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ABSTRACT BOOK

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3Rs IN ACTION: FROM ETHICS TO IMPLEMENTATION SESSION

INFRAFRONTIER.GR: ADVANCING THE 3RS IN DISEASE MODELLING

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Recent innovations in genomics, non-invasive phenotyping technologies, advanced *in vitro* and microphysiological systems, as well as regulatory and legislative shifts have accelerated the adoption of the 3Rs (Replacement, Reduction, Refinement) across biomedical research. INFRAFRONTIER.GR, the Greek Research Infrastructure for archiving and phenotyping of model mammalian genomes and the Hellenic node of INFRAFRONTIER ERIC, has played a central role in translating these advances into practical solutions that improve animal welfare while strengthening scientific outcomes.

Through its network, INFRAFRONTIER.GR supports the development, harmonization, and deployment of next-generation disease models and methodologies. Key achievements include optimized cryopreservation strategies that enable efficient archiving and global distribution of mouse lines, substantially limiting the need for breeding and transport of live animals. Standardized sperm and embryo preservation protocols, combined with refined quality control approaches, allow repositories such as the European Mouse Mutant Archive (EMMA) to maximize resource sharing while minimizing animal use. In parallel, INFRAFRONTIER.GR has advanced systemic and disease-oriented (e.g. chronic inflammation, degenerative diseases, etc.) phenotyping services by refining experimental designs, introducing non-invasive and non-terminal tools, improving housing and handling practices and providing standardized, role-specific training pathways. These measures enhance data robustness while reducing severity, supporting more ethical and reproducible *in vivo* studies. Importantly, INFRAFRONTIER.GR is expanding beyond animal-based models by integrating complex *in vitro* systems into its service portfolio. 3D cell cultures, such as organoids, are being leveraged to develop biologically relevant alternatives to mouse models, particularly in gastrointestinal, respiratory and cancer research. Ongoing projects aim to establish reliable *in vitro* platforms for diseases, reducing and complementing animal use in these areas.

Together, these activities demonstrate how coordinated Greek research infrastructures can drive innovation, promote collaboration, and embed the 3Rs at the core of disease modelling.

EVALUATION OF REPLACEMENT METHODS IN NON-TECHNICAL PROJECT SUMMARIES (NTS)

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Article 37 of Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes requires that Member States shall ensure that an application for project authorisation is submitted by the user or the person responsible for the project. The application shall include at least the following: (a) the project proposal; (b) a non-technical project summary. Both documents shall provide a demonstration of compliance with the requirements of replacement, reduction, and refinement. The Directive also requires Member States of the European Union (EU) to publish non-technical project summaries (NTS) of all authorized projects involving animals (Article 43).

The study aims to evaluate the alternative methods described by the applicants at the institutional level (University of Debrecen) project proposal and non-technical summaries (NTS), as well as in the international level in animal use reporting EU system available NTS database.

During the study were examined that the person responsible for the project in the replacement part of NTS “explains what partial and/or full replacements were considered – or used – prior to deciding to use animals, and these may include in silico, in vitro, or ex vivo approaches, and explains why they were not (yet) suitable” (1).

References:

1. Taylor K, Weber T, Alvarez LR. Have the non-technical summaries of animal experiments in Europe improved? An update. ALTEX (2024). 41(3):382-394. doi: 10.14573/altex.2310181.

ULTRASOUND-GUIDED REFINEMENT OF BOVINE RESPIRATORY DISEASE COMPLEX STUDIES

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Introduction: Bovine Respiratory Disease Complex (BRDC) remains one of the most significant health and welfare challenges in cattle, resulting from complex interactions between viral and bacterial pathogens, host immunity, and environmental stressors. Experimental BRDC challenge models are essential for evaluating vaccine efficacy and advancing understanding of disease pathogenesis. However, variability in baseline pulmonary health can compromise animal welfare and scientific outcomes.

Aims: This work aimed to refine an existing *in vivo* BRDC challenge model by integrating non-invasive diagnostic imaging to improve baseline uniformity, enhance animal welfare, and support evidence-based decision-making, while maintaining scientific quality and reproducibility.

Materials and Methods: Transthoracic ultrasonography was systematically incorporated into animal inclusion and monitoring procedures. Calves underwent comprehensive lung ultrasound screening to identify pre-existing pulmonary lesions or consolidations associated with increased risk of adverse outcomes following challenge. Animals with significant baseline pathology were excluded. Following challenge, ultrasound examinations were repeated at defined time points to assess lung pathology and guide decisions regarding animal retention, intervention, or removal from the study. In parallel, ongoing research is investigating the development of non-invasive microRNA (miRNA)-based diagnostic tests for earlier detection of BRDC.

Results: Ultrasound-guided refinement resulted in more uniform and healthy study cohorts and improved consistency in disease expression across treatment groups. The proactive use of transthoracic ultrasonography enabled early identification of animals at risk of severe disease and has virtually eliminated post-challenge mortalities. In addition, ultrasound provided a rapid, non-invasive method for monitoring disease progression and supporting objective clinical assessment.

Conclusion: Integrating transthoracic ultrasonography represents a meaningful refinement of an existing *in vivo* BRDC challenge model. The use of non-invasive diagnostic imaging reduces suffering and avoids unnecessary mortality by enabling evidence-based decision-making, while improving baseline uniformity, scientific quality, and reproducibility. Complementary miRNA biomarker research offers future opportunities to further refine respiratory disease models and reduce disease burden.

STOP THE COLD BEFORE THE FALL: HEATED ANESTHESIA INDUCTION IN MICE

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Introduction: Peri-anesthetic hypothermia is a common and critical complication of rodent anesthesia, negatively affecting animal welfare, physiologic stability, and experimental reproducibility. Although active warming is routinely applied during anesthetic maintenance, the induction phase (when thermoregulatory defenses collapse and heat loss is most rapid) often remains unprotected.

Aims: To evaluate a cost-effective Arduino-based heated anesthesia induction as a refinement strategy for early hypothermia in mice and to assess its effects on thermal stability and anesthetic physiology.

Materials and Methods: A precisely temperature-controlled, low-cost heated induction chamber was developed to provide active warming at the onset of inhalant anesthesia. Bench testing assessed thermal stability and surface temperature accuracy. In a randomized, paired, repeated-measures design, ten adult C57BL/6 mice (5 females/5 males) served as their own controls and underwent isoflurane induction under two conditions: unheated induction and heated induction (37.5 °C). All animals then underwent a standardized 10-minute anesthetic maintenance phase on a heated surgical platform. Core body temperature was measured using intraperitoneal RFID transponders, with concurrent monitoring of physiologic parameters and anesthesia recovery time. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC).

Results: Heated induction rapidly achieved thermal stability and maintained normothermia during anesthesia induction. In contrast, unheated induction produced a rapid and clinically relevant decline in core body temperature. Mice undergoing unheated induction did not regain baseline temperature until several minutes into anesthetic maintenance and stabilized at approximately 1 °C below those receiving heated induction. Physiologic parameters and recovery times were comparable between conditions.

Conclusion: Heated anesthesia induction effectively prevents early peri-anesthetic hypothermia in mice without compromising physiologic stability. By addressing heat loss at its most vulnerable stage, this approach represents a practical refinement that improves animal welfare and reduces temperature-related experimental variability. Preventing hypothermia during induction should be considered a best-practice refinement in rodent anesthesia.

CO_2, CO, AND N_2 EUTHANASIA IN LABORATORY MICE: A MULTISYSTEM WELFARE ASSESSMENT

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Millions of laboratory mice are euthanized worldwide every year, most often with carbon dioxide (CO_2). However, CO_2 raises major animal welfare concerns, because it is aversive and induces strong respiratory distress, which may cause anxiety and pain.

Currently, insufficient data are available to assess and classify the impact of CO_2 and other gases proposed as alternatives. In this context, it is crucial to accurately determine when animals lose consciousness as the humaneness of an euthanasia method depends on the experience of pain or distress before loss of consciousness (LOC).

Here, we analyzed and compared the effect of CO_2, N_2, and CO euthanasia in female and male C57Bl/6N mice, one of the most widely used mouse strains. Mice were implanted with transmitters to record neurological and cardiovascular signals while assessing also respiration, behavior by video-tracking, and measuring biochemical stress parameters. This multi-modal assessment enabled us to determine the time points of loss of posture (LOP) and LOC.

CO_2 induced gasping and muscle fasciculations before and after LOP whereas CO and N_2 caused severe seizures and strong agitation, and delayed cessation of electrical brain activity. LOC, induced by the anesthetic properties of CO_2, was the fastest with a high-volume displacement rate of CO_2. Multi-modal assessment revealed that CO and N_2 led to a hypoxia-induced death that is less humane than with CO_2 euthanasia. CO and N_2 cannot be recommended for a more humane euthanasia of laboratory mice.

INHALANT ANESTHESIA BEFORE EUTHANASIA WITH CO2 IN LABORATORY MICE: IMPROVEMENT OR CHANGE FOR THE WORSE?

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Carbon dioxide (CO_2) remains the most widely used method for euthanasia of laboratory mice. However, its use is associated with relevant animal welfare concerns, since it is aversive and induces respiratory distress, which may result in anxiety and pain. In our recent multisystem welfare assessment, we showed that CO_2 at a 30% volume displacement rate (VDR) produced the lowest overall distress burden, whereas CO_2 at 70% VDR induced faster loss of consciousness (LOC) but was associated with increased distress beforehand.

Building on these findings, the present study addresses a key refinement question: whether the welfare burden associated with CO_2 (30% VDR) can be further reduced by blunting the conscious phase through anesthetic induction. Although anesthesia before euthanasia has been proposed to limit aversion and distress, inhalational anesthetics itself can provoke respiratory irritation aversion and therefore distress, anxiety or even pain. It therefore remains unclear whether anesthetic LOC induction reduces the overall distress or merely shifts it to an earlier phase – compared to CO_2 only.

We investigated three commonly used anesthetic agents for induction prior to CO_2 (70% VDR) euthanasia: nitrous oxide (N_2O), isoflurane, and sevoflurane. N_2O provides anxiolytic and analgesic effects, but suppression of visible distress does not necessarily indicate LOC. Isoflurane and sevoflurane reliably induce unconsciousness but have been associated with excitation and respiratory irritation during induction. Using our multisystem framework, we quantified behavioral events, respiratory and cardiovascular responses, EEG/EMG endpoints, and biochemical stress indicators in female and male C57BL/6J and BALB/c mice.

This design not only complements our previous work by assessing sex- and strain-specific responses under CO_2 (30% VDR) alone, but also enables direct comparison with anesthetic-assisted protocols. By doing so, we determine whether anesthetic induction provides a measurable welfare benefit during euthanasia, and identify which strategies improve mouse welfare.

Keywords: Gas Euthanasia, Laboratory Mice, CO2, Refinement, Distress

THE EVOLVING FRAMEWORK OF STANDARDS FOR ACCREDITATION OF ANIMAL CARE AND USE PROGRAMS

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A combination of legal requirements and other adopted standards forms the foundation of the AAALAC International (AAALAC) accreditation process. AAALAC has accredited more than 1,150 animal care and use programs in 52 countries and regions, including 131 programs in 22 European countries. Accredited programs must comply not only with applicable legislation but also with a defined set of international standards categorized according to their importance within the accreditation process.

At the top of the hierarchy of non-legal accreditation standards are the so-called *Primary Standards*. Two key Primary Standards are the *Guide for the Care and Use of Laboratory Animals* (the Guide) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS 123). The Guide is currently undergoing revision, and the outcome is expected to be a substantially different type of document, with more frequent topic-specific updates. In contrast, although ETS 123 remains valid—since many housing and accommodation standards in Directive 2010/63/EU are based on Appendix A of ETS 123—the Council of Europe has no current plans to revise it. Consequently, ETS 123 may eventually become outdated in relation to other European legal instruments.

Within this evolving international context, and as a part of its most recent Strategic Plan, AAALAC is restructuring its accreditation standards framework. As an initial step, new Position Statements—representing the second level of standards after the Primary Standards—have been published on the 3Rs and Culture of Care, and the Position Statement on Veterinary Care has been revised. In addition, many existing Frequently Asked Questions are being transformed into a new category of standards known as *Guidance Statements*. The evolution and implementation of this updated accreditation standards framework will be described in detail.

CHARACTERIZATION OF A NEW AGGRESSIVENESS MODEL IN GOTTINGEN MINIPIGS

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Background: The development of an aggressiveness model in pigs supports translational drug development by providing a large-animal system with human-relevant anatomy, physiology, and social behavior. Such a model enables evaluation of pharmacological interventions targeting social stress, impulsive aggression, and associated neuroendocrine responses under controlled yet ethologically relevant conditions. The resident–intruder paradigm offers a valuable approach to bridge the gap between rodent models and clinical application, improving prediction of efficacy and safety for compounds aimed at modulating social conflict and stress-related psychopathology.

Methods: Four uncastrated male Göttingen minipigs (residents) were single-housed. Two castrated male minipigs (intruders) were single-housed in a separate room. On day 1, each intruder was introduced sequentially to a resident for 10 minutes, and interactions were video-recorded. Dominant behavior, biting, lifting, and contact interactions were quantified. Forty minutes after interaction, animals underwent an open-field test. Blood and saliva samples were collected for stress biomarker analysis. Next, for model validation, the resident animals were treated with risperidone (1 mg/kg, p.o.) 2.5 hours prior to the exposure to the intruder.

Results: Residents immediately engaged intruders, displaying dominant behaviors such as mounting. The number of dominant behavior episodes was 28 ± 3. Risperidone treatment reduced dominant behavior by approximately 50% ($p < 0.05$). In the open-field test, risperidone-treated animals showed reduced locomotor activity, as expected. In the approach test, treated animals exhibited prolonged latency to approach the entering researcher compared with vehicle-treated controls.

Conclusion: The resident–intruder paradigm represents a robust and translational model for studying aggression in Göttingen minipigs. Key endpoints include dominant behavior, locomotor activity, and approach behavior, supporting its utility for the evaluation of novel psychiatric drugs targeting aggression.

REFINING XENOTRANSPLANTATION RESEARCH: UTILIZING RETIRED SOWS FOR LUNG PERFUSION TO PROMOTE RESPONSIBLE ANIMAL USE

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Xenotransplantation, the transplantation of non-human organs into humans, is rapidly becoming clinical reality with initial FDA-approved trials. Nevertheless, further research is still needed. At Hannover Medical School – one of the largest transplant centers in Germany, a multidisciplinary team is working to bridge knowledge gaps. Utilizing complex genetically modified pigs as organ donors holds significant promise to reduce immune response in kidney transplantation. However, the lung remains particularly susceptible to rapid dysfunction upon contact with human blood. To address this challenge, ex vivo lung perfusion (EVLP) experiments are crucial enabling research on isolated organs. Organ retrieval and EVLP must be performed under sterile conditions, using established clinical protocols and equipment normally applied in human lung transplantation. Therefore, juvenile pigs are usually used as donors.

In order to maximize resource utilization and reduce the number of animals required, we aimed at including retired breeding sows in these analyses as refinement strategy. This presented logistical challenges regarding housing adaptation and surgical organ procurement. Specifically, the implementation of inhalational anesthesia with intubation and thoracotomy itself in large sows (350-400 kg) required careful consideration. A collaborative, multidisciplinary team – including scientists, animal caretakers, veterinarians and a cardiothoracic surgeon experienced with porcine thoracotomy – was assembled. Extensive planning, incorporating contingency measures and direct consultation with regulatory authorities guided the process. Procedures encompassed acclimation, pre-sedation, monitored transport, anesthesia including intubation, analgesia, thoracotomy and preparation of the lungs for EVLP with human blood. Managing intubation and adequate ventilation proved critical. All challenges were overcome and excellent lungs were used in EVLP experiments.

This study demonstrates the viable and ethical utilization of retired breeding sows for EVLP. The procedure proved comparable in feasibility and organ quality to younger swine, offering a valuable option for maximizing the use of genetically defined animals and promoting the refinement and reduction in xenotransplantation research.

References:

1. Stoerzer S, Kruszona S, Wand P, Linge H, Zlatev H, Hoeffler K, Singh J, Roters N, Muth V, Tavil S, Saipbaev A, Cvitkovic K, Kues WA, Zardo P, Ius F, Mengwasser J, Splith K, Schmidt-Ott KM, Goecke T, Schwinzer R, Niemann H, Ruhparwar A, Schmelzle M, Ramm R, Felgendreff P. Advances in Xenotransplantation: Evaluation of αGal-KO Porcine Livers and Lungs Using Normothermic Machine Perfusion in a Collaborative Perfusion Hub. Transpl Int. 2025 Mar 7;38:13781. doi: 10.3389/ti.2025.13781. eCollection 2025.

EDUCATION and TRAINING FOR THE FUTURE: SKILLS, ETHICS & MENTORSHIP

ONE WELFARE: AN ATTEMPT TO LINK EVERYTHING

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The concept of One Welfare recognises the interconnectedness of animal welfare, human wellbeing, and environmental sustainability. Building upon the principles of One Health, One Welfare encourages a holistic and collaborative approach to challenges that arise at the interface of humans, animals, and the environment.

Within Laboratory Animal Science, the One Welfare approach offers an opportunity to strengthen dialogue and collaboration between researchers, veterinarians, animal care staff, policy makers, and society at large. By breaking down traditional disciplinary boundaries, it promotes a culture in which scientific quality, animal welfare, staff wellbeing, and societal responsibility are considered together rather than in isolation.

This keynote presentation will explore how One Welfare can contribute to a more sustainable and ethically responsible framework for the use of animals in science. It will reflect on the opportunities and challenges of integrating welfare, health, and environmental considerations, while striving to achieve the best possible balance between scientific progress, animal wellbeing, human wellbeing, and care for the environment.

THE EUROPEAN COLLEGE OF LABORATORY ANIMAL MEDICINE: TRAINING, CERTIFICATION, AND PROFESSIONAL IMPACT

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Veterinary specialization in Laboratory Animal Medicine plays a pivotal role in advancing animal welfare and scientific quality across biomedical research institutions. The European College of Laboratory Animal Medicine (ECLAM), recognized and governed by the European Board of Veterinary Specialisation (EBVS), establishes and maintains high professional standards within this field through rigorous education, examination, and continuing professional development.

This presentation provides an overview of ECLAM's structure, objectives, and certification pathways, outlining the requirements for residency training and board qualification within the College. Key aspects of the residency program—including eligibility criteria, training supervision, learning objectives, and assessment of competencies—will be discussed, emphasizing how residents acquire advanced expertise in laboratory animal science, legislation, ethics, and research management. The talk will also describe the ECLAM certifying examination and the evaluation process used to ensure fairness, consistency, and alignment with EBVS standards.

Beyond credentialing, ECLAM fosters a professional network that supports continuous learning and collaboration across academia, industry, and regulatory environments. Being a Diplomate of ECLAM not only provides access to expert communities and recognition as a European Specialist in Laboratory Animal Medicine but also offers opportunities to mentor colleagues across Europe, contribute to the training of the next generation of specialists, and further develop leadership skills and expertise in education. Membership benefits thus extend from career development and professional credibility to direct contributions to the refinement, reduction, and replacement (3Rs) of animal use in research.

By contextualizing ECLAM within the broader framework of European veterinary specialization, this talk aims to highlight its unique contribution to harmonizing education and professional excellence among laboratory animal veterinarians, ultimately advancing both scientific integrity and animal welfare across Europe.

References:

1. Home – European Specialists in Laboratory Animal Medicine https://eclam.eu/

DISENTANGLING POSTGRADUATE EDUCATION FOR LABORATORY ANIMAL VETERINARIANS

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Postgraduate education will transition a general veterinary practitioner into a Laboratory Animal Veterinarian who may also fulfil the responsibilities of a Designated Veterinarian or Named Veterinary Surgeon.

A number of formal training routes are available in Europe and the UK, including Board Diplomas, Professional Certificates, and Postgraduate Degrees. These programmes will provide structured learning on the key elements of laboratory animal medicine and lead to a formal qualification. However, these programmes vary, for example, in syllabus, requirements, intensity and duration. The ECLAM Diploma is considered the highest qualification. Continuous professional development courses are topical and useful for developing core competencies and maintaining knowledge and skills, and rely on the veterinarian to make informed choices regarding their learning needs and knowledge gaps. Mandated training as per a country's legislation e.g., EU Functions A, B, C, D (Article 23 of EU Directive 2010/63) will be covered elsewhere and therefore not by this presentation. Examples of postgraduate programmes will be used to compare and contrast their respective benefits aiding new entrants in their choice of learning routes.

References:

1. Poirier GM, Bergmann C, Denais-Lalieve DG, Dontas IA, Dudoignon N, Ehall H, et al. ESLAV/ECLAM/LAVA/ EVERI recommendations for the roles, responsibilities and training of the laboratory animal veterinarian and the designated veterinarian under Directive 2010/63/EU. Lab Anim. 2015;49(2):89-99.
2. Home – European Specialists in Laboratory Animal Medicine https://eclam.eu/

MAPPING LABORATORY ANIMAL VETERINARIANS' EDUCATION IN EUROPE AND 3RS INTEGRATION: A 2026 PERSPECTIVE

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Veterinary education in Europe operates within a well-established quality-assurance framework coordinated by the European Association of Establishments for Veterinary Education (EAEVE). Of the 123 veterinary education establishments (VEEs) registered in Europe, 96 are EAEVE members and 84 currently hold accredited or approved status. Central to this framework is the ESEVT Standard Operating Procedure (SOP) 2023, which defines 38 Day One Competences (D1Cs) required to ensure that graduates can practise veterinary medicine safely and effectively. Laboratory Animal Medicine and the principles of Replacement, Reduction and Refinement (3Rs) are explicitly embedded within these competences, notably through addressing scientific research methods and 3R implementation (D1C 1.2), and focusing on the biology, welfare and behavioural needs of laboratory-housed animals (D1C 2.3).

This study aims to provide an updated and comprehensive overview of how Laboratory Animal Medicine and the 3Rs are integrated into European veterinary curricula in 2026, with particular emphasis on the translation of regulatory expectations into clinical and practical training. A dual methodological approach is employed. First, a systematic review of Self-Evaluation Reports from EAEVE-member VEEs based on SOP2023 is conducted to assess the structure and learning outcomes related to LAM and integration of 3Rs. Second, a targeted questionnaire is distributed to VEEs to collect current data on dedicated LAM training tracks, exposure to the "Designated Veterinarian" role, and the implementation of the "never the first time on a live animal" principle.

The findings are expected to reveal substantial variability in Laboratory Animal Medicine education and delivery models, alongside a growing reliance on skills labs and alternative training methods to reduce early-stage animal use. By mapping current practices, this study provides a strategic overview to support further harmonisation of 3R-based education across Europe. The findings invite participants to share experiences and reflect on transferability to their contexts.

MAPPING DESIGNATED VETERINARIANS' EDUCATION AND TRAINING REQUIREMENTS AD IN EUROPE

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Directive 2010/63/EU (1) classifies all personnel involved in the use of animals for scientific purposes and related functions. Art. 23 of the Directive states that personnel responsible for animal care, performance of procedures (including culling) and designing procedures, must possess appropriate education and training. In addition, the Directive requires establishments to appoint a 'designated veterinarian with expertise in laboratory animal medicine, or a suitably qualified expert where more appropriate.' However, specific requirements for education, training, competence and continuous professional development are determined at the level of individual Member State.

The presentation aims to provide a comprehensive overview of education and training requirements across different European countries for veterinarians to cover the role of Designated Veterinarian. It will examine how these requirements shape the professional roles and responsibilities of Designated Veterinarians.

By highlighting similarities and differences between national approaches, the presentation seeks to contribute to a broader discussion on the mobility of veterinarians within Europe and the potential for greater harmonization and competency standards.

References:

1. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes

FEDERATION OF EUROPEAN LABORATORY ANIMAL ASSOCIATIONS WORKING GROUP RECOMMENDATIONS ON “TRAINING FOR REPLACEMENT”

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Replacement is embedded in Directive 2010/63/EU as the long-term objective for scientific research involving animals. Despite rapid advances in non-animal technologies (NATs) and new approach methodologies (NAMs), the systematic implementation of Replacement remains limited and inconsistent across institutions, disciplines and career stages. Researchers, animal welfare bodies, ethics committees and project reviewers frequently report insufficient expertise in identifying, evaluating and adopting non-animal methods. Existing education and training resources are fragmented, often outdated, and tend to prioritise reduction and refinement rather than early and explicit consideration of replacing animal use altogether. Guidance on how to search for, assess and transparently document alternatives is similarly limited, constraining progress toward human-relevant research practices.

To address these gaps, FELASA (Federation of European Laboratory Animal Associations) Working Group on “Training for Replacement” developed a standardised, competency-based syllabus to support harmonised, high-quality education in replacement strategies. The syllabus defines core learning outcomes for participants, aligned learning objectives for tutors, and practical guidance for course structure, delivery, assessment and evaluation. It is organised into five interconnected sections: (1) introduction to replacement and the scientific, ethical and regulatory foundations of the 3Rs; (2) development and reframing of research questions to enable non-animal approaches; (3) identification of information sources and effective searching strategies, including bibliographic databases, grey literature and expert networks; (4) critical assessment of search results for scientific relevance, credibility and regulatory suitability; and (5) a practical, skills-based short course introducing selected NAMs in specific research contexts.

The framework is intended for a broad, multidisciplinary audience. This syllabus aims to strengthen confidence and competence in non-animal approaches, accelerate adoption of human-relevant methodologies, and support progress toward the long-term goal of phasing out animal use in research and testing.

DESIGNING FOR WELFARE: FACILITIES, TECHNOLOGY & SUSTAINABILITY

ACUTE MORTALITY IN IMMUNOCOMPROMISED MICE LINKED TO *PAENICLOSTRIDIUM SORDELLII*-INDUCED CYTOLYSIN-MEDIATED SEPTICEMIA

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Immunocompromised mouse strains, such as NOD.Cg-Prkdcscid-Il2rgtm1Wjl/SzJ (NSG) and NOD.Cg-Rag1tm1Mom-Il2rgtm1Wjl/SzJ (NRG), are invaluable in biomedical research. However, these strains pose significant health management challenges due to their susceptibility to infections with opportunistic bacteria and common pathogens. While breeding in strict hygienic barrier units is a prerequisite for maintaining the health integrity, these mice remain at risk after transportation to experimental barriers for research procedures.

