood out in studies with animals; however, only from the early nineteenth century that there was a significant increase in the use of animals in scientific experiments [2] due to the publication of the principles of animal use as study models by Claude Bernard [3].

The principles of the "3 Rs" (replace, reduce and refine) [4] established the care that must be taken to conduct research with animals and are now internationally accepted as criteria for humane animal use in research and testing. According to these principles: (1) animals must be replaced by alternative methods; (2) the number of animals used should be reduced in order to increase the methodological quality and enhance the statistical analysis of data; (3) the refinement of the technique used must have the purpose of alleviating pain and discomfort of animals during experiments [5]. Therefore, the practice of the "3 Rs" is essential to gradually reduce the suffering and number of animals used in experiments [6].

Currently, several higher education institutions around the world have invested heavily in research to create *in vitro* and *in silico* models that could be alternatives to using animals in teaching and research [2]. However, there are no alternative scientific methods that allow the full replacement of animals in research studies.

Biomedical research with animals are distributed in various lines of development, usually related to drug toxicity testing, production of vaccines and serum, biological and molecular mechanisms of diseases and findings in the growing field of genetic engineering [1].

In several areas of medical sciences, especially in dentistry, many studies using animals are conducted. A recent systematic review showed that the animals most commonly used in dental research are dogs, monkeys, rabbits, sheep, mini-pigs, rats, cats, goats and mice and the key interventions involve peri-implantitis treatment, distraction osteogenesis, orthodontic movement, bone regeneration around the surface of the dental implant and research with biomaterials [7]. However, it appears that there are few studies with this focus in the Brazilian [8] and international literature [7,9].

Given the increasing use of animals in research in the dentistry field, this study aimed to identify the

types of animals commonly used in experimental research in dentistry in Brazil.

MATERIAL AND METHODS

The Brazilian Society for Dental Research (SBPqO) is the Brazilian division of the International Association of Dental Research - IADR and gathers a significant amount of graduate and undergraduate students, teachers and researchers from public and private Schools of Dentistry from various regions of Brazil [10]. Each year, there is a meeting in which the main studies developed in the country are presented. Given the importance of this meeting for the Brazilian scientific community, the papers presented at the 2013 Annual Meeting were chosen as the material to be analyzed in this study because they represent the excellence of Brazilian research. Of the 2721 papers presented, 379 (13.9%) used animals and therefore composed the study sample. A specific form was developed to record data and information on the area of knowledge, type of study performed, type of animal used, number of animals per study, occurrence of sacrifice, body part studied and presence of financing.

Data were organized using the SPSS® software (Statistical Package for the Social Sciences), version 18.0 and presented using descriptive statistics (absolute distributions, percentage, mean and standard deviation). To verify the association between type of study and group of animals used and the presence of financing and type of study, the chi-square test was used, with significance level of 5 %.

RESULTS

Most studies involved the areas of Dental Materials (38.8 %) and Surgery, Anesthesiology and Implantology (25.6 %), as shown in Table 1.

Table 1. Distribution of studies according to the area of knowledge.

Scientific Group	n	%
Dental Materials	147	38,8
Surgery, Anesthesiology and Implantology	97	25,6
Periodontal Research	68	17,9
Oral Medicine and Pathology	28	7,4
Orthodontics and Orthopedics	11	2,9
Cariology Research	15	4,0
Diagnostic Sciences	9	2,4
Prosthodontics Research	4	1,1
Total	379	100,0

According to Table 2, *in vivo* studies prevailed (60.2 %), followed by *in vitro* (39.1%) and *in situ* (0.7 %) studies. Among the animals used, rats stood out (82.7 %), as well as *in vivo* studies and the use of bovine teeth in *in vitro* (95.3 %) and *in situ* studies (100 %).

The analysis of the area of knowledge and type

of animal used (Table 3) showed that while rats were predominant in the areas of Surgery, Anesthesiology and Implantology, Periodontal Research, Oral Medicine and Pathology, Orthodontics and Orthopedics, Diagnostic Sciences and Prosthodontics Research, bovine teeth were used primarily for research in Dental Materials and Cariology Research.

