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Conference Report

XXVIII Annual Meeting of the Italian Association for Laboratory Animal Sciences †

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Abstract: The Italian Association for Laboratory Animal Sciences (AISAL) held its annual meeting in September 2022. The main topic of the meeting was the section 'Quality Control of the Components in the Study' of the PREPARE guidelines. Among the audience, all stakeholders of AISAL were present: animal caretakers, technicians, facility managers, veterinarians, scientists, and animal welfare officers. One session was also dedicated to the round table on education and training, with the aim to discuss the legislative requirements as per Italian Decree 5 August 2021.

Keywords: PREPARE; animal welfare; education and training; laboratory animal science

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1. Introduction

The Italian Association for Laboratory Animal Sciences (AISAL) held its XXVIII Annual Congress in September 2022. The Congress was focused on the section 'Quality Control of the Components in the Study' of the PREPARE Guidelines [1], with the aim to share valuable information for experimental reproducibility important not only for researchers but also for technical staff (animal caretakers, technicians, and veterinarians) involved in the care of the experimental animals. We believe that this approach also fosters a culture of care within the lab animal science setting.

The Congress was divided into three sessions: 'Characteristics of the experimental animals essentials for the study and reporting'; 'Quarantine and health monitoring'; and 'Housing and husbandry'.

We hereby report the white paper on staff training according to the Italian legislation and abstracts of both oral and poster presentations.

2. AISAL White Paper on Staff Training According to the Italian Ministerial Decree, 5 August 2021, Resulting from the Round Table 'One Year after the Decree of 5 August 2021: Where We Are', Organized at the XXVIII Annual Meeting of the Italian Association for Laboratory Animal Sciences, Trieste, Italy, 30 September 2022

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2.1. Premises

The Ministerial Decree of 5 August 2021 and the following Directorial Decree of the Directorate General of Animal Health and Veterinary Drugs of the Ministry of Health, published on 18 March 2022 (hereinafter "Training Decrees"), identifies, in compliance with Legislative Decree No. 26 of 4 March 2014, Article 23, paragraph 2, the modalities for acquiring, maintaining and demonstrating an adequate level of education and training of the staff involved, in different capacities and roles, within projects involving the use of animals.

Nevertheless, the decree of 5 August 2021 attributes to the training an enabling characteristic that may de facto lead to a potential interruption to both the worker's activity and the implementation of the research project in which he or she is involved. This aspect is particularly relevant not only from an ethical and legal point of view linked to the application of the 3Rs but also because of the constitutional guarantees applied to scientific research, as inferred from the combined provisions of Articles 9 and 33 of the Italian Constitution.

2.2. Aim

We hereby identify possible solutions for education and training in Italy (Table 1). This will provide Italy with the level of pre-clinical research necessary to preserve its competitiveness among EU countries, in which the same Directive 2010/63/EU applies.

Table 1. Overview of the critical issues identified and proposed actions.

Critical Issue	Proposed Action
Assessment of staff competence: risk of interruption/suspension of activities in projects authorised under Decree 26/2014 due to overload of the central structure (Ministry of Health)	For an implementation of the recognition process, it would be appropriate to allow the AWB to autonomously assess the competencies of the staff in the individual facilities, relevant to their professional role. The Ministry/ISS would exercise supervision through periodic audits or verifications.
Recognition of training acquired in accordance with Directive 63/2010/EU and certified by the relevant authority in the country of origin, for persons from other European countries	It would be advisable to allow the involvement of the AWBs, at the peripheral level, which, having prepared a table of correspondence/equivalence (in terms of content, timetables, and CPDs) between the courses taken abroad and those envisaged by Italian regulations, sends its assessment to the Ministry.
Staff training and retraining costs are often not included among the eligible expenses in funded research projects	It would be appropriate if the budgets for this type of grant were also intended to cover the costs of training personnel involved in animal experimentation for scientific purposes.
Access to financial resources allocated for the organisation and delivery of courses in accordance with Article 41(2)(c) of Legislative Decree 26/2014	An implementation of information and a uniformity of allocation criteria would be desirable and decisive in solving this critical issue.

Table 1. *Cont.*

Critical Issue	Proposed Action
Use of animals for theoretical and practical courses	The Ministry of Health could approve theoretical–practical courses on carcasses obtained from ordinary activities and on alternative materials (animals and plastic materials, virtual reality applications) by entrusting the most important practical part to the practical training at the institutional level within the authorized research projects.
It is difficult to provide all those working in the sector with the opportunity to train in a professionally and scientifically appropriate manner and within a timeframe that does not create further delays in authorisation procedures.	It would be of great help to create a stable network project, an organisational form of collaboration that has as one of its objectives the development of forms of cooperation on training projects.
Find complete and up-to-date information on the accredited training offerings available in the country.	AISAL is the perfect candidate, in continuous dialogue with the Ministry, to become a point of collection and sharing of all information on the training offerings available, functional for the full sustainability of the system. AISAL would make available to users a real 'training portal' that is always up to date and aimed at the real-time verification of the state of the art of the 3Rs. https://www.aisal.org/corsi-convegni-attivita/corsi-accreditati-ai-sensi-del-dm-5-agosto-2021-in-aggiornamento/ (accessed on 9 September 2022)

To this end, we proposed the establishment of a constructive dialogue between the Italian Association of Laboratory Animal Science (AISAL), which is the national reference point for the personnel involved (designated veterinarians, animal welfare officers, scientific members, personnel with functions A, B, C, and D), and the Institutions concerned (Ministry of Health, Istituto Superiore di Sanità, and Ministry of Research).