We recently observed severe diarrhea and sudden deaths in three cohorts of NSG and NRG mice housed in an individually ventilated cage (IVC) system within an experimental barrier unit. Occasionally individual mice of immunocompetent, genetically engineered mouse strains showed also clinical symptoms. Both genders and all age groups were affected. Gross pathology revealed edematous to hemorrhagic intestinal inflammation, while histopathological analysis showed massive cell lysis of the intestinal epithelium. Intestinal content from affected mice was transferred into healthy NSG recipients via oral gavage, inducing a similar disease phenotype within 4-6 days, suggesting an infectious etiology.

Extensive microbiological and virological diagnostics ruled out an infection with common murine pathogens. However, *Paeniclostridium sordellii* was isolated from the blood, livers, kidneys, and intestinal contents of 13 out of 23 affected mice. PCR analysis revealed that the isolated strains consistently expressed a cholesterol-dependent cytolysin, indicating that fatal septicemia resulted from toxin-induced damage to the intestinal epithelium and subsequent intestinal hyperpermeability.

These findings highlight *Paeniclostridium sordellii* and other *Clostridiaceae* as emerging pathogens causing infectious diseases in immunocompromised mouse colonies, underscoring the need for enhanced surveillance and biosecurity measures in such high-risk populations.

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HEALTH SCREENING: GOING ANIMAL-FREE

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In support of the 3Rs and best practice in laboratory animal veterinary medicine, the University of Aberdeen reviewed its use of live animals for routine rodent health monitoring. The primary aim was to assess whether animal-free screening methods could replace sentinel animals while maintaining, or improving, the quality of diagnostic information required for veterinary oversight, colony health management and regulatory reporting.

Following evaluation of several alternative approaches, an adapted shake-cage method using a molecular sampling system (Ghost Sentinel®, FERA Science) was selected for in-house trials. This approach allows direct environmental soiled bedding sampling from cages, providing material suitable for comprehensive pathogen detection without the use of live sentinel animals.

Two comparative trials were conducted, comparing the existing soiled bedding sentinel programme with the Ghost Sentinel® method. Samples were collected from both open cages and individually ventilated cages (IVCs) and analysed by two independent diagnostic laboratories. An extensive panel of viral, bacterial and parasitic agents was tested in line with FELASA recommendations.

All agents previously identified through sentinel monitoring were also detected using the Ghost Sentinel® system. Importantly, direct molecular sampling showed greater consistency and sensitivity, enabling earlier and more reliable detection of pathogens. One agent not previously reported using sentinel animals was also identified. These findings have clear implications for veterinary risk assessment, outbreak management, health status reporting and decision-making regarding biosecurity and colony management.

This presentation will discuss the validation process, interpretation of results and subsequent implementation. The data demonstrate that animal-free health monitoring can enhance veterinary diagnostic confidence while supporting the 3Rs, particularly replacement. This approach has been successfully implemented at the University of Aberdeen since January 2024.

WHEN WELFARE HIDES IN PLAIN SIGHT: LATENT STATE MODELS FOR SEVERITY MONITORING

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Introduction: Current severity assessment in laboratory animal research relies on static thresholds or subjective scores that may fail to capture the dynamic nature of animal welfare[1].

Aims: We propose a Hidden Markov Model (HMM)[2] a probabilistic framework, that infers an animal's true welfare condition from continuous observations over time, enabling dynamic, uncertainty-aware monitoring.

Materials and Methods: Using the Relative Severity Assessment (RELSA) score[3] as input, which integrates body weight change, heart rate, heart rate variability, activity, and core temperature, the HMM estimates the likelihood of being in one of three welfare states (Well, Transition, Impaired). These estimates are converted into an intuitive traffic-light system with continuous risk curves and alarms for humane endpoints. We validated the framework using computer simulations (B = 50 replications, 300 animals each) across three scenarios and applied it to published dextran sodium sulfate (DSS)-colitis and transmitter-implantation mouse data.

Results: In controlled simulations, classification accuracy exceeded 90%, and confidence estimates were well-calibrated. Under high-noise conditions with overlapping state distributions, accuracy dropped to 46%, confirming that the model signals uncertainty rather than overconfident misclassifications. Application to experimental data revealed biologically plausible severity patterns: risk curves rose during disease flares and post-surgical periods, while controls remained predominantly in the Well state. Chi-squared analysis confirmed significant differences in state distributions between treatment groups (DSS: $\chi^2 = 38.4$; transmitter: $\chi^2 = 54.9$; both $p < 0.001$). Sensitivity analysis showed alarms remained stable across varying thresholds.

Conclusion: This framework enables real-time severity monitoring at the individual-animal level, providing probability-based risk curves and automated alarms to support timely intervention decisions. The approach extends from monitoring individual animals to laying the groundwork for broader welfare oversight within automated home-cage systems, providing a consistent basis for large-scale, ongoing welfare evaluation that advances the principles underpinning the 3R concept.

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LISTEN TO YOUR RABBITS WHEN DESIGNING FLOOR PENS FOR SOCIAL HOUSING NAIVE RABBITS

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In 2024, Inotiv UK created a new facility for social housing of New Zealand White rabbits. Prior to creating the new facility, our practice, in our breeding barrier, was to wean rabbits at five weeks of age into pairs or trios and maintain them in cage housing until the point of sale.

The new facility allowed rabbits to be housed in floor pens, with rabbits weaned directly into groups of forty rabbits. The pens were designed to allow rabbits to interact with each other across a 60,000 cm^2 area. A variety of enrichment is provided. Rabbits remain in these social groups until the point of sale, which is generally between ten and sixteen weeks of age.

Whilst floor pens have gained popularity in research facilities in recent years, we demonstrate their successful use by a commercial breeder. Rabbits are gregarious animals and are capable of forming complex social relationships with each other. Social housing has been shown to reduce stress-related behaviours.

In the two years since we began using the floor pens, we have seen both a positive effect on the rabbits and feedback from customers who have noted a calmer behaviour in the animals they have purchased.

From the original pen design to the current pen set-up, it has been a long dynamic process with continuous changes and improvements to meet the rabbits´ needs, learning from the rabbits their likes and dislikes. Examples of the changes include the pen material, pen height, food hoppers, hay nets, enrichment, and increased platform size and huts.

INTEGRATED ENVIRONMENTAL AND BIOLOGICAL MATERIAL MONITORING TO ADVANCE ETHICAL PRINCIPLES IN RESEARCH RODENT COLONIES

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Traditional rodent health monitoring using soiled bedding sentinels (SBS) suffers from low sensitivity, inconsistent performance, and animal welfare concerns. Strong supporting evidence for SBS effectiveness exists for only a limited range of pathogens, making it increasingly insufficient for today's laboratory animal programs. In contrast, Environmental Health Monitoring (EHM), including exhaust dust testing, sentinel-free soiled bedding, and direct colony sampling, provides a more sensitive, consistent, and efficient monitoring strategy. EHM is supported by a broader and more robust peer-reviewed evidence base and delivers clear operational advantages, such as reduced labor and cost, while eliminating the need for sentinel animals.

Simultaneously, the expanding use of biological materials, particularly patient-derived xenografts and diverse cell lines, has increased the risk of introducing microbial and viral contaminants into research animal colonies. When insufficiently monitored, these contaminants can compromise animal health, disrupt experimental outcomes, and undermine data validity and reproducibility. Such failures not only waste animals and resources but also threaten the integrity of the scientific work itself.

Integrating EHM with rigorous screening of biological materials represents a practical and impactful application of the 3Rs principles. Refinement is achieved through more accurate and proactive health monitoring; reduction results from preventing study failures driven by undetected infections; and replacement occurs through the elimination of sentinel animals. Together, these complementary strategies reinforce animal welfare, enhance scientific rigor, and strengthen institutional research integrity.

COMMUNICATION & TRUST: GLOBAL PERSPECTIVES FOR SCIENCE, SOCIETY

COMMUNICATING THE 3RS TO YOUNG PEOPLE

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The use of animals in research is controversial and often emotive. Young people develop values and opinions from parents, teachers and peers during adolescence when worldviews are actively formed and challenged. School curricula in many countries include studies in philosophy and ethics. Animal research is frequently discussed here because of its contentious nature. Anti-vivisection organisations often provide educational material for schools, however, input from those working in laboratory animal science is usually absent, which may result in unbalanced information.

Our institution strongly supports openness and transparency, as these are essential for building public trust. We believe opinions should be informed by accurate and sufficient information, therefore we have developed links with local schools and public institutions to inform teachers, young people and families about the animals we use in research and how we apply the 3Rs.

Our outreach activities include a science centre event for primary-school children (5-11), featuring interactive activities to help them understand how rodents perceive the world. These include following an ultraviolet trail to simulate urine marking and completing a jigsaw while wearing goggles smeared with petroleum jelly to demonstrate rodents reduced visual acuity. They also observe live wax-moth larvae under the microscope as an example non-vertebrate model.

We deliver workshops for secondary-school pupils (11-17), encouraging discussion around species choice, limitations of non-animal alternatives and refinement of housing and procedures. We run sessions for work-experience students aged 16+, covering the ethics of animal research, including the Mouse Exchange[1] as an interactive activity, and provide tours of our animal facilities. We assess students' views before and after activities using anonymous voting software. We consistently observe a shift towards increased support for animal research and reduced concerns following outreach events.

We are currently working with a Dutch institute to connect UK and Dutch students for discussions on animal research.

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ANIMAL RESEARCH, NAMS AND PUBLIC TRUST: WHERE ARE WE HEADING?

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New Approach Methodologies (NAMs) are rapidly advancing and gaining scientific, regulatory, and political attention. Organoids, organs-on-chips, AI, in silico approaches, and other human-relevant models are ready to provide valuable insights, and their development should already be seen as part of the 3R strategic implementation. However, the current debate often presents NAMs as a near-term replacement for animal research, neglecting their limitations and the ongoing need for animal studies in crucial biomedical areas.

This presentation will argue for a balanced approach. NAMs should be seen, by scientists and society alike, as part of a broader research toolbox, supporting replacement where possible and even answering some initial biological questions that once required animal research, making animal use much more targeted. Nevertheless, contrary to widespread misinformation, they cannot fully replace animal studies: they are not able to either reproduce whole-body responses or complex interactions among organ systems, which are essential in many fields such as basic research in immunology, neurosciences, endocrinology, infectious diseases, nor to validate translational research and safety assessment before clinical trials.

Misinformation shapes public and policy narratives. The talk will address distortions such as the “95% failure rate” claim and contrast them with public-ready evidence showing substantial concordance between animal and clinical findings. It will also include recent policy trends that risk reducing the 3Rs to replacement alone, potentially compromising responsible science and animal welfare if decisions go beyond scientific evidence.
The research sector must respond with clarity and openness. EARA is a global advocacy and communications association that aims to enhance public understanding of the continued necessity of animal research, the progress in welfare and the 3Rs, and the complementary nature of NAMs and animal research. We support our members in transparent communication and position it as the only way to proactively combat public misinformation about animal research.

Keywords: Animal research, NAMs, 3Rs, transparency, openness, science communication, policy, animal welfare, Europe+

A STEP FURTHER TOWARDS A TRANSPARENCY AGREEMENT IN GREECE

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National Transparency Agreements on animal research are important tools for promoting openness and strengthening public trust in the biomedical sector. To date, ten countries have established such agreements under the guidance of the European Animal Research Association. These initiatives bring together researchers and communication professionals to reduce barriers and encourage proactive communication about the use of animals in research.

We conducted a nationwide survey among public and private institutions in Greece that use animals for scientific purposes, in order to assess their motivation to participate in a Greek Transparency Agreement (TA). Facility managers were asked whether they would be willing to support the four core commitments of the TA, which focus on enhancing both internal and external communication, improving openness, and promoting the exchange of experiences and best practices.

The survey revealed strong positive feedback and a clear willingness to move towards the establishment of a national agreement. These preliminary results were presented at the FELASA 2025 Congress and at the EARA meeting in Berlin in 2025. Building on this momentum, the Hellenic Society for Biomedical and Laboratory Animal Science (HSBLAS) has joined this initiative, and discussions have begun regarding the practical steps required for the development of a Greek TA. In February, the first joint meeting was convened, bringing together representatives from EARA, HSBLAS, and Greek animal facilities. At least five facilities have already committed to participating in the initial implementation phase.

At the CELASC meeting, we will present the progress achieved towards the establishment of a Transparency Agreement in Greece, along with additional data from our survey regarding the species used in research and their relative frequency. These findings represent an important step towards fostering a culture of openness and coordinated communication at the national level.

LINKING PLANNING, PRACTICE, AND REPORTING: THE EQIPD QUALITY SYSTEM AS A FRAMEWORK FOR HIGH-QUALITY ANIMAL RESEARCH

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Planning as the foundation for quality animal research

High-quality animal research begins with robust planning, supported by early and structured dialogue between research groups and animal facilities. Such engagement is essential for aligning scientific objectives with animal welfare considerations, refining experimental design, and anticipating operational risks. Guidance such as PREPARE[2] provides a strong foundation by promoting shared responsibility and transparency at the outset of animal studies. However, planning alone cannot ensure consistent quality throughout study execution and reporting.

The EQIPD Quality System for research practice

The EQIPD Quality System[1] was developed as a flexible framework to assure quality during research practice, where many critical decisions influencing scientific rigor and animal welfare are made. It embeds structured yet proportionate processes for study conduct, documentation, data integrity, and risk-based decision-making. By translating well-prepared study plans into traceable everyday practices, the EQIPD Quality System supports consistency, scientific robustness, and feasibility in academic and pre-clinical animal research environments. In doing so, the EQIPD Quality System has been implemented across diverse laboratory types and research settings worldwide, demonstrating its adaptability and relevance in different institutional and cultural contexts.

Enabling transparent reporting and long-term impact

Complete and transparent reporting is essential to maximize the scientific value of animal studies and avoid unnecessary duplication. The EQIPD Quality System facilitates alignment with ARRIVE Guidelines[3] by ensuring that key methodological and analytical details are systematically captured during the study rather than reconstructed retrospectively.

By linking planning, practice, and reporting, the EQIPD Quality System strengthens rigor, supports the 3Rs, and provides laboratory animal science with a coherent framework for sustainable, high-quality research.

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2. PREPARE https://norecopa.no/PREPARE
3. ARRIVE https://arriveguidelines.org/

HUNGARIAN PLATFORM OF INSTITUTIONAL ANIMAL WELFARE BODIES: MISSION, STRUCTURE, AND NATIONAL IMPACT

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The Hungarian Platform of Institutional Animal Welfare Bodies (HUPAWB) is a nationwide umbrella organization that was established in 2022 with the aim of serving academic and industrial institutional AWBs to harmonize and improve laboratory animal science across the country. HUPAWB enables the exchange of experiences and best practices between member AWBs, fostering mutual learning and methodological development. As such, HUPAWB members have created a unified operating framework, defined harmonised guidelines at the national level, and took the leading role in promoting modern laboratory animal science emphasizing the 3Rs and new alternative methods.

Currently HUPAWB has around one hundred members who are appointed representatives of the local AWBs of their institutions and are directly involved in the first-stage ethical review process of the (ethical) project licence applications involving procedures on laboratory animals. Members may also act as advisors for researchers throughout the application process. The platform regularly holds online meetings and an annual conference in person. Recently, a new website has been launched (www.omab.hu), which serves to inform scientists and technical staff on advancements in the field and support advocation and communication on animal science and welfare. In this context, members actively participate in the nationwide *Researchers' Night* program series and in similar events. In 2024, the HUPAWB joined the European Network of National Networks of Animal Welfare Bodies (ENAWB), to strengthen our national and international partnerships, expand outreach and communication especially to students of high-schools and universities and to the general public.

THE POLISH NETWORK OF ANIMAL WELFARE BODIES: EARLY EXPERIENCE AND FUTURE DIRECTIONS

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Animal Welfare Bodies (AWBs) are entrusted with a broad range of responsibilities related to the welfare of animals used for scientific purposes, including advisory and oversight functions. In practice, their effective fulfilment is often challenged by a high workload of AWB members and the absence of formal institutional recognition for animal-welfare-related duties. As a result, AWB structures and practices vary significantly across Polish institutions.

In April 2025, 10 years after the implementation of Directive 2010/63/EU in Poland, the Polish Network of Animal Welfare Bodies (Polska Sieć Zespołów do spraw Dobrostanu Zwierząt) was established as an informal, bottom-up initiative to strengthen AWB performance nationwide and facilitate integration into European structures. To date, more than 40 AWBs – approximately one third of those operating in Poland – have joined the network voluntarily.

The network's core activities focus on facilitating the exchange of experience and good practices, harmonising operational procedures, providing mutual scientific, ethical, and regulatory support, and acting as a coordinated platform for dialogue with national authorities. As an initial step, a detailed survey was conducted among member AWBs to assess current practices, identify effective organisational models, and determine key barriers to optimal functioning. The survey findings will inform the establishment of thematic working groups tasked with developing practical tools to support AWBs in their statutory responsibilities. These activities will culminate in the first hands-on workshop for AWB members, organised in cooperation with the Polish Laboratory Animal Science Association (PolLASA) in September 2026.

Shortly after its formation, the Polish network joined the European Network of National Networks of Animal Welfare Bodies (ENAWB). Participation in the ENAWB provides a framework for strengthening national systems, harmonising practices across the EU, and promoting the 3Rs through cooperation.

EUROPEAN NETWORK OF NATIONAL ANIMAL WELFARE BODY NETWORKS (ENAWB): SUPPORTING COLLABORATION AND CULTURE OF CARE IN EUROPE

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Animal Welfare Bodies (AWBs), mandated by Directive 2010/63/EU, play a key role in animal welfare oversight, implementation of the 3Rs (Replacement, Reduction and Refinement), and promotion of a strong Culture of Care within establishments using animals for scientific purposes. Experience from several European countries shows that structured national networks of AWBs strengthen these functions by enabling exchange of best practices, improving internal processes, supporting staff wellbeing, and contributing to scientific quality. However, national AWB networks are not yet established in many countries, limiting opportunities for collaboration and shared learning.

At the 2022 FELASA Congress, a dedicated session highlighted the value of national AWB networks in fostering a Culture of Care across Europe. Representatives of the existing networks subsequently initiated the European Network of National Animal Welfare Body Networks (ENAWB) as an informal umbrella organisation to facilitate bottom-up, voluntary collaboration among national AWB networks. Currently, thirteen countries are represented in the ENAWB.

ENAWB, with the support of the European Commission, aims to promote the establishment of national AWB networks where they do not yet exist, support the further development of established networks, and encourage greater consistency in AWB practices across Europe. The network provides a platform for exchange of information and experiences between AWBs, sharing of good practices and innovations, and joint reflection on common challenges related to animal welfare, the 3Rs, scientific quality, and staff wellbeing. ENAWB also contributes to the dialogue with animal welfare professionals, Competent Authorities, National Committees, and relevant European organisations.

By strengthening collaboration between AWBs at national and European levels, ENAWB supports effective implementation of Directive 2010/63/EU and reinforces a shared, sustainable Culture of Care across European animal research establishments, ultimately serving both animal welfare and scientific integrity.

ENAWB's website is currently hosted by Norecopa: https://norecopa.no/ENAWB

3Rs IN ACTION: FROM ETHICS TO IMPLEMENTATION

LIP SERVICE, OR REAL IMPROVEMENT? USING GUIDELINES INTERACTIVELY ON THE PATH TO BETTER SCIENCE

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Are scientists merely paying lip service to funders and journals when they cite guidelines for planning, conducting and reporting animal studies? And if so, can we make them more aware of (and more enthusiastic for) the immense amount of guidance that the Laboratory Animal Science community can now offer?

A casual glance at publications describing animal research shows that the existence of guidelines for planning, conducting and reporting animal studies is, unfortunately, no guarantee of research quality. How can we encourage scientists to look upon these resources as tools for better science, rather than buzz words to mention, or hurdles to pass, on the road to funding and publication?

Norecopa has spent considerable resources on developing generic guidelines for planning studies which appear to involve animal use, and then directing scientists to more specific guidance for their individual needs.

This presentation will describe how the PREPARE guidelines can be used from day 1 of planning to implement all three Rs (Replacement, Reduction, Refinement), in such a way that scientists realise the value of paying more than lip service to these principles.

The presentation will also present novel ways in which Artificial Intelligence can be combined with recognised resources within Laboratory Animal Science to raise scientists' awareness of their need for improved quality control, and to provide them with the guidance they need to achieve this.

References:

1. https://norecopa.no/PREPARE

EFFECTS OF REPEATED VOLATILE ANESTHESIA IN LABORATORY MICE AND RATS

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Volatile anesthetic gases are widely used in *in vivo* research due to their rapid induction, controllability, and favorable safety profiles. Isoflurane is the most commonly used agent, yet its pungent odor has raised concerns regarding aversion and discomfort, particularly during repeated exposures. Sevoflurane, which lacks a pungent smell, has been proposed as a potential alternative, although its suitability for repeated anesthesia in mice and rats remains insufficiently characterized.

In this study, we investigated the effects of repeated exposure to isoflurane and sevoflurane, in laboratory mice and rats. Male and female individuals of both species (BALB/c, C57BL/6J, Wistar and Sprague Dawley) aged ten weeks were exposed three times to the same anesthetic gas. Aversion and respiratory parameters were assessed using whole body plethysmography during baseline, induction, maintenance, and recovery phases. Loss of posture was used as an indicator of anesthetic depth. Blood samples were collected after each exposure, and animals were euthanized after the final session for tissue and organ analyses.

Our preliminary results in mice showed no sex related differences in time to loss of posture. Strain dependent differences were observed during the first exposure to isoflurane and sevoflurane, but these differences were no longer present during subsequent exposures.

Our upcoming findings could highlight important strain dependent responses to repetitive volatile anesthetics exposure and their potential distress induced in mice and rats. Ongoing analyses of behavioral, respiratory and stress related parameters will further inform potential refinement strategies for repeated volatile anesthesia in laboratory mice and rats.

DEVELOPMENT OF MONITORING SHEETS FOR SHEEP AND PIGS UNDERGOING CARDIAC SURGERY

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Correct follow-up of research animals is essential to refine experiments and reduce pain and suffering. Standardized sheets facilitate this process. Although the term score sheet is more often used, we have decided to use the term monitoring sheet as score sheets often imply a numerical scoring system which is not always easy to use. Besides, our primary aim was to define actions to take when a single parameter deviated, and not on a sum of scores.

To develop the monitoring sheets for pigs and sheep undergoing cardiac surgery the following people were involved: the caretakers, technicians and researchers closely involved in these experiments, and the designated veterinarian. Together, we defined which clinical signs can be seen, how often these should be assessed and which action should be taken when a parameter deviates from normal.

The following parameters were included for pigs and sheep: food intake, general condition/activity, pain, bleeding, infection, breathing, and oedema. For the pigs we also included cyanosis as this is sometimes seen in the model of myocardial infarction. As in sheep valves and cardiac assist devices are implanted, these are also included for the sheep.

These monitoring sheets were then presented to the Animal Welfare Body and subsequently approved by the institutional Ethical Committee. The monitoring sheets must now be used in all projects involving this type of experiments.

A RETROSPECTIVE: SEVERITY ASSESSMENT IN MICE USING BODY WEIGHT AND VOLUNTARY WHEEL RUNNING

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Since the entry into force of Directive 2010/63/EU, the legal classification of procedure severity has become mandatory, both for prospective project authorization and for retrospective reporting of the actual severity experienced by animals. Although Annex VIII of the Directive provides examples of expected severity categories, it offers limited guidance on how these were derived. To enable an evidence-based comparison between prospective and actual severity, this study retrospectively analyzed body weight and voluntary wheel running (VWR) data from several mouse models.

Data from approximately 300 C57BL/6 sub-strain mice subjected to interventions such as blood sampling, surgery, and disease models (pancreatic cancer, colitis) were analyzed. Severity was assessed using clinical score sheets including changes in body weight and VWR monitoring. An algorithm (RELSA) was then applied for severity quantification and comparison with both the prospective severity classification from the authorization process and the actually reported severity.

The retrospective analysis revealed a more differentiated classification of procedures than provided by the legal framework. As expected, mice that were only handled exhibited the lowest severity scores, whereas animals subjected to invasive surgeries showed the highest scores. Although all reviewed procedures had been prospectively classified as “moderate” according to official requirements, the analysis identified transitions between low and moderate severity that are not explicitly addressed in current guidelines. This transition zone is particularly relevant for timely definitions of humane endpoints and discontinuation criteria. Although the prospectively assumed maximum severity was confirmed, this was not consistent across all animals.

Overall, these findings suggest that current legal severity classifications may not fully reflect the actual severity experienced by animals. The integration of objective behavioral readouts and algorithm-based tools into routine severity assessment could improve the accuracy of project evaluation, support refinement strategies, and strengthen the practical implementation of the 3Rs.

SHAPING LAS EDUCATION – FELASA'S COMMITMENT TO THE 3RS

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FELASA (Federation of European Laboratory Animal Science Associations) accreditation is a key driver in improving the quality, ethical integrity, and consistency of laboratory animal science training across Europe and beyond. It ensures that courses and training programs in laboratory animal sciences align with Directive 2010/63/EU and the latest expert working group recommendations, which require all personnel involved in animal research to be properly trained.

FELASA has updated its accreditation framework to strengthen the importance of constructive alignment between learning outcomes, teaching methods, and assessment, while promoting high ethical standards and robust internal and external quality-assurance processes. These measures foster transparency, mutual confidence, and the seamless recognition of qualifications across institutions. The new approach also underscores how accreditation supports the 3Rs (Replacement, Reduction, Refinement). It does so by encouraging tiered training pathways, realistic and ethically responsible learning environments, and structured assessments that help learners build and demonstrate essential skills.