Table 2. Distribution of types of study in relation to the animal used.

Type of study	n	%
In vivo		
Rat	187	82.7
Rabbit	16	7.1
Mouse	14	6.2
Dog	5	2.2
Sheep	2	0.9
Mini-pig	1	0.4
Hamster	1	0.4
Total*	226	100
In vitro		
Bovine (teeth and pelvic bone)	141	95.3
Rat	4	2.7
Mouse	1	0.7
Dog	1	0.7
Pig	1	0.7
Total	148	100
In situ		
Bovine teeth	3	100
Total	3	100

* In two studies, the type of animal used was not informed.

The average number of animals used in *in vivo* studies was 44.3 (SD = 33.3), with minimum of four and maximum of 192 animals. Only 141 (61.8 %) studies contained information on the number of animals used, totaling 8,367 animals. In relation to the sacrifice of the animal, 61.6 % of studies mentioned having sacrificed animals during or at the end of the experiment. In relation to the body part studied, the majority of studies used bovine teeth (44.2 %), as described in Table 4.

There was no statistically significant difference between type of experimental design and the presence of financing ($p > 0.05$). However, there was statistically significant association between type of experimental design and group of animals used ($p < 0.001$) (Table 5).

DISCUSSION

Surveys are developed from the human need to improve the existing knowledge or to obtain new information. In experimental research, it is no different. Every experiment must carefully establish its goals and provide reasons for the choice of the animal model: species, number of animals used, randomization method and statistical analysis. Therefore, planning the experimental design is essential [5].

Several authors discuss the importance of the use of animals in the teaching process and in biomedical

Table 3. Distribution of areas of knowledge according to the animal used

Scientific Group	n	%
Dental Materials		
Bovine (teeth)	126	85.7
Rat	19	12.9
Mouse	2	1.4
Total	147	100
Surgery, Anesthesiology and Implantology		
Rat	74	77.9
Rabbit	16	16.8
Sheep	2	2.1
Mouse	1	1.1
Dog	1	1.1
Pig	1	1.1
Total	95	100
Periodontal Research		
Rat	59	86.8
Mouse	6	8.8
Dog	3	4.4
Total	68	100
Oral Medicine and Pathology		
Rat	22	78.6
Mouse	5	17.9
Hamster	1	3.6
Total	28	100
Orthodontics and Orthopedics		
Rat	6	54.5
Dog	2	18.2
Bovine (pelvic bone)	1	9.1
Mouse	1	9.1
Mini-pig	1	9.1
Total	11	100
Cariology Research		
Bovine (teeth)	14	93.3
Rat	1	6.7
Total	15	100
Diagnostic Sciences		
Rat	7	77.8
Bovine (teeth)	2	22.2
Total	9	100
Prosthodontics Research		
Rat	3	75.0
Bovine (teeth)	1	25.0
Total	4	100.0

Table 4. Distribution of studies in relation to the body part used

Body part used	n	%
Teeth	163	44.2
Periodontium	65	17.6
Skull	42	11.4
Back	18	4.9
Mandible	15	4.1
Tibia	13	3.5
Maxilla	9	2.4
Femur	7	1.9
Others	37	10.0
Total*	369	100

* In ten (10) studies, the body part used was not informed.

Research [5,11-14]. If not for the use of animals in experiments, much of what is known today about vaccines, new techniques, diagnostic tests and therapeutic methods would not have been possible. Furthermore, the use of animals during surgical experimentation is very important as it provides the surgeon greater dexterity and safety in his actions, as well as familiarity with the risks and making decisions about unexpected events, something difficult to be reproduced by artificial computer models [13].

The main limitation on the use of animals in experiments is the extrapolation of data obtained for humans. The study of pathological conditions in animals brings a number of limitations due to the complexity of the pathogenesis and pathophysiology [15]. Therefore, regardless of the animal species chosen, one must critically analyze the inherent limitations of biological, anatomical and physiological differences in relation to man and the diseases studied [16].

This study aimed to describe the use of animals in experimental dental research developed in Brazil. The information about the types of animals most commonly used in dentistry and their distribution according to the area of knowledge enables further discussion on the use of animals in research and could guide the designing of studies on this topic.

It was found that the areas of knowledge that most used animal in research were Dental Materials and Surgery and Anesthesiology and Implantology. In relation to the area of Dental Materials, *in vitro* studies and the use of teeth predominated. In relation to the area of Surgery, Anesthesiology and Implantology, *in vivo* studies were more frequent, and are reflexes of the best anesthetic support, sophistication of material infrastructure for continuous preoperative monitoring and constant search for models that reproduce manifestations of human diseases with fidelity [17].

There was a prevalence of experimental *in vivo* studies, regarding experimentation conducted within or on the living tissue of the chosen animal. Regarding the type of animal used, rodents were the most used, and rats were the animals most commonly used. Other animals; however, such as rabbits, bovines and dogs

Table 5. Association between type of experimental design and the following variables: Presence of financing and group of animals used.

animals used:			
	Experimental Design		
Variable	<i>In vivo</i>	<i>In vitro/In situ</i>	<i>p</i> -value
	n (%)	n (%)	
Financing			
Present	121 (65.1)	65 (34.9)	0.07
Absent	108 (56.0)	85 (44.0)	
Animal Group			
Rodent mammals	202 (97.6%)	5 (2.4)	<0.001
Non-rodent mammals	25 (14.7)	145 (85.3)	

have also been used, confirming previous findings [14]. In South Africa, the main experimental animals used in dental research are rats and vervet monkeys, although other species such as dogs and baboons have also been used [9].

The selection of the animal species depends of type, reasons and objectives of the study. Other criteria include: cost to acquire and care for animals, availability, acceptability to society, tolerance to captivity and ease of housing [18]. Regardless of the chosen animal, it is essential that the researcher use it in a suitable, comfortable and ethic manner [19].

The average number of animals used per study was forty-four. The precise definition of the number of animals for experimental groups and sub-groups is extremely important [5]. Furthermore, for ethical reasons, studies should be designed so as to use the minimum number of animals as possible and to obtain conclusive results, saving them from suffering [8].

Regarding the sacrifice of the animal, more than half of studies reported having sacrificed animals during or at the end of the experiment. According to the Brazilian Federal Council of Veterinary Medicine, euthanasia should be indicated when the animal is the object of research. In addition, the choice of the euthanasia method should be according to the animal species used, stage of life, physiological conditions, forms of containment, technical ability of the researcher and number of animals [20].

Regarding the body part under study, the majority of studies have used the teeth, especially bovine. The expressive use of bovine teeth is justified because they are morphologically and histologically similar to human teeth and present some advantages, such as: they can be obtained from slaughterhouses and have large size and large surfaces [21]. Moreover, they are easier to be obtained in larger amounts, in better condition and with a more homogeneous composition compared to human teeth, and their use has shown a marked increase over the past three decades [22].

Financing agencies require the full adherence to ethical guidelines and welfare of research animals to provide financial support [14]. In this context, the review of research projects involving animals by Ethics Research Committees becomes very important, since it can contribute both to ensure the rights of animals as for researchers to obtain financial resources for the implementation of their studies.

The use of animals has raised and still raises many discussions in the epistemological, social, economic and religious spheres [13]. However, considering animals as partners of humanity, rather than victims, may be the first step along the path to a peaceful coexistence of the various currents of thought that permeate the world [23]. We must face our doubts and believe that the worst of all perspectives is not to face challenges or not to offer space for discussion of new issues [1].

Finally, teachers, graduate and undergraduate

students involved in biomedical research must know and practice the ethical principles of animal protection in compliance with international principles governing animal experimentation [23].

CONCLUSION

Areas of Dental Materials and Surgery, Anesthesiology and Implantology are those that most used animals in research, with the prevalence of rodents being sacrificed during or at the end of the experiment.

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