While fully respecting the role and prerogatives of the Institutions, it is indeed believed that the solutions identified by AISAL could help, in full compliance with the current regulations, to implement the provisions of the Training Decrees, therefore avoiding possible heavy repercussions on the various strategic research sectors in our country.

2.3. Research Resources

A fundamental prerequisite for maintaining and improving the level of preclinical research (and thus of the research and production chain dependent on it) is to ensure adequate economic and human resources allocated to the system.

The economic resources for carrying out research projects are ensured through funding provided by sources including (but not limited to) the Italian Government (e.g., Ministry of Research, Ministry of Health, Regional), the European Union, or research foundations. Funding, granted based on a competitive process, must be used within a predetermined timeframe, and must also be financially and scientifically accounted for. One of the elements of the funding bodies' assessment of the research project is the presence of adequate and competent personnel to carry out the research project. **Every research project has eligible and ineligible expenses; in fact, in most cases, expenses for the training and further training of personnel are not included among the reportable expenses.**

Research projects involving activities with animals for scientific purposes in order to achieve their scientific objectives require the submission of a project application (Art. 3(1)(b) of Legislative Decree 26/2014) followed by authorisation based on the Legislative Decree 26/2014 by the Ministry of Health.

Human resources are necessary and functional to carry out research projects, to achieve the planned objectives, and to account for the allocated resources.

2.4. Acknowledgement of Prior Training of Staff

The above-mentioned factors delineate the general picture in which the Ministerial Decree of 5 August 2021 and the subsequent Directorial Decree (Training Decrees) fall. These Decrees determine the level of required training, how it is achieved, and the continuing professional education necessary for the personnel.

According to the two aforementioned decrees, training and refresher courses should be organized; moreover, transitional measures should be implemented, to ensure the continuity of the projects authorised under Decree 26/2014, thus avoiding any interruption and suspension. This outcome, in fact, would entail negative consequences for the entire system, and it is in contrast with the constitutional dictate on the freedom of scientific research (Article 33 of the Italian Constitution.), i.e., (1) the suspension of all personnel activities, given the enabling function of the decree of 5 August 2021 and following (Training Decrees); (2) the extension of the project's timeframe beyond the time limit set by the ministerial authorisation; (3) non-compliance with the 3Rs principle in relation to the number of animals used or in breeding, if transgenic colonies with a suffering phenotype are involved; (4) non-compliance with the timeframe of the entire funded project; (5) negative impact on the reporting and sustainability of the project itself.

Indeed, a key factor is having the adequate and continuously updated competence of personnel involved in animal experimentation, which guarantees the achievement and the standard of scientific training relevant to the work and the species used. However, it is believed that it would be appropriate to allow the Animal Welfare Body (AWB) to independently assess the competences of the personnel involved, according to the role performed. This role of the AWB falls within the spirit of the Training Decrees art. 7 paragraph 1, Ministerial Decree of 5 August 2021. The Ministry could implement the supervision of the AWB through periodic audits or verifications.

An autonomous AWB on this matter would, on one hand, lighten the **workload of the central structure (Ministry of Health, ISS)**, thus freeing up resources, and, on the other hand, create a greater accountability of the institutes carrying out research, by improving their quality.

Furthermore, in relation to the personnel described above, special mention should be made of individuals from other European countries who possess the necessary skills to carry out the research project and who have therefore been selected to participate in it, usually employed under a fixed-term contract. In case they participate in activities regulated by Legislative Decree 26/2014, they must have obtained a training certificate pursuant to the Training Decrees before starting their work; alternatively, they must obtain, from the Ministry of Health, recognition of the training acquired under Directive 63/2010 and certified by the relevant authority in their country of origin.

Currently, the evaluation and recognition of certificates is carried out centrally, on a case-by-case scenario, by the Ministry of Health. An implementation of the aforementioned recognition process could see the involvement of the AWBs at the peripheral level. With a table of correspondence/equivalence (in terms of contents, timetables, and CPDs) between the courses taken abroad and those envisaged by the Italian regulations, the AWB could evaluate the certificates and notify the Ministry about its evaluation. A division of workload and responsibilities between the peripheral level (AWBs) and the central level (Ministry) would lead to an optimisation of the time needed to recognise the competencies of personnel who were already trained in different EU countries, in accordance with the same Directive 63/2010/EU, and are essential to research activities.

2.5. Specific Resources Related to Training

The **economic resources for the organization of training and CPDs** by institutions come from funds allocated by the Ministry of Health to the regions by means of an annual allocation decree.

In reality, however, important critical issues are emerging: the lengthening of the time it takes for institutions to organise courses; the lengthening of the time it takes to release the funding allocated for training; the accreditation decrees issued by the Ministry of Health; and costs associated with staff participation in the courses themselves.