FELASA WORKING GROUP RECOMMENDATIONS FOR THE HUMANE KILLING OF LABORATORY FISH

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The Directive 2010/63/EU lists some methods to kill laboratory fish and was amended to add hypothermic shock, in specific conditions, for zebrafish only. Scientific projects may require the use of other techniques to achieve their scientific needs. However, environmental impact, health and risks, developmental stages and impact on animal welfare must also be considered when choosing the method of euthanasia. Following the first technique to kill fish, to comply with the directive, it is essential to perform a second method to complete and confirm the death of the animal. The FELASA Working Group on methods of humane killing of laboratory fish published their recommendations, reflecting on what would constitute an acceptable death, proposing comparisons between methods, and describing practical approaches to comply and refine according to the species, developmental stages, and context. Here, we will review experimental data pilar to these recommendations (such as efficacy and aversion of anaesthetics, hypothermic shock, electrical stunning, and concussion – percussive blow to the head), compare the methods, and focus on common and useful considerations for zebrafish according to developmental stages. Noticeably, we will differentiate between the termination of a colony when samples are not required, versus examples of killing a fish to obtain and culture biological material.

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3Rs IN ACTION: FROM ETHICS TO IMPLEMENTATION

THE MI-RAT, A NOVEL MURINE MODEL FOR THE STUDY OF OSTEOARTHRITIS

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Osteoarthritis, the leading cause of chronic joint pain, is studied through different animal models, but none of them is ideal in terms of reliability, reproducibility and translational value. The OA rat model has been used more extensively in the past years because it offers structural and functional pain measure outcomes. However, there are a plethora of models available. There is no consensus on which is the most be translational model The surgical models that are the most widely used are destabilization of the medial meniscus (DMM) and anterior cruciate ligament transection (ACLt). The objective of the first study was to evaluate the structural, functional and molecular (spinal neuropeptides) outcomes of 3 murine surgical models of osteoarthritic pain (DMM, ACLt and DMM/ACLt) and 1 chemical model (MIA). The DMM, ACLt and MIA models induced temporary alterations but not convincing structural changes. The DMM/ACLt combination induced more persistent functional alterations, correlated to structural and molecular changes[1]. Subsequent studies using this model confirmed the influence of sexual hormones, particularly estrogen, in pain control, and the necessity to work on ovariectomized females. The introduction of calibrated regular exercise to the DMM/ACLt led to the Montreal Induction of Rat Arthritis Testing (MI-RAT) as being translationally validated for homogeneous structural alterations, and corresponding functional and molecular pain signals[2,] including epigenetics[2.] Moreover, the application of quantitative sensory testing to MI-RAT offers a non-invasive assessment of facilitatory/inhibitory pain processes[3,] In conclusion, these recent developments and validation of the murine MI-RAT enlarged our perspectives in a refined translational osteoarthritis model.

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BEFORE THE MOUSE, THE MODEL: HYPER-REALISTIC SIMULATION AS A RODENT TRAINING REFINEMENT STRATEGY

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Introduction: Training in rodent biomethods and surgical techniques has traditionally relied on live animals or cadavers, raising ethical concerns and limiting opportunities for repeated, standardized practice. This approach conflicts with the principles of the 3Rs and can expose both animals and trainees to avoidable risk during early skill acquisition.

Aims: To evaluate hyper-realistic simulation-based training as a refinement strategy for rodent biomethods and surgical education, to improve competency while reducing reliance on live animals.

Materials and Methods: A series of anatomically accurate, task-specific rodent simulators was designed and validated to support training in commonly performed biomethods and surgical procedures. Models were developed using a combination of 3D printing, silicone molding, and soft plastic casting to achieve high anatomical fidelity, durability, and low cost. The modular design allows task deconstruction, repeated practice, and progressive skill development. Simulators were integrated into a structured training program through learning objectives and disseminated through hands-on workshops in Europe, Latin America, and the US.

Results: To date, more than 500 technicians, researchers, and veterinary professionals have been trained using these models. Participants consistently reported increased confidence and preparedness prior to performing procedures in animals. Quantitative assessments conducted for selected models demonstrated improvements in procedural accuracy, reduced error rates, and improved task efficiency following simulator-based training. Qualitative feedback highlighted the perceived anatomical realism of the models and the value of a safe, repeatable learning environment that supports early skill development without animal use.

Conclusion: Hyper-realistic rodent simulators represent an effective refinement for training in rodent biomethods and surgery, reducing and in some cases replacing the need for live animals during early training stages. Their implementation promotes ethical practice, standardizes instruction, and supports global dissemination of best practices aligned with the 3Rs. Simulation-based training should be considered a core component of modern, competency-based laboratory animal science education.

ULTRASOUND GUIDED SERRATUS PLANE BLOCK IN PIGS: ANATOMICAL STUDY AND DESCRIPTION OF THE TECHNIQUE

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Experimental pigs undergoing thoracic surgery require a multimodal analgesic approach. The serratus plane (SP) block is a fascial anaesthetic technique that, in humans and in dogs[1], provides effective analgesia of the chest wall[2].

The aim of this study was to evaluate the normal and the ultrasound anatomy of the serratus plane in pigs, and to describe the technique for performing the SP block.

Seven commercial hybrid pig cadavers (mean weight 35.6kg) were included. An anatomical study was first performed in one cadaver to define the anatomical features and to identify the anatomical landmarks. In the six remaining cadavers the ultrasonographic examination and the injection of the staining solution were performed in one hemithorax. With the animals in lateral recumbency, a linear probe was positioned on a line parallel to the spine and at the level of the scapulo-humeral joint as a reference.

The deep SP was then identified as a hyperechoic line above the external intercostal muscles; ropivacaine (0.25%) 0.3 ml/kg diluted with methylene blue (3:1 ratio) was injected at the 6th and 9th intercostal spaces. The anatomical dissection highlighted the distribution of the injectate between the intercostal muscles, and both beneath and superficial to the SP. In two cases the spread of the dye into the pleural space was observed.

The described technique was feasible in pigs, and the spread of the solution suggests that it might provide thoracic wall analgesia trough sensory block of the lateral cutaneous branches of the thoracic spinal nerves and the long thoracic nerve as described in other species[1]. Strict adherence to the reference landmarks is pivotal for the success of the block. Further studies are needed to understand the in vivo effectiveness of this technique for thoracic analgesia, thereby providing a significant refinement for perioperative pain management in pigs.

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NEW APPROACH METHODOLOGIES (NAMS): A THREAT OR A CHALLENGE FOR LABORATORY ANIMAL VETERINARIANS (LAVS)

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NAMs are an umbrella term for any technology or approach that can provide information on chemical hazards or biological effects without using animals, or by reducing their number or refining experimental procedures. Cell & tissue models, organoids, organs-on-chips, in silico models, and human-based data may be used to obtain data that are more human-relevant, faster, and often cheaper, while improving animal welfare.

The rise of NAMs presents several existential and practical challenges for the LAV community, which could be considered as a professional threat. These are linked to: (a) Career Displacement: The most obvious fear is the reduction in the "patient" population. (b) Skill Gap Re-tooling: Many LAVs spent a decade mastering animal surgery, anesthesia, and colony health. NAMs require knowledge and skills not traditionally taught in veterinary school. (c) Funding Shifts: Research grants are increasingly earmarked for "animal-free" innovation. (d) Identity Crisis: There is a deeply rooted professional identity in being the "voice for the animals" within a research institution. If there are no animals, the veterinarian must redefine their value proposition to the scientific team. Although this is a hard truth, there is also another option, much more promising for LAVs.

New Approach Methodologies (NAMs) can be considered as an invitation for LAVs to play a broader role as ethical decision-makers, helping determine when animal use is truly necessary and advising on 3Rs implementation. They can also serve as translational science experts, interpreting NAM data in the context of whole-organism physiology and guiding study design to maximize welfare and scientific rigor. Furthermore, NAMs open new career avenues in organoid research, computational biology, regulatory science, and 3Rs leadership. Rather than being replaced, veterinarians may become the main players in integrating NAMs responsibly, ensuring both ethical and high-quality biomedical research.

ANAESTHETIC PROTOCOL SELECTION IN LABORATORY ANIMAL RENSEARCH: ALIGNING ANIMAL WELFARE AND SCIENTIFIC REQUIEREMENTS

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The selection of anaesthetic and analgesic protocols in laboratory animal science is a major refinement opportunity, as anaesthetic agents exert wide-ranging effects on physiology that can influence both animal welfare and the validity of scientific outcomes. Across commonly used laboratory species, anaesthesia should therefore be regarded as a biologically active component of the experimental system rather than a neutral technical prerequisite.

Different categories of scientific procedures impose distinct physiological constraints that should guide protocol selection. In studies involving the central nervous system, including neurosurgery, neurophysiology, and imaging, anaesthetic depth and agent choice can markedly affect cerebral blood flow, neurovascular coupling, and neuronal network activity, with potential consequences for data interpretation if these effects are not carefully controlled. Similarly, in cardiovascular and cardiorespiratory research, many routinely used anaesthetic agents alter heart rate, vascular tone, blood pressure, and respiratory function in a dose and species-dependent manner, risking confounding of primary endpoints if haemodynamic stability is not prioritised. Anaesthetic choice is also increasingly recognised as relevant to immunology, inflammation, and infectious disease models. Both volatile and injectable agents have been shown to modulate innate and adaptive immune responses, influence cytokine release, and interact with peri-procedural stress pathways. Where immune parameters constitute key experimental outcomes, the consistency of anaesthetic exposure and the explicit justification of the protocol choice are therefore essential.

Adopting a procedure-led approach to anaesthetic selection, informed by the dominant biological system under investigation, supports refinement by improving welfare during induction, maintenance, and recovery, while simultaneously enhancing scientific robustness and reproducibility. Aligning anaesthetic practice with the specific physiological vulnerabilities of each experimental model provides a pragmatic framework for advancing both ethical and scientific standards in laboratory animal research

REPEATED ISOFLURANE ANAESTHESIA AS A WELFARE AND SCIENTIFIC VARIABLE IN MICE

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Introduction: General anaesthetics are widely used in biomedical research, yet increasing evidence shows that their effects on the brain and related systems extend beyond transient loss of consciousness. Although the impact of single anaesthetic exposures has been extensively examined, the biological consequences of repeated anaesthesia remain poorly defined.

Aims: This study aimed to determine how brief, repeated isoflurane exposures influence behaviour, cerebrovascular function, and microglial phenotype in adult mice, and to identify sex-dependent differences in these outcomes.

Methods: Adult male and female CD1 mice underwent a series of short isoflurane exposures replicating common laboratory protocols. Behaviour was assessed using open-field locomotion, nesting, and burrowing tests. Cerebral blood flow, endothelial markers and glial states were analysed to characterise vascular and immune responses.

Results: Repeated anaesthesia induced distinct behavioural, vascular, and cellular changes. Females showed reduced locomotor activity, whereas both sexes exhibited impaired nesting and burrowing performance. Cerebrovascular responses were affected by anaesthesia, but not directly by the repeated exposure. But repeated anaesthesia altered endothelial marker expression and shifted microglia toward a hyporeactive phenotype.

Discussion/Conclusions: These findings show that even short, routine anaesthetic exposures lead to meaningful alterations in neural, vascular, and immune systems. Anaesthetic regimen is therefore an important experimental variable that warrants careful consideration to improve reproducibility and interpretation in preclinical research.

WEB-BASED PLATFORMS ARE A VALUABLE TOOL FOR MANAGING PRECLINICAL ANIMAL STUDIES

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Web-based platforms offer features such as data capture, reporting, and collaboration, all of which are crucial to the success of preclinical research. These platforms are keen to reduce administration and fit compliance with regulatory standards.

The contemporary ecosystem of web-based systems for preclinical research can be divided into five categories: (1) GLP-grade preclinical study suites, (2) enterprise Electronic Laboratory Notebook (ELN)/Laboratory Information Management Systems (LIMS) with in-vivo modules, (3) non-GLP operational workflow managers, (4) academic preregistration registries, and (5) web-based analytical/visualization engines.

Across categories, EU on animal use (Directive 2010/63/EU) gives the 3Rs legal force. Digital systems can operationalize the 3Rs in concrete ways: preregistration reduces duplication (Reduction) and sharpens design details that promote humane, bias-minimized study conduct (Refinement); enterprise platforms with integrated vivarium links, dosing histories, and threshold alerts help codify humane endpoints and severity tracking (Refinement); and modules that facilitate justified use of historical controls can lower concurrent control animal counts where scientifically appropriate (Reduction). Software cannot deliver Replacement on its own. Modern digital platforms increasingly support Reduction by enhancing planning, data sharing, and analytical power—helping research become both more humane and more scientifically efficient.

Together, these categories are reshaping academic preclinical research by aligning digital practice with reproducibility, 3R obligations, and, where pursued, regulatory readiness.

Modern preclinical research has become increasingly data-intensive, regulation-heavy, and collaborative, making traditional spreadsheet-based or paper-based workflows insufficient. Web-based platforms now play a growing role in ensuring study integrity, reproducibility, and operational efficiency across animal research. As preclinical studies continue to grow in scale and complexity, these tools provide the backbone for efficient, ethical, and scientifically robust animal research.

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SUPPORTING THE 3RS BY INCREASING CATHETER PATENCY DURATION IN RODENTS

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Vascular access is a key element in many preclinical studies. In chronic protocols involving repeated administration of compounds, blood sampling, or both, maintaining a prolonged catheter patency duration is essential. Several factors influence the catheter functionality, the most critical being the accurate positioning of the catheter tip at an optimal anatomical site. The use of a closed system is another crucial determinant; closed systems incorporating transcutaneous buttons have been shown to significantly extend catheter patency performance. Catheter material and tip design also play important roles. Polyurethane is widely considered the material of choice, while a rounded tip minimizes endothelial damage and reduces the risk of thrombus formation. Strict adherence to aseptic technique and the use of sterile solutions are essential to prevent infection and biofilm formation, thereby promoting an unobstructed catheter flow. In addition, employing a positive pressure technique during catheter locking decreases the likelihood of intraluminal clot formation. Collectively, these factors must be carefully considered and consistently applied to achieve optimal catheter patency duration.

Beyond extending the openness period of the catheter, the transcutaneous button offers additional advantages that support the principles of the 3Rs. With respect to Refinement, the protective metal cap covering the button enables group housing, improving animal welfare and respecting rodents' natural social behaviors. The transcutaneous button also reduces animal handling and associated stress, contributing to enhanced well-being and better data quality. In terms of Reduction, prolonged catheter patency duration decreases the number of animals subjected to surgical procedures and enrolled in studies, thereby supporting more ethical and efficient chronic research designs.

EDUCATION and TRAINING FOR THE FUTURE: SKILLS, ETHICS & MENTORSHIP

YOU CAN'T CHOOSE WHAT YOU'VE NEVER SEEN: REINTRODUCING LABORATORY ANIMAL MEDICINE TO VETERINARY EDUCATION PROGRAMS

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Introduction: Laboratory animal medicine is underrepresented in veterinary education, as curricula are predominantly focused on companion and food animal practice. Consequently, veterinary students complete their education with little to no exposure to the field. This limited visibility contributes to persistent misconceptions and stigma surrounding animal research, including misunderstandings about animal care, regulatory oversight, and the veterinarian's role in safeguarding animal welfare. These factors contribute not only to declining recruitment into laboratory animal medicine but also to broader gaps in professional understanding among veterinary graduates.

Aims: To develop and evaluate a 4-week rotation in laboratory animal medicine designed to increase student exposure, address misconceptions about animal research through direct, mentored experience, and improve understanding of the ethical, regulatory, and clinical frameworks governing laboratory animal care.

Materials and Methods: A 4-week final-year clinical rotation in laboratory animal medicine was developed for students from our institution's college of veterinary medicine. The rotation fulfilled core professional activities while providing comprehensive exposure to the specialty. Learning objectives, adapted from the ACLAM role delineation document, included IACUC protocol review, clinical care, anesthesia, surgery, necropsy, and facility management. Implementation required institutional support, strategic scheduling, and iterative refinement based on student feedback. Targeted outreach emphasized hands-on experiences and exposure to diverse species.

Results: Over three years, enrollment increased by 78%. Most students reported no prior exposure to laboratory animal medicine. Post-rotation feedback demonstrated high satisfaction, improved understanding of the field, and strong appreciation for the emphasis on animal welfare and respectful animal care. Hands-on clinical engagement and mentorship were consistently identified as key drivers of positive perception.

Conclusion: Distributive clinical education models can be leveraged to expand exposure to laboratory animal medicine while addressing misconceptions and improving recruitment. Structured, competency-based rotations offer a scalable strategy to educate veterinary professionals and strengthen the workforce pipeline in modern veterinary curricula.

OPTIMIZING RESOURCES: DO WE REALLY NEED A DIRTY NURSE IN EXPERIMENTAL SURGICAL PROTOCOLS?

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One of the obligations of a researcher is the responsible conduct of research. To attain this, one must try to optimize resources, may they be financial, material and/or human. A frequent and divergent discussion in research facilities is the use of dirty nurses in research protocols. A dirty nurse is defined as a person dedicated to the dirty (not sterile) part of the realization of an experimental surgical protocol in which we find a strict sterile part. A dirty nurse will realize the anesthesia, preparation, positioning of an animal in a surgical protocol, not to forget the postoperative recovery. The sterile nurse or surgeon will only perform the sterile part of the protocol including material set-up, surgery and preparation of the next surgery (new material, suture, instruments, etc.).

Should we include dirty nurses in studies or is a single person efficient at realizing all steps of an experimental protocol? I believe that if we want to optimize resources, the use of a dirty nurse is imperative. Instead of performing 6 surgeries in one day, we can perform 24 to 30 when using a dirty nurse. I will illustrate with a real time video, the choreography of the tasks performed by a team of 2 in a research setting using the destabilization of the medial meniscus (DMM) model in mice.

In this fashion, the best of care is conducted by the dirty nurse, including recovery surveillance, The cross contamination is minimized, thus reducing the post-operative infections. The realization of surgeries in one day also refines the post-operative evaluations which can all be performed at the same timepoints, minimized the bias of time in experimental protocols.

REFINE TO REDUCE: ANAESTHESIA AND TECHNIQUE

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Safe, well-designed anaesthetic protocols represent one of the most effective refinement strategies capable of directly reducing animal numbers in preclinical research. Mortality during anesthesia in healthy laboratory rodents is generally expected to be exceptionally low and is typically reported to be well below 1% (1). Yet preventable losses still occur due to inadequate preparation, insufficient monitoring, and avoidable physiological disturbances. Implementing structured refinement—beginning with the PREPARE and ARRIVE frameworks—reduces periprocedural mortality, improves data quality, and ensures that no animal life is unnecessarily wasted.

Three domains are central to achieving Reduction: optimised anaesthetic protocols, comprehensive monitoring, and proactive prevention of vagal inhibition. Modern anesthesia requires balancing the anesthetic triad—hypnosis, analgesia, and muscle relaxation—while recognizing that different agents contribute unevenly to these components. In laboratory rodents, inhalational agents such as isoflurane provide rapid titration but limited analgesia; therefore, thoughtful premedication and robust perioperative pain management are essential. The appropriate use of sedatives, opioids, and local anaesthetics minimizes stress responses, stabilizes physiological parameters, and reduces experimental variability, thereby lowering the number of animals required to achieve reliable outcomes.

Monitoring forms the second pillar of refinement. Continuous assessment of reflexes, respiratory and cardiovascular function, body temperature, and depth of anaesthesia—supported by pulse oximetry, temperature probes, ECG, or respiratory pillows—enables early recognition of complications. Preventing hypothermia, hypoxia, and hypovolemia is fundamental to both animal welfare and scientific validity.

Finally, vagal inhibition is an under-recognised but critical cause of anaesthetic mortality. Common stimuli such as laryngeal manipulation, ocular pressure, visceral traction, or α_2-agonist premedication may provoke severe bradycardia or asystole. Understanding these triggers, maintaining adequate anaesthetic depth, and having anticholinergic or reversal agents readily available are essential measures to prevent fatal events.

Refining anaesthesia, monitoring, and technique not only safeguards welfare but directly supports Reduction, ensuring that every animal contributes meaningful scientific value.

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CTRL+ALT+DELEGATE: LEVERAGING AI TOOLS TO BOOST NACWO, NIO, NTCO AND VETERINARIAN ROLES AT LABORATORY ANIMAL FACILITIES

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Introduction: Named Animal Care and Welfare Officers (NACWOs), Named Training and Competency Officers (NTCOs), Named Information Officers (NIOs) and Veterinarians are the cornerstone roles at each facility conducting animal research. These professionals ensure regulatory compliance, champion animal welfare and the 3Rs, overseeing staff training and competency and promoting a Culture of Care. Yet these vital professionals face mounting challenges: escalating administrative workloads, the demand for clear and engaging training content, and the complexity of maintaining effective communication across diverse stakeholder group which can all reduce the time dedicated to their core missions. Here we present concrete examples on how AI tools can help address these issues.

Material and Methods: At AstraZeneca, we embarked on an exploration of a set of different AI-powered tools to streamline operational demands and enhance the role of our named officers: automatically generate comprehensive compliance meeting minutes, accelerate the review and modernisation of regulatory training, and create diverse educational materials (including presentations, interactive quizzes, procedural checklists, and FAQ documents) directly from legislation, institutional policies and standard operating procedures. Additionally, these tools can synthesise complex animal welfare regulations and accreditation requirements into accessible summaries for the broader *in vivo* community. Building on these successes, we have expanded our AI toolkit to include further capabilities. AI-generated training videos provide information and training with engaging visual narratives, while specialized AI agents can assist in writing, review and improve regulatory documents (ex: Licences and permits, SOPs, Work instructions) consistent with national and European legislation and AAALAC accreditation requirements.

Conclusion: However, AI tools works best as a smart assistant, not a replacement for professional judgment. AI tools don't replace named officers but, when used thoughtfully with robust oversight, they free capacity for meaningful work requiring human compassion, expertise, and judgment, ultimately advancing animal welfare and the 3Rs.

IMPLEMENTING A HARMONIZED CONTINUING PROFESSIONAL DEVELOPMENT FRAMEWORK TO ENHANCE COMPETENCE OF ANIMAL RESEARCH PROFESSIONALS

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A structured continuing professional development (CPD) process for professionals involved in animal research is essential to ensure sustained competence, high standards of practice, and ongoing improvements in animal welfare and laboratory animal science. FELASA – Federation of European Laboratory Animals Sciences Associations – has published recommendations for a lean, transparent, and harmonised framework designed to improve CPD across all functions, roles, and responsibilities defined under Directive 2010/63/EU. Continuing professional development is conceived as a career-long process that begins after completion of basic training and achievement of initial competence.

Central to the framework is the creation of an individual continuing professional development portfolio, aligned with a personal development plan and regularly reviewed by the person responsible for training and competency. The portfolio provides a structured method for documenting learning activities, reflecting on professional growth, and assessing maintained competence. Institutions are encouraged to integrate this system into routine monitoring processes, with formal competency reviews recommended at intervals not exceeding 5 years.

The recommendations further address the scope and diversity of eligible development activities and discuss expectations regarding the minimum level of engagement required to demonstrate ongoing professional growth. To promote consistency and mutual recognition, essential information that should accompany certificates of participation is defined, enabling transparent evaluation of continuing professional development across organisations and countries.

Overall, implementation of a harmonised strategy for planning, recording, and reviewing continuing professional development supports professional mobility, strengthens institutional quality assurance, motivates staff, and fosters a strong culture of care, ultimately contributing to responsible, ethical, and high-quality animal research practices.

EMERGING JOINT REGIONAL 3RS CENTER IN SPLIT, CROATIA – COST ACTION (CA) IMPROVE INITIATIVE

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The CA IMPROVE initiative's goal in the last year of its scientific mandate (2026) is to establish a joint regional 3R center at the Medical Faculty Split (MEFST), University of Split, Croatia. The 3Rs centres and platforms are very important points of contact and play an important role in their respective countries as 'on the ground' facilitators of Directive 2010/63/EU. The joint 3R center aims to serve the Balkan region and its non-EU and EU member countries under the umbrella of European network of 3R Centers (EU3Rnet) by provision of continual professional development (CPD) opportunities in biomedical and Laboratory Animal Science (LAS) field. With its state-of-the-art laboratory animal facility and congress center, MEFST can facilitate international collaboration and act as a regional networking hub.

The joint 3R center aims to accelerate the implementation of the 3Rs and animal-free new approach methodologies (replacements) through expert advice and provision of 2010/63/EU aligned education and training tailored to the specific research and training needs and aims of each country or region, enabling conduct of high-quality scientific research throughout Europe. Furthermore, it aims to provide its members with expert consultations to help them steer through *in vivo* Project Applications' and Authorisations' requirements and *in vivo* project ethical evaluations. The center aims to host international and diverse stakeholders from academia, industry, legislative bodies, biomedical associations and like and they all stand to benefit from the center's short-term and long-term goals and deliverables compliant with 2010/63/EU requirements and best scientific and (laboratory animal) veterinary research practices.

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SMARTER SUBMISSIONS: AI-POWERED PRE-ETHICS REVIEW TO ENHANCE PROTOCOL QUALITY

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Introduction: Ethical review of animal research protocols is essential, yet committees often receive submissions with inconsistent quality, missing methodological details, and variable adherence to the Israeli Animal Welfare Law and the *Guide for the Care and Use of Laboratory Animals*. These gaps create delays and repeated revision cycles. AI tools offer an opportunity to improve early-stage review. Etiq-Tech aims to pair deterministic data validation with grounded AI guidance so that protocols arrive aligned with regulatory and refinement expectations while preserving committee authority.

Aims: To develop an AI-based co-reviewer that evaluates animal-use ethics applications, identifies omissions and non-compliance, and returns immediate, structured, evidence-based corrections linked to the relevant regulation or refinement principle, improving protocol quality before committee submission.

Materials and Methods: Etiq-Tech uses a layered design. A parser converts RTL-formatted protocol exports into a unified structured object for programmatic checking. A deterministic linter and renderer apply word limits, reconcile animal numbers, enforce monitoring according to severity, verify analgesia and housing details, activate relevant sections when needed, and generate compliant output. A metadata catalog stores exemplars and prompt templates that integrate schema rules, linter findings, and grounded text. An evidence layer indexes refinement literature, Israeli regulatory clauses, and institutional guidance to provide traceable citations. Batch and regression tools maintain consistency across updates.

