INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH & DEVELOPMENT

ISSN: 2278 – 0211 (Online)

Considerations For Use Of Experimental Animals In Biomedical And Periodontal Research

Dr. Amit Arvind Agrawal
MDS (Periodontics), Department Of Periodontics And Implantology
KBH-MGV Dental College And Hospital, Panchvati, Mumbai-Agra Road, Maharashtra, India

Abstract:
Human biology is very much like that of many other animals. That is why results from animal experiments apply to people. Animal models have been used to evaluate the pathogenesis of periodontal diseases and various periodontal treatment modalities. However, animals are used more as a matter of tradition, not because animal research has proved particularly successful or better than other modes of experimentation. The claim that animals are necessary for biomedical research is unsupported by the scientific literature. One must avoid direct extrapolation of results of studies in laboratory animals to the human. However, the welfare of human being will always outweigh the welfare of non-human beings and the quest for knowledge must never be hindered by what may be interpreted as ignorance or sentimentalism.
Scientists, that work on experimental animals should keep them well and treat them with respect. They should be deeply concerned about the rational and humane use of animals in research. They should be concerned about avoiding unnecessary pain or suffering or injury to animals during holding, experimentation and post–experimentation period by monitoring and improving their housing, environment, feeding and veterinary care. This review intends to discuss the importance and controversies of animal experimentation; guidelines for animal welfare; legal implications; selection of appropriate species; problems inherent in animal experimentation and their possible solution.

Key words: *animal experimentation, periodontal research, animal welfare, animal models*

1.Introduction

Galen (129-200 AD), used animals to demonstrate that the arteries contained blood and not air. We have come a long way since then and specially bred laboratory animals consisting of mice, rats, hamsters, guinea pigs, rabbits, cats, dogs, monkeys, higher farm animals and a variety of birds and other lower forms are now integral part of biomedical research. Research, in reality, involves three facets: acquisition of new knowledge, use of animals in teaching exercises, and the testing of compounds, chemicals or devices for safety and effectiveness. There must be reasonable expectation that research-involving animals will contribute significantly to present and future knowledge, which may eventually lead to the protection and improvement of the health and welfare of either humans or animals. Human biology is very much like that of many other animals. That is why the results from animal experiments apply to people. Most laboratory animals have the same set of organs - heart, lungs, liver, and so on which work in the same way as they do in human. The scenario in India, with respect to laboratory animals is far from satisfactory. Even though Indian Council of Medical Research (ICMR) made a head start as early as 1960 along with other countries, their progress has been tardy due to various reasons. Usually one can recognize two main sources of suffering of laboratory animals: husbandry and environmental conditions, including the effect of restricted space, diet, social isolation or unusual social grouping, the noise and smell of other animals; and scientific procedures involved in experimental research like handling, oral dosing, injections, withdrawal of body fluids, withdrawal of food, withdrawal of specific nutrients etc.

Contemporary discussions involve issues as: What constitutes an essential experiment? What is appropriate conduct when using animals in research? What alternatives to sentiment animals are available? It is doubtful that any issue in science has generated as much emotion as animal experimentation. This review intends to discuss both sides of the coin. The importance of animal experimentation, guidelines for conducting the same, selection of appropriate species, problems inherent in animal experimentation and the relevant clinical significance.