In fact, upon the entry into force of the aforementioned Decrees, difficulties immediately emerged relating to the economic aspects, both for those who have to attend accredited training courses—especially young researchers—and for those who could organise them in their own premises, should they possess the required characteristics.

The difficulties encountered by researchers are often linked to two main aspects: (i) the lack of funds for training and CPDs in the institutions where they work; and (ii) the type of grant programmes that fund their research, which do not allow the use of funding for training activities but only to attend conferences. Among these grant programmes, there are also those provided by the Ministry of Health. Therefore, it would be advisable that the funding provided by these grants also cover the costs of training personnel involved in animal research.

For course organisers, it is not always easy to find the economic resources to offer a quality product. For instance, the Ministry of Health delegates the allocation of funding to regions, but among regions, there is no uniformity in award criteria, and it is not always easy to find related references. An implementation of information (e.g., access modalities, allocation criteria, rapid and certain disbursement times) as well as the uniformity of allocation criteria would be desirable and decisive in resolving this critical issue.

Another just as important resource for carrying out training courses is represented by the animals, the subject of the course itself. Regarding practical courses, in order to save animals, the Ministry of Health could approve theoretical–practical courses on cadavers, obtained from ordinary activities, and on alternative materials (animals and plastic materials, simulations through virtual reality). This would leave the most important practical parts to be covered during practical training, which is already planned. During practical training, the goal is to identify a few elements on which to train staff in normal activities within the projects and practices; the discomfort of animals during supervised learning will certainly be less than the sole use of animals for the purpose of training, in compliance with the 3Rs principle.

2.6. Final Remarks: Networking to Improve Organization

The current Italian legislative framework on training defines the criteria and procedure for the accreditation of training courses, the number of credits required for the fulfilment of training and continuing professional development obligations, the modalities for the management of the competence record, the modalities for the recognition of CPD Credits, and the modalities for the inclusion of the training and continuing professional development certificates of the designated veterinarian, the research project manager, the animal welfare and care manager, and the scientific member, in the ministerial VETINFO platform.

In such a complex legislative framework, it is difficult to provide all those working in this sector with the opportunity to train in a professionally and scientifically appropriate manner and within a timeframe that does not create further delays in authorisation procedures.

Moreover, it is also difficult for people involved in this field to access complete and up-to-date information on the accredited training offerings available in the country.

Therefore, it would be of great help, on one hand, to create a stable network project, an organisational form of collaboration that has as one of its objectives the development of forms of cooperation on training projects. This would promote the exchange of information and training experiences; value the respective competencies, resources, and positive

attributes of the individual institutions; optimise the use of resources; and also create forms of collaboration and learning thanks to IT tools and new multimedia technologies. On the other hand, it would be essential to provide the staff involved with all the continuously updated information on access to the accredited courses available in the country.

AISAL is the perfect candidate, in continuous dialogue with the Ministry, to become a point of collection and transmission of all that information. All those who in various ways use animals for scientific purposes will be able to have at their disposal a real 'training portal' that is always up to date and aimed at a real-time verification of the state of the art of the 3Rs.

Currently, AISAL is already providing on its website (https://www.aisal.org/corsi-convegni-attivita/corsi-accreditati-ai-sensi-del-dm-5-agosto-2021-in-aggiornamento/, accessed on 9 September 2022) the information related to licensed courses, specifically, the following: the course title, the organiser institution, the link to the course page, the species the course is accredited for, the course modules (where possible), event's dates, registration deadline date, attendance modality (face-to-face, synchronous distance learning (FAD), asynchronous FAD, other), whether the course is reserved for personnel within the institution, and registration fees. Close collaboration with the Ministry for the acquisition of information on accredited courses in real time would be (1) essential to enable professionals to access the training offerings available and (2) functional for the full sustainability of the system.

3. Oral Presentations

3.1. The 4th S: The Importance of Sex in the Lab

L. Gioiosa, University of Parma, Parma, Italy (laura.gioiosa@unipr.it)

Scientific evidence indicates that sex and gender affect health and disease susceptibility in both animal models and humans. Despite several official calls recommending the inclusion of both sexes and/or the report of sex as an experimental variable, animal research continues to preferentially use males over females. As a result, female subjects are still under-represented in basic and preclinical research. This is unfortunate considering that biological sex differences have been observed at multiple levels and in different phenotypes, from behaviour to physiology to susceptibility to stressors and diseases. This talk reviews several studies on sex differences in different fields of neuroscience, from social and emotional behaviour to response to stress, with particular emphasis on the sex-dependent effect of common experimental procedures. I will highlight the following: (a) the importance of sex as a biological variable when designing an experiment; (b) how, during development and later in life, social and environmental factors, and in particular common laboratory and experimental procedures, can differentially affect male and female behavioural and physiological responses; (c) the importance of unravelling factors contributing in sexual differences that confer differential vulnerability to disease; and finally (d) how, in the perspective of good science, is it particularly important to carefully consider not only species/strain/genotype but also the sex of experimental animals.