Results: The system reliably detects missing information and non-compliance, improving clarity and completeness before committee review. Users report better understanding of expectations and faster revision. Parsing, issue detection, rendering, and automated prompting function consistently. The evidence engine provides structured corrections with governing citations and confidence metadata.

Conclusion: An AI-based co-reviewer can strengthen the quality, compliance, and refinement of animal research protocols, supporting ethical integrity and committee efficiency.

ETHICS, 3R PRINCIPLES AND SIMULATION-BASED LEARNING FOR ZOONOTIC PREPAREDNESS IN VETERINARY EDUCATION

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Introduction: Veterinary education requires ethical decision-making, animal-welfare responsibility and preparedness for managing zoonotic risks, while training systems are increasingly expected to align with the 3R principles (replacement, reduction, refinement). This study presents an integrated curriculum model in which ethics, the 3Rs and One Health reasoning function as measurable competencies, avoiding the routine use of laboratory animals.

Aim: To develop an integrated, simulation-based and research-oriented education model that strengthens zoonotic preparedness, biosafety behaviour and analytical reasoning, while embedding the 3Rs as operational professional competencies across an 11-semester veterinary curriculum.

Materials and Methods: The model is implemented within the Integrated Master of Veterinary Medicine programme (5.5 years; 347 ECTS) and emphasises a One Health approach using a backward-design framework. Ethics-, 3R- and zoonosis-related outcomes guide the selection of learning activities and assessment tools. Simulation-based training utilises task trainers, synthetic models, virtual cases and standardised scenarios addressing specimen collection, containment, PPE use, infection control, triage and risk communication, and is assessed through OSCEs and structured checklists. Research-oriented learning is based on slaughterhouse-derived diagnostic materials, archival resources, authentic surveillance datasets and data-driven analysis in biostatistics, bioinformatics and GIS. Workflows from the SRNSFG project FR-22-6780 are integrated as authentic learning resources. Portfolios and longitudinal monitoring support progressive competency development.

Results: The model generates (i) an ethics- and 3R-competency map; (ii) a portfolio of simulation activities with measurable OSCE outcomes; and (iii) a non-animal research learning trajectory linking fieldwork, diagnostic reasoning and computational analysis. Engagement with surveillance workflows improves biosafety awareness, analytical judgement and zoonotic risk communication.

Conclusion: The model enhances zoonotic preparedness and evidence-based competence while avoiding routine laboratory-animal use, offering a transferable approach that supports animal welfare, educational quality and public trust.

Acknowledgements: *The work was supported by Shota Rustaveli National Scientific-Research Foundation of Georgia (SRNSFG) [Grant Number FR-22-6780]*

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FROM COMMUNITY TO COMMUNITY: STRENGTHENING KNOWLEDGE AND COMPETENCE ACROSS EUROPE

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The Education and Training Platform for Laboratory Animal Science (ETPLAS) aims to strengthen excellence and harmonisation in laboratory animal science across Europe by connecting people, improving knowledge and promoting competence. Through the development and sharing of high-quality education and training resources, collaborative networks, and best practices, ETPLAS supports professionals, institutions and regulators in advancing consistent standards and promoting continuous professional development (CPD). By fostering cooperation between stakeholders and facilitating access to innovative learning opportunities, the platform contributes to improved skills, ethical practice and scientific quality.

ETPLAS offers training solutions for authorities, accreditors, establishments, course organisers and individuals. E-learning modules, that can be used for blended learning, competence assessment tools, a course directory of registered LAS courses, a secure online examination platform are available. These resources facilitate consistent standards, mutual recognition, and trustworthy competence across Member States, while supporting CPD requirements and lifelong learning. Through shared materials and standardised assessments, ETPLAS helps ensure robust, reliable and continually improving training outcomes.

This presentation will outline ETPLAS's services, impact and future priorities, and highlight opportunities for community engagement in shaping the next generation of LAS education and training across Europe.

3Rs IN ACTION: FROM ETHICS TO IMPLEMENTATION

SEX DIFFERENCES IN THE PHARMACOKINETICS OF BUPIVACAINE SHOULD BE CONSIDERED WHEN TREATING PIGS

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Background: Bupivacaine is a widely used local anaesthetic, clinical and preclinical studies have suggested potential sex-related differences in its pharmacokinetics, particularly in absorption and elimination phases, though the findings remain inconsistent. In rodent models, females often display lower peak plasma concentrations and slower elimination compared to males[1]. However, limited data exist in pigs, a key translational model for evaluating new local anaesthetic formulations. This study aimed to assess sex-related differences in bupivacaine pharmacokinetics and local tissue characteristics following wound infiltration in Göttingen minipigs.

Methods: Male and female Göttingen minipigs were anesthetized, and a standardized 7 cm flank full skin incision was made[2,3]. Bupivacaine (8 mg/kg) was administered into the wound space. Blood samples were collected at defined time points for quantification of plasma bupivacaine levels. Skin samples from the injection site were processed for histological examination (H&E and IHC staining) to measure epidermal, dermal, and subcutaneous fat layer thickness as well as blood vessels density.

Results: Plasma bupivacaine concentrations were significantly higher in females compared with males (6480 ng/mL vs. 4600 ng/mL; $p < 0.001$). The time to reach peak concentration was 30 minutes in females and 15 minutes in males, indicating slower absorption in females. Histological analysis revealed no sex-related differences in epidermal or dermal thickness; however, females exhibited a thicker subcutaneous fat layer (2929 ± 167 µm vs. 2326 ± 1394 µm; $p < 0.01$). No correlation was observed between body weight and fat layer thickness. Ongoing analyses are evaluating potential sex differences in local vascular density to further elucidate absorption kinetics.

Conclusion: Female minipigs exhibited higher systemic exposure and delayed peak levels following local bupivacaine administration which may put them in higher risk for cardiovascular toxicity. Differences in tissue composition may underlie the observed pharmacokinetic sex related difference.

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SMALL STEPS, BIG IMPACT: IMPROVING ASEPSIS TIPS FOR RODENT SURGICAL PROCEDURES

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The principle of aseptic surgery, established at the end of the 19th century, allows to prevent contamination with microorganisms during a surgery and associated post-operative complications.

This fundamental principle applies to human and veterinary medicine. In the context of research projects, strict aseptic conditions do not only allow preventing healing issues of operated individuals but also avoid bias in collected scientific data.

Strict aseptic conditions are usually respected for large laboratory animals' surgeries. However, it remains significantly improvable when it comes to rodents. Rodent surgeries carry their own challenges and some of the large animals' aseptic techniques are not easy to implement in rodents. But specific strategies can be established, without impairing surgery in series cost and efficiency.

During this presentation, pragmatic and affordable tips regarding prevention of contamination from potential sources (environment, animals, instruments, and surgeons) will be presented.

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FELASA WG ON BENCHMARKING TECHNICAL STAFF RATIOS FOR LABORATORY MOUSE CARE: PRELIMINARY FINDINGS

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Laboratory mice husbandry and care primarily rely on technical personnel. Across facilities, differences in housing conditions, husbandry practices, and technological advancements require varied operations management and staffing levels. The FELASA Working Group conducts a benchmarking exercise to understand staffing practices according to personnel's role and husbandry conditions.

We developed two surveys to collect data on staffing numbers and task-based time allocation. The first survey explored general management practices, while the second focused on task-based workload. Initial exploratory findings revealed substantial variation in facility operations and perceptions of optimal staffing. Absenteeism and increased workload were identified critical challenges; these issues significantly affect continuity of care and operational stability. Preliminary data indicate that 65% of respondents perceived a workload increase, while 72% identified absenteeism as a major issue.

The follow-up survey was designed to investigate specific operational requirements for animal caretakers, animal technicians and animal technologists[1]. Its purpose is to capture workload associated with animal care and supporting activities, categorized by task types and various technical staff roles.

We will present the preliminary results from both surveys. Our analysis aims to provide evidence-based guidance for appropriate technical staff workforce design for laboratory mouse housing and care. These findings may support facilities assess staffing levels, identify workforce gaps, implement corrective actions, and harmonized staffing practices in mouse laboratory animal facilities, ultimately enhancing animal welfare and scientific quality.

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NAMS IN ACTION: REDUCING ANIMAL TESTING THROUGH INTEGRATED IN SILICO STRATEGIES

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The integration of in silico toxicology into early-stage drug development marks a paradigm shift toward more ethical, efficient, and predictive research. This study explores the strategic application of computational methods – including quantitative structure-activity relationship (QSAR) modeling and activity prediction algorithms (PASS), along with pharmacokinetic and pharmacodynamic simulations (SwissADME, GUSAR) – for evaluating novel active pharmaceutical ingredients (APIs). Leveraging these tools during lead optimization allows effective prioritization of compounds with favorable safety profiles and eliminates high-risk candidates prior to any in vivo experiment. Our case studies demonstrate that this systematic implementation reduces animal use in preclinical safety testing by 40–70%, directly advancing the 3Rs principles. We describe frameworks that address model transparency and applicability to ensure regulatory acceptance, highlighting integration pathways where computational predictions complement targeted in vitro assays. The methodology has been successfully applied during the development of a series of pharmaceutical products for external use, which are now progressing to clinical studies.

This successful translation from in silico prediction to clinical candidates underscores a broader transition in toxicology, which is being supported by an evolving regulatory landscape that increasingly endorses validated computational approaches. Ultimately, the purposeful adoption of predictive toxicology establishes computational methods as critical gatekeepers before animal studies begin. This not only accelerates development timelines and lowers costs but also defines the future of ethical drug development through the harmonious integration of computational methods and responsible use of laboratory animals.

REFINING IN ACTION: A COMPARISON OF HYPOBARIC AND NORMOBARIC EXPERIMENTAL HYPOXIA MODELS IN PERINATAL RATS

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Reducing Animal Use: Sharing Resources, Avoiding “Wasted Life”

A variety of animal models have been developed to study perinatal hypoxic–ischemic (HI) brain injury and evaluate therapeutic interventions. The Rice–Vannucci model, involving unilateral carotid ligation in P7 rats, has been the gold standard for decades but is invasive and non-physiological. Hypoxia-only models provide ethically refined alternatives, allowing study of milder, clinically relevant injury while reducing animal distress.

This study aims to compare hypobaric hypoxia (HH) and normobaric hypoxia (NH) induced on postnatal day 1 (P1) in rats, and to compare their translational relevance for modeling mild perinatal hypoxic events.

In HH, 52 Wistar Han pups (RccHan: WIST) (3 females and 3 males per experiment) were exposed for 2 hours to hypobaric (pO_2 9.7kPa; pATM 46.7kPa) or control (pO2 21.2kPa; pATM 101.3kPa) conditions1. In NH, 76 pups (4 females and 4 males per experiment) were exposed for 2 hours to hypoxic (8% O_2 in 92% N_2) or control (21% O_2) conditions, with continuous monitoring of oxygen, temperature, pressure, and humidity2. Following exposure, pups were assessed for acute metabolism or returned to dams for subsequent behavioral testing of exploration, sociability, and learning during adolescence (P33–P43).

Acute metabolic analysis confirmed hypoxic stress in both models, with reduced base excess and bicarbonate, increased blood lactate, and preserved systemic pH. Perinatal hypoxia consistently increased exploratory rearing. HH induced broader, sex-specific effects, including increased locomotion, reduced sociability in females, and transient learning impairment in males. NH produced subtler but reproducible behavioral changes without impairing learning.

NH was identified as the mildest perinatal intervention capable of inducing measurable neurobehavioral alterations. By avoiding invasive ischemic procedures while maintaining translational relevance, NH represents an ethically refined, scientifically robust model. This approach exemplifies practical 3Rs implementation, particularly Refinement and Reduction, maximizing the scientific value of each animal used.

Acknowledgements: *Croatian Science Foundation: HRZZ-IP-2024-05-4135 and NPOO 10106-25-2906; PK.1.1.10.0009.*

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SEX AS A BIOLOGICAL VARIABLE IN LABORATORY RESEARCH: OBSERVATIONS FROM COLLABORATIVE RESEARCH ENVIROMENTS

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The failure to consider sex as a biological variable (SABV) in preclinical and clinical research has led to clinically relevant consequences, particularly for women. Biological sex differences influencing pharmacokinetics and pharmacodynamics have resulted in women being systematically exposed to higher effective drug doses, increasing the risk of chronic overmedication and adverse drug reactions (ADRs)[1]. Reports of certain drugs withdrawn by the U.S. Food and Drug Administration (FDA) due to safety concerns suggest they may pose a greater risk in women, highlighting potential issues with testing therapies primarily in male subjects.

Several arguments have historically been used to justify sex bias in biomedical research. In preclinical studies, the estrous cycle in females has often been cited as a reason to exclude them from studies. In contrast, in some cases, researchers have avoided using male animals due to concerns about aggression. In clinical research, the protection of women of reproductive age has long used to justify excluding them from early-phase trials, further reinforcing sex bias in evidence generation. Such practices have sometimes persisted due to methodological inertia or misinterpretation of the 3Rs principles[2],[3].

Recognition of this problem has led to policy changes, including the National Institutes of Health (NIH) requirement (NOT-OD-15-102) to address SABV in grant applications. These changes are increasingly visible at the local level, reflected in sex-inclusive preclinical and translational studies conducted within our Institute and collaborating institutions, demonstrating that implementation of SABV is both feasible and scientifically informative.

Despite these advances, sex bias remains prevalent. Addressing this gap requires continuous education, interdisciplinary dialogue, and changes in everyday research practice. Initiatives such as COST Action CA24168 – *European Initiative to Enhance the Current SABV Policy in Preclinical Biomedical Research (EU-SABV)* support these efforts and promote the integration of SABV into laboratory animal research.

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ONE WELFARE

CULTURE OF CARE: REALISTIC IMPLEMENTATION IN THE EUROPEAN FRAMEWORK

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Implementing a culture of care in research animal facilities has become essential over the past decades – not only to safeguard animal welfare, but also to uphold the integrity of scientific research and ensure the wellbeing of all personnel involved.

A culture of care is defined as a shared commitment to the ethical treatment of animals, encompassing attitudes, behaviors, and practices that prioritize animal welfare and responsible research.

This presentation will provide a comprehensive overview of practical and effective strategies for fostering a culture of care within European research animal facilities, illustrated through real-world examples drawn from recent AAALAC International site visits across Europe.

We will examine the impact of Directive 2010/63/EU, which sets standards for the protection of animals used for scientific purposes, and explore how it has shaped institutional policies and practices.

Key elements such as staff training, ethical review processes, continuous improvement, and the practical application of the 3Rs will be discussed. The critical role of Animal Welfare Bodies (AWBs) and Ethics Committees (ECs) in promoting and monitoring a culture of care will be highlighted, emphasizing their importance in ensuring compliance and driving ongoing progress.

Additionally, we will address the importance of teamwork and the active involvement of all stakeholders—from researchers to animal care staff—in creating a supportive and collaborative environment.

Attendees will gain valuable insights into best practices and innovative approaches that have been successfully implemented across diverse institutions.

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WHAT HAVE SEA-KAYAKING AND WORKING IN ANIMAL RESEARCH IN COMMON?

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At first glance, sea-kayaking and animal research appear to have little in common. However, both depend on effective leadership, clear communication and shared responsibility to ensure safety, welfare and successful outcomes. This presentation uses a sea-kayaking journey as a metaphor to explore how leadership and communication practices from this demanding activity can be translated into the culture of animal research.

Leadership and communication are two-way processes. An effective sea-kayak leader must encourage individuals to develop their skills through appropriate challenge while preventing fear and maintaining safety. Support is provided when needed, but individuals are also given the opportunity to achieve independently. These principles closely mirror good leadership practice within animal research environments.

Teamwork is critical. Leaders must understand the skills, limitations and personalities of all group members and know how best to use these in an emergency. Imagine a howling wind and a choppy sea, with two kayakers capsized near rocks. Everyone must understand their role in keeping those in the water, and the wider group, safe—whether through rapid recovery, stabilisation or recognising when external rescue is required. Training prepares teams for such scenarios, but clear and timely communication is equally essential.

Individuals also carry responsibility for accurately assessing their own abilities. Overestimating competence can place the entire group at risk. Respect for the skills and experience of others is vital, and all must feel empowered to raise concerns. This presentation translates these lessons into the animal research environment, highlighting their relevance to leadership strategies for ethical culture change, culture of care, training, welfare, teamwork, mutual respect and the consequences of getting it wrong, and recognising that animal welfare and human well-being are deeply interconnected.

EXAMINATION OF MENTAL WELL-BEING AMONG LABORATORY ANIMAL RESEARCH WORKERS

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Introduction: In addition to the ethical and professional concerns surrounding animal experimentation, increasing attention is being directed toward the psychological burdens experienced by professionals directly involved in such research. Personnel working in animal facilities—technicians, caretakers, researchers, and veterinarians—interact closely with experimental animals daily; in the case of prolonged studies, personal attachments may even develop, and they are often involved in the animals' euthanasia. This duality—the provision of care for the animals contrasted with their sacrifice for scientific purposes—is termed the "caring-killing paradox" in the literature and constitutes a primary source of compassion fatigue (CF), secondary traumatization, and moral distress.

The concept of compassion fatigue was originally described in helping professions (e.g., healthcare and social work), where prolonged exposure to clients' suffering can lead to heightened psychological exhaustion. Over the past decade, however, a growing body of studies has demonstrated that CF is also highly prevalent among laboratory animal research staff.

Materials and Methods: The objective of our study is to map the construct of compassion fatigue among Hungarian laboratory animal research professionals and to assess the psychological burden on these workers. The questionnaire items were grouped into seven dimensions: Moral Distress, Ethical Conflict, Euthanasia Distress, Animal Suffering Disposal, Compassion Fatigue, Role Conflict, and Compassion Satisfaction, with six questions per dimension. To refine the questionnaire and enhance its validity, five experts evaluated the compiled material based on conceptual clarity and comprehensibility: two psychologists, two mid-career professionals working with laboratory animals, and one assistant with 15 years of experience in animal care, research, and teaching. The revised questionnaire will be distributed to as many institutions as possible to capture the phenomenon of compassion fatigue across all levels of laboratory animal staff. At the conference, we aim to present both the questionnaire and preliminary findings.

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HANDLING METHOD OF LABORATORY MICE HAS EFFECT ON THEIR EXPLORATORY BEHAVIOUR

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Routine work with laboratory animals involves handling that cause discomfort and stress. The tail picking is the method most commonly used with mice. Response of the mouse to the tail picking is a diminished interest in exploring their surroundings. This can have a significant impact on the results of behaviour research and the behaviour of animals in various experiments, but also on the results of research. The introduction of non-aversive method of handling, tunnel handling, can help to reduce differences and deviations. During the study, we investigate how the constant presence of a tunnel in a cage affects behaviour and how long it takes for mice to get used to handling. In the study we use three different mouse lines: FHI, FLI and BALB/c and each mouse line consisted of six animals, including three males and three females. Each line was handled in all three different ways (tail, home tunnel, foreign tunnel). Before, during, and after handling, we performed an open field test and an elevated plus maze test with all animals. From the results, it appears that mice handled by the tail during the open field test spent more time immobile in the centre of the arena, whereas mice handled using a tunnel were much more active and explored the periphery more extensively; they also showed reduced urination and defecation. In the elevated plus maze test, we observed immobility and reduced exploration of the open arms in mice handled by tail picking. No differences were detected between mice that had continuous access to a tunnel in the cage and those that were exposed to the tunnel only during handling. The results indicate that tunnel handling reduces anxiety-like behaviour and promotes exploratory activity compared to tail picking, while only minor differences related to sex and mouse line were observed.

CHALLENGES IN USING AGED ANIMAL MODELS FOR NEURODEGENERATIVE DISEASE RESEARCH

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Introduction: Neurodegenerative diseases are challenging for modelling – they rarely occur spontaneously in animals. Advanced age is the most significant risk factor for conditions such as Parkinson's and Alzheimer's disease, the use of aged animals in preclinical research remains limited due to experimental, time, and cost-related challenges. Cats and dogs develop a similar condition to Alzheimer's disease, yet drug testing is limited to clinical patients for their own benefit rather than for advancing general scientific knowledge. Few studies have demonstrated age-related beta amyloid plaques and neuronal loss in rodents; however, researchers rely on induced models that cannot fully recapitulate the slow progression and complex pathology observed in humans.

Aims: Find best rodent model for preclinical testing of butyrylcholinesterase inhibitors after in vitro modelling.

Material and Methods: Ageing C57BL/6 and Balb/c mice of both sexes were compared to chemically induced cognitive impaired mice. Male 15–16-month old C57BL/6 mice were given the effective dose orally and tested in elevated plus maze, novel object recognition and modified Barnes maze. Surplus mice were killed between 18 and 24 months of age and whole brain collected.

Results: The drug crossed the blood-brain barrier but failed to induce significant improvement in behavioural te ntreated mice a decrease in brain cortex thickness, hippocampal area, and an increase in amyloid plaques and nNOS-positive neurons were observed[2], raising questions about the choice of using younger mice for drug testing.

Conclusion: Studies performed on mice aged 2 years or older are extremely rare. According to a large animal supplier, 24-month old mice correspond approximately to 70-year old humans, with senescent changes in almost all biomarkers in all animals. In our case, the study design simply failed to account for the prolonged developmental timeline required to observe the full spectrum of neurodegenerative pathology. Later on testing (re)turned to induced and transgenic mice models[3].

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ARE FRAUDULENT STUDIES IN PRECLINICAL MEDICINE LEADING US ASTRAY?

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There is a growing awareness of fabricated studies being passed off as real ones in scientific journals. The practice is not new, but with the ever-increasing pressures to publish, combined with a greater availability of computer software capable of aiding fraudsters (most recently, the explosive adoption of generative artificial intelligence - GenAI), the numbers of faked publications are reaching new highs. How will this affect our ability to make sense of scientific literature in our field going forward? And to which degree is laboratory animal science/preclinical medicine affected?

In the course of carrying out systematic reviews of rat studies in depression and pain, we have had the misfortune to uncover a great number of problematic – potentially fraudulent – studies. Having sifted through hundreds of papers across two unrelated fields of laboratory animal studies, we have found that 10-20 % of studies are unreliable; possibly even fabricated. These are shocking numbers to us – numbers that cannot be ignored.
In this talk, I will present what we found in our investigations. I will show what a problematic study looks like and explain why we sometimes suspect foul play. We have also established how a systematic review can be affected by the inclusion of problematic studies. I can offer no simple solutions to the problem, rather, my goal is to raise awareness of these issues, and to demonstrate that they are hiding in plain sight. We may all have read a peer-reviewed publication recently describing a study that never actually took place. Most likely we trusted that study. This begs the question: How do we make sure that our own research is not led astray by mistakenly believing in fraudulent studies?

DESIGNING FOR WELFARE: FACILITIES, TECHNOLOGY & SUSTAINABILITY

REFINING HUMANE ENDPOINT IDENTIFICATION THROUGH TIME-SERIES FORECASTING

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Introduction: Improving the severity assessment in animal research is crucial for refining animal research in line with the 3R (Reduce, Replace, Refine) principle. Objective and model-specific parameters, in combination with robust statistical and algorithmic procedures, are essential for enhancing the evaluation of animal distress.

Aim: Therefore, we want to expand the application of the RELative Severity Assessment (RELSA) procedure with our AutoRegressive Integrated Moving Average (ARIMA)-based foRcast tool. This tool is designed to identify individual animals at risk of humane endpoint thresholds by predicting future RELSA scores from previous observations.

Materials and Methods: To validate and evaluate the performance of this function, we re-analyzed the published data of, in total, 14 male and female C57BL/6 mice over five distinct animal models and interventions, including a sepsis model, two dextran sodium sulfate (DSS)-induced colitis models with different stressors, a pancreatic cancer model, and a neurosurgical intervention. The RELSA scores were calculated for each model with different outcome measures. These measures comprise transmitter-generated variables, such as heart rate, and behavioral parameters, such as voluntary wheel running.

Results: Humane endpoint RELSA scores ranged between 0.63 and 1.03, with the foRcast predictions achieving an overall root mean square error (RMSE) of 0.08 and a prediction interval coverage probability (PICP) of 91.67 %.

Conclusion: The results indicate an overall precise prediction of the RELSA scores at the humane endpoint and the general direction of the animal's development. At the same time, the foRcast function shows robust predictive behavior across the different animal models. Nevertheless, the results also highlight the intrinsic limitation of ARIMA models, that drastic changes are not reliably predictable. We assume higher-frequency measurements to reduce the impact of this limitation and achieve better predictions through incremental changes. Overall, the foRcast function demonstrates robust, precise predictive ability and is a valuable addition to 3Rs.

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PRECLINICAL IMAGING AT THE IPHYS BIOIMAGING FACILITY: THE CZECH-BIOIMAGING AND EURO-BIOIMAGING NODE MEMBER

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The IPHYS Bioimaging Facility is a strong partner in preclinical imaging, providing comprehensive support ranging from assistance with ethical protocol preparation and approval to access to advanced imaging techniques and methodologies. In collaboration with the Czech-BioImaging infrastructure, the facility offers a broad range of state-of-the-art technologies, including miniscopes, *in vivo* optical imaging and optogenetics (with 2P and 3P options), PET-CT modalities, and advanced MRI systems.

Research projects supported by the facility cover a wide spectrum, from behavioural studies using miniscopes, imaging of acute brain sections, and optical imaging of the brain through cranial windows, to metabolic studies of bone marrow using PET-CT scanning. In addition to research support, the facility organizes an annual course in preclinical imaging for researchers seeking to expand their knowledge of *in vivo* animal imaging.

Facility activities will be demonstrated through several case studies and user projects, illustrating how the IPHYS BioImaging Facility helps researchers enhance project effectiveness and efficiency while improving animal welfare. By enabling non-invasive, longitudinal imaging, the facility actively supports the 3Rs (particularly Refinement and Reduction): procedures are gentler and better monitored, the number of animals required can be reduced, and welfare is safeguarded through earlier detection of issues and more precise, data-driven interventions.

THE IMOUSE SYSTEM – VISUAL INSPECTION MEETS AI-BASED LONGITUDINAL ANALYSIS OF STANDARDIZED BEHAVIOURS

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In translational research, experimental animals remain standard for assessing the efficacy and safety of potential therapeutics. However, comprehensive longitudinal datasets are often not acquired, as manual data collection must be minimized to comply with the 3R principles. Moreover, visual observations and behavioural testing can disrupt resting periods, elevate stress levels, and introduce artificial outcomes.

We aim to tackle these unmet needs by implementing of the camera based digital monitoring system (iMouse) for animal husbandry and experimental use cases by upgrading existing laboratory equipment (retrofit). Here, we examine the impact of manual handling towards mice with no visible phenotype. Therefore, we recorded 6w of manual handling. We used 2w pre-experimental video material from 3 perspectives to train AI algorithms, followed by a time series analysis of standard and unusual behaviours during the 4w experimental data set.

In result, we showed that recorded video material in the pre-experimental phase was capable to train our existing models to detect behaviours for the specific strain with over 90% precision. Furthermore, we identified that unusual phenotypic behaviour during the experimental phase was induced directly by manual handling.

In summary, our results demonstrate that digital visual inspections of experimental animals are capable to identify a visible phenotype which has been overlooked by human based manual inspection for years. Thus, our findings lead to a better understanding of mouse models, reducing the bias through human handling. At the same time, we increase the data density and provide contact-free continuity of video surveillance.

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FIXING WHAT SLOWS US DOWN: SIMPLE DIGITAL SOLUTIONS FOR SMARTER ANIMAL CARE PROGRAMS

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Introduction: Oversight of laboratory animal facilities generates large volumes of operational and clinical data; however, these data are often fragmented across paper records, spreadsheets, or isolated software platforms. This fragmentation limits timely communication, hinders longitudinal tracking, and reduces the ability to make data-driven decisions that support animal welfare and program efficiency.

Aims: To implement and evaluate cost-effective, cloud-based digital solutions designed to improve data consolidation, communication, and veterinary care oversight in a laboratory animal program.

Materials and Methods: We developed and implemented low-cost digital tools using widely available cloud services, QR codes, and online survey formats. QR codes were deployed at the point of care to enable rapid access to standardized digital forms for veterinary notifications, husbandry operations, recharge tracking, pathology reporting, and procedural documentation. Submitted data were automatically consolidated into centralized cloud-based repositories, generating time-stamped records and searchable archives. These systems enabled real-time notifications to veterinary and research personnel and supported longitudinal tracking of clinical events, service utilization, and operational metrics.

Results: Implementation of these tools resulted in marked improvements in data accessibility, completeness, and timeliness. Veterinary notifications and researcher communications became standardized and traceable, reducing delays and information loss. Centralized data capture enabled reliable tracking of service recharges, pathology incidence, recurrence, and trends. Searchable archives facilitated retrospective analyses and supported data-driven adjustments to veterinary care, staffing, and resource allocation. All solutions were implemented at minimal to no financial cost and required no specialized hardware or proprietary software.

Conclusion: Low-cost, cloud-based digital innovations can substantially refine veterinary care delivery and program oversight by improving data consolidation, communication, and decision-making. By leveraging simple technologies such as QR codes and online forms, laboratory animal programs can enhance efficiency, transparency, and animal welfare without significant financial investment. These approaches represent a scalable refinement supporting proactive, data-driven veterinary care and continuous program improvement.

FROM PROCEDURE TO PRODUCTION: CREATING THE BIOEMTECH VEIN ACCESS INJECTION PLATFORM

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BIOEMTECH's "Vein Access Injection Platform" is an example of how a procedure implemented in the daily operation of a preclinical laboratory can lead to the creation of a novel commercial product. The setup for intravenous injections to the lateral tail vein of rats and mice includes various materials and equipment: intravenous catheters, syringes, gauze, heatpad, warm water and either a restrainer or anesthesia mask. Administration of radioactive materials requires additional provisions for radioprotection such as a lead syringe case. Daily administrations became an inspiration for creating a platform which accommodates all needs into a handy device. The use of a tailor-made purpose-specific injection platform promotes 3 important goals: (1) operator comfort and safety, (2) uniform setup and standardized procedure steps which ensure successful vein access and repeatability with significantly fewer deviations for more robust results, and (3) a refined approach for animal welfare.

Having a manufacturing department within the company makes the realization of the idea easier. Manufacturing and producing a device for preclinical research involves several risks, such as the cost and effort of production versus the market price, the small target customer pool and limited laboratory budgeting for certain products. Communication between departments, marketing and corporal support can lead to the launch of a new product despite the challenges. Such devices, designed by users for users, promote refinement and reproducibility of animal procedures and ultimately fill in a significant market requirement.

RELOCATION WITHOUT DISRUPTION: DO'S, DON'TS AND LESSONS LEARNED

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The Netherlands Institute for Neuroscience (NIN) recently completed a major renovation of its rodent facilities, comprising a mouse breeding unit and a rodent experimental unit, including a biosafety level 2 (BSL-2) area.

The NIN does not own the premises in which it operates, and the maximum capacity of the facility is ca 2100 cages (mice and rats). Modernization of rooms and equipment was required, but the key challenge was to ensure continuity of research activities throughout the renovation process.

The renovation was conducted in three phases. First, the breeding unit was closed and essential breeding activities were temporarily relocated to the old experimental unit to maintain experimental continuity. This phase was also used as an opportunity to improve genetic quality and health status of the mouse strains through rederivation or re-establishment via commercial vendors. Second, following renovation and disinfection though an external company, the new animal facility- comprising both breeding and experimental units - was commissioned, after which experimental equipment was cleaned, disinfected and moved. Finally, the old facility was closed, disinfected and returned to the property owner.

The entire process, from closure of the initial unit to full transition into the new facility and decommissioning of the old experimental unit, was completed within eight months.

Throughout this period, multiple operational and organizational aspects required careful management, including decisions on outsourcing services, allocation of personnel, negotiation of new researchers' requests for experimental space and time to continue their research, coordination with construction companies on technical requirements, management of the psychological impact of the relocation on PhD students and their research projects, and resolution of damages, errors, and unforeseen issues during the move.

This presentation will provide a stepwise overview of the process, highlighting key lessons learned and practical recommendations that may support other research institutions facing similar renovations challenges.

THE IMPACT OF PRE-EXPERIMENTAL HOUSING CONDITIONS ON SONG PRODUCTION IN CANARIES

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Introduction: Canaries are a powerful model for studying motor sequence learning due to their complex and flexible song repertoires. Successful neurophysiological recordings in singing birds require that birds resume singing shortly after electrode implantation and reliably produce high daily song output (~100 songs). Prior to experimentation, canaries are housed either in aviaries, which provide environmental enrichment and flight opportunities, or in smaller cages. A potential concern is that transferring birds from a large aviary to a confined acoustic recording chamber may disrupt song behavior more than transfer from smaller cages, whose conditions resemble the experimental setup.

Aims: We examined whether pre-experimental housing conditions influence song onset and production following transfer to acoustic recording chambers.

Materials and Methods: Using a double-blind design, canaries were housed for 90 days either in small cages (n = 11; 3–5 birds per cage) or in aviaries (n = 12; 6 birds per aviary). Birds were then transferred to individual recording chambers for an 8-day recording period. We quantified latency to song onset and daily song rate.

Results: Pre-experimental housing condition did not affect the day of song onset, with a median onset of day 2 in both groups (Wilcoxon's signed-rank test: $p = 0.25$). Across the experimental epoch, the daily average number of songs produced by aviary-housed birds was consistently equal to or greater than by cage-housed birds. Significant between-group differences were observed on two days (days 2 and 8), with aviary-housed birds producing more songs, and two additional days showed a similar trend (area-under-the-curve bootstrap test: $p < 0.05$ and $p < 0.1$, respectively).

Conclusions: These findings indicate that pre-experimental housing in aviaries does not impair song production following transfer to experimental conditions and may support higher song output. Our results have implications for experimental design, animal welfare, and optimization of neurophysiological data collection.

THE POWER OF STARTING SMALL – A WIN WIN SITUATION OF REFINEMENT AND SUSTAINABILITY

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The Central Animal Facility (CAF) at Hannover Medical School manages approximately 25,000 animals across various species, including mice, rats, gerbils, hamsters, guinea pigs, rabbits, Xenopus, sheep, pigs, and cats. These animals are housed under different hygiene standards, ranging from germ-free units to BSL 2 areas, utilizing both open-top cages and Individually Ventilated Cage systems. Large animals are housed in environments varying from farm like group settings to highly controlled Specific Pathogen-Free conditions. Because animal numbers at the CAF have halved over the past five to ten years, comprehensive restructuring of the housing for all species became possible.

The primary aim of this study was to implement a refinement and enrichment strategy tailored to the specific needs of each species, considering the facility's complexity, size, financial constraints, and sustainability. Over the last decade, a multi-faceted approach was adopted, focusing on species-specific solutions. Key interventions included introducing tunnel handling for mice, procuring 10,000 tunnels, and training personnel to use these tunnels for cage changing instead of traditional tail handling. For rats, Type IV cages were replaced with systems akin to those used for guinea pigs or rabbits. These are better suited to their behavioral needs and have, e.g., an increased height. Guinea pigs were transitioned to adapted rabbit stellages, while rabbits were transferred to floor housing. Pig pens were enlarged, incorporating rooting areas and ample straw to promote natural behavior.

Despite challenges like increased cleaning times and the need for adaptive strategies, these changes significantly enhanced animal welfare. Feedback loops between caretakers, researchers, and management enabled continuous adaptation, ensuring practical and effective solutions. The involvement of animal caretakers improved job satisfaction and integrated their insights into the refinement process.

3Rs IN ACTION: FROM ETHICS TO IMPLEMENTATION

STUDY OF THE RELATIONSHIP BETWEEN BEHAVIOR AND SEXUAL MATURATION IN FISHES

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A key limitation of toxicological studies on fish animal models is that the behavioral effects of tested chemicals are typically evaluated on group-level analysis using average behavioral parameters. This approach obscures individual variability and potentially significant sex-specific differences. Also, in many cases this requires the use of more animals than necessary to obtain sufficient data.

My doctoral research aims to reduce this methodological limitation and facilitate the identification of more refined mechanisms, by applying two fish models – zebrafish (*Danio rerio*) and paradise fish (*Macropodus opercularis*) – and integrating approaches from behavioral science, toxicology and genetics.

During the current, early phase of the project, we concentrated on zebrafish. In my previous work, I established a partially transparent transgenic zebrafish line, that enables detailed, non-invasive, *in vivo* monitoring of ovarian development. This line is also used in this present project, as it is expected to be informative for the analysis of sex-specific behavioral differences. In the later phase, I will expand my studies to paradise fish as well. This latter species has been recently reintroduced as a complementary model species for behavioral studies by the ELTE Fish Genetics Research Group led by Dr. Máté Varga.

Using these two species, our goal is to develop an artificial intelligence- and deeplearning-based system capable of high-throughput, individual-level behavioral analysis. The system is expected to identify sex-specific differences in behavioral responses to various chemical treatments. Short-term isolation is often a prerequisite for such treatments, but such isolation is known to alter the behavioral responses in zebrafish, a quintessentially social species. Our lab's recent results suggest that the paradise fish, in contrast, tolerates well individual housing, therefore, using this species results in more robust, reproducible results, enabling more accurate data evaluation using less individuals.

SEX-SPECIFIC VULNERABILITY IN A TWO-HIT MODEL OF STRESS: IMPLICATIONS FOR 3RS AND TRANSLATIONAL VALIDITY

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Stress-related psychiatric disorders arise from complex interactions between early-life adversity and later environmental challenges. Two-hit models of stress in rodents aim to mimic this cumulative vulnerability by combining early-life stress exposure with a subsequent adult stressor. However, despite strong sex differences in stress-related psychopathology in humans, many preclinical stress paradigms continue to rely predominantly on male subjects. In our studies, we implemented a two-hit stress model in Wistar rats of both sexes, combining early-life stress with a later-life challenge to assess long-term behavioral and neuroendocrine consequences in both sexes. Behavioral phenotyping included anxiety- and depressive-like measures, accompanied by assessment of hypothalamic–pituitary–adrenal (HPA) axis reactivity. We observed distinct sex-specific trajectories in stress responsivity. While both sexes exhibited altered behavioral outcomes following cumulative stress exposure, females showed heightened HPA axis activation and differential behavioral adaptation compared to males. Importantly, the interaction between early programming and later challenge differed qualitatively between sexes, suggesting divergent mechanisms of vulnerability and resilience. These findings indicate that biological sex critically shapes the outcome of cumulative stress exposure. Had only one sex been included, the interpretation of stress susceptibility and resilience mechanisms would have been incomplete and potentially misleading. From a 3Rs perspective, incorporating sex-balanced designs in complex stress paradigms represents a refinement of experimental validity and supports reduction by minimizing unexplained heterogeneity and replication driven by inconsistent results. Addressing sex bias in two-hit stress models is therefore essential for improving reproducibility, translational relevance, and responsible implementation of animal research.

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IMPROVING WELFARE AND REDUCING ANIMAL WASTAGE IN GA MOUSE BREEDING COLONIES BY CENTRALISATION TO A DEDICATED SERVICE

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This poster presents a genotyping and colony management system implemented by our institution which has demonstrably improved accuracy and efficiency of genotyping, reducing animal wastage and improving welfare. It has enhanced communication between researchers, facility managers, the veterinarian and animal care staff and has allowed technicians to use their expertise, leading to better job satisfaction.

The system centralises responsibility for genotyping and colony management wholly to Biological Services. Researchers discuss their planned use of animals at regular meetings with a colony manager (CM) who then decides on the best breeding strategy to achieve these aims and develops a written colony management agreement. Animal technicians collect ear tissue samples and place them into 96-well bar-coded plates. A trained genotyping technician enters animal and line identification information into an online submission system. Plates are sent to a trusted external provider, where genotyping is performed using a highly accurate and precise robotic system. Results are uploaded to the online system, checked for inconsistencies by the genotyping technician and then made available to researchers and the CM. Thereafter, the CM selects appropriate animals for breeding, based on genotype information, examination of animals to confirm their health and discussion with the Designated Veterinarian as needed, and allocates animals to required experimental purposes.

This new system has been widely adopted and handles >900 samples monthly, from approximately 100 genetically altered mouse strains. It has allowed a number of welfare- and research-related improvements. The turnaround time for results is typically less than five days, allowing prompt culling of unwanted animals and early social grouping of experimental animals. The re-biopsy rate has reduced four-fold. Genetic screening of background strains has identified anomalies in new lines, allowing corrective measures. Use of backcrossing has increased, with backcrossed lines producing 5% more pups to weaning than those that aren't backcrossed.

ANTITUSSIVE EFFECTS OF THE SELECTIVE NAV1.8 BLOCKER A-803467 IN GUINEA PIG MODEL OF ALLERGIC RHINITIS COMPARED WITH HEALTHY ANIMALS

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Background: Allergic rhinitis is a common chronic inflammatory disease of the upper airways frequently associated with cough and heightened airway sensory responsiveness. Increasing evidence suggests that cough hypersensitivity in allergic conditions is mediated not only by immune mechanisms but also by neuronal pathways. Voltage-gated sodium channel NaV1.8, selectively expressed in nociceptive sensory neurons, has emerged as a potential target for antitussive therapy. The presented study investigated the effect of repeated intranasal ovalbumin (OVA) challenge on cough sensitivity and evaluated the antitussive efficacy of the selective NaV1.8 inhibitor A-803467.

Material and methods: Guinea pigs were systemically sensitized and repeatedly challenged intranasally with OVA for six weeks to induce chronic allergic rhinitis. Citric acid-induced cough was assessed 1 h after the 1st, 4th, and 6th nasal challenges. In a second experimental phase, animals were pretreated with inhaled A-803467 (3 mM) or vehicle prior to citric acid exposure. Allergic rhinitis symptoms, cough frequency, and respiratory rate were recorded.

Results: Repeated intranasal OVA challenges significantly increased citric acid-induced cough compared with control animals. Pretreatment with inhaled A-803467 markedly suppressed cough responses during both the 4th and 6th OVA challenges by approximately 70%, without affecting the respiratory rate. OVA-sensitized animals exhibited pronounced eosinophilic and neutrophilic airway inflammation with mucous cell hyperplasia, whereas control animals showed no pathological changes.

Conclusion: Chronic allergic rhinitis is associated with enhanced cough sensitivity mediated by airway sensory pathways. Selective inhibition of NaV1.8 effectively attenuates citric acid-induced cough without compromising respiratory function, highlighting NaV1.8 as a promising therapeutic target for cough associated with allergic airway inflammation.

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QUANTITATIVE ASSESSMENT OF NON-EVOKED PAIN IN PIGS USING SPONTANEOUS BEHAVIOR AND NATURAL RESPONSES

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Background: Non-evoked (spontaneous) pain in pigs remains a major challenge for translational pain research and for evaluating novel analgesic therapies. Current approaches rely on condition-specific behavioral scoring, posture, and automated measures of movement or social interaction, but standardized methods are still limited.

Aim: This work presents three complementary methods to detect discomfort and pain-associated responses in pigs based on spontaneous and minimally constrained behavior.

Methods:

- Open field behavior:

The open field test, widely used in rodents to assess anxiety-like behavior and locomotor changes, was adapted to pigs to characterize spontaneous pain-related behavior. In a previously validated neuropathic pain model, pattern changes in locomotor activity were demonstrated despite preserved total distance, and here these measures are extended using improved computerized tracking and higher-resolution behavioral analysis.

- Computerised force distribution using Zebris apparatus:

A force-detection based apparatus was used to quantify weight and force placement on each limb following unilateral hindlimb injury, enabling objective detection of spontaneous altered load-bearing without evoked mechanical stimulation.

- Approach test:

A home-pen approach test, first described in 2022 (1), was used to quantify distress- and pain-related changes in social approach to a familiar researcher. Under baseline conditions, pigs typically move away when a person enters the pen and then rapidly approach to initiate play, whereas under pain or distress conditions, the latency to approach increases significantly, reflecting altered motivation and affective state.

Conclusion: Pig distress and pain can be detected quantitatively by integrating their natural and spontaneous behaviors in open field and home-pen environments with objective limb loading measures. These non-evoked readouts provide a translationally relevant framework for assessing analgesic efficacy while minimizing reliance on externally applied noxious stimuli.

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SUPPORTIVE CARE IN SPINAL CORD INJURY MODEL IN RATS

Kigel-Zur, K., Nchlieli, M., Slosman, G., Finkelstein, L., Weksler, A., Aran, J., Ofir, Y., Castel, D., Meilin, S.

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Supportive care in a rat spinal cord injury (SCI) model is critical for the success of the model and for minimizing post-injury morbidity and mortality[1].

Postoperative care:

- Place animals in a clean, warm recovery cage with easily accessible food and water.
- Provide fluids: 3–5 ml sterile 0.9% NaCl SC at the end of surgery, then daily for the first 2–3 days.
- Add dry and wet food on the cage floor.

Medication:

- Start antibiotics per your IACUC and chief veterinarian officer recommendation.
- Use MULTIMODAL ANALGESIA (e.g., buprenorphine plus an NSAID) to control post-laminectomy pain.
- To prevent autophagy, treat with Pregabalin at a dose of 10 mg/kg orally, daily.

Urinary care:

- Urinary retention after SCI, requires regular manual expression.
- Monitor for urinary track infection (UTI): hematuria, cloudy/whitish urine, abdominal discomfort, reduced activity.

Nutrition, housing, and wound care

- Ensure access to water and daily monitoring of body weight; >10–15% loss or continued decline beyond day 3–4 warrants intervention.
- Inspect the incision daily and apply povidone-iodine or similar. Remove sutures/clips around day 7–10 post surgery.

Physiotherapy:

- Aquatic therapy: Fill a large bath with water maintained at 37 °C and generate gentle bubbles to encourage movement. Fit animals with a Styrofoam float and allow them to swim for several minutes. Perform this procedure 2–3 times per week.
- Enrichment related, physiotherapy: Allow animals to explore and play with enrichment in a large enclosure (minimum floor area 1 m × 1 m) for 10–15 minutes, three times weekly.

Monitoring and humane endpoints

- Monitor the animals for: appearance, posture, grooming, body weight, food/water intake, respiratory pattern, fecal output, and behavioral indicators of pain/distress.
- Red flags: persistent autophagia, unresponsive weight loss, wound infection, pain despite rescue analgesia, or refractory UTI.
- Keep communication with the attending veterinarian to improve welfare.

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COMPARISON OF IN VIVO AND IN VITRO RESULTS OF MEDICAL DEVICES SENSITIZATION TESTING

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Sensitization is an obligatory prerequisite of biocompatibility assessment of medical devices. The recently amended standard ISO 10993-10:2021 allows only in vivo test methods on animals or humans, but includes also a list of non-animal methods, validated for neat chemicals, which might be used after verification of applicability for complex mixtures as medical devices or their extracts. Our pilot study aimed to compare results of regulatory testing of 10 real life medical devices by Local Lymph Node Assay (OECD TG 442A), performed in compliance with the ARRIVE guidelines, with results of a battery of assays covering all three key events of the Sensitization Adverse Outcome Pathway (DPRA, OECD TG 442C; LuSens, OECD TG 442D; h-CLAT, OECD 442E), conducted according to GCCP guidelines. The samples were extracted or diluted in saline solution, DMSO and/or culture medium. The 4 positive samples in the in vivo test were correctly classified by the battery of non-animal tests using “the 2 of 3" principle of the Defined Approaches for Skin Sensitisation (OECD TG 497). One sample (rubber glove) was negative in vivo and positive in vitro, showing the high sensitivity of the in vitro methods to latex and similar compounds. The presented results confirm the feasibility of non-animal methods for skin sensitization of medical devices and provide information on the optimization of these methods in accordance with the current European Commission initiative to define a Roadmap to phase out animal testing in the EU.

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A REFINED PRECLINICAL MODEL OF ALZHEIMER'S DISEASE WITH COMORBID NEUROPATHIC PAIN: IMPLICATIONS FOR TRANSLATIONAL THERAPEUTICS

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Introduction: Alzheimer's disease (AD) is frequently accompanied by chronic pain, which can exacerbate cognitive decline and reduce quality of life. Preclinical research often investigates neurodegeneration and chronic pain separately, limiting translational relevance and increasing animal use. Refining models to incorporate clinically relevant comorbidities may enhance scientific validity while supporting the 3R principles and opens perspectives for the development of complex biologics, biomarker validation and personalized therapies.

Aims: This study aimed to develop a refined preclinical model integrating cognitive impairment and neuropathic pain and to evaluate multi-target therapeutic strategies on pain, neuroinflammation and neuronal integrity while minimizing experimental burden and improving translational potential.

Materials and methods: A rat model combining scopolamine-induced cognitive impairment with unilateral sciatic nerve ligation–induced neuropathic pain was used to reflect AD-associated pain comorbidity. An EU-GMP certified *Cannabis sativa L.* strain (Cannabixir® Medium Flos) administered alone or in combination with donepezil and tramadol, was evaluated. Outcomes included thermal and mechanical nociceptive testing, clinical monitoring and immunohistochemical analyses of markers related to neuroinflammation, apoptosis and neuronal integrity in central and peripheral tissues.

Results: The combined model allowed simultaneous assessment of cognitive and pain outcomes within the same animals, increasing data yield per subject and supporting Reduction. Cannabixir® Medium Flos produced sustained analgesic effects and reduced astrocytic and microglial activation, inflammatory and apoptotic markers, and preserved hippocampal neurons and peripheral nerve structure. Combination therapy enhanced analgesic efficacy compared with conventional analgesics alone.

Conclusion: Integrating neurodegeneration and neuropathic pain within a single preclinical paradigm represents a refined, translational strategy that improves clinical relevance and supports the 3R principles. Such models can inform the development of complex biologics, enable biomarker validation and support personalized therapies, reducing reliance on multiple disease-specific models while promoting ethical and efficient preclinical research.

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DIGITALIZATION AND AI IN PRECLINICAL STUDIES: FROM EFFICIENT DRUG DISCOVERY TO ETHICAL ANIMAL USE

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Introduction: Digitalization is transforming preclinical research, reshaping experimental design, data acquisition and interpretation. At Advanced Research and Development Center for Experimental Medicine "Prof. Ostin C. Mungiu" – CEMEX, artificial intelligence (AI), automation and digital monitoring are integrated across the drug discovery pipeline and animal studies, offering opportunities to improve both scientific rigor and animal welfare in line with the 3R principles (Replacement, Reduction, Refinement), while opening perspectives for complex biologics development, and personalized therapies.

Aims: This contribution aims to demonstrate how AI-driven tools and digital technologies implemented at CEMEX optimize preclinical workflows, reduce animal use, support ethical study conduct and facilitate translational research for advanced therapeutics.

Materials and methods: Recent CEMEX projects employing AI-assisted virtual screening, predictive modeling, and high-throughput analysis were reviewed. Digital monitoring technologies included computer vision–based behavioral analysis, telemetry and continuous physiological monitoring in rodent models of neurodegenerative and metabolic disorders. Workflow adaptations, including adaptive study designs and cohort minimization strategies, were evaluated for impact on animal welfare, experimental efficiency and translational potential.

Results: AI-assisted compound selection reduced low-probability candidates entering in vivo testing, improving study efficiency. Automated behavioral and telemetry monitoring enhanced data precision, enabling early detection of pain or distress and supporting refinement strategies. These digital approaches allowed meaningful results to be obtained from smaller animal cohorts, increased statistical power and improved longitudinal characterization of complex disease models. Overall, integration of AI and digital monitoring at CEMEX contributed to a measurable reduction in animal use while enhancing welfare, scientific robustness and translational relevance for complex biologics development.

Conclusions: The application of AI and digital technologies at CEMEX strengthens preclinical research by improving reproducibility, ethical responsibility and 3R implementation. Digitalization complements, rather than replaces, animal studies, increasing their scientific value and supporting translational strategies for complex biologics, biomarker validation, and personalized therapies.