2.Controversy Regarding Animal Use For Biomedical Research

The animal rights movement, which originated in Europe and America does not seem to be due to any religious beliefs. On the other hand, oriental and particularly the Indian attitude to animals is one that is tinged with religious and ethical colours. Animals and birds are thought not only as 'Vahanas' or Vehicles on which God rides, but much more useful as well. Over the centuries this has brought about a very healthy respect in the Indian mind for all forms of life. The cow is sacred not because it is a divine vehicle alone, but because it has an overall utility value. Buddhism and Jainism carry this attitude further, leading to vegetarianism and respects for all living beings. Even pigs, boars, buffalos and monkeys are referred in holy books and the Indian mindset can become easily sensitive when it comes to these animals. These religious sentiments could be one major reason why the animal activism in this country has found firm roots, while in the West it may be because of the writings of some secular philosophers. The foremost in this group was, Jeremy Bentham an English Barrister who in 1780 questioned the lack of moral regard of humans to fellow animals. But the actual animal rights movement took root from the uncompromising ideas of Tom Regan who wrote the famous Thea case for animal rights' (1983).[1] Regan believed that all humans and most animals have inherent rights, and that their bodies cannot be transgressed, no matter how much good might there result is. He took the extreme view that animals cannot be experimented on, because they are not really means to an end. This view is supported by two arguments; firstly, that the animals have similar rights to humans and that experimenting on them without their consent is indefensible as it would be with people. Darwinism theory of evolution[2] clearly shows that all animal life is related through evolutionary process and some species such as apes are sibling species to humans. We are clearly related to chimpanzees as tigers to cats and horses to donkeys. The theory of evolution teaches further that animals have feelings, are capable of suffering and experiencing pain and pleasure, because we share much of our inherited emotional make up with them. Thus, the people who use animals for research and other purposes should keep them well and treat them with respect. According to animal activists, any animal research project implies a potential violation of six widely subscribed moral norms and values[3]. These are as follows: respect to animals as they are, perform good science, be a good citizen, have responsibility for future generations, have responsibility for environment and show respect for life style and religious orientation of people.

3.Role Of Scientists

The scientists should be deeply concerned about the rational and humane use of animals in research. The ethics committee should be functional in institutions. They should be concerned about avoiding unnecessary pain or suffering or injury to animals during holding, experimental and post–experimentation period of monitoring and improving their housing, environment, feeding and veterinary care. This can be achieved by providing accreditation services to laboratories by constituting, National Accreditation Board of Testing and Calibration Laboratories (NABL), [4] having membership of the International Laboratory Accreditation Cooperation. Such accreditation of animal facilities would demonstrate their commitment to responsible animal care and use and good science since such an accreditation is an indicator of an institution's ability to comply with its assurances. Animals free of clinically recognizable or latent disease and of appropriate genetic stock of the strain should be chosen. The working objective and ethical requirement for the use of animals in research dictates the need to use the best methods, on the smallest number of appropriate animals required, in order to obtain valid scientific information. Animals' lives should not be wasted because of inadequate knowledge of the requirements of the species being used, incorrect management or handling techniques, inappropriate or incorrect experimental design, or lack of surgical knowledge and experience. If meaningful scientific results are to be accrued through the use of animals in research, teaching and testing, the scientific community in every country must ensure the humane and ethical treatment of animals is achievable and worthy of consideration before the use of animals is permitted.

4.Guidelines For Animal Welfare

The guidelines are uniform throughout the world, and strict adherence to these guidelines is made compulsory by appropriate animal welfare laws. These guidelines insist on standard environmental conditions to be provided to the animals in terms of light, temperature, humidity, ventilation, noise levels etc. and also the standard nutritive diet to be provided to them. On a large scale this can be achieved by minimizing the use of animals in scientific research, testing and education by resorting to the three `R' principles of Replacement, Reduction and Refinement advocated by British Zoologist William M.S. Russel and the microbiologist Rex L. Burch in 1959. Replacement of conscious, living vertebrates by non-sentient alternatives; Reduction in number of animals needed; Refinement of procedures to reduce to a minimum the incidence or severity of suffering.

There is an International Committee for Laboratory Animal Science (ICLAS),[5] which has taken up the job of setting up international guidelines for animal husbandry, experimental procedures, teaching and training of researchers and professionals in the field. In India such guidelines were published by Indian National Science Academy (INSA) in 1992 and recently revised in 2000.[6] India enacted an animal law as early as 1960's called the `Prevention of Cruelty to Animals Act'[7] amended in 1982 currently undergoing revision. Under Chapter 4 of the same Act there is provision for the control of experimentation on animals. The act also provides the constitution of an Animal welfare board to take care of the welfare of the animals in general, and also provides that the Animal Welfare Board constitute a Committee for the Control and Supervision of Experiments on Animals. This Committee is empowered to take care of the legal and ethical aspects of experimental animals being used in research and enact preventive measures wherever there is violation of the law. In recent times, the CPCSEA has become more active and it is now made mandatory that every animal facility in the country be registered and the facilities have a local Institutional Ethical Committee to monitor the experimental work going on in their respective institutes.

Some of the important strategies for periodontal research are as follows:
Avoid unnecessary repetition of experiments.
Use in vitro methods, which include sub-cellular fractions, tissue-slices, cell suspensions and perfused organs, and tissue culture proper (cell and organotypic cultures), including human tissue culture, in toxicity testing and for preliminary screening.
Use lower organisms with limited sentience/ or not protected by legislation controlling animal experiments, which include invertebrates (Drosophila, the earthworm, the horse shoe crab etc.) plants, fungus, bacteria (Salmonella) and viruses.
Use early developmental stages of vertebrates before they reach a point at which their use in experiments and other scientific procedures is regulated. Use human tissues and volunteers, wherever possible to avoid the problem of interspecific extrapolation from animals to humans and to get more mechanistic information.

5.Selection Of Animal For Periodontal Research

Animal models have been used to evaluate the pathogenesis of periodontal diseases and various periodontal treatment modalities. From the viewpoint of comparative biology, non-human primates are similar to humans, having comparable periodontal tissue structures and healthy and diseased periodontal states, as observed in humans[8]. However, most non-human primates used for research purposes are large, expensive and difficult to handle. Furthermore, the genetic background of many of these animals has not been established, because animals used in research are often wild-captured animals, with heterogeneity in age, body weight, and oral and general health conditions[8]. Among the species of non-human primates, squirrel monkeys and marmosets are small in size and relatively easy to handle, but unfortunately do not exhibit an inflammatory profile characteristic of human periodontal disease. Periodontal tissue specimens from these animals, unlike humans, exhibit very limited numbers of lymphocytes and plasma cells[9-11]. Rodents, belonging to the cohort Glires, such as mice, rats and hamsters, have been used widely for periodontal research because of specific advantages such as small size, low cost, known age and genetic background, controllable microflora and ease of handling and housing. However, anatomical structures of periodontal tissues and histopathological features of periodontal disease of rodents are different from those of humans[12]. Several non-human primate species, varying in size from the small bushbabies (Galago senegalensis) to the big mountain gorilla (Gorilla beringei), have been used in studies of periodontal disease pathogenesis.
In an electronic search done by Dannan and Alkattam[13], it was shown that dogs were most used in periodontal research as biological models (31.16%). In view of their docile temperament and natural susceptibility to periodontal disease, dogs, particularly beagles, are used in dental research for the study of periodontal disease progression, guided tissue regeneration, tissue wound healing and dental implants. The etiologic factors of periodontal disease seem to be identical in humans and dogs[14]. However, dogs were not preferred in implant studies and bone regeneration investigations. Monkeys came at a second level (15.58%) and were most considered in periodontal regeneration studies and to investigate periodontitis and/or gingivitis models. Due to the possibility of obtaining block biopsies, the rhesus monkey, cynomolgus monkey, and baboons have been used to study osseointegrated oral implants. Fritz et al[15] suggested that ligature-induced periodontitis around teeth and ligature-induced peri-implantitis follow similar destructive patterns, namely alteration of microbiological flora. However, in the fields of bone healing investigations, laser application and bone regeneration investigations, monkeys were not preferred. Rats came at the third level (10.66%) and were used in periodontal tissue regeneration studies, in bone healing investigation studies and to describe periodontitis models. One of the major differences is that the gingival sulcular epithelium of the rat is keratinised. The most frequently used rat strain in periodontitis studies is the Sprague-Dawley strain. The rat is predominantly lymphocytic, therefore, the granulocyte-related first line of defense against periodontal pathogens may be less efficient in the rat than in man, and the lymphocyte-related immune response relatively more important. There are, however, three areas in which the rat model is particularly valuable. These are: 1) pathogenicity studies; 2) studies on the importance of immunological dysfunction; and 3) immunization studies.[16]
Rabbits came at the forth level (4.1%) and were most used in periodontal tissue regeneration studies. Pigs, ferret and sheep came at the fifth level as animal models in periodontal research (1.64%). Pigs where used in peri-implant tissue studies[17,18] and ferrets were used in two studies to evaluate the clinical and histological characteristics of ligature induced periodontitis[19,20]. A ferret is a suitable model for the study of calculus because of its resemblance to human calculus and the fact that formation of calculus is not diet dependent as in the rat and hamster.
Two studies introduced sheep as new animal models; one of them[21] described a model suitable for training for several periodontal surgical methods. The other study[22] evaluated the sheep as a suitable animal model in which to compare periodontal wound healing after furcation defects were treated with different guided tissue regeneration membranes and showed a successful use of the sheep animal model for guided tissue regeneration research. Only one study (0.82%)[23] used goats to evaluate the clinical applicability and biological behavior of a newly developed injectable calcium phosphate (Ca-P) cement as bone filler for gaps around oral implants. Tooth size of a 'swine' is comparable to man and the edentulous jaws of a larger strain of swine have been used with human sized dental implants. The swine is relatively resistant to systemic infections with proper immunizations and is amenable to surgical and anesthetic treatment. The immune system and the gingival characteristics of swine make it an acceptable model to longitudinally evaluate the changes in microbial flora within the periodontium.[24]

6.Problems Inherent In Animal Experimentation

Scientists use animals in biological and medical research more as a matter of tradition, not because animal research has proved particularly successful or better than other modes of experimentation. In fact, animal 'models' have never been validated, and the

claim that animals are necessary for biomedical research is unsupported by the scientific literature.[25] Instead, there is growing awareness of the limitations of animal research and its inability to make reliable predictions about human health.
Animal studies do not reliably predict human outcomes.
Nine out of ten drugs that appear promising in animal studies go on to fail in human clinical trials.
Reliance on animal experimentation can impede and delay discovery.
Animal studies are flawed by design.
In addition to these there are several factors, which could affect the result of animal experimentation (Table-1). The presentations by Drs. Caton Wikesjö and Lynch illustrated the need for further discussion of some of the problems inherent in animal experimentation for periodontal research, including: standardization of periodontal defect configuration; adequate recording of preoperative attachment level; minimizing the effect of biological variability; use of surgically-created versus naturally-developed periodontal lesions; choice of observation period; and regenerative potential of the model chosen. Considering all the variables beyond our control, do the "animal models" we are currently using really qualify as model systems in a scientific context, or should they merely be regarded as convenient substitutes for human experimentation?[26] The biomedical community would instead be better served by promoting increased funding and research efforts for the development of non-animal models.

7.Discussion

In designing any medical or dental animal study, it is often advantageous to select an animal that is phynogenetically similar to humans. The wide range of animal species allows appropriate selection of bio-models for different investigations. Choosing a gold animal model, which suits all fields of application is a current goal in research though seems to be very difficult or impossible.
In the years to come there will be a need for more and better laboratory animal houses in developing countries because of an increased demand for biomedical research, sera, vaccines and drugs. With strict enforcement of animal welfare laws, the animal testing can now be totally conducted within the developing countries. However, due to constraints in finances, any availability of specialized strains of animals (mutants, transgenics) and alternative technologies of drug testing is still beyond the reach of developing countries. Till such time the situation improves, these countries need to import all these animals from abroad. And since these strains have special requirements for their maintenance, provisions also should be there for infrastructure development and trained professionals. There should be harmonization of the existing rules prevalent in different countries and both should have commitment to undertake projects, which are of universal appeal to humans as well as animals and the benefits should be shared in an equitable manner.
There are over 400-500 animal facilities in our country spreading over research institutions; public and private pharmaceutical companies; universities; medical and veterinary collages (Table-2). Many of the animal facilities, especially the ones attached to universities, medical and veterinary collages, have no proper infrastructures in terms of building, housing etc. Proper record keeping and quality control measures are also lacking. Many of them do not have well defined inbred strains and the country is yet to develop its own specialized mutants, transgenic and knock out animals, that would have improved the quality of the work and also reduce the number of animals used in research. Yet, compared to Western countries our intake of laboratory animals is very low, (approximately around one tenth). The existing inferior situation is mostly due to lack of enough funds rather than lack of awareness of good laboratory animal husbandry practices.
There is a distinct lack of trained manpower, at the senior level in many of the facilities. Laboratory Animal Sciences is an emerging new field and it is not covered in the curriculum of any of graduate courses in the country be it veterinary, medical, dental, pharmaceutical or any biosciences course. It should be made compulsory, that every person working with the animals either at the management level or for experimentation should have a certificate in laboratory animal care and procedures. It can be of short duration for research workers who need to orient specifically for the experimental procedures, which they may likely to use in the course of their work. But those who are managing the facilities need to have a longer training (6 months to one year).
In India and other developing countries national bodies concerned with laboratory animal sciences conduct short-term training courses ranging from 6 to 12 weeks for those who are involved in the husbandry of the animals and to those who use them. In many developed countries, every scientist starting research on animals has been required to take a three-week orientation course. They have to learn hands-on procedures, proper anesthesia, specification of inbred strains and so on, as well as the three R's. In India National Centre for Laboratory Animal Sciences (NCLAS) at Hyderabad under the ICMR and Central Drug Research Institute (CDRI), Lucknow under the Council for Scientific and Industrial Research (CSIR) administer such training programs regularly.

8.Conclusion

Animal experimentation is necessary at the current level of knowledge for studying the pathogenesis of different disease, undertake drug trials, and generate a variety of biologicals such as immuno-diagnostics, vaccines to alleviate suffering in the human and animals themselves. In-vitro alternate methods cannot replace animal experimentation totally but can work only as adjuncts and reduce the number of animals to the extent possible. This is why the use of animals continues to be mandatory to meet the statutory requirements. However, efforts to develop alternate methods should continuously be made so that the day will be reached when no more animals are used for experimentation.
One must avoid direct extrapolation of results of studies in laboratory animals to the human. Investigations should be predicated on the premise that, in light of present knowledge, the most that one can hope to accomplish is the establishment of patterns of similarity in the disease between the two species, and then, having done this , top evaluate, item by item, responses in each species to

predisposing factors responses during the course of the disease, responses during treatment, and, finally, response to preventive techniques.[27] A gold animal model in periodontology does not exist since every application requires a model that fills specific needs. "You do not settle whether an experiment is justified or not by merely showing that it is of some use. The distinction is not between useful and useless experiments, but between barbarous and civilized behavior. Vivisection is a social evil because if it advances human knowledge, it does so at the expense of human character."

George Bernard Shaw.
"Unseen they suffer,
Unheard they cry,
In agony they linger,
In loneliness they die."

Sr. No.	FACTORS	Watch for
1.	Genetic quality	Strain / stock Breeding system Quality breeder / supplier
2.	Biological status	Sex Age Body weight
3.	Health status	Quality breeder / supplier Constant level of quality Hygiene barrier in maintenance
4.	Nutrition	Quality supplier Constant composition (lot-nr.) Quality drinking water
5.	Maintenance	
a.	Cage	Type (dimensions) Bedding Number of animals per cage
b.	Animal room	Ventilation Temperature Relative humidity Lighting Noise Other animals
6.	Transportation	Means of transportation Transport cage Food supply
7.	Animals care	Qualification of Animal Care Taker
8.	Experimental techniques	Qualification of animal technician Sandardisation of techniques Time of intervention

Table 1: Factors, Which Could Influence The Results Of Animal Experiments

Sr. no.	ANIMAL SUPPLIERS	ANIMALS AVAILABLE
1	Indian Institute of Science, Bangalore 560 012	Rats, Mice, Guinea Pigs, Rabbits, Hamsters, Goats
2	Field Research Laboratory, C/o 56 APO, Bharat Sarkar Raksha Mantralaya, Anusandhan Tatha Vikas Sangthan	Cattle, Sheep, Goats, Yak, Poultry, Birds, Squall
3	Industrial Toxicology Research Centre, Mahatma Gandhi Marg, P. B. No. 80, Lucknow 226001 UP	Mice, Rat, Guinea Pigs, Rabbit, Sheep, Goat and Fish
4	Regional Research Laboratory, canal Road, Jammu Tawi 180 001	Rats, Mice, Guinea Pigs, rabbits, Sheep, Gerbils
5	Annamalai University, Annamalai Nagar-608 002	Rats, Mice, Guinea Pigs, Rabbits, Hamsters, Frogs
6	Govt. College of Pharmacy, Karad, Vidyanagar-415124	Rats, Mice, Rabbits, Guinea Pigs, Frogs
7	Bombay Veterinary College, Parel, Mumbai 411 012	Mice, Rats, Rabbits, Guinea Pigs
8	National Research Centre on Mithun, Indian Council of Agricultural Research, Jharnapani, Medziphema, Nagaland 797106	Bulls, Cows
9	Project Directorate on Poultry, Rajendranagar, Hyderabad-500030	Gallus Domesticus, Supply hatching eggs and day old chicks
10	College of Veterinary Sciences & Animal Husbandry, C.S.A. University of Agri. & Technology, Mathura 281 001 UP	Cow, Buffalo, Sheep, Goat, Horse, Rabbits, Mice, Rats, Guinea Pigs
11	Indian Veterinary Research Institute Campus Muketeswar, Kumaon, Naintal, Uttaranchal	Goat, Sheep, Rabbit, Mice, Guinea Pigs, Calves, Cows, Ponies, Bulock
12	Rajiv Gandhi College of Veterinary & Anima Sciences, Kurumbapet, Pondicherry-605009	Cows, Buffalo, horses, Goat, Sheep, Pigs, Poultry, Rabbits, Rats
13	Reliance Life Sciences Pvt. Limited, Sir H. N. Hospital & Research Centre, New Tower Bldg. V. Fllor, P. G. Chowk, Raja Ram Mohan Roy Road, Mumbai 400 004	Mice, Rat, Rabbits, Hamsters, Guinea Pigs, Mini Pig, Goat, Sheep, Cow, Buffalo.
14	Raee Horticulturist and Garden Contractors, 6, Vikram Apartments, II floor, Krishnad colony, 91/2, Kothrud, Pune 411 038	Fish, Guppy, Carp, tilapia, Pigeon, Chicken.
15	University College of Medical Sciences an Guru Teg Bahadur Hospital, Delhi 110095	Mice, Rats, Guinea Pigs, Rabbits, Sheep
16	Central Duck Breeding Farm, Hessarghatta, Bangalore 560 088	Ducks
17	Raj Biotech Pvt. Limited, Talekar vasti, opp. Suessen Asia, P. O. Village Wng, Taluk Khandala, Dist. Satara 412 801 (Maharasthra)	Hamsters

Table 2: Short List Of Animal Suppliers In India

9.References

1. Tom Regan. (1983) An essay 'A bird in the Cage'. URL http://www.tomregan-animalrights.com/regan_bird2.html [accessed 1st March 2010]
2. Darwin's Theory Of – A Theory in Crisis Evolution. URL http://www.darwins-theory-of-evolution.com [accessed on 9th April 2010]
3. Giridharan N.V. (May 2000). Use of animals in scientific research. URL http://icmr.nic.in/bioethics/Animals_biomedical%20research.pdf, accessed on 25th February 2010.
4. National Accreditation Board of Testing and Calibration Laboratories. URL http://www.nabl-india.org/ [accessed on 28th February 2010]
5. International Committee for Laboratory Animal Science (ICLAS). URL http://www.iclas.org/ [accessed on 28th February 2010]
6. Indian National Science Academy (INSA). (1992) URL www.**insa**india.org/, [accessed on 15th March 2010]
7. Prevention of Cruelty to Animals Act. (1960) URL http://envfor.nic.in/legis/awbi/awbi01.pdf [accessed on 2nd March 2010]
8. Schou S, Holmstrup P, Kornman KS. (1993). Non-human primates used in studies of periodontal disease pathogenesis: a review of the literature. J Periodontol, 64(6),497-508.
9. Adams RA, Zander HA, Polson AM. (1981). Interproximal and buccal cell populations apical to the sulcus before and during experimental periodontitis in squirrel monkeys. J Periodontol, 52(8), 416-9.
10. Novak MJ, Polson AM. (1989). Effects of levamisole on experimental periodontitis. J Periodontol. 1989, 60(3),137-44.
11. Schectman LR, Ammons WF, Simpson DM, Page RC. (1972). Host tissue response in chronic periodontal disease. 2. Histologic features of the normal periodontium, and histologic and ultrastructural manifestations of disease in the marmoset. J Periodontal Res,7(3),195-212.
12. Jordan HV. (1971). Rodent model systems in periodontal disease research. J Dent Res,50(2), 236-42.
13. Dannan A and Alkattan F. (2008). Animal models in Periodontal Research: A mini- review of the literature. The Internet Journal of Veterinary Medicine, 5(1).
14. Ericson I, Lindhe J, Rylander H, Okamoto H. (1975). Experimental periodontal breakdown in the dog. Scand J Dent Res,83(3),189-192.
15. Fritz ME, Braswell LD, Koth D, Jeffcoat M, Reddy M, Cotsonis G. (1997). Experimental peri-implantitis in consecutively placed, loaded root-form and plate-form implants in adult Macaca mullata monkeys. J Periodontol, 68(11), 1131-5.
16. Klausen B. (1991). Microbiological and Immunological Aspects of Experimental Periodontal Disease in Rats: A Review Article. J Periodontol,59,73.
17. Singh G, O'Neal RB, Brennan WA, Strong SL, Horner JA, Van Dyke TE. (1993). Surgical treatment of induced peri-implantitis in the micro pig: clinical and histological analysis. J Periodontol,64(10),984-9.
18. Hickey JS, O'Neal RB, Scheidt MJ, Strong SL, Turgeon D, Van Dyke TE. (1991). Microbiologic characterization of ligature-induced peri-implantitis in the microswine model. J Periodontol,62(9),548-53.
19. Fischer RG, Klinge B. (1994). Clinical and histological evaluation of ligature-induced periodontal breakdown in domestic ferrets immunosuppressed by Cyclosporin-A. J Clin Periodontol,21(4),240-9.
20. Fischer RG, Klinge B. (1994). Clinical and histological evaluation of ligature-induced perodontitis in the domestic ferret. J Clin Periodontol,21(4),230-9.
21. Al-Qareer AH, Afsah MR, Muller HP. (2004). A sheep cadaver model for demonstration and training periodontal surgical methods. Eur J Dent Educ,8(2), 78-83.
22. Danesh-Meyer MJ, Pack AR, McMillan MD. (1997). A comparison of 2 polytetrafluoroethylene membranes in guided tissue regeneration in sheep. J Periodontal Res, 32, 20-30.
23. Comuzzi L, Ooms E, Jansen JA. (2002). Injectable calcium phosphate cement as a filler for bone defects around oral implants: an experimental study in goats. Clin Oral implants Res,13 (3), 304-11.
24. Hickey JS, O'Neal RB, Scheidt MJ, Strong SL, Turgeon D and VanDyke TE. (1991). Microbiologic Characterization of Ligature-Induced Peri-Implantitis in the Microswine Model. J Periodontol,548 – 553.
25. Matthews, R.A.J. (2008). Medical Progress Depends on Animal Models – Doesn't It? Journal of the Royal Society of Medicine,101,95-98.
26. Selvig KA. (1994). Animal Models in Reconstructive Therapy. J Periodontol, 1169 – 1172.
27. Shafer WG. (2006). Use of animals for periodontal studies. Annals of the New York Academy of Sciences,153,223-229.