3.2. The Impact of Genetic Background on Animal Model: Phenotypic Differences between B6J and B6N MID1 KO Mice

Baldini R., Mascaro M. and Meroni G., University of Trieste, Trieste, Italy (gmeroni@units.it)

The Opitz G/BBB syndrome is a genetic malformation pathology characterized by midline defects; patients mainly present with hypertelorism, laryngo-tracheo-esophageal defects, hypospadias, and cerebellar hypoplasia, but other clinical signs may be present with very variable expressivity. The MID1 gene is responsible, when mutated, for the X-linked form of this syndrome, and in our laboratory, we are interested in studying the pathogenetic mechanisms that lead from an altered MID1 gene to the clinical signs of the disease. To study the mechanisms affected by alterations during embryonic development in the syndrome, we generated a mouse model bearing the inactive Mid1 gene, orthologue of the human MID1 gene. The mouse model recapitulates the neurological aspect of the

disease by showing a cerebellar defect in Mid1-/Y males (and Mid1-/- females). Mid1-/Y males, our experimental model, show defects in the median part of the cerebellum with the absence and malformations of the anterior lobes of the cerebellar vermis, defects that are histologically visible in adult animals but which arise during embryonic development. The generation of the Mid1 knock-out mouse model was performed using strain C57/B6N, and the colony was maintained for years on this genetic background in which histological and molecular studies were carried out. For logistical/organizational reasons, more recently, we have 'moved' the line to the C57/B6J background in which, however, we have noticed a reduced penetrance and severity of the cerebellar defect. This aspect is very relevant, especially in the case of models of malformations characterized by variable expressivity, and it is a very interesting topic to study experimentally in order to identify modifier genes that can also have relevance from a clinical point of view.

3.3. Strategies to Reduce the Number of Surplus Animals

P. Bugnon, University of Zurich, Zurich, Switzerland (phibug@access.uzh.ch)

The 3Rs principle has long been known in animal testing, but it is often forgotten that this principle is also applicable to the production of laboratory animals. In this case, the "Reduce and Refine" principle plays an important role in breeding. Not all animals born in our animal houses can be used in experiments. The usual explanation for this issue is that some animals do not have the required genotype for the experiment. But there are many other explanations for these surplus animals that give us the opportunity to act and reduce this still too large number.

Breeding planning is crucial to choose the strategy that will most closely match the number of animals required for the experiment, including, of course, some reserve animals.

If the number of produced animals is lower than the minimum number of animals required for the experiment, these animals cannot be used and are eliminated. On the other hand, if this number is too high, animals will also be eliminated. The challenge is to find the right balance, keeping in mind that the breeding conditions also play a role in making the theory (strategy) reflect the reality (breeding).

The challenge for the scientist is to be able to calculate the strategies in order to compare them and identify the best ones in terms of animal numbers. Surprisingly, there is no software available to calculate and compare such strategies. Our institute has started a project-to-program software that will allow for the better planning of breeding and thus reduce the number of surplus animals. Part of the software is already available online for the scientific community.

3.4. Cryopreservation, the ICGEB Experience

S. Vodret, ICGEB, Trieste, Italy (simone.vodret@icgeb.org)

The advent of genetic recombination technologies such as CRISPR/Cas has revolutionized the processes of generating genetically modified animals, causing a consequent increase in the number of laboratory animals. Cryopreservation represents an important tool for the control of essential resources by reducing the number of animals within animal facilities. With this method, it is possible not only to archive transgenic lines for indefinite periods but also to exchange material between different laboratories, operating in a simple, ethical manner and within reasonable times and costs. The commitment to establishing a Cryopreservation unit and the results of the ICGEB in this field show the effectiveness of the technique and can serve as an example for the management of resources and for the concrete promotion of the application of the 3Rs principle.

3.5. Transport of Laboratory Animals. Legal and Technical Aspects

F. Giorietto, UVAC PIF Veneto-FVG-Trentino Alto Adige, Verona, Italy (f.giorietto@sanita.it)

Animals used for scientific purposes, given their peculiarity, require specific needs to be met to guarantee their biological status and physiological needs. With only few exceptions, these animals are subject, throughout their lives, to being transported, at

least once, to and from breeding establishments or users, including through imports or exchanges between Member Countries. Transportation can also have a significant impact on the scientific nature of the experimental study. For this reason, the Italian Legislative Decree 26 of 2014 requires that all necessary measures must be taken to ensure that animals are transported in conditions that minimize suffering and stress in relation to the species, the transport's duration, and the vehicle used.

It is therefore necessary, especially in consideration of the provisions of Regulation (EC) 1 of 2005, to have a clear understanding of the specifications to be observed, the technical requirements to be adopted, and the necessary authorizations. However, it must be highlighted that the transport of animals between user establishments is not always related to an economic activity and therefore sometimes does not fall within the scope of Regulation (EC) 1 of 2005.

It is important to know the best procedures to manage this type of transport. We will therefore try to convey some arguments on this fundamental matter to apply the principle that "no one is authorized to transport or have transported animals in conditions that expose them to unnecessary injury or suffering."