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REFINEMENT THROUGH IMPLANTABLE DEVICES: MINIMIZING HANDLING AND STRESS IN EXPERIMENTAL ANIMALS

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Introduction: Repeated animal handling associated with chronic compound administration represents a major source of stress, variability, and welfare burden in preclinical research. Such stress-related factors can negatively influence both animal well-being and experimental outcomes, particularly in long-term studies relevant to surgery and neuroscience.

Aims: In accordance with the 3Rs principle of refinement, this work aims to present the methodological development and application of implantable osmotic pumps as an innovative strategy to reduce handling-related stress in experimental animals, with particular relevance for surgical and neuroscience-based research.

Materials and Methods: Implantable osmotic pumps were employed to enable continuous and controlled delivery of bioactive compounds over extended periods, thereby eliminating the need for repeated restraint and manual dosing. Optimised surgical implantation procedures, perioperative care strategies, and long-term maintenance protocols were developed and implemented. Emphasis was placed on minimizing surgical invasiveness, ensuring stable pump performance, and integrating the devices seamlessly into complex experimental designs commonly used in neurology and chronic disease research.

Results: By reducing the frequency of animal handling, the use of implantable osmotic pumps significantly refined experimental procedures, leading to decreased stress-induced physiological responses and improved animal welfare. From a methodological perspective, this refinement contributed to enhanced data consistency, reduced confounding effects related to handling-induced stress, and improved reproducibility of experimental outcomes.

Conclusion: The presented approach demonstrates how surgical innovation can be effectively leveraged to advance refinement within in vivo research. Implantable osmotic pumps represent a practical and ethically responsible solution for long-term studies requiring chronic administration, supporting the alignment of scientific objectives with animal welfare considerations and current European regulatory expectations. Integrating refined surgical methodologies into neuroscience and preclinical research offers a clear pathway toward more humane and robust experimental practices.

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INTRACRANIAL CANNULATION AS A TARGETED DELIVERY STRATEGY IN PRECLINICAL ALZHEIMER'S DISEASE MODELS

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Introduction: In preclinical Alzheimer's disease (AD) research, intracranial cannulation represents a key methodological approach for the acute or chronic delivery of neurotoxic agents, viral vectors, and therapeutic compounds directly into defined brain regions. Because the blood–brain barrier restricts central nervous system penetration of many biologically active molecules, intracranial cannulas are essential for targeted administration of pathological proteins, such as amyloid-β, into structures including the hippocampus or lateral ventricles, enabling robust modeling of AD-related cognitive dysfunction.

Aims: Intracranial cannulation in preclinical AD models aims to achieve precise, localized delivery of experimental compounds to specific brain regions, thereby improving dosing accuracy, reducing systemic variability, and supporting reliable mechanistic and pharmacological investigations.

Materials and methods: Standard cannulation systems consist of a guide cannula anchored to the skull using dental or cranioplastic cement, allowing repeated, precise intracerebral injections in awake or anesthetized animals. Optimized stereotactic techniques and robust stabilization strategies are applied to maintain experimental integrity. Comprehensive perioperative management, including appropriate analgesia and post-operative monitoring, supports rapid recovery and stable baseline behavior. Effective fixation methods ensure long-term cannula stability during repeated intracranial administrations, minimizing data loss due to implant displacement.

Results: Intracranial administration via cannulation improves delivery efficiency by enabling precise deposition of compounds at defined target sites, reducing variability associated with systemic distribution and peripheral metabolism. Effective local concentrations can be achieved using lower doses, limiting off-target effects and unnecessary systemic exposure. Multiple studies indicate that dorsal hippocampal cannulation performed using standardized protocols does not significantly affect locomotor activity, motor coordination or emotional behavior, allowing behavioral outcomes to be attributed to experimental interventions rather than surgical artifacts.

Conclusion: Advances in cannula design, surgical methodology, and perioperative management reinforce intracranial cannulation as a reliable and versatile tool for investigating AD mechanisms and evaluating localized therapeutic strategies in preclinical models.

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KEEP THE HEAT THROUGH IT ALL: COMPARING CONTEMPORARY THERMAL SUPPORT STRATEGIES IN MOUSE ANESTHESIA

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Introduction: Peri-anesthetic hypothermia remains one of the most consistent and consequential confounders in mouse anesthesia, adversely affecting animal welfare, physiologic stability, and experimental reproducibility. While outdated warming approaches such as phase-change materials and disposable heat packs are still used in some settings, contemporary thermal support options vary widely in design, mechanism, and reported efficacy. Direct, standardized comparisons among current standards remain limited.

Aims: To systematically compare contemporary mouse surgical thermal support strategies for their ability to (A) restore core body temperature after acute heat loss during standard isoflurane induction and (B) maintain normothermia following heated anesthetic induction, in order to inform best practices and procedural refinement in mouse anesthesia.

Materials and Methods: This ongoing, controlled, repeated-measures study evaluates multiple thermal support modalities, including circulating water blankets, infrared heating systems, and heated surgical platforms, while excluding outdated approaches such as phase-change materials and disposable heat packs. Ten adult C57BL/6 mice (5 females, 5 males) serve as their own controls and undergo standardized anesthesia consisting of a 3-minute isoflurane induction followed by a 15-minute maintenance phase. Core body temperature is continuously monitored using intraperitoneal RFID temperature sensors with concurrent rectal probes. Physiologic parameters, including heart rate, respiratory rate, and oxygen saturation, are recorded throughout the maintenance phase under standardized environmental and anesthetic conditions. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC).

Results: Data collection is currently in progress. Planned analyses will compare thermal efficiency, time to normothermia, temperature stability, and physiologic consistency across thermal support modalities.

Conclusion: This study is designed to address critical gaps in comparative evidence regarding modern thermal support strategies for mouse anesthesia. By directly evaluating commonly used systems under standardized conditions, this work aims to support harmonization of best practices, improve procedural refinement, and reduce temperature-related variability in preclinical research.

THERMONEUTRAL ENVIRONMENT IMPROVES MOUSE WELFARE AND REDUCES STRESS IN METABOLIC CAGES

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Metabolic cages (MCs) are often used to collect feces and urine samples. However, the housing of mice in MCs can be stressful, potentially affecting parameters of interest. We compared our standard protocol for individual MC housing (4 days at 23°C: 3 days of permanent acclimatization followed by 24h sampling (MC23)) with a short-term intermittent acclimatization protocol (3h of MC housing for 3 days plus 24h sampling (accMC23)), the provision of a nest (4 days at 23°C in MC (nest-MC23)) and MC housing at thermoneutrality (4 days at 30°C, MC30)). C57BL6/N mice were implanted with telemetric transmitters to collect ECG, blood pressure, core body temperature and activity data. Single-housed mice in the MC at 23°C had lower core body temperatures and higher heart and respiratory rates than mice in the MC30 group. Mice housed in MCs at 23°C exhibited increased food consumption and weight loss, combined with significantly increased expression of mRNAs of key molecules in brown fat compared to mice housed in MCs at 30°C. They also showed increased corticosterone levels. Some male mice of the MC23 and accMC23 groups exhibited episodes of reduced core body temperature, and reduced blood pressure and heart rate. Our study demonstrates that housing mice in MCs at 23°C has a substantial impact on their physiology and welfare due to a substantial cold stress. MC housing at thermoneutrality (30°C) provides a simple solution to improve mouse welfare. Furthermore, the results showed that a single acclimatization period had the same effect as repeated exposure to the MCs, and therefore provided no additional benefit.

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RESPIRATORY SENSITISATION: EXPLORING THE ADVERSE OUTCOME PATHWAY USING 3D HUMAN TISSUE MODELS

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Sensitisation in general is a specific immune response that may occur after contact with a sensitising agent, leading up to contact dermatitis, asthma, rhinitis or other adverse systemic reactions. Chemicals, ingredients of cosmetics, medical devices and other consumer products or environmental factors can trigger the process of sensitisation in the sensitive population. Skin sensitisation is described in detail and officially tested by many *in vivo* and *in vitro* methods included in the OECD guidelines. In contrast, very little is known about the principles, key factors and events involved in the respiratory sensitisation process, where some features appear to be similar to skin sensitisation. Further investigation of the respiratory sensitisation mechanism and completion of the Adverse Outcome Pathway (AOP) is urgently needed. Epithelial human-derived 3D tissue models of the respiratory tract are being incorporated into respiratory sensitisation and irritation tests providing more complex insights into the long-term and multiple exposure experiments essential for initiating the sensitisation process in an ethical way without the use of animals conducted according to GCCP guidelines. In this extended study, a set of reference potential respiratory sensitisers/non-sensitisers and two consumer products were tested using an *in vitro* 3D bronchial epithelial model MucilAir (Epithelix) in a multiple exposure design in order to investigate the process of respiratory sensitisation, mimicking human exposure in the air-liquid interface (ALI). Following on from a previous study, the production of a wide set of interleukins (IL-4, IL-6, IL-8, IL-13 and RANTES/CCL5) was determined during a 3 month period including repeated seven exposures with promising expression levels especially of RANTES/CLL5, IL-6 and IL-8. These results were complemented by the inclusion of transepithelial electrical resistance (TEER) measurement and microscopy analysis during the experiments.

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ETHICAL ASSESSMENT OF EXPERIMENTS ON MONKEYS

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Introduction: Animal testing on nonhuman primates represents a major and central component of biomedical research. Due to their strong genetic, anatomical, and cognitive similarity to humans, primates are used in crucial areas of research such as neuroscience, toxicology, and infectious disease studies.

Aims: The aim of the research was to critically examine the ethical aspects of such experiments and to explore public perception regarding this highly sensitive topic.

Materials and Methods: Through an online questionnaire distributed to the general population, attitudes, current levels of knowledge, and moral beliefs concerning animal testing on primates were collected. The results indicate that a large proportion of respondents view primate testing critically and report being emotionally affected by the topic. While many acknowledge the scientific value of such experiments, they simultaneously call for stricter regulations, greater transparency, and the promotion of alternative research methods. However, the literature shows that, despite ethical concerns, primate experiments remain indispensable in many areas of research. Although the 3Rs principle (Replacement, Reduction, Refinement) serves as the ethical foundation of animal experimentation, international differences in implementation and moral standards persist.

Conclusion: The findings of this research demonstrate that societal trends are increasingly shifting toward the development of alternative methods. However, complete replacement remains challenging, as primates are still essential to certain fields of research. A balance between scientific benefit and ethical responsibility is therefore required. Key steps toward this goal include transparent communication, stricter oversight, and the continued advancement of viable alternatives.

IMPACT OF TOPICAL LIDOCAINE PRILOCAINE ANALGESIA ON PAIN INDUCED GROOMING AFTER EAR NOTCHING OF MICE

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Introduction: Finnish animal welfare legislation (Government Decree 1165/2023) effective as of January 1, 2024, requires establishments to apply "appropriate pain relief" in association with ear notching identification method of laboratory rodents[1]. However, there is a lack of scientific evidence whether the benefits of applying analgesia outweigh the additional stress related to handling and drug administration.

Aims: Our study aimed to determine if acute pain potentially linked to ear notching increases self-grooming behavior of the affected body area and if topical application of lidocaine-prilocaine ointment has the potential to mitigate such pain.

Materials and Methods: A total of 103 12 – 19 weeks old, group-housed male and female C57BL/6JRj mice were randomized to the following 4 groups (n=25 – 26): 1, topical analgesic ointment + ear notch, 2, topical analgesic ointment + handling, 3, vehicle ointment + ear notch, 4, vehicle ointment + handling. Treatments were applied 30-60 minutes prior notching or handling only, then at specific timepoints (30, 60, 120, 240, 360 minutes and 24, 48, 72 hours) we utilized the Grooming Transfer Test[2] under ultraviolet-A light conditions with fluorescent oil applied to the ear during notching and associated handling or handling only, to score the grooming behavior of the animals by a blinded observer.

Results: According to preliminary data, mice treated with analgesic ointment showed significantly higher Grooming Transfer Test scores at 240- and 360-minute timepoints (Fisher's exact test: P=0.0214, and P=0.0017, respectively, n=25 – 26 animals/group) compared to groups receiving vehicle ointment (n=26 animals/group).

Conclusion: The increase in self-grooming behavior in analgesic-treated groups suggests a pharmacological effect of the treatment, possibly the offset of the topical analgesic, given that all animals received analgesic or vehicle topical treatment. The data provides valuable insight into potentially refining rodent care in the scope of new legislation.

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REFINING INDIRECT CALORIMETRY EXPERIMENTS BY DEFINING HABITUATION REQUIREMENTS IN MICE

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Acclimatization to new housing and experimental conditions is essential in mouse studies to minimize stress- related confounding effects. However, there is no general consensus on the duration required to achieve stable physiological readouts, as acclimatization depends on multiple factors including facility conditions, experimental setup, prior procedures, and animal handling.

In this study, we investigated the acclimatization process in an indirect calorimetry system with the aim of determining the time required to obtain stable metabolic measurements. Because total daily energy expenditure is strongly influenced by ambient temperature, we examined whether housing at thermoneutrality at 30°C compared to standard housing at 23°C affects acclimatization dynamics. Male and female C57BL/6J and BALB/c mice aged 15 to 16 weeks were continuously housed in the indirect calorimetry system for 14 days. During the first 7 days, animals were maintained at either 23°C or 30°C, followed by a temperature switch to the alternate condition for the remaining 7 days.

Preliminary results indicate that mice adapted to changes in housing temperature from 23°C to 30°C and from 30°C to 23°C within one day. In addition, metabolic parameters stabilized within less than two days after initial placement in the indirect calorimetry system. These findings suggest that a short acclimatization period is sufficient to obtain reliable metabolic data, enabling higher experimental throughput without compromising animal welfare.

REFINEMENT AS A KEY ELEMENT OF SCIENTIFIC RELIABILITY AND ANIMAL WELFARE: A CASE STUDY FROM THE PRECLINICAL RESEARCH CENTER OF THE UNIVERSITY OF PÉCS

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The Preclinical Research Center at the University of Pécs Medical School in Hungary follows a research strategy based on 3R principles (Replacement, Reduction, Refinement), emphasizing Refinement to enhance animal welfare while minimizing pain and stress.

We ensure representativeness by including both sexes, reduce animal numbers through biostatistics, and optimize breeding. Surplus animals are used for education and training to avoid unnecessary termination. Our experimental designs operate within an interdisciplinary framework that promotes collaboration among animal caretakers, researchers, and physicians, supported by continuous training. Our protocols include species-specific enrichment, stress-reducing procedures, customized lighting, and special bedding requirements. We plan to implement dynamic endpoints from human clinical research, maximize sample utilization, and apply preventive *in vitro* models in future studies. We have implemented an online lifetime animal registry system that documents animal conditions and experimental participation. We plan to use artificial intelligence to optimize the experimental design, reduce the number of animals, and enhance the results. Our institution combines scientific excellence with animal welfare considerations as a model for other centers. We collaborate with manufacturers and distributors to test new equipment, bedding materials and enrichment devices. Staff complete mandatory courses and gain pathological knowledge using surplus animals. We test 3D-printed surgical models and alternative training methods, utilizing slaughterhouse organs and surplus animal cadavers. The Center's interdisciplinary staff ensures high-quality and ethical experiments. We use modern anesthetic machines, monitoring systems, and diagnostic devices with saturation, ECG, and blood pressure monitoring. We maintain animal health through veterinary supervision and provide health training for non-veterinary staff. Our institution works with the Institutional Animal Welfare Committee, and our director serves as a member.

Our Center implements a comprehensive program based on Refinement principles. Through scientific rigor, animal welfare, and interdisciplinary collaboration, we provide a model for international, preclinical research.

OPERATIONALIZING THE 3RS IN PRECLINICAL BIOSAFETY ASSESSMENT THROUGH REGULATORY HARMONIZATION

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The implementation of the 3Rs principle in preclinical biosafety assessment requires not only the development of alternative methods, but also their structured integration within existing in vivo study frameworks operating under regulatory constraints. This contribution presents a case study describing a stepwise harmonization process implemented within a multidisciplinary preclinical testing unit performing a broad range of non-clinical studies, including biosafety-related assessments.

Starting from an established operational setting involving rodents (mouse and rat) and a non-rodent species (rabbit), currently used for acute and repeated-dose toxicity and genotoxicity testing, a systematic review of procedures, species use and documentation practices was undertaken. This analysis aimed to identify opportunities for refinement, reduction and improved integration of alternative approaches, while maintaining alignment with internationally accepted regulatory frameworks.

Procedural alignment with relevant OECD test guidelines supported the standardization of in vivo methodologies, whereas guidance from the International Council for Harmonisation informed strategic decisions related to species selection, study duration and test combinations appropriate for non-clinical safety evaluation. In parallel, exploratory in vitro approaches were assessed as complementary tools for early hazard identification and study prioritization, supporting a reduction in animal use and improved experimental design.

Particular emphasis was placed on procedural refinement, including standardized clinical observations, stress minimization measures and enhanced data traceability, as part of a structured preparation for Good Laboratory Practice accreditation. This case study illustrates how incremental regulatory harmonization can operationalize the 3Rs principle within preclinical testing units, enabling ethical and scientifically robust biosafety assessment without disrupting existing research infrastructures.

While previous contributions to 3Rs implementation have largely focused either on the development of isolated alternative methods or on regulatory advocacy at institutional level, the present work is distinctive in addressing all three dimensions concurrently: the stepwise integration of alternative approaches within an active, multi-species testing program; the simultaneous pursuit of GLP accreditation as a quality framework that reinforces rather than conflicts with 3Rs goals; and the specific operational context of a preclinical unit in Central and Eastern Europe, a region underrepresented in published harmonization case studies. Together, these features position this work as a practically replicable model grounded in real institutional constraints, rather than an idealized framework developed outside of routine testing conditions.

This approach may serve as a transferable model for preclinical testing units in Central and Eastern Europe.

REFINEMENT OF A RABBIT OSTEOARTHRITIS MODEL THROUGH IMPROVED SURGICAL AND ANESTHETIC APPROACHES

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Refinement of surgical and peri-procedural techniques represents a key component of the 3Rs principle, particularly in experimental *in vivo* disease models requiring repeated interventions. This study describes the implementation of refined surgical access and anesthetic management in a rabbit model of osteoarthritis, with the aim of improving tissue visualization, procedural control and animal welfare.

Osteoarthritis was induced through anterior cruciate ligament transection using a modified surgical approach. Enhanced visualization of the ligament was achieved by performing a medial parapatellar incision followed by lateral patellar dislocation. With the limb maintained in flexion, this technique allowed clear exposure of the anterior cruciate ligament, facilitating precise transection while minimizing unnecessary tissue manipulation. Following ligament sectioning, the patella was repositioned, and wound closure was performed using a two-layer suturing technique involving the joint capsule and skin.

Postoperative management included the administration of anti-inflammatory and antibiotic treatment to support recovery and reduce pain and inflammation. A further refinement was introduced during intra-articular administration of test substances in the osteoarthritis model. A short and targeted anesthetic protocol was employed, consisting of intramuscular xylazine (5 mg/kg) followed by maintenance with isoflurane (2%) during the injection procedure. This approach allowed rapid induction and recovery, minimizing handling stress and reducing overall exposure to anesthesia during repeated joint injections.

The described refinements demonstrate how targeted modifications of surgical access and anesthetic protocols can enhance both scientific quality and animal welfare in experimental osteoarthritis models. This case study illustrates the practical application of the Three Rs principle through refinement, supporting ethically responsible in vivo research while maintaining experimental robustness.

A MULTICOLOR TRANSGENIC ZEBRAFISH MODEL (CHILI) FOR IN VIVO STUDY OF XENOESTROGENIC EFFECTS

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Introduction: Endocrine-disrupting compounds (EDCs), particularly estrogenic chemicals, heavily contaminate surface waters. Fish, as the only vertebrates completing their full life cycle in water, are highly exposed to these pollutants. While short-term effects can be modeled *in vivo* under laboratory conditions, long-term organ- and tissue-level effects are difficult to study, as they typically require animal sacrifice. The Casper-based *Chili* line, a transparent multicolor transgenic zebrafish, enables *in vivo* monitoring of both short- and long-term estrogenic effects without euthanasia, providing a powerful alternative for more ethical and detailed investigations.

Aims: We aimed to characterize the *Chili* line, a double-recessive multicolor transgenic zebrafish, and evaluate its suitability for: life-cycle documentation, *in vivo* monitoring of short- and long-term estrogenic effects, and tracking ovotestis development *in vivo* in males.

Materials and Methods: All experiments were performed in accordance with Hungarian animal welfare legislation (permits: PE/EA/350-7/2019, PE/EA/731-7/2019). The *Chili* line was generated by crossing Casper, Tg(*vtg1*:mCherry), and Tg(βact:YFP) lines. Short-term exposure studies (5 days) used E2 and EE2 on embryos; long-term studies involved 4-week EE2 exposure of adult males. Fluorescent signals (mCherry, YFP) were monitored *in vivo* via microscopy to assess vitellogenin expression and ovotestis formation.

Results: The *Chili* line successfully tracked both short- and long-term estrogenic effects *in vivo*. Short-term EDC exposure induced mCherry fluorescence within 48–72 hours. Long-term EE2 treatment led to observable ovotestis formation in adult males without animal sacrifice. A life-cycle atlas provides reference for evaluating phenotypic changes across both sexes.

Conclusion: The *Chili* line is a robust model for *in vivo* assessment of estrogenic compounds, enabling concurrent short- and long-term monitoring while supporting the 3Rs: reducing animal numbers (Reduction), avoiding euthanasia (Refinement), and replacing more invasive models (Replacement). It offers promising applications for toxicology, developmental biology, and aquaculture research.

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AI-BASED MODEL FOR ANIMAL WELFARE: EVALUATING GPT-5 FOR RABBITS FACIAL EXPRESSION EVALUATION

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Introduction: Animal welfare, handling and performing procedures in animal research changing approach, has gradually changed since last decades in breeding and animal use facilities [1,2]. Nowadays, animal emotional welfare and state becomes the most important factor to grow trust in sciences. Measures for ensuring the welfare of laboratory animals are now applied across all species [3,4]. The use of artificial intelligence (AI) in experimental animal facilities is still a relatively inovative approach; however, promising results are observed [5]. Aim of this study was to evaluate two different evaluators and AI for facial expressions scoring.

Methods: In the analysis, a generative AI-model (ChatGPT 5.2) was used to assess the same set of rabbit (n=55) facial images for potential signs of pain. AI was provided with a standardized prompt describing rabbit facial pain indicators and asked to classify each image using the same three-point ordinal scale applied for human assessors as well. AI-generated scores were compared with human ratings using weighted agreement statistics.

Results: A total of 55 rabbit facial images were independently scored by three evaluators using a three-point welfare scoring scale. Overall inter-rater agreement among the three evaluators was moderate, as indicated by Fleiss' kappa ($\kappa = 0.55$). Friedman's test revealed no statistically significant differences in overall scoring tendencies between the veterinarian, animal caretaker, and AI evaluator ($\chi^2(2) = 4.47$, $p > 0.05$). This suggests that, at the group level, none of the evaluators consistently assigned higher or lower welfare scores across images. Study results reflect a reasonable level of agreement beyond facial-expression-based welfare assessment across evaluators with different backgrounds.

Conclusion: AI–based analysis of animal facial images showed an objective and valid approach to assessing pain and distress. Overall use of AI enhances animal welfare monitoring, reduces human observer bias, and directly supports refinement within the 3Rs principle.

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WHEN THE STRAIN MATTERS – THE IMPORTANCE OF A PROPERLY CHOSEN IMMUNODEFICIENT STRAIN FOR CANCER RESEARCH

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Introduction: Selecting a suitable mouse strain is crucial for good results. Moreover, the right choice supports the implementation of the 3Rs.

We aimed to compare the pros and cons of immunodeficient mouse strains in cancer research.

Results: T-cell deficiency makes athymic mice suitable for subcutaneous xenografts and lung colonization via i.v. injection induced by commercial tumour cell lines. But preserved immune system components can reduce engraftment rates. Moreover, breeding and production of mice are ineffective. A proportion of animals must be excluded from use in experiments due to unsuitable phenotypes. SCID (SCID/bg, NOD/SCID) mice possess higher immunodeficiency, enabling better engraftment of xenografts. Breeding is easy, and all progeny can be used. Their drawback is a high frequency of spontaneous lymphomas. In our study, we detected lymphoma in 11 out of 54 SCID/bg mice. The differences in tumour cell engraftment between athymic and SCID/bg mice can be demonstrated by our study with TNFα-overexpressing tumour cells. They were not tumorigenic in athymic mice, whereas in SCID/bg mice small xenografts were detected[1]. NSG mice exhibit severe immunodeficiency, making them an excellent host for patient-derived tissues. They reproduce readily, and adult animals do not develop spontaneous malignancies. They seem to be a perfect model for most cancer research projects, but we should be aware of tumour-stroma interaction studies due to differences in the stromal compartment. We showed that the proliferation of carbonic anhydrase I overexpressing carcinoma cells was significantly reduced unless supported by the addition of the ECM. However, this effect was observed only in SCID/bg mice, not in NSG mice.

Conclusion: Researchers should carefully consider all characteristics of available mouse strains to obtain trustworthy outcomes.

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EVALUATION OF BETA-GLUCAN–OXALIPLATIN CONJUGATES IN PATIENT-DERIVED XENOGRAFTS OF COLORECTAL CANCER

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Colorectal cancer (CRC) is a major global health problem. It is the third most common type of cancer and the second most common cause of cancer-related death. Combination chemotherapy regimens often include oxaliplatin (OX). The recent introduction of immunotherapy based on immune checkpoint inhibitors (pembrolizumab, nivolumab) in the treatment of CRC has fundamentally changed the treatment landscape of microsatellite instability–high colorectal cancer. Given the limited efficacy of immunotherapy in microsatellite-stable colorectal cancer, there is a critical need for approaches that can potentiate antitumor immune responses in combination with cytotoxic therapy.

The aim of the study was to explore alternative ways to bring immunotherapeutic approaches to the treatment of CRC tumors using precisely modified beta-glucans as immunostimulatory carriers of OX. Beta-glucans (BG) are polysaccharides found in the cell walls of fungi and yeasts with immunoregulatory and antitumor effects. One of the most effective BG is schizophyllan (SCL) from *Schizophyllum commune*. This unique SCL/OX conjugate with combined cytostatic and immunostimulatory effects is aimed at the treatment of microsatellite-stable colorectal cancer (MSS CRC) or CRC without a base mismatch repair defect (pMMR CRC), in which immunological treatment has failed.

Patient-derived xenografts (PDX) were used to evaluate antitumor efficacy *in vivo*. PDX were generated by implantation of colorectal carcinoma tissues into athymic nude mice. Mice were randomly divided into several groups and intraperitoneally injected with selected drug conjugates and standard of care (OX). Antitumor efficacy was assessed by monitoring tumor size (caliper measurements, sonographic imaging) and by analyzing animal survival. The health and weight of the animals were monitored throughout the experiment.

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DOES MINIMALLY INVASIVE ALWAYS MEAN REFINED? HANDLING-ASSOCIATED STRESS IN ZEBRAFISH GENOTYPING METHODS

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Genotyping is a routine procedure in zebrafish colony management. Skin swabbing is increasingly promoted as a refinement technique because it avoids tissue removal and anesthesia. However, refinement should be evaluated based on the overall burden experienced by the animal, including handling duration, restraint, repeated interventions and procedural predictability.

We compared two sampling techniques — fin clipping under anesthesia and non-anesthetized skin swabbing — under routine facility conditions.

Skin swabbing required prolonged manual restraint of actively moving fish and was highly dependent on the operator's experience, particularly during initial training. Insufficient or incorrectly positioned sampling frequently resulted in repeated attempts. In addition, swabbing involves direct contact with the body surface and temporary disruption of the mucus layer, which serves as a primary protective interface between the fish and the aquatic environment. In contrast, anesthetized fin clipping allowed rapid immobilization, standardized execution, and reliable genotyping success after a single intervention.

Although swabbing eliminates tissue removal, its variability increased handling duration and cumulative exposure to stressful stimuli. These findings emphasize that refinement should not be defined solely by the invasiveness of a single action but by predictability, repeatability, and total burden of the procedure. In routine zebrafish colony management, a short standardized procedure under anesthesia may represent a lower overall stress load than a minimally invasive technique requiring prolonged restraint and repetition.

ESTABLISHMENT OF A RAT PRIMARY TRABECULAR MESHWORK CELL MODEL TO INVESTIGATE THE PROTECTIVE EFFECTS OF SIGMA-1 RECEPTOR AGONIST IN HYPERGLYCAEMIA-INDUCED OCULAR FIBROSIS

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Introduction and aims: Diabetes (DM) is associated with a doubled risk of ocular complications caused by trabecular meshwork (TM) fibrosis that is aggravated by chronic hyperglycaemia. Here, we developed a primary rat TM cell model and evaluated the protective potential of our Sigma-1 receptor (S1R) agonist against DM-induced TM fibrosis, while adhering to the 3Rs principles.

Materials and Methods: Primary TM cells (prTM) were isolated by magnetic bead injection from two DM and two healthy Wistar rats. Seven days post-injection, TM cells that had phagocytosed the beads were isolated using a magnetic field. After enzymatic digestion, their identity was verified by a dexamethasone treatment followed by myocilin detection. To evaluate the effect of S1R agonists, prTM cells isolated from healthy and DM rats were treated with fluvoxamine (FLU) (10 μM, 48 hours). S1R levels and localisation were assessed by immunocytochemistry, and the accumulation of fibrotic elements was determined by Western blot.

Results: The magnetic bead isolation method successfully yielded pure, viable primary rat TM cell cultures. S1R was mainly localized in the endoplasmic reticulum of prTM. S1R protein level was reduced in prTM cells from DM rats, whereas FLU treatment significantly increased that. In parallel, more intense fibronectin and higher level of α-smooth muscle actin were measured in prTM cells isolated from DM rats, suggesting the development of TM fibrosis.

Conclusion: These findings highlight the potential of S1R activation as a promising therapeutic target to alleviate hyperglycaemia-induced TM fibrosis. By establishing a robust primary TM cell culture model, we significantly reduced the required number of animals and provided a strong basis for further drug testing to investigate DM-induced ocular fibrosis.

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SYSTEMATIC REVIEW OF 177LU-PSMA MOUSE STUDIES: COMPLIANCE WITH 3RS AND REPRODUCIBILITY

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Introduction and aims: New treatment strategies for advanced-stage cancer are under development. Radioligand therapy is emerging as one of the most promising targeted treatment approaches in oncology. Two lutetium-177 (177Lu) labelled radiopharmaceuticals are already approved for human use ([177Lu]Lu-PSMA-617 and [177Lu]Lu-DOTA-TATE). However, the field is still evolving, and there are many strategies to improve the efficacy and safety of the radiopharmaceutical. Animal studies using 177Lu–labelled PSMA-targeted radioligands for prostate cancer enable the assessment of biodistribution, dosimetry, and therapeutic efficacy. These studies have directly supported the translation of ^{177}Lu-PSMA radioligand therapies into clinical trials. Therefore, we systematically reviewed published mouse studies using 177Lu–labelled PSMA-targeted radioligands for prostate cancer and assessed their consistency with the state-of-the-art knowledge.

Methods: A search for mouse studies using 177Lu–labelled PSMA-targeted radioligands for prostate cancer was conducted in literature databases (PubMed). Data were extracted from 33 relevant articles published in 2015-2025; 8 articles published in the first 5-year period (2015-20) and 25 publications from the second 5-year period (2020-25).

We checked the articles for the reporting of details of the tumor mouse models (mouse strain, source, sex, type of tumor cell lines, amount of tumor cells, s/c, i/v, ortotopic injection), type of a study (biodistribution, dosimetry, therapeutic efficacy), measures to reduce bias (randomization and blinding), and information on clinical assessment, tumor size measurement, refinements, and humane endpoints.

Results and conclusion: Our analysis demonstrates similarity in experimental design across published 177Lu-PSMA tumor mouse studies, while revealing significant deficiencies in the reporting of essential methodological parameters required for transparency and reproducibility. In particular, the lack of reporting the use of randomisation and blinding, and critical variables such as animal sex, age, and baseline tumour burden, refinements and humane endpoints were frequently omitted or insufficiently described. We identify these limitations and propose concrete improvements.

A METHOD FOR THE INTRAVITAL ISOLATION OF PERITONEAL MACROPHAGES IN A MOUSE MODEL OF INFLAMMATORY BOWEL DISEASE

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Peritoneal macrophages play an important regulatory role in the chronic inflammation pathogenesis. Analysis of the ratio of proinflammatory to antiinflammatory phenotypes is important for assessing the pathogenesis of chronic inflammatory diseases. Published protocols for the isolation of peritoneal macrophages in mouse models setups it as a terminal procedure. Here we tried to develop a protocol for the intravital isolation of peritoneal macrophages in mice. First, this method allows for a significant reduction in the number of animals required, and second, it allows for tracking the individual dynamics of the macrophage phenotype ratio in each animal, providing higher-quality data on disease dynamics and its diversity.

We initially tested the intravital peritoneal macrophages isolation procedure on intact C57Bl/6J male mice. Firstly, we demonstrated that our procedure yields a sufficient number of cells for subsequent cytometry analysis. Secondly, we assessed the impact of the procedure on animal welfare and demonstrated that its effect on the general condition of the mice was no different from that of anesthesia alone. Finally, we demonstrated that the resulting macrophage phenotype ratios were comparable to those obtained using the classical terminal isolation method.

We performed our developed procedure on a mucin2-deficient mouse model that reproduces IBD symptoms. We demonstrated that the individual dynamics of the macrophage phenotype ratio in this mouse strain correlated well with the dynamics of clinical signs. Thus, our proposed method of intravital selection of peritoneal macrophages provides a new tool for assessing pathogenesis in this model, as well as in other models of chronic inflammatory diseases.

REFINEMENT THROUGH INNOVATION: A NON-SURGICAL TROCAR TECHNIQUE FOR TUMOUR IMPLANTATION IN RODENTS

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The 3Rs principles underpin best practice in laboratory animal science, with refinement focused on minimising pain, distress, and procedural burden while maintaining scientific robustness. Conventional tumour fragment implantation in rodent models typically requires a surgical approach involving skin incision, tissue manipulation, suturing, and post-operative care, all of which can adversely affect animal welfare and introduce avoidable variability.

Here we describe a refined, non-surgical method for tumour fragment implantation using a trocar. The technique enables subcutaneous placement of tumour material through a single percutaneous insertion, eliminating the need for incisions, sutures, and wound closure. The procedure is quick, minimally invasive, and requires reduced handling and restraint when compared with traditional surgical methods.

Implementation of the trocar-based approach has resulted in clear animal welfare benefits, including reduced tissue trauma, rapid recovery, and the absence of surgical wounds or associated complications. Animals resume normal behaviour shortly after the procedure, with minimal requirement for post-procedural monitoring or intervention. Tumour establishment and growth remain consistent with outcomes achieved using surgical implantation, ensuring continued scientific validity.

This technique represents a significant refinement within the 3Rs framework and provides a practical, reproducible alternative to surgical tumour implantation in rodents. Its adoption supports a culture of care and continuous improvement in laboratory animal procedures, aligning closely with the aims of CELASC to promote innovation, welfare-focused refinement, and best practice in animal research.

APPLICATION OF THE 3R PRINCIPLES IN A RABBIT MODEL FOR A FOCUSED ULTRASOUND ABLATION TRIAL

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Focused Ultrasound (FUS) has increasingly expanded its application in surgical and therapeutic settings, particularly for precise, non-invasive tissue ablation. FUS delivers concentrated ultrasound energy to a well-defined target, inducing thermal and mechanical effects that lead to cellular death while preserving surrounding tissues[1]. The present study aimed to evaluate the safety, tolerability, and optimal operating parameters of a FUS device in a rabbit model, focusing on kidney and skeletal muscle as target tissues. The study also sought to establish a robust preclinical basis for future clinical applications and to characterize preliminary biological effects on treated tissues.

The experiment was designed to maximize the application of the 3R principles, in accordance with European and National Legislations (D. Lgs. 26/2014)[2] to reduce the number of animals and to refine experimental procedures. Twenty male rabbits (n = 20), aged 8–10 weeks, were included (Italian ministerial approval no. 331/2025-PR). All animals arrived at the facility at 4 weeks of age and underwent a structured acclimatization and training program. Training included daily handling, supervised access to an exercise area to enhance musculoskeletal development, and interaction with technicians. This preparation allowed follow-up ultrasound assessments and blood sampling without the need for sedation.

The two-week trial included one intermediate blood sampling and ultrasound evaluation. During the mid-point assessment, no excessive restraint or sedation was required. Direct arterial catheterization enabled rapid blood collection with minimal impact and without vascular injury. No clinical signs were observed following lesion induction; structured analgesia was limited to the day after the procedure, and animals with muscle lesions showed no motor deficits. In conclusion, a model-specific training program is critical for reducing animal stress, improving experimental handling, and enabling the effective application of the 3R principles. These results support the feasibility and ethical implementation of preclinical FUS studies in rabbit models.

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GLUCOSE CONCENTRATION-DEPENDENT INTERSPECIES HEMORHEOLOGICAL CHANGES, AN IN VITRO STUDY

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Introduction: The deformability of red blood cells varies depending on the osmolarity of the microenvironment. Previous studies have shown differences in osmotic gradient deformability between humans and laboratory animals.

Aims: During the investigation of the micro-rheological effects of glucose, the question arose as to how glucose concentration influences the modulation of red blood cell deformability under varying osmolarity in different species.

Materials and Methods: The study was performed on blood samples from ten volunteers, 6 pigs, and 10 rats. The animal samples were drawn from parallel non-recovery experiments. For the osmotic gradient deformability measurements, a series of polyvinylpyrrolidone solutions with four different glucose concentrations was prepared. Under constant shear stress and osmolarity gradient, the elongation index (EI) values were determined for all four glucose concentrations.

Results: By analysing the notable points of the elongation index-osmolarity curves, such as maximum EI, minimum EI, and EI measured in the hyperosmolar range, as well as the corresponding osmolarity data, we observed differences between species and variations in different directions depending on glucose concentration. A significant difference in the EImin value measured during the osmoscan between human, pig, and rat samples was detected. A significant change in the EImax value was observed in the human group compared with concentrations of 0 mmol/L. The elongation index-osmolarity curves typically shifted right and downward in humans, to the left and downward in rats, and to the left in pigs.

Conclusion: An increase in glucose concentration impairs the deformability of the osmotic gradient in red blood cells. However, the extent and dynamics of the impairment show interspecies differences. The experimental design allowed for a reduction in the number of experimental animals, and the inclusion of human samples created the possibility for direct comparison in this in vitro study.

IMPACT OF TUMOR IMPLANTATION DEPTH ON THE IMMUNE RESPONSE IN MOUSE MELANOMA MODELS

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Introduction: Ensuring reproducibility and rigor is very important in research involving animal models. The immunogenicity of tumors can depend on depth of their implantation. Thus, the site of tumor implantation can significantly influence the experimental results.

The aim of this study was to investigate the influence of tumor implantation depth on immune response. We assessed the distribution of lymphocyte populations and Natural Killer (NK) cell phenotype in two experimental tumor models.

Materials and Methods: Syngeneic B16-F10 melanoma cells were implanted subcutaneously (SC) or intradermally (ID) into the skin overlying the right flank of C57BL/6 mice. All experiments were conducted according to recognized principles of laboratory animal care in the framework of EU Directive 2010/63/EU (project authorization no. 475/04.11.2019). After 14 days, spleens were harvested and immediately assessed using flow cytometry analyses for a large panel of cell surface markers.

Results: Analysis of lymphocyte populations showed a slight increase in the percentage of T and NK cells in SC tumor-bearing mice, but without statistical significance. The percentage of B cells was significantly lower in SC tumor-bearing mice compared to ID tumor-bearing mice. Evaluation of NK cell phenotype showed a decreased expression of NK cell activation and maturation markers in mice with SC tumors. Analysis of NK cell subsets also indicated a decrease in the mature subset and an increase in the immature NK cell subset in SC tumor-bearing mice.

Conclusion: Overall, this study showed that differences in depth of tumor implantation can translate into differences in the immune response. These data highlight the need to consider tumor implantation sites when performing in vivo studies.

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DESIGNING FOR WELFARE: FACILITIES, TECHNOLOGY & SUSTAINABILITY

FIXING WHAT SLOWS US DOWN: SIMPLE DIGITAL SOLUTIONS FOR SMARTER ANIMAL CARE PROGRAMS

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Introduction: Oversight of laboratory animal facilities generates large volumes of operational and clinical data; however, these data are often fragmented across paper records, spreadsheets, or isolated software platforms. This fragmentation limits timely communication, hinders longitudinal tracking, and reduces the ability to make data-driven decisions that support animal welfare and program efficiency.

Aims: To implement and evaluate cost-effective, cloud-based digital solutions designed to improve data consolidation, communication, and veterinary care oversight in a laboratory animal program.

Materials and Methods: We developed and implemented low-cost digital tools using widely available cloud services, QR codes, and online survey formats. QR codes were deployed at the point of care to enable rapid access to standardized digital forms for veterinary notifications, husbandry operations, recharge tracking, pathology reporting, and procedural documentation. Submitted data were automatically consolidated into centralized cloud-based repositories, generating time-stamped records and searchable archives. These systems enabled real-time notifications to veterinary and research personnel and supported longitudinal tracking of clinical events, service utilization, and operational metrics.

Results: Implementation of these tools resulted in marked improvements in data accessibility, completeness, and timeliness. Veterinary notifications and researcher communications became standardized and traceable, reducing delays and information loss. Centralized data capture enabled reliable tracking of service recharges, pathology incidence, recurrence, and trends. Searchable archives facilitated retrospective analyses and supported data-driven adjustments to veterinary care, staffing, and resource allocation. All solutions were implemented at minimal to no financial cost and required no specialized hardware or proprietary software.

Conclusion: Low-cost, cloud-based digital innovations can substantially refine veterinary care delivery and program oversight by improving data consolidation, communication, and decision-making. By leveraging simple technologies such as QR codes and online forms, laboratory animal programs can enhance efficiency, transparency, and animal welfare without significant financial investment. These approaches represent a scalable refinement supporting proactive, data-driven veterinary care and continuous program improvement.

REFINING SOCIABILITY TESTING: A WELFARE-ORIENTED HOME-CAGE APPROACH TO STUDYING SOCIAL BEHAVIOR IN MICE

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Social behavior of laboratory rodents is commonly assessed using short-term tests in novel environments, such as three-chambered or reciprocal social interaction tests, which may alter the natural behavior of animals. Home-cage monitoring represents a refinement approach that allows the assessment of spontaneous social interactions under low-stress conditions. Despite the importance of sex hormones, research on estrogens, particularly estradiol, in regulating social behavior remains limited, and female animals are still underrepresented in experimental research.

Therefore, this project investigated the effects of low estradiol concentrations induced by ovariectomy (OVX) or aromatase inhibition (letrozole, LET) on social behavior in adult female C57BL/6J mice using home-cage PhenoTyper systems.

Two-month-old females (n=40) underwent either OVX (OVX, n=20) or sham surgery (F, n=20) and were assigned to four treatment groups: sham-operated females treated with either LET (1mg/kg, F+LET, n=10) or olive oil vehicle (1ml/kg, F, n=10) and OVX females treated with either 17β-estradiol (10μg/kg, OVX+E, n=10) or olive oil vehicle (1ml/kg, OVX, n=10). Treatments were administered subcutaneously once daily for two weeks. During the final three days, locomotor activity, indirect social interaction, and reciprocal social interactions were assessed directly in PhenoTyper cages, minimizing stress associated with environmental novelty.

OVX females showed lower uterus weight than F females, while estradiol supplementation increased uterus weight in OVX mice, confirming effective hormonal manipulation. OVX did not affect locomotor activity but reduced social disinterest in the reciprocal social interaction test, compared to F mice. Automated indirect measures of social behavior showed no group differences, indicating limitations of current software (EthoVision XT 10) and the need for manual analysis.

In conclusion, this study combines female-focused research, surgical and pharmacological estrogen depletion, and home-cage testing, providing a refined, welfare-oriented approach consistent with 3R principles.

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DESIGN AND DEVELOPMENT OF A RABBIT ACTIVITY TRACKING MODEL FOR ORTHOPEDIC PAIN ASSESSMENT

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Rabbits have been employed as a model species in preclinical orthopedic research[1]. In human patients, pain and reduced activity are typical clinical signs for patients with osteoarthritis (OA). Rabbit activity tracking, in comparison to rodents or zebrafish, is not a thoroughly defined method in the literature. In this study we aim to assess pain in a surgically induced OA model through the tracking of animals' activity and free movement using automated software. This activity-based model will be able to account not only for the change in gait as possible sign of pain, but also for the change in behavior[2].

9 Female, NZW rabbits (>20 weeks old, Charles River) were included. Rabbits were recorded in a dedicated pen where they were allowed to roam freely. Ethovision XT v17 (Noldus) was used to analyze the activity of the animals.

The first study involved 4 rabbits acclimatized once to the pen before the baseline recording. Activity outcomes were acquired at different timepoints after surgical intervention inducing OA. In a second study, 5 unoperated animals entered the tracking pen daily for 5 days with no prior acclimatization.

Results showed a high variability between individuals in both experiments. Operated rabbits showed a peak of activity in week 4 that had a decreasing tendency until week 11. For unoperated animals, there was a marked decreasing tendency in activity from day 1 to day 3 and a slight increase in the tendency from day 4 to 5.

In conclusion, a decline in activity with advanced OA was observed. Further, there seems to be an acclimatization effect of the rabbits to the tracking pen. Variability seems to decrease with time in unoperated animals. Further testing must be conducted to assess best acclimatization protocols and to correlate activity tracking with macroscopic and histological assessment of OA.

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THE REFINED STANDARD: ENRICHED RAT HOUSING THAT RESPECTS FACILITY HYGIENE

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One of the main concerns repeatedly raised relates to the housing conditions of laboratory rodents. Although in line with minimal standards given by legislation, standard rat housing conditions do not fully meet the species-specific needs of these animals. Therefore, laboratory rats often face monotony to limited space and stimulation. Due to the small cage size, playing and chasing behavior can be rarely observed but signs of boredom or even apathy instead.

So far, most efforts have focused on providing taller multi-level cage designs (often rabbit cages), however, with the drawback of not being compatible with standard rodent housing and cage processing regimes. To address this issue, we developed a 3D-printed cage connector system that enables the modular (inter)linking of standard rat cages. The implementation of this connector system resulted in the housing of larger group sizes with an increased total available space per animal. In addition, it allowed us to use a variety of enrichment configurations including diverse elements like hammocks, hemp ropes, nesting and burrowing materials, gnawing items, cognitive challenges and different shelters, providing the animals with a larger variety of options for exploration and social interaction. For the first time we observed playing and chasing behavior in our 6 – 12 weeks old male and female Wistar and Sprague Dawley rats and the rats appeared to be more curious and less cautious.

To integrate this system into our regular housing structure, we designed it (connectors as well as enrichment materials) in way to ensure full compatibility with existing hygienic and procedural standards in our experimental housing facility (all items are washable and autoclavable). With our system we demonstrate that refined housing conditions can be successfully implemented within standard infrastructure while offering substantial welfare benefits for laboratory rats.

DEVELOPMENT OF PATIENT-DERIVED XENOGRAFT AND ORGANOID MODELS FOR STUDY OF EFFICACY OF NEW TAXANE-BASED CHEMOTHERAPEUTICS

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Pancreatic cancer is an extremely fatal malignancy with increasing incidence and mortality due to late diagnosis and low sensitivity to treatments. Search for the most appropriate drugs and therapeutic regimens is the most promising way to improve the treatment outcomes of the patients. The aim of this study was to implement the patient-derived xenograft (PDX) *in vivo* models of pancreatic carcinomas for study of efficacy of experimental Stony Brook taxanes (SB-Ts) and establish patient-derived organoids (PDO) as 3D miniaturized versions of tumors with functions resembling their *in vivo* counterparts.

We successfully created three PDX models with various subtypes of pancreatic cancer (ductal, acinar, and adenosquamous adenocarcinomas) and used these models for comparison of conventional paclitaxel- and SB-T-based chemotherapy regimens. Combination of new taxane analog SB-T-121606 with paclitaxel *in vivo* was effective in suppressing tumor growth and well tolerated at small doses (≤3 mg/kg), providing a novel promising therapeutic regimen. In addition, we established and optimized the growth of organoids from tumor cells of pancreatic carcinoma patients. Optimization of organoids in pharmacotyping of drug efficacy is currently underway, together with molecular characterization of underlying changes in tissues after successful treatment.

In conclusion, the established PDX or PDO models serve as useful models for testing of new and optimal therapeutic regimens and future discovery of signaling pathways behind the aggressiveness of pancreatic carcinoma.

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SUSTAINABILITY AND SCIENTIFIC IMPACT OF ELECTRONIC CAGE LABELING IN RODENT FACILITIES

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Introduction: Paper-based cage cards and fragmented record-keeping can contribute to transcription errors, delayed updates, and unnecessary time spent inside rodent rooms – factors that may compromise both operational efficiency and animal welfare. Aligned with the principles of the 3Rs and with facility-level sustainability objectives, this work presents a lab-wide digitalization approach based on individual microchipping, routine scanning, and electronic/QR-coded cage labeling to strengthen traceability and centralize animal-related data in mouse and rat facilities.

Aims: The proposed workflow integrates permanent individual identification via RFID-compatible microchips, rapid cage- and animal-level data access through QR codes and scanner-based retrieval, and centralized digital records enabling near real-time updating of colony status, experimental procedures, and husbandry information.

Materials and Methods: The implementation framework is based on standardized electronic labeling, digital data capture via scanning, and centralized management of animal- and cage-level information, ensuring consistency across users and work shifts. Emphasis is placed on reducing manual transcription, improving data integrity, and minimizing the frequency and duration of staff interventions required solely for administrative updates.

Results: From a sustainability perspective, electronic labeling supports the progressive reduction of paper consumption and prevents repeated re-printing of cage cards following minor changes in experimental or housing conditions. From a scientific and welfare standpoint, improved traceability and rapid access to up-to-date information can reduce avoidable handling, lower the risk of misidentification, and support more consistent daily practice within animal facilities. Additionally, centralized digital records facilitate retrospective data verification and enhance transparency in colony management.

Conclusion: Overall, electronic cage labeling – combined with microchipping and centralized data management – represents a practical and scalable strategy that links technological innovation, welfare-oriented facility design, and sustainable operations in modern rodent research environments. This approach supports harmonized workflows while contributing to refinement-driven improvements in laboratory animal care and management.

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VIABILITY TESTING OF VITRIFIED MOUSE EMBRYOS AFTER LONG-TERM STORAGE

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Introduction: Vitrification is a widely used method of embryo cryopreservation and a proven archiving technique for genetically modified mice. The literature primarily provides data on freshly frozen samples or samples stored for up to a few years, with little information available on the effects of long-term storage lasting up to decades.

Aims: The aim of our study was to analyze the effects of long-term cryopreservation on the survival rate, *in vivo* development, and viability of offspring of vitrified preimplantation mouse embryos. We thawed vitrified embryos from mouse lines with different genetic backgrounds, most of which were genetically modified, including both freshly vitrified and samples stored for more than two decades. The embryos were grouped according to several criteria, including the time elapsed since vitrification, the stage of development, the genetic background, and the presence of transgenesis. We examined the revival rate after thawing and then implanted the embryos into recipient females. After embryo transfer, we analyzed the pregnancy rate and the number of live births.

Results: Our studies confirmed that despite long-term storage, embryos are capable of implantation and producing healthy offspring, but there is a strong downward trend in pregnancy and birth rates over time. The survival rate of embryos around 20 years old showed a significant decrease compared to samples less than 10 years old. The embryos appear to be fine after thawing, but they develop in lower numbers both *in vitro* and, especially, *in vivo*.

Conclusion: In the case of long-term storage, therefore, many more vitrified embryos need to be stored in order to have enough embryos to thaw and implant for successful line regeneration.

Keywords: vitrification, mouse embryo, long-term cryopreservation, GA mouse lines, embryo transfer, assisted reproduction

DAILY ANIMAL CARE OBSERVATIONS SUPPORTING ANIMAL WELFARE

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While early laboratory animal welfare research was ocused primarily on housing conditions and experimental variables, the contribution of animal care staff to welfare assessment and refinement has gained increasing recognition since the late 20th century. Animal welfare is widely influenced by providing conditions that allow species-specific behaviours and approximate natural environments[1].

In our animal facility, animal care staff are central to refinement through daily observations and active involvement in environmental enrichment. Their role goes beyond routine application of standard enrichment items and includes locally developed solutions, as well as ideas inspired by webinars, conferences, and exchanges with other facilities.

Routine observations by caretakers have guided changes to improve mice welfare. For example, stereotypic behaviour was mitigated by alternating toys and obstacles. Platforms were introduced for mice with diabetes, while gnawing materials supported animals on soft diets. In cages housing aggressive males, additional nesting material, chewable wooden elements, and at least two tunnels were provided to reduce competition. Inspiration from other facilities, such as using glass jars as shelter, was implemented locally, producing clear positive effects on behaviour[2]. Awareness that rodents' thermoneutral temperature is ~30 °C, not 20–24 °C, led caretakers to adjust nesting materials[3]. By selecting and providing appropriate nesting resources, staff enabled mice to build enclosed nests that retain heat and offer improved protection for pups.

These examples illustrate how the experience and daily presence of animal care staff directly support refinement and welfare. Their careful decisions, engagement, and empathy are central to effective and humane animal care.

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PARVOVIRUS, LYMPHOCRYPTOVIRUS AND RHADINOVIRUS REACTIVATION DURING IN VIVO PRECLINICAL EXPERIMENTATION IN *MACACA FASCICULARIS*

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Macaca fascicularis is the preferred non-human primate species for preclinical toxicology studies. Those from Mauritius offer a favorable serological profile, as the island is free of major zoonotic viruses such as Herpes B, Rabies and immunosuppressive retroviruses. They are also routinely vaccinated against Measles and Hepatitis A.

Immunocompetent animals may carry latent viruses that generally do not affect research outcomes. However, in studies testing immunomodulatory compounds, silent viruses may reactivate during the *in vivo* phase. Reactivation of Simian Parvovirus, Lymphocryptovirus (LCV), and Rhadinovirus (RhV) are known to occur, which can impact research activities.

In our facilities, we observed compound-related parvovirus-induced hematological abnormalities presenting as non-regenerative anemia. Hematology monitoring showed gradual decline in red cell parameters, but the *in vivo* phase was completed as planned. Bone marrow cytology and histopathology were consistent with parvoviral infection, which was confirmed by positive in situ hybridization for Simian parvovirus in terminal bone marrow samples. Compound-related LCV and RhV reactivation resulted in the occurrence of lymphomas, with variable clinical signs that required premature euthanasia in high-dose animals. Widespread lymphoproliferative lesions were present in multiple organs, including lungs, kidneys, pancreas, and lymph nodes. In situ hybridization confirmed LCV and RhV in the affected lymphoid tissues.

Clinically affected, compound-treated animals were unable to mount an immune response to the virus and therefore serology results were unreliable. The altered immune status induced by the compounds prevents accurate immunological testing during treatment and for the detection of infection during treatment. Serology assays should thus be limited to initial screening before these studies.

Additionally, preclinical studies with immunomodulatory compounds can be challenging and entail sanitary and safety issues given the potential for manifestation of subclinical infections with pathological and opportunistic agents (e.g., amebae, ciliates, mycobacteria, Campylobacter and Adenoviridae), some of which may be zoonotic.

ADVANCING RODENT WELFARE IN IVC: FROM METABOLIC ASSESSMENT TO NOVEL MONITORING TECHNOLOGIES

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Introduction: Accurate phenotyping of genetically modified rodents and ensuring their welfare remain central challenges in biomedical research.

Aims: To implement non-invasive technologies at the animal facility supporting the 3R principles (Replacement, Reduction, Refinement) of humane research.

Materials and Methods: Funded through the National Recovery and Resilience Plan, the Rodent Facility (RF) at the International Institute of Molecular and Cell Biology in Warsaw (IIMCB) installed a state-of-the-art metabolic platform capable of measuring gas exchange, calorimetry, and methane production, enabling comprehensive metabolic profiling of newly generated mouse lines. Separately, a Digital Ventilated Cage (DVC) system was deployed, utilising sensor technology embedded in individually ventilated cages (IVC) to continuously track locomotor activity, feeding behaviour, and welfare indicators without animal handling.

Results: Both systems are being incorporated into the RF service portfolio, with the expectation that each independently demonstrates the capacity to characterise mouse phenotypes through entirely non-invasive approaches. The metabolic platform with methane analysers is expected to reveal subtle metabolic and gastrointestinal differences among novel mouse models. The DVC system provides uninterrupted behavioural and welfare data, enabling early detection of distress or disease. Each system reinforces 3R compliance—refining experimental procedures, reducing unnecessary interventions, and potentially lowering animal numbers through improved data sensitivity.

Conclusion: The adoption of these two independent, non-invasive technologies at IIMCB establishes a modern framework for rodent phenotyping and welfare assessment, demonstrating that 3R principles and scientific excellence are mutually achievable.

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ONE WELFARE

WHICH IS THE BEST ANIMAL MODEL TO STUDY OSTEOARTHRITIS (OA)?

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Osteoarthritis (OA) is a crippling disease. It affects more than 50% of the population over 50 years old. It has been studied for years using numerous animal models. Several models are available, but which one should be used, which one is the best model for OA studies? Easy question, easy answer: the best model is a human suffering from OA! In the meantime, we must concentrate on a specific question: What is the purpose of the study? Once that question is answered, it becomes easier to select an appropriate model.

Moreover, this lecture will review the most frequently used models, will outline the advantages and disadvantages of each model and will try to illustrate what are the most common means of evaluating function and pain in small and large animal models of OA using various methodologies. These tools were developed and validated in laboratory animals. They were then adapted for use in animals with spontaneous osteoarthritis in a research setting, for example, in dogs and cats. These tools are complementary in our understanding of this condition. These tools allow us to objectively evaluate the effectiveness of therapeutic approaches.

Furthermore, this lecture will present three research proposals for which the thought process to select the best model for each proposal will be illustrated. The means of measuring outcomes will also be presented and discussed.

The translation of our results is important. We can document the presence of osteoarthritis, which is very similar across all species. We were able to observe the temporal evolution of osteoarthritis: no progression, slow progression, or rapid progression (RPOA), which is what is also observed in humans. We can assess the impact of treatments in several species. This will allow us to adapt our therapeutic strategies and propose new ones.

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HOW MOVING TO VOLUNTARY ADMINISTRATION OF SUBSTANCES SUPPORTS HUMAN AND ANIMAL WELFARE

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The University of Dundee established a 3Rs Champions Group (3RCG), comprising animal technicians, to advance its institutional 3Rs strategy. Working alongside research groups and the Named Veterinary Surgeon, the group focuses on refining experimental and husbandry procedures. One major initiative was the development of a voluntary oral dosing method for mice to replace oral gavage, a commonly used but stressful procedure for both animals and staff.

Building on previous research[1], two technicians were tasked with exploring palatable liquids and semi-solid carriers that mice would consume voluntarily. Successful identification of suitable carriers enabled mice to be trained to accept test substances. This approach was validated through a pilot study involving long-term drug administration, followed by a full study in which mice were voluntarily dosed three times weekly for six weeks, consistently consuming the full dose.

The method was shared at an internal training event, prompting further collaboration. Research and animal technicians jointly developed a robust voluntary dosing protocol for protozoan parasite infections, now used routinely and being adapted for helminth parasites. The approach was also applied to oral glucose tolerance testing (OGTT). Technicians demonstrated that mice would voluntarily consume glucose solutions, producing results comparable to gavage while reducing stress. Additionally, hypoglycaemic mice undergoing insulin challenge were shown to voluntarily consume condensed milk to restore blood glucose, avoiding invasive intervention.

This stepwise, technician-led refinement resulted in broad application of voluntary dosing at our organisation, with an estimate that over 3,000 mice per year could benefit. The method enhances animal and human welfare, by reducing the stress for both and improves scientific date quality. Collaborative working between researchers and technicians has built trust and improved our culture of care. The technicians leading this work have felt supported and empowered and were recognised with the EPAA 2025 Refinement Prize.

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TELL YOUR STORY IN A POSTERETTE – STRENGTHENING SCIENTIFIC ENGAGEMENT, TRAINING, AND WELFARE LEADERSHIP AMONG ANIMAL TECHNOLOGISTS

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Introduction and Aims: Animal Technologists (ATs) are central to animal welfare, daily monitoring, and the reliability of research data. Yet increasing regulatory expectations, complex care responsibilities, and the emotional demands of caring roles can leave ATs feeling under-prepared to engage in scientific planning or to recognise the scientific value of their work. Strengthening ATs' communication skills and research engagement is essential to sustaining a progressive culture of care. Within this environment, the designated veterinarian holds a pivotal leadership role. Vets are well positioned to support ATs in understanding study design, interpreting welfare indicators, shaping humane endpoints, evaluating refinements, and recognising how their observations directly influence research quality. They also facilitate transparent, bidirectional communication across Named Persons, researchers, technicians, and institutional teams, ensuring alignment between welfare considerations and scientific aims.

Methodology: We describe the development of a practical resource designed to enhance ATs' self-awareness, confidence, and engagement in scientific dialogue. Central to this initiative is the Tell Your Story workshop, which uses scenario-based exercises to explore communication challenges encountered when planning, executing, and presenting a scientific poster. Through guided activities, participants gain theoretical and practical skills in poster design, scientific structuring, and presenting welfare-relevant information in formats appropriate for research environments. They also practice articulating their technical expertise to research teams. These practical components run alongside facilitated reflection on ATs' care and welfare responsibilities, supporting them in navigating caring pressures while strengthening their professional identity.

Results and Conclusions: This resource was evaluated at two sites, receiving overwhelmingly positive feedback. By integrating veterinary leadership with communication and research-presentation skills, the workshop empowers ATs as scientific contributors and welfare advocates, enhancing research quality and embedding a collaborative and resilient culture of care.

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IMPLEMENTING THE 3RS IN VETERINARY EDUCATION USING ARTIFICIAL MOUSE AND RAT MODELS

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Background: The 3Rs must be an integral part of education, especially in veterinary practical education, where the use of live animals is unavoidable. As part of the implementation of the 3Rs at the University of Veterinary Medicine Budapest, students have access to artificial mouse and rat models. Early practical experiences can be stressful for both animals and students, especially when students encounter handling, sampling, and administration techniques for the first time.

Aims: The aim of this study was to evaluate the role of artificial mouse and rat models in laboratory animal science education as tools for implementing the 3Rs, improving animal welfare, and supporting student well-being during practical training.

Methods: Laboratory animal science education starts in the third semester, so most students are trying different sampling and administration methods on animals for the first time. Basic practical techniques can be practiced on model animals until students can perform them confidently and correctly, before moving on to carcasses and ultimately live animals. Student feedback regarding learning experience, confidence, and stress levels was collected and evaluated.

Results: Student feedback indicated that the use of artificial models improved confidence and reduced anxiety before performing procedures on live animals. Students reported feeling better prepared and more competent, which resulted in calmer and more controlled handling during subsequent live animal sessions. The use of models also contributed to a reduction in the number of carcasses and live animals required for training purposes.

Conclusion: Artificial training models are effective tools for supporting all three principles of the 3Rs. They facilitate replacement by providing alternatives to live animals during early training, reduction by decreasing the number of animals required, and refinement by minimizing stress and discomfort during live animal procedures. In addition, these models positively impact student well-being and represent a valuable refinement of laboratory animal science education.

WHAT CAN WE LEARN FROM THE PAST? LESSONS FROM THE HISTORY OF ANIMAL EXPERIMENTATION

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Background: Knowing the past helps us understand the present and prepare for the future. The history of animal experimentation can be traced back to antiquity, with animals being studied as early as the 6th century BC to advance anatomical and physiological knowledge. These early practices raised ethical concerns and encouraged philosophical reflection on the moral status of animals and the justification of their use in scientific research.

Aims: The aim of this poster is to provide a concise historical overview of animal experimentation and to highlight how scientific progress and ethical considerations have shaped current regulations and animal welfare standards.

Historical development: In Europe, animal experimentation largely declined during the Middle Ages, while in the Middle East, various surgical techniques were tested on animals as early as the 13th century. A significant turning point occurred during the Renaissance, when scientific interest in animal experimentation was renewed. In the following centuries, experiments became increasingly widespread. From the 17th century onward, animal experimentation gained a stronger medical focus; however, animals were also used for entertainment purposes, often in a cruel and unregulated manner.

Ethical awareness and legislation: Physiological knowledge gained from animal experiments gradually revealed similarities between animals and humans, challenging earlier views that sharply separated the two. This awareness contributed to the emergence of the animal protection movement and ultimately led to the first legislation regulating animal experimentation, including the On the Cruelty to Animals Act of 1876.

Conclusions: During the 19th century, the discovery of microbes and the development of vaccines and antitoxins increased the number of animals used in experiments, and many previously unused species were included in studies. This put increasing pressure on both scientists and legislators, leading to a growing number of guidelines and legislation. Over time, this resulted in the well-regulated scientific sector we know today.

PROMOTING MULTIDISCIPLINARY 3RS TRAINING THROUGH FELASA SEVERITY WORKSHOPS IN GREECE

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Hellenic Society of Biomedical and Laboratory Animals Science (HSBLAS), Governing Board 2024-2026

The Hellenic Society of Biomedical and Laboratory Animal Science (HSBLAS), is actively involved in the delivery of FELASA Severity Workshops (SW), with 2 independent trainers and 3 prospective trainers who have attended a train-the-trainer workshop. Between February 2024 to February 2026, HSBLAS offered 4 SWs, in 3 major cities with significant academic and research activity: Athens, Thessaloniki and Heraklion. In total 124 delegates were trained to classify and report the prospective and actual severity using interactive examples. The main models used in all workshops were the mouse tumour and rat neuropathic pain, while the zebrafish genotyping and calf cryptosporidiosis were selectively presented depending on the audience's research interests. Sponsorship from hosting institutions, commercial suppliers and a well appreciated grant from Laboratory Animals Limited (LAL) reinforced HSBLAS towards organizing the SWs. Laboratory animal professionals: technicians, researchers, veterinarians, managers, new users as well as competent authority officers and regulators completed the training. An online questionnaire was used by HSBLAS to assess the delegates' benefit of knowledge level and confidence in classifying and reporting the severity of procedures and their feedback on the quality of delivered SWs. The overall positive responses support HSBLAS goal for continuing the organization of SWs and making an effort to host future SWs in research institutions of other cities and deliver them in Greek language, to enable new users and personnel to attend. Being a part of the FELASA Severity Workshop community has given the opportunity to HSBLAS to complement its educational activities promoting multidisciplinary training in replacement, reduction and refinement, with a significant standardized Workshop.

THE EUROPEAN SOCIETY OF LABORATORY ANIMAL VETERINARIANS – EMPOWERING VETERINARIANS THROUGH EDUCATION, SUPPORT AND ADVOCACY

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European Society of Laboratory Animal Veterinarians (ESLAV)

The European Society of Laboratory Animal Veterinarians (ESLAV) was founded in 1997 to support all veterinarians working within the field of laboratory science.

With more than 200 members within and outside Europe, ESLAV's main aim is to provide continuing professional development and training activities for veterinarians working in all areas of laboratory animal science. ESLAV promotes high standards and shares developments in the field through conferences, training events, webinars. ESLAV brings together veterinarians interested in the field and represents them at international level, as FELASA Associated member, representation in EVERI, AAALAC international, and thanks to active presence at the biannual National Contact Point meetings at the European Commission.

ESLAV has developed partnership with the European College of Laboratory Animal Medicine, the University of Copenhagen, Aachen University and the Autonomous University of Barcelona for educational activities of its members.

ESLAV is also active in discussing and promoting the role of veterinarians to ensure animal welfare outside the Lab Animal Science world, such as the congress of the Federation of European Neuroscience Societies (FENS).

OPTIMIZATION OF COLORECTAL CANCER CELL-LINE DERIVED XENOGRAFTS AND COMPARISON OF SCREENING APPROACHES

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Colorectal cancer (CRC) is the third most commonly diagnosed malignancy worldwide and the second leading cause of cancer-related mortality.[1] Its high biological heterogeneity causes a significant challenge for effective treatment. Traditional two-dimensional (2D) cell line models fail to recapitulate the spatial architecture and physiological complexity of native tumors. In contrast, three-dimensional in vivo models, such as cell line–derived xenografts (CDXs) and patient-derived xenografts (PDXs), represent physiologically relevant tools for studying tumor growth, metastasis, and therapeutic efficacy. CDX models play a pivotal role in the early stages of drug development by enabling the identification of promising therapeutic candidates in the preclinical setting.

The aim of this study was to optimize the establishment of colorectal cancer CDX model using the murine colon adenocarcinoma cell line MC38 in C57BL/6 mice and to assess the antitumor efficacy of candidate drugs using multiple readout strategies.

To establish the optimal tumor inoculation protocol, mice were subcutaneously injected with varying numbers of MC38 cells. Higher cell numbers resulted in faster and more aggressive tumor growth, whereas inoculation with lower cell numbers provided a longer experimental window, which is more suitable for drug efficacy assessment. During tumor progression, multiple screening methods were evaluated to monitor tumor growth and detect potential necrosis, including digital calliper measurements with visual inspection, sonographic imaging, and additional assessment techniques. These methods were compared to determine the optimal approach in terms of data quality and time efficiency. The optimized establishment of CDX model and screening strategy provide a robust and practical platform for preclinical evaluation of therapeutic candidates in colorectal cancer.

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WHERE DOES THE TROLLEY GO? USING ETHICAL DILEMMAS IN VETERINARY EDUCATION

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Background: Bioethical issues have long been an integral part of animal science, especially when we talk about laboratory animal science or veterinary medicine. That is why we pay special attention to preparing students at the University of Veterinary Medicine Budapest to think and discuss about such issues.

Aims: The aim of this poster is to present an educational approach that integrates classical ethical thought experiments into the teaching of laboratory animal science, and to demonstrate how this method supports ethical awareness and critical thinking among students.

Methods and educational approach: The trolley dilemma and related thought experiments have long been part of ethical discourse. Four years ago, these dilemmas were incorporated into the teaching of laboratory animal science in the form of a hand-in assignment. Student responses are collected and analysed anonymously on an ongoing basis. In the assignment, students are presented with four partially overlapping scenarios and are asked to decide how they would respond to each situation and to explain the reasoning behind their decisions.

The first scenario presents the original trolley dilemma, where students must choose between saving five people by diverting a train and sacrificing one individual. In the second scenario, instead of pulling a lever, students are required to actively intervene by killing one person. The third scenario focuses on a medical decision related to the treatment of seriously ill individuals, while the fourth scenario involves decision-making in a transplant case.

Educational relevance, conclusions: These dilemmas effectively engage students in reflecting on animal welfare, ethical principles, and legal considerations relevant to laboratory animal science. Based on our experience, this teaching method has proven to be highly useful for encouraging discussion and critical thinking. Consequently, the ongoing evaluation of student responses provides valuable insight into the attitudes of younger generations toward animal experimentation, supporting the development of ethically responsible future professionals.

MINIMALLY INVASIVE FOOT VEIN BLOOD SAMPLING AS A REFINEMENT OF CLASSICAL TECHNIQUES IN RATS

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In recent years, increasing emphasis has been placed on refinement strategies in animal experimentation, particularly for procedures that are performed repeatedly. Repeated blood sampling in laboratory rats is a common requirement in biomedical research, yet some sampling techniques require anesthesia (vena sublingualis or cava) while restraint and hair clipping is suggested for others (tail or saphenous vein), making all of them stressful for the animal.

To address these limitations, we developed and implemented a refined method for repeated blood collection from the dorsal digital veins. The procedure is performed by two operators and involves only mild restraint. Optionally, the rat's foot can be briefly warmed in a water bath at a maximum temperature of 42°C to promote vasodilation, after skin disinfection, the foot is gently dried and one of the dorsal digital veins pinched with a 24G needle to allow blood collection with a Microvette 300 (Sarstedt, Germany). Hemostasis is achieved by short manual compression with a cotton swab, after which the animal is immediately returned to its home cage.

This method offers several advantages over classical techniques. No shaving or anesthesia is required, sufficient blood volumes (up to 0.2 ml) can be obtained repetitively, and serum/plasma samples are rarely hemolytic. The procedure is rapid and minimally invasive, and no hematoma formation was observed. Importantly, repeated sampling can be performed without adverse effects. Our approach represents a practical refinement of routine blood sampling that improves efficiency while reducing animal burden and supporting high welfare standards in laboratory rat studies.

EUROPEAN COLLEGE OF LABORATORY ANIMAL MEDICINE: FACTS AND FIGURES

Zacharioudaki, A., Williams, R.

ECLAM, UK

The European College of Laboratory Animal Medicine (ECLAM) is one of 27 European Board of Veterinary Specialisation (EBVS®) recognized veterinary specialist colleges. ECLAM adopts EBVS® standards and ensures excellence and consistency in education, training and veterinary specialisation in Laboratory Animal Medicine in Europe. ECLAM was provisionally recognized in 2000, received full recognition in 2008, while the first certification exams were held in 2005. Since then 135 veterinarians have received the title of ECLAM Diplomate and European Veterinary Specialist in Laboratory Animal Medicine, of whom 61 are founding and de facto, 4 accepted as equivalent and 70 by examination. Currently ECLAM Membership comprises 84 certified Diplomates, 19 non-certified and 32 retired. Also, there are 31 active Residents, of whom 11 attend the 4 standard residency programs and 20 follow the alternative training route. The majority 60% of ECLAM Diplomates are employed in academia, 30% in industry and 10% in government positions. Membership data uncover a rise in Diplomates who are certified by examination in parallel with the retirement of de facto Diplomates, and a slow rise in standard residencies while the alternative route remains the dominating route to specialisation.

ECLAM is governed by the Council, consisting of the President, Past President, President Elect and Ordinary Members, guiding strategy, while operation is run by the Education Committee, credentialing exam candidates, training programs and new residents, the Exam Committee, delivering the certification examinations, and the Mock Exam Committee, preparing a mock exam to enable candidates' preparation. ECLAM offers multiple routes to specialisation, including a standard and an alternative residency, plus the internationally recognized expert route, while ensuring all requirements remain relevant through periodic review. Furthermore, ECLAM promotes lifelong learning via continuing education programmes such as ESLAV-ECLAM Summer and Winter Schools, an annual scientific meeting, and other events.

IN VITRO RECONSTRUCTED 3D MODEL OF HUMAN SMALL INTESTINE EPITHELIUM FOR ASSESSMENT OF SUBSTANCE BIOAVAILABILITY AND SAFETY

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Animal models have been widely applied for assessing bioavailability and toxicity of orally administered drugs and other substances. However, such testing can be expensive, time consuming and limited in terms of translatability to human conditions. Hence, in vitro models are needed to guide the design of molecules or dosing schedules that mitigate safety risks in humans. We have developed an organotypic in vitro model of small intestine that closely mimics morphology and physiology of intestinal epithelium.

To test the usability of this in vitro model as a gastrointestinal (GI) toxicity prediction tool, we have selected a panel of drugs with known effects on GI tract and therapeutic compounds for which animal toxicity studies were not predictive of human toxicities.

In the assays aimed at the assessment of bioavailability of selected compounds we have shown that test drugs with human absorption of >80% (high permeability drugs) had an in vitro apparent permeability coefficients (Papp) of >2 x106 cm sec-1 and drugs with <80% human absorption (low permeability drugs) had in vitro Papp values of <2 x106 cm sec-1. Using these criteria, the tissue model categorized the test drugs as high permeability and low permeability with a high sensitivity and specificity compared to historical human absorption data. Drug-drug interactions were also examined using efflux transporter inhibitors. The inhibitors increased drug bioavailability while decreasing the efflux ratio. Efflux ratios for substrates talinolol, digoxin, and loperamide were reduced by 45%, 40%, and 60%, respectively, in the presence of the P-gp inhibitor, verapamil. Results from drug metabolism studies also showed midazolam (CYP3A substrate) was metabolized by the intestinal tissue model.

In conclusion, the in vitro human primary cell-based small intestinal tissue model may serve as a promising tool to predict safety, permeation, metabolism of orally administered substances in humans.

HIGHLY DIFFERENTIATED 3-DIMENSIONAL VAGINAL TISSUE MODEL FOR TESTING OF FEMININE HYGIENE PRODUCTS AND ITS REPRODUCIBILITY

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When making risk and hazard assessments of new ingredients or finished products, reproducibility of the toxicology test system is of extreme importance to regulators, in-house toxicologists, and formulation scientists. One of the advantages of in vitro test systems vis-à-vis animal-based systems is that the in vitro systems can be highly reproducible while in vivo models typically exhibit high variability. The commercially available, in vitro vaginal tissue model, EpiVaginal has been proposed as an in vitro replacement for the rabbit vaginal irritation test for assessing the safety of spermicides and other feminine care products. In the current study, reproducibility of the quality control data and testing results for feminine care products were each compared in 2 labs. For testing of end-use products (test articles or TAs), tissues were produced by MatTek Europe (Bratislava) and testing was performed at MatTek Europe and at the Centre of Toxicology and Health Safety (Prague). Two concentrations of 5 feminine care products or ingredients were applied to the apical tissue surface for 24 hrs. Tissue viability for the TAs was assessed using the MTT assay and transepithelial electrical resistance (TEER) measurements. Values obtained for TAs were compared to the negative control tissues exposed to ultrapure water. The results for the 2 labs were compared, determining the difference in % tissue viability and % barrier integrity. The average difference for the % tissue viability for the 10 TAs was 10.1%; only 2 TAs had a difference >15%. The average difference for the %barrier integrity was 9.0%; only 2 TAs had a difference >15% demonstrating a high level of interlaboratory reproducibility. The lot-to-lot and lab-to-lab reproducibility in two different geographical locations indicate that the vaginal model can serve as a valuable and reliable alternative method to screen feminine care products and reduce the use of animals.

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