ISRAEL LABORATORY ANIMAL FORUM

2ND EAST MEDITERRANEAN REGIONAL ICLAS MEETING. JERUSALEM, DECEMBER 2008

Chairmen: Waner, T. and Kalman, R.

The Israel Laboratory Animal Forum (ILAF) was privileged to host the 2nd East Mediterranean Regional International Committee for Laboratory Animal Science (ICLAS) Meeting held in Jerusalem, on the 3rd and 4th December 2008. As ICLAS is dedicated to advancing human and animal health worldwide by promoting the ethical care and use of laboratory animals, the theme of the meeting was "**IMAGING, GENETIC CONTROL, AND PROTOCOL ETHICAL EVALUATION: TOOLS TO PROMOTE THE 3Rs**". Our goals for this meeting were to give those working in the field of laboratory animal science an opportunity to discover and discuss the state of the art in laboratory animal science. The program was designed to give scientists performing research on animals a better insight into the many considerations that need to be taken in account when planning animal experiments. Emphasis was placed on the ethical considerations, which are essential in order to raise animal care standards and improve animal welfare. The programme included state of the art invited lectures.

The meeting was held at Jerusalem's Mishkenot Sha'ananim, an international cultural, ethics and conference center. This was an ideal venue where a mixture of history, religion, ethics and science all have a special meaning. The meeting attracted individuals from all over the world.

Dr. Trevor Waner, Chairman of the Organizing Committee and Chairman of ILAF.
Dr. Rony Kalman, Chairman of Scientific Committee and Past Present of ILAF.

THE IMPACT OF INTERNATIONAL HARMONIZATION OF GUIDELINES ON ANIMAL WELFARE

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Since 2000, the International Council for Laboratory Animal Science (ICLAS) has taken practical initiatives to bring members of the international scientific community together to identify and recommend acceptance of guidelines considered to be suitable as international reference documents in the area of animal care and use in research. ICLAS believes in the harmonization of animal care and use guidance as a reflection of the globalization of research. However, harmonization must be distinguished from standardization (one worldwide set of regulations), since ICLAS believes that each country should be able to maintain an oversight system for animals used in science that reflects its cultures, traditions, religions, laws and regulations.

Other international (OIE, IACLAM), regional (FELASA) and national (ILAR, CCAC) organizations have been involved in different ways to harmonize animal care and use practices internationally without creating problems at the national level. A brief review of the ICLAS work on harmonization was provided and the impact on animal welfare of several harmonization initiatives will discussed.

THE THREE R'S AND ANIMAL CARE AND USE

Howard, B.
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This lecture briefly reviews the progress with each of the three R's of William Russell and Rex Birch, discussed in their book, published in 1959.

Replacement is the use of non-sentient material to replace conscious living vertebrates and may be *relative* or *absolute*. Relative replacing techniques involve conditions under which animal cannot suffer, including studies done under anaesthesia or decerebration, or increasingly by using hen's eggs or tissues or organs removed after humane killing. Absolute replacing technologies use non-living physical and chemical approaches such as analytical equipment, computer modeling or work on archived tissues, and also include higher plants, micro-organisms (e.g. the Ames test) and some very primitive metazoan such as *C. elegans* (studies of gene regulation and function).

Reduction involves minimizing the numbers of animals required by applying mathematical techniques to carefully determine how many are needed. Animal populations should be kept uniform by keeping them healthy and stress free in optimal environmental conditions. Unfortunately, measures to minimize genotypic variation are not universally applied.

Refinement involves the humanist possible treatment of each animal; it is actually a prerequisite for good animal experimentation. It also means conducing the science to the highest standards so as to ensure the validity of findings.

We have made considerable advances in Refinement and some in Replacement, but a great deal remains to ensure that Reduction is consistently applied. The three Rs need to be seen as a process rather than an end in itself.

PERSPECTIVES ON LABORATORY ANIMAL SCIENCE IN ISRAEL

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Animal experimentation is controlled in Israel by law: "Law for Prevention of Animal Suffering (experimentation on animals), 1994". The system in Israel is very similar to the major systems in Europe and the USA.

Several veterinarians working for research institutes in Israel initiated local courses of animal care as early as 1968 – 1972: Prof. Asher Meshorer, Prof. Jonathan Adler, Dr. Meki Shenbaum, Dr. Naam Kariv and Dr Daniel Mordechovich. Their initiation led to a voluntary code by the Israeli Academy of Science (1987). The Israeli Law followed this initiation. According to this law the government, (Ministry of Health) control animal experimentation through the Israeli Council for Experimentation on Animals.

The Council consists of 23 members: 6 representatives of the National Academy of Science: (2 from the sphere of the life sciences or medicine, 4 from the spheres of social science, liberal arts, exact science and law), 6 representatives from the Medical, Veterinary and Industrial Associations, 8 representatives of the 8 Governmental Ministries and 3 representatives recommended by the umbrella organization for the Prevention of Cruelty to Animals societies.

The Council is in charge of the issue of experiments on animals. It prescribes rules for the grant of permits to carry out experiments on animals, and the way the experiments are to be carried out, all in order to ascertain that suffering caused to animals is minimized, and to prevent the conduct of superfluous experiments. It prescribes rules about training in the sphere of minimizing the suffering of laboratory animals. It initiates information programs on subjects within the scope of its activity, as well as training and guidance programs for scientists on subjects connected to experiments on animals.

The council regulates the experiment in 62 research institutes through National Animal Care and Use Committee and 29 Institutional Animal Care and Use Committees. The Council employs a scientific advisor and veterinarian who are responsible for the supervision of the experiments and the institutions.

THE VITAL ROLE OF ANIMAL CARE TECHNICIANS: ENSURING SUCCESS IN LABORATORY ANIMAL RESEARCH

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There are six reasons why training of animal care staff and technicians is important:
1. Scientific requirements

2. Animal welfare requirements
3. Regulatory obligations
4. Employer obligations
5. Personal expectations
6. Public expectations

By satisfying the requirements, fulfilling obligations and meeting expectations, trained animal care staff and technicians reduce the costs to experimental animals. In doing so, the quality of experimental data is enhanced by improving the amplitude and/or intensity of the signal elicited from the animal and reducing the level of background noise associated with the captive environment and the experimental procedure. As a result, everyone (the experimental animal, science, researcher, institution and society) benefits because good animal-based research, testing and teaching is performed by skilled and knowledgeable animal care and use staff and technicians. In doing so, the care and use of laboratory animals for research, testing and teaching becomes humane and complies th societal expectations.

RECOMMENDATIONS ABOUT THE IDEAL COMPOSITION OF ETHICAL COMMITTEES IN EUROPE

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Ethical evaluation committees have been established in various countries all over the world including Europe, USA, Canada, Brazil, Argentina and Middle East on a national, institutional, legal or voluntary basis. Best practice guidelines regarding their composition have already been published.

As far as the European legislation is concerned, the revision of Directive 86/609/EEC looks on ethical evaluation procedures as a legislative prerequisite, since this process will become obligatory for all projects. The composition of these committees will be defined in the forthcoming revision. Existing committees comprise of various categories of participants, the selection relying on experience acquired through this process and on best practice principles. Minimum competences of an ethical committee are recommended as follows:

1) Competences that are required to participate:
- Chairman
- Scientists with knowledge and expertise in biological and biomedical research
- Veterinarians
- Statisticians

2) Competences that may fruitfully contribute to the process:
- Lay persons or persons without affiliation to the requesting organization
- Ethicists
- Lawyers
- Experts on animal welfare
- Animal technicians
- Representatives of animal protection organizations-
- Representatives of patient organizations
- Experts on alternatives to the use of animals

According to the specific experimental protocol under review, more than one expert of the same specialty from the above mentioned categories might be requested and, in the meantime, some other might not be asked to contribute. The participation of categories such as lay persons has also been considered and remains a controversial issue.
In any case, all participants in ethical review processes should be aware of the principles to be followed in the process and their specific role. The imposition of suitable and challenging questions to applicants as well as the presentation of issues of common concern and relevant experience is thought to be crucial for the accomplishment of a fruitful meeting and review of experimental protocols.

DESIGNING FOR REFINEMENT

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When designing and planning new laboratory animal facilities many factors need to be taken into consideration, the requirements of both the building and regulatory authorities must be met, the building also needs to accommodate the specific requirements of the animals, those of the researchers, veterinary and animal care staff.

Because of the time taken to plan, design, raise the finances, gain approvals and finally construct and equip a new facility, flexibility of design is of paramount importance as the needs of the research community linked with equipment developments can change quite rapidly.
When designing and constructing a new multi-species facility for the University of Oxford we tried to provide

a facility which met all the above requirements. One of our major goals was to design animal holding space which provided an improved and refined environment especially for sensitive species that require complex space. It was important to provide a range of procedural space to accommodate minor non-invasive procedures to complicated recovery surgery, also linking the long term animal holding area with behavioral testing and imaging equipment.

However carefully designed a building its space and financial constraints mean that not every requirement of research can be accommodated within the building footprint so that suitable systems of animal delivery and return need to be put into place to allow procedures to be conducted in specially equipped external laboratories.
This presentation attempts to explain how these issues were addressed and solutions developed, when planning to accommodate for a range of species from fish to non-human primates.

IMPLEMENTATION OF 3RS IN TURKEY

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During 1990's, rapid increase in the number of studies using laboratory animals has prompted the universities to establish their ethics committees to meet the requirements of the journal editors. In the meantime, the Turkish Scientific Research Council (TÜBITAK) has required the researchers to seek ethics committee approval prior to review grant applications. Starting from 2002, Turkish-EU integration efforts have gained momentum and the reforms to incorporate "Acquis Communautaire" have paved the way to the present structure.
In 2004, the Ministry of Agriculture and Rural Works issued a regulation on the breeding and husbandry of laboratory animals, which requires that all facilities have to meet specific technical and operational criteria. Subsequently, in 2006 the Ministry of Environment and Forestry promulgated the regulation on Laboratory Animals Ethics Committees and established the "National Ethics Committee for Animal Experiments", a watchdog to monitor local committees.
Present situation:
- All institutions using laboratory animals should appoint a local committee to monitor the usage, breeding and husbandry of the laboratory animals.
- The committee should review all demands for laboratory animal use and approve experimental protocols in line with the 3R principles.
- The committee should supervise the living conditions of the animals, monitor anesthesia and euthanasia protocols.
- All individuals who wish to use laboratory animals should receive a training of minimum 80 hours.
Local ethics committees should report their activities to the National Committee, which acts as the highest competent authority to implement the policies on animal use and to resolve the disputed issues.

THE AAALAC APPROACH TO THE ETHICAL REVIEW PROCESS

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AAALAC (Association for Assessment and Accreditation of Laboratory Animal care) International is a voluntary, peer-review and independent system that evaluates and accredits programs of laboratory animal care and use throughout the world. It assesses the main programmatic areas: Institutional Policies and Responsibilities; Animal Environment, Housing and Management; Veterinary Medical Care; and also the Physical Plant. The Ethical Review Process (ERP) is one of the main Institutional Responsibilities. The ERP can be performed in a variety of manners, though there are some basic requirements that should be met: having support from management; having specialized and well trained people to perform the ERP, including veterinarian(s) and scientists; ensuring the prospective review of animal research protocols avoiding potential conflicts of interest; monitoring the post-approval development of the protocols; performing periodic inspections of the program; and engaging the Institutional Official in the process. The evaluation of the whole process is carried out by the members of the AAALAC Council on Accreditation using performance based standards which are outcome oriented, focused on expected results rather than the process used to achieve them. This is why different approaches can be accepted when the outcome is positive. The professional judgment and peer review process will assure a proper evaluation of the ERP. Findings after the evaluation, if there are any, are classified as either "Suggestions for Improvement" that the institution can address in a voluntary manner; or "Mandatory" issues that must be satisfactorily addressed in order to obtain the accreditation. A priori, the only certain "must" is that there must be an effective ERP on site.

THE 3RS AND THE CONTRIBUTION OF INBRED STRAINS TO BIOMEDICAL RESEARCH

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The first inbred strains of mice, rats and guinea-pigs were developed nearly 100 years ago. Their contribution to biomedical research has been enormous. More than 20 Nobel prizes have been awarded for work which would have been impossible or very much more difficult without them. And they continue to be of immense value in modern genetical research. So what are their properties which make them so useful? Their most useful property is that of isogenicity, with all individuals within an inbred strains being genetically identical. However, it is the property of homozygosity which makes them immortal, with offspring being genetically identical with the parents. Other useful properties, such as phenotypic uniformity which means that fewer of them are needed to achieve a given level of statistical power, flow from these two. Yet there are still disciplines such as toxicity testing which resist their use. Is this scientifically justified, or is it a case where misunderstanding of their properties and of the properties of outbred stocks has led scientists up a blind alley?

THE NC3RS: ITS ROLE IN THE ETHICAL EVALUATION OF ANIMAL RESEARCH

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The NC3Rs is an independent scientific organization established by the UK Government to develop, validate and promote scientific and technological advances in the 3Rs. The Centre funds research projects, organizes workshops and symposia, and produces guidelines on all 3Rs.

The NC3Rs collaborates with the major UK research councils and charitable funding bodies of bioscience research using animals to improve the ethical evaluation of such research and to raise standards of animal welfare. For example, the Centre reviews all research proposals to the Biotechnology and Biological Sciences Research Council, Medical Research Council, Royal Society, Wellcome Trust and other member charities of the Association of Medical Research Charities which involve the use of non-human primates, cats, dogs or horses. All research involving non-human primates must comply with the NC3Rs Guidelines 'Primate accommodation, care and use'.

The NC3Rs has recently published guidance on 'Responsibility in the use of animals in bioscience research'. The principles for good practice and the sources of advice in this document are intended to help researchers, veterinarians, animal care staff, ethics committees and peer reviewers apply the 3Rs effectively during the design, evaluation and conduct of research using any vertebrate species. Implementation of the principles is a condition of receiving funds from the NC3Rs and other funding bodies.

These initiatives, together with steps to improve the quality of information provided for ethical evaluation of research using animals, have resulted in greater attention being given to the 3Rs in academia, with concomitant reductions in the numbers of animals used and improvements in animal welfare.

IN VIVO TWO-PHOTON CORTICAL IMAGING IN THE MOUSE

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Classically, electrophysiological recordings were used to characterize the sensory response profiles of the cortical neurons. Recent advances in optical imaging now allow live imaging of sensory responses, *in vivo*. Specifically, I will present a recent method based on two-photon excitation and laser scanning microscopy that allows us to image sensory responses of cortical neurons. Specifically, we use calcium transient imaging to decipher receptive fields of cortical neurons in the mouse neocortex. This method is relatively non-invasive and has the advantage of imaging the physiology of hundreds of neurons in a single animal. In addition, by imaging multiple neurons simultaneously it is possible to extract the precise spatio-temporal interaction between neurons in small networks at microscopic resolution. These capabilities are not possible using traditional electrophysiological methods and are a useful addition to the physiologist's toolkit.

OVERVIEW OF IMAGING MODALITIES AND CONTROL OF SMALL ANIMAL PHYSIOLOGY DURING LIVE IMAGING

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In the last decade, small animal (rat and mouse) imaging methods have become recognized as useful tools in basic science research and phenotyping genetically engineered mice. An overview of currently utilized imaging modalities applicable to small laboratory animals will highlight the advantages and disadvantages of MRI, PET, SPECT, optical and ultrasound imaging. These imaging modalities permit the acquisition of three- and four-dimensional data that can ultimately result in the reduction in numbers of experimental animals needed for studies. The successful imaging of live small animals requires control of animal physiology while simultaneously providing the respiratory synchronization and cardiac gaiting sometimes necessary to acquire high resolution images. Issues associated with anesthesia, heat control, motion, and monitoring will be presented with suggested solutions for maintaining stable physiology during live animal imaging.

COST-B24: THE MANUAL OF THE BEST PRACTICE. REPORT FROM THE WORKING GROUPS

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COST ("Co-Operation in Scientific and Technical research") is an EU initiative supporting nationally funded research. Its role is to develop new understanding by critically reviewing existing knowledge and to disseminate this as best practice. Action B24 is directed at increasing ethical and scientific knowledge underpinning the sustainable use of laboratory animals in research.

Four working groups have been established: housing of animals and scientific integrity; refinement of procedures; genetically-modified animals and other new models; ethical evaluation and cost-benefit analysis. A fifth working group is collating material collected by the other four in relation only to rodents and rabbits and will publish this as the Manual of Laboratory Animal Care and Use: Refinement Reduction and Research. The text will not deal with Replacement because the scientific disciplines differ substantially.

COST-B24: THE MANUAL OF THE BEST PRACTICE.WG1: HOUSING OF ANIMALS AND SCIENTIFIC INTEGRITY

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Improvement of animal welfare combined with the same or, whenever possible, better scientific outcome are the ultimate goals of Cost Action B24 Working Group 1. The work covers three topics: 1) Facilities and facility planning, 2) Housing and care, and 3) Animal needs and environmental improvement. Under these topics, the WG is preparing chapters for the COST Manual.

During facility planning, broad expertise is required and the design team should include different specialists. It is important to consider the purpose of the facility, the nature of the research, the species maintained and the number of animals. Furthermore, it is necessary to know whether animals will be bred in-house, whether genetically modified animals will be maintained and whether work with infections, chemicals or isotopes will be carried out. More detailed planning includes plans for rooms, barriers, corridor systems, locks, storages, laboratories and distribution systems. Structures for environmental control, monitoring and security system must be considered.

Laboratory animal housing has undergone major changes towards more complex and furnished environments within the containment. The focus is to find out the golden practises for everyday housing based on scientific results and type of research. Both primary (cages) and secondary (racks, IVC's, isolators) enclosures are reviewed. Environmental factors are discussed with a special attention how the animal itself is sensing the world.

Animal motivations and needs are reviewed, and recommendations will be given how to improve quality for life of animals, physical environment, how to assess validation of improvement and well-being, and finally the impact on scientific results. Golden practices aim for improvements that benefit animals and improve science.

COST-B24: THE MANUAL OF THE BEST PRACTICE.WG2: REFINEMENT OF PROCEDURES

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The task of COST B24 Working Group 2 is to review the 2Rs (Refinement & Reduction) potential for the procedures, produce critical reviews and write COST Manual chapters on the topics selected. While implementing the 2Rs we should aim at going beyond the threshold set by Russell and Burch, however with the same scientific outcome, *i.e.* look at those methods that result in better science. We should not only concentrate in painful procedures, but rather understand that small changes in the common procedures, to which large numbers of animals are exposed, may equally give in return considerable overall improvement. Furthermore, the group has tackled the problems associated with *ad libitum* feeding, and come up with a solution allowing restricted feeding in groups without derailing the normal diurnal rhythms of the animals. This solution is based on work for food principle, where work is gnawing wood in order to reach food. All this will be translated to the following COST Manual chapters: Visions for the future, Animal selection and preconditioning, Handling, Sampling, Telemetry, Anesthesia and analgesia, Euthanasia, Administration of substances, Pain and distress, Imaging, Experimental design and Humane endpoints.

COST-B24: THE MANUAL OF THE BEST PRACTICE.
WG3: GENETICALLY MODIFIED (GM) ANIMALS AND OTHER NEW MODELS

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Genetically modified animals have been used to create unique characteristics in animals through careful breeding and selection for desired characteristics. Modern science has accelerated the process, whereby genes of interest are placed early in development so that the gene becomes incorporated into the genetic material, the DNA of the organisms. While this was a breakthrough, it was paled by the ability to completely remove the targeted gene through recombination events, resulting in a "knockout"!
Since the first transgenic mouse was reported at the beginning of the eighties and the first knockout mice a decade later; genetically modified mice have become an indispensible tool in biomedical research.
Although, the use of knockout and transgenic rodents (especially mice and rats) represent the two important model organisms in biomedical research, other model organisms (like Drosophila, *C. elegans* etc) that can be genetically modified, are also useful in early studies of a particular genetic target or developmental process. The use of these non-mammalian has thus reduced the number of mammals used in research. Furthermore, these later organisms collectively contribute to minimize the suffering of rodents, and therefore, play a major role on the 3R's (Reduction, Refinement, Replacement) paradigm to reduce animal use in research. But, these alternative organisms cannot replace the mammalian "mimics" for human physiology, and thus the mouse remains a necessary model in biomedical research.

im of WG3

The purpose of the WG3 is to stimulate thought-provoking considerations prior to the use of transgenic or knockout mice and highlighting both the advantages and disadvantages of the alternative model organisms.
The following important aspects of the use of genetically modified organisms are covered:
a) the use of other species than mice for initial experiments
b) general aspects in the production of genetically modified mice
c) line management and security of gm mice
d) phenotyping of gm mice, a forgotten issue that has gained resurgent importance in research in the current post genomic era
e) some aspects regarding harmonization and legal issues in the gm production, pointing out the important issues of animal welfare, local regulations and laws

COST-B24: THE MANUAL OF THE BEST PRACTICE. WG4: ETHICAL EVALUATION AND COST-BENEFIT ANALYSIS

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Ethics review of experiments involving animals is an essential step in ensuring animal welfare both by the general public and by specialists. Most countries with active biomedical research have some system for ethics review in place. In the document, prepared by COST B24 based on existing literature and on the wide and international experience of the working group members, we present a checklist of issues to consider in an ethics evaluation. These issues include:
- Description and purpose of the study

- Replacement
- Refinement
- Reduction
- Retrospective information from similar studies
- Information about the researcher and institute
- Experts and competent persons
- General aspects
- Composition and mode of action of an ethics committee

In several sections each corresponding to a particular point in the check list we briefly discuss various aspects of these issues and to what extent their evaluation is possible and productive for the purpose of achieving a responsible use of animals respecting the 3Rs. Special attention is given to the following topics: harm-benefit analysis, compliance with the 3R principles, composition and functioning of ethical committees, communication with the society, tension between extensive control (resulting in a heavy administrative burden for scientists and committees) and trust in a culture of responsibility (with the risk of that responsibility not being taken seriously).
This document is intended as a working tool for committees and individual committee members, in carrying out the review process, in initial training of new members and in continuing education programs for both committee members as well as for researchers and students.

APPLICATION OF MAGNETIC RESONANCE: MICROSCOPY IN TOXICOLOGY AND TOXICOLOGIC PATHOLOGY

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Magnetic resonance (MR) microscopy is a high resolution magnetic resonance imaging (MRI) modality applicable to *ex vivo* and *in vivo* imaging of rodents, embryos, and small tissues. The highest resolution for whole animals is achieved utilizing contrast enhancement in perfused fixed specimens (*ex vivo* imaging). With appropriate animal restraint and physiological monitoring, *in vivo* imaging allows assessment of organ function and lesion progression. Examples of *ex vivo* applications of magnetic resonance microscopy will include phenotyping genetically engineered mice, assessment of hepatic vascularity in liver toxicity, potential use of MR microscopy for teratology studies, and applications in carcinogenicity studies. *In vivo* applications will touch on pulmonary function and monitoring the progression of hepatic carcinogenesis in mice (4-dimensional imaging). The superb soft tissue contrast, ability to obtain quantitative data, and the non-destructive nature of MR microscopy provide a useful adjunct to the conventional optical microscopy typically done in rodent toxicology and cancer studies.

THE ROLE OF DIET AND FEEDING IN LABORATORY ANIMAL WELFARE

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Diet and feeding can improve animal welfare through refinement and reduction. But careful management is needed to avoid complications.
Refinement is predominantly the development of methods to reduce distress to laboratory animals. One good example, particularly for primates, is environmental enrichment, where the standard nutritionally-complete diet is supplemented by palatable foodstuffs differing in texture/consistency, appearance, and flavour. Unfortunately many commonly selected supplementary foodstuffs are nutritionally imbalanced and may lead to nutritional disorders.
Reduction means obtaining the most accurate and precise information using the fewest number of animals.

In toxicology studies, survival and pathology in control animals is improved by restricting the amount of food eaten (diet restriction, DR): greater survival means the use of fewer animals. DR may also make the test more sensitive for the detection of treatment-induced adverse pathology. Additionally, the standard deviations of body and organ weight are decreased, allowing better interpretation. But DR studies require single housing of animals that may adversely impact on the refinement aspect of animal welfare.

Reduction can also be achieved by decreasing variability of nutrient composition, for example by the use of purified diets rather than natural ingredient diets. But purified diets may create abnormal physiological conditions, including diabetes and obesity.

Natural-ingredient diets may contain non-nutrients, e.g. phyto-oestrogens; nitrosamines, that vary in concentration and may increase variation or interfere in experimental results. Avoidance or minimization of specific raw materials and the use of fixed-formula diets may do much to avoid such complications, decrease inter-study variation and achieve a reduction in animal use.

ZEBRAFISH AS A RESEARCH MODEL AND THEIR HUSBANDRY

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The use of Danio rerio, commonly known as Zebrafish, has significantly increased over the last decade. During this presentation the unique characteristics of the species, advantages of use in research and fields of application will be analyzed in relation to the growing attention to the 3 R's, attempting to enlighten how Zebrafish can match replacement, reduction and refinement concepts. In the international research community, the need for better definition of standardized ideal housing conditions has just begun. Because of experimental needs, in most of the cases housing systems were designed and implemented by researchers or institutions themselves, with little attention to basic rules of aquaculture and mechanical engineering aspects. If from one hand water quality represents the driving factor for successful housing, the care of the various colonies is sometimes very basic and still unknown. Zebrafish was born as a laboratory model no more than 30 years ago, its use is now becoming wider and wider, spreading from basic research into pharmacology.

THE PROTECTIVE EFFECT OF DIFFERENT OPHTHALMIC VISCOELASTIC DEVICES ON CORNEAL ENDOTHELIAL CELLS DURING PHACOEMULSIFICATION IN A RABBIT MODELS

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Purpose:
To evaluate the protective effect of different Ophthalmic Viscoelastic Devices (OVDs) on corneal endothelial cells during phacoemulsification in a rabbit model.

Method:
18 New Zealand White (NZW) rabbit eyes were randomly assigned to 3 equally sized groups. Endothelial cell count and pachymetry were performed in all eyes prior to study initiation. In Group A the aqueous humor was replaced with Biolon™ (Bio-Technology General Ltd.). In group B the aqueous humor was replaced with a combination of Viscoat® (Alcon) and Provisc® (Alcon) using the soft shell technique. In group C the aqueous humor was replaced with a combination of Visiol® (TRB CHEMEDICA) and Biolon™ using the soft shell technique. The eyes were exposed to alternating 10 seconds of phacoemulsification and a 10 second pause until a total exposure time of 2.5 minutes was reached. Endothelial cell count and pachymetry were repeated 3 days post-surgery.
Results: Group A (Biolon™) showed the highest endothelial cell loss -13%, followed by group B (Viscoat® and Provisc®) -7%, and group C (Visiol® and Biolon™) -4%. The difference between groups C (Visiol® and Biolon™) and A (Biolon™) was found to be statistically significant, p=0.037. Accordingly, Group A (Biolon™) demonstrated the highest decrease in corneal thickness -8%, followed by group B (Viscoat® and Provisc®) -7%, and group C (Visiol® and Biolon™) -2%.

Conclusion: The soft shell technique using a combination of Visiol® and Biolon™ demonstrated better protective effect on the corneal endothelial cell during phacoemulsification comparing to Biolon™ alone.

GENETIC MONITORING: DO WE REALLY HAVE WHAT WE THINK?

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Increasing evidence demonstrates that phenotype of transgenic animals is not only the result of the new genetic information, but genetic background of the host also plays a role. These two factors: transgene and host background should be considered in the management of transgenic mice colonies.

Development of a new model has to be carefully planned in advance. Several factors should be considered in order to refine the procedure, thus reducing the number of animals involved in an experiment. To generate founders using inbreed strains or not so efficient ES lines diminish the efficiency of transgenic production, but it reduces sensibly the number of generations needed to achieve the correct mode. This approach also avoids the extra burden of creating a congenic strain.

Once the transgenic line is established, it is of utmost importance to guarantee its genetical stability. This involves not only monitoring the transgene, it is also necessary to periodically check the genetic background to avoid unexpected contaminations. Monitoring systems based on detection of genome sequences as SNPs or microsatellites are the best choice.

At last it is necessary a strategy to ensure the permanence and genetical soundness of the genetically modified line.

Cryopreservation at early stages of the line development is the best choice since it serves as backup in case of transgene inactivation, disease outbreak, and mistakes in the management of the colony or the stochastic appearance of mutations that sometimes compromise the reproductive performance. Cryopreservation also guarantees the disposability of the line to the scientific community.

ETHICS COMMITTEES AND THE CULTURE OF CARE.

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The use of animals for scientific purposes is highly contentious and it is essential that scientists using animals engage with this principled concern and proceed with great caution. Welfare should underpin the way animals are sourced, cared for and used.

The term "ethics committee" is an unfortunate one; no committee can be an ethical arbiter, and even if it could, its conclusions would apply only to its members. What is needed is an institution-wide awareness and consensual buy-in to a moral "culture". A committee may promote and co-ordinate this but no more. Most committees are distrusted by those who they are expected to serve. Those opposed to animal experiments may see them as a way of deferring change, concealing facts or rubber-stamping activities, whereas scientists often regard them as unnecessary bureaucracy. To be effective they must comprise a forum for discussion and debate which is based on consistent ethical positions and is rigorous, transparent, and effective. Those involved must represent a wide spread of different attitudes and expertise; often a senior member of the establishment is involved to demonstrate commitment and to ensure that decisions are carried through. Other key persons are the veterinarian, a senior member of the animal care team and an active scientist representing the "user". Often too, a lay person, someone with expertise in study design and possibly an animal welfare specialist are involved; other inputs can be helpful, providing the group is not too large.

REDUCING THE NUMBER OF ANIMALS USED IN RESEARCH BY IMPROVEDEXPERIMENTAL DESIGN AND STATISTICS

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Most laboratory animals are used in formal controlled experiments, but scientists are not always taught how to design a good experiment. Experiments need to be sufficiently sensitive to detect subtle treatment effects without biases leading to false positive results. Just the right number of animals should be used. Too few and important effects may be missed and the experimental animals wasted, too many and animals will also be wasted. So formal methods of determining sample size should be adopted, rather than relying on tradition. But optimum sample size also depends on controlling the variability of the experimental material. The use of inbred strains, design techniques like the use of randomized block and crossover designs, and methods of reducing measurement error such as the use of telemetry may mean that far fewer animals are needed. Are the responses the same in males and females or in different strains of animals? The range of applicability of the results across gender and strain can also be explored using factorial experimental designs without increasing the number of animals. These provide extra information at no extra cost. And any experiment must be amenable to a statistical analysis in order to quantify uncertainty in the results. This means correct identification of the experimental unit. The use of good experimental design techniques and correct statistical analysis will ensure that the minimum number of animals is used in achieving the scientific objectives of the study.