3.6. Health Monitoring and Microbiota Analysis in Mouse Colonies Using Metagenomic Sequencing: A New Approach

F. Scavizzi, EMMA Infrafrontier CNR, Roma, Italy (ferdinando.scavizzi@cnr.it)

The need to monitor the health status of genetically modified mouse colonies has always been important to guarantee the health of the animals and the quality of the research. In recent years, the importance of the microbial component (or microbiota) in many aspects of animal physiology and behaviour has also been understood. The knowledge of microorganisms is fundamental for understanding microbiota/host interactions and must be considered in the interpretation and evaluation of the phenotype of mutant lines. This study presents a new approach which, thanks to Next Generation Sequencing, allows us to carry out a careful health monitoring plan and, at the same time, qualitatively and quantitatively analyse the microbiota. The advantage of this Shotgun Metagenomic Sequencing approach is that it also allows the identification of microorganisms (pathogenic or otherwise) that are not specifically searched for. This approach respects the 3R principle because it does not require the sacrifice of sentinel animals (since faecal samples are used). Furthermore, the latest developments seem to demonstrate that it can also be used on filters, as environmental microbiological monitoring.

3.7. The Hows and Whys of a Zebrafish Health Monitoring Program

N. Pereira, Oceanario de Lisboa, Lisbon, Portugal (npereira@oceanario.pt)

Recent advances in the refinement and standardization of zebrafish husbandry and health management strategies were documented through pivotal publications, including international guidelines and recommendations. This talk addresses the main rationale for health programs' implementation, namely, animal welfare, infectious and non-infectious disease containment, safe animal exchanges within facilities, and robust experimental results. After a brief description of the relevant pathogens in zebrafish facilities, both published and unpublished examples of microorganisms that may impact research outcomes are provided. These include pathogens such as some mycobacteria species, *Pseudoloma neurophilia*, and nematodes like *Pseudocapillaria tomentosa*, which can be associated, even in subclinical presentations, with histological lesions, inflammatory responses, autofluorescence, and disturbances of the gut microbiome that can interfere with research results. Health control programs rely on robust biosecurity and screening protocols, which are detailed in this talk with emphasis on the recently published FELASA-AALAS recommendations. Zebrafish health and microbiological status assessment depend on facility-adapted screening patterns (frequency, test type, sample number) of sentinels, sick, and surplus animals as well as of live feed and environmental samples. Pathogens are classified and screened according to expected prevalence, transmissibility pattern, fish and personnel,

and research impact. Screening testing methodology comprises multimodal clinical and analytical techniques—daily animal observation, clinical live fish exams, necropsies, histology, microbiology, and molecular biology (PCR)and should not be confined to the latter. Reports accompanying fish transfers between facilities include screening, husbandry, and biosecurity details, providing a health and microbiological status enabling a comprehensive infectious risk assessment. Finally, this talk addresses examples of therapeutic and/or corrective approaches to potential infections in zebrafish facilities caused by pathogens like mycobacteria, *Pseudoloma neurophilia*, and *Pseudocapillaria tomentosa*.

3.8. Preclinicatrials.eu, Free Database of Preclinical Studies

V. Galligioni, Netherlands Institute for Neuroscience, Amsterdam, the Netherlands (v.galligioni@nin.knaw.nl)

Human clinical trial protocols must be registered on an EU-based platform that is publicly available (https://www.clinicaltrialsregister.eu/ctr-search/search, accessed on 16 August 2022). Its aim is to protect clinical trial participants and harmonize the supervision of clinical trials in the EU.

In the preclinical setting, many guidelines (ARRIVE, PREPARE) have been adopted by funding agencies and scientific journals to improve methodological rigor and reproducibility. However, systematic reviews and meta-analyses in different fields have highlighted the poor predictive value and lack of methodology in preclinical science.

Preclinicaltrials.eu (https://preclinicaltrials.eu/about-pct, accessed on 16 August 2022) is a free database of registered studies from all biomedical fields with following aims: (i) increase transparency; (ii) avoid the unnecessary duplication of animal studies; (iii) reduce reporting bias, such as publication bias and bias induced by selective outcome reporting, p-hacking, and HARKing; (iv) increase data sharing, by allowing fellow researchers and reviewers to access information on the study design, often lacking in publications, and by providing a platform to share the details and data of otherwise unpublished animal studies; (v) create opportunities for collaborative research.

There are currently 123 registered protocols belonging to several fields, from oncology to neonatology/paediatrics, neuroscience, virology, cardiology, immunology, infectious diseases, and critical care medicine. This database is freely accessible and can be used not only by researchers but also by animal welfare bodies' members as a reference to identify any possible duplication of animal study and to review procedures, their severity, and related outcomes.

3.9. How to House the Laboratory Mouse: Let's Learn about Well-Being from Mus Musculus

M.A. Marconi, Konrad Lorenz Institute of Ethology, Wien, Austria (mariaadelaidemarconi@gmail.com)

House mice (Mus musculus) are one of the most widespread mammals in the world, and their global distribution was a result of hitch-hiking with human migration. These rodents are often called 'human commensals', but these agricultural pests are better labelled 'kleptoparasites,' since they have been eating human crops and food stores throughout recorded history. House mice are a very popular 'model organism' for the biomedical sciences and are perhaps the single most intensively studied species on the planet. Yet, most of what is known about mice comes from research on domesticated laboratory mice. Lab mice were artificially created by crossing three Mus subspecies originating in Asia and Europe, and some authorities therefore consider them to be a separate species, Mus laboratorius (or Mus gemisch), especially since they have many differences from their wild cousins. The first goal of our talk is to summarize the differences in the behaviour (genetics, morphology, and physiology) between wild versus laboratory mice. We will examine studies on housing conditions, social experience (pre- and post-weaning), environmental enrichment, and animal handling. Our second goal is to summarize studies on the behaviour of mice carried out in both laboratory and semi-natural conditions and explain why studies in semi-natural conditions are so important. Finally, we will suggest

how the welfare of house mice in captivity might be improved by using knowledge about the behaviour of wild and wild-derived house mice in more natural conditions.

3.10. Environmental and Social Needs in Relation to Different Life Stages in Rodent Models

A. Wirz, Fondazione Santa Lucia, Roma, Italy (a.wirz@hsantalucia.it)

The last few decades have seen major changes in the use of animal models in scientific research: changes in regulations, technology, but above all, in the approach to animals. The sensitivity and attention of both researchers and the public has increased, and more and more people are talking about safeguarding animal welfare. It is therefore essential to start from a knowledge of the model that one is working with and its biological and ethological needs. This translates into the careful care of the animals from the moment they arrive at a facility and are housed to before, during, and after their use in experimental procedures.

In refining and harmonising the care and use of laboratory animals, effects on welfare must be taken into account by considering not only the species but also the strain, the age, and the sex, affecting the potential increase in the variability of scientific data and their reproducibility. It is also necessary to reflect on the scarcity of precise and detailed information in publications about housing conditions and the strategies implemented to make the animals' living environment more suitable in order to reduce their suffering and discomfort. Indeed, pain and stress, besides being a serious ethical concern, are a potential source of experimental error, as many physiological, immunological, endocrine, and behavioural parameters may change as a result of such conditions.

In recent years, for example, all facilities have tried to comply with European regulations by including cages and nesting material in the mouse models' cages. But now, questions are already being raised as to whether this is sufficient.

Therefore, a solid programme for animal care and use becomes important for several reasons concerning ethical aspects, compliance with current legislation, quality of scientific results, communication, and transparency towards the public. Developing such a programme must involve a team of trained people.

4. Poster Presentations (in Alphabetical Order of the First Author)

4.1. Hair Steroids Quantification in Laboratory Rats as Potential Tool for Welfare Assessment

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Rats (*Rattus norvegicus*) are the second most popular rodent species employed for biomedical research [2], and increasing attention is given to their behavioural welfare. Indeed, appropriate enrichment is pivotal to satisfy rats' eagerness to explore [3]. Housing conditions influence their behaviour, which is strictly related to steroid hormones [4,5]. Corticosterone is the main steroid related to chronic stress in rodent species, and it can be measured using several matrices [5]. Corticosterone quantification from hair can provide long-term retrospective information through a minimally invasive procedure [6,7].

The aim of this preliminary study was to quantify rats' hair steroids to evaluate the potential behavioural effect of a widespread form of environmental enrichment, the plastic tunnel, used in two different manners. Twenty Wistar rats housed at the Comparative Medicine Unit of Trinity College Dublin were included. The "NORMAL" group (n = 10: sex ratio 1:1; n2 cages) was provided with a traditional plastic tunnel placed on the bedding; the "EXTRA" group (n = 10: sex ratio 1:1; n2 cages) had the tunnel suspended to the grid of the cage. Hair was shaved from rats' backs (neck to tail) two days before weaning (T0 = Baseline; before enrichment) and after three months (T1; after enrichment). Steroids (corticosterone, cortisol and dehydroepiandrosterone) were extracted and subsequently quantified as previously described, by either ELISA or radioimmunoassay [6]. A two-way ANOVA followed by Tukey's Post Hoc test showed a difference in males' corticosterone (concentration/mg of hair), with lower values in NORMAL-enriched animals. The same comparison was not significant in females. The discrepancy between sexes was relevant

considering both corticosterone and dehydroepiandrosterone. Cortisol was not quantifiable, confirming its minor role in rodents. Despite the relatively low sample size of this preliminary study, the data obtained seem to support further research for the evaluation of hair corticosterone as a potential and non-invasive biomarker for the improvement of rodents' housing practices.

4.2. Study on Gut Microbiota Modification upon a High-Salt Diet in Spontaneously Hypertensive Stroke-Prone Rats

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The gut microbiota (GM) is the set of microorganisms living in the bowel, which are capable of influencing the host's health and the functionality of physiological processes [8]. Alterations to the GM may induce variability into animal model responses and their internal and external validity. The Spontaneously Hypertensive Stroke-Prone Rat (SHRSP) represents a suitable model for the study of hypertension-related stroke [9]. SHRSPs spontaneously develop cerebral ischemia, but stroke onset is accelerated upon a high-salt Japanese-style diet (JD) [10,11]. Gastrointestinal complications frequently follow stroke, and several studies have focused on the relationship between the GM and acute ischemic stroke, confirming the existence of a bidirectional microbiota–gut–brain axis [12]. The aim of this research was to evaluate the effect of the JD on the GM composition in SHRSPs with Spontaneously Hypertensive Rats (SHR) as the control group.

Six-week-old SHRSPs (n = 16) and SHRs (n = 14) were randomly divided in two subgroups which received JD or a regular diet (RD) following standardized and published work [10]. Rats were sacrificed between 28 (T28) and 67 (T67) days of the diet. In order to analyse the GM composition, DNA was extracted from colon contents and the V3–V4 regions of the 16S rRNA gene were sequenced through the MiSeq Illumina platform [13]. Bioinformatic analysis was performed with R software. β-diversity estimated by Bray–Curtis showed a significant difference between the GM profiles of the different subgroups ($p = 0.022$). In SHRSP, in response to JD, *Firmicutes* relative abundance increased, while *Bacteroidetes* decreased. Those results, along with further alterations at the genus level, demonstrated that shifting to JD had an effect on the SHRSPs GM.

4.3. Set-Up of an In Vitro Protocol to Complement In Vivo and In Silico Studies of the Mammary Epithelial Barrier in the Göttingen Minipig Animal Model

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The risks associated with the use of drugs in pregnancy and breastfeeding have not been investigated for most marketed drugs [14]. Using the framework of the European project ConcePTION [15], a non-clinical platform will be defined to predict the transfer of maternal medication into the human breast milk in the Göttingen minipig animal model. This will complement in vitro, in vivo, and in silico models to ensure the standardization of the animal model and to achieve a reduction in the use of animals for experimentation, in full compliance with the 3Rs principle [16]. The present research aimed to characterize the monolayer integrity of primary cultures of mammary epithelial cells (MECs) derived from Göttingen minipig, to develop a robust in vitro model for the study of mammary epithelial barrier function experimentation.

Three primary cell lines of minipig (mp)MECs were isolated, characterized and expanded following the protocol previously set up on commercial hybrid sows [17]. The three primary cell lines were then cultured at different cell seeding in the 24-well PET inserts, and the ability to create a barrier was evaluated by measuring trans-epithelial electrical resistance (TEER) and sodium fluorescein (SF) transport. The results showed that mpMECs, seeded at 0.15×10^6 cells, reached high TEER values and SF < 0.3% on day 3 of culturing and were stable until day 4, verified by confirming the formation of a tight monolayer. In conclusion, in the present research, we have reported an efficient method to study epithelial

barrier function. A pre-mixed pool of mpMECs, resulting in reduced variability, can be utilized to rapidly screen a wide array of drugs before proceeding with in vivo trials. The insights obtained will be highly relevant for translational purposes.

4.4. Refinement of Management, Care, and Welfare of the Gunn Rat in 2022

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The Gunn rat originated from a Wistar colony in 1936 through the onset of a spontaneous mutation in the UGT1A1 enzyme responsible for bilirubin clearance. They have hyperbilirubinemia and icterus, triggering neurological damage (jj rats). Until the 1980s, the strain presented variable behavioural deficits. Later on, behavioural deficits disappeared due to development of resistance to hyperbilirubinemia or through selection imposed by breeders.

Since 2015, in our colony, we have observed large phenotypic variability in jj rats, in which behavioural abnormalities may be assessed only through tests; behavioural abnormalities are identified in animals with evident balance problems, tremors, or wobbly gait (SP: severe phenotype). The housing and breeding of the colony require special care. Animals are bred in an SPF facility in IVCs (individual ventilated cages). Appropriated enrichment (cellulose wading, sterile objects, building nest material) guarantees species-specific ethological requirements and animal welfare. After the birth of a new litter, cages are checked daily, with special attention to the identification of SP subjects, with the phenotype appearing at 20–25 days of age. SP pups are then monitored twice a day by a trained animal caretaker but without the frequent opening of the cages. Weaning is delayed until the full achievement of SP animal autonomy. Special care in the safe manipulation of animals is performed by the technicians during the changing of the box and animal monitoring. Due to reduced motor abilities, some pellet is put down on the cage to facilitate food access, and SP subjects are housed within the same cage with up to two individuals after weaning. The phenotype, inherited up to generation F7, agrees with the clinical presentation of hyperbilirubinemic human infants, making our Gunn colony a relevant translational model. Efforts to fix the phenotype and genomic studies to identify potential mutations responsible for it are ongoing.

4.5. Use of the Octopus (Octopus Vulgaris Cuvier, 1797) for Scientific Research Activities in Controlled Environments

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The octopus has some very interesting biological characteristics, such as a short life cycle, high growth rate, food conversion, fecundity, easy adaptation to captivity, and a vast behavioural repertoire. This last aspect is of fundamental importance, as the observation and interpretation of it, even on a few specimens, becomes a valid and effective indicator of the state of health of the animals in the tank. The expression of body patterns is related to the complexity and variety of the habitat [18–20]. This paper aims to provide some indication on how to manage octopuses in a controlled environment for scientific purposes, considering all aspects from capture to reproduction.

This being said, a series of precautions must be taken concerning tank characteristics (rectangular, 700–1500 L, grey beige vs. blue, grids, covers, surface area/octopus), environmental enrichment (sand, rocks, stones, shells vs. NO), den (plastic vs. terracotta, size), ethological/behavioural needs (predator, solitary, territorial, cannibalism), eco-physiological conditioning programmes (temperature, photoperiod, salinity), feeding (ad libitum vs. rationed, crustaceans vs. fish, live vs. unfrozen) during the animal's various life stages (male/female, growth, sexual maturity, reproduction, senescence), water quality (pH, ammonia, nitrite), and animal handling (anaesthesia, confinement) [21]. Parallel

to housing conditions, all the preceding phases are equally important, in particular, the catching method (trap vs. hand line), the transport system (type of tank, isolation, number of subjects, water quality, temperature), and timing (<24 h>), as if this is carried out in an unsuitable manner, it can cause stress/distress to the animal which is difficult to reverse and has repercussions in the relaying environment, even if all other conditions are optimal [22].

Therefore, all operations must be carried out by competent and experienced personnel to manage the various design procedures and determine any endpoints.

4.6. A New Scaffold for Bone Regeneration: In Vivo Study on Rabbit

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Polybutylene succinate (PBS) has high potential as a scaffold in bone tissue engineering due to its capacity in cell proliferation and differentiation, biocompatibility, and bioresorbability. This study aimed to evaluate in vivo bone regeneration promotion ability and the osteointegration potential of electrospun microfibrillar PBS-based scaffolds surgically implanted in critical-size cranial bone defects experimentally created in a rabbit model. Two circular defects (8 mm in diameter) were formed on the left and right calvarial (frontal) bones of nine New Zealand white rabbits, followed by the implantation of the scaffold (8 mm $\times$ 2.5 mm) at the site of only one defect (treated). The contralateral defect underwent spontaneous repair (control). Bone formation was evaluated by in vivo computed tomography (CT) imaging and histological analysis at 4 (Group 1, n = 3), 12 (Group 2, n = 3), and 24 (Group 3, n = 3) weeks post-implantation. At 4 and 12 weeks post-surgery, we observed new bone tissue infiltration at the implantation site, with a small amount of fibrous tissue formation; on the other hand, in the contralateral untreated defect, we detected the presence of fibrous tissue with small areas of bone growth at the defect margins. At 24 weeks post-implantation, the defect area was replaced with new bone tissue in all rabbits, although with reduced wall thickness. In particular, in the untreated defect, the examination revealed the presence of poorly mineralized, thinned, and fragmented trabeculae in the newly formed bone, with numerous areas of osteonecrosis. In conclusion, our study shows that this PBS-based scaffold effectively promotes the healing of calvarial defects and in situ bone regeneration and osteointegration in rabbits.

4.7. Evaluating the Evidence of Sentience of Decapod Crustaceans and Their Protection in eu Legislation

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Despite research that uses decapod crustaceans as laboratory models, there is a general lack of concern for their welfare, and compared to vertebrates, they are often maintained with minimal animal care. Current European legislation covering the protection of animals used for scientific purposes excludes them because they are not considered sentient and, therefore, are considered incapable of suffering. However, recent scientific evidence suggests that decapods have a capacity to experience pain, suffering, and distress, and, consequently, protecting these animals is required through a revision of European legislation. After all, there is current pressure to cut the use of vertebrates and to use invertebrates instead, to partly satisfy one criterion of the "3Rs" principles (Replacement) in animal welfare. This requires an evaluation of sentience so that an animal with "higher" sentience is replaced by one that is "lower" (often unprotected by legislation, i.e., embryos of vertebrates before of the last third of their normal development or invertebrates except live cephalopods). To this end, the EU Directive 63/2010 states that animals "with the lowest capacity to experience pain, suffering, distress or lasting harm" should be used. Nevertheless, in 2005, the Animal Health and Animal Welfare Panel of the European Food Safety Authority recommended their inclusion in the legislation that provides protection

for animals. However, decapods were excluded from the subsequent Directive 2010/63/EU due to the refutation of the conclusiveness of the studies cited by the panel.

Considering more recent evidence on the complexity of life and behaviour, learning ability, the functioning of the brain and nervous system, indications of pain or distress, the biological basis of suffering and other feelings such as fear and anxiety, and indications of awareness based on observations and experimental work, we argue that their welfare should be taken into account, and they should be included in ethical review processes that aims to minimize potential pain. Lastly, we recommend the development of decapod welfare guidelines and a clearer regulation.

4.8. Preclinical Research Strategies to Assess the Role of Estrogen Deficiency Osteoporosis in the Development of Breast Cancer Bone Metastasis

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Breast cancer is the most diagnosed disease in women. At diagnosis, 5–6% of cases present with metastases, and the skeleton constitutes the most widespread site of metastasis. Alterations in bone metabolism, such as osteoporosis (OP), cause disturbances in monocyte and macrophage functions and a consequent alteration in the immune functions and pro- and anti-inflammatory regulators that could promote breast cancer spread to bone. Although some evidence already exists, preclinical and clinical proof of the link between OP and the occurrence of bone metastasis are sketchy and fragmented. We set up a platform of preclinical studies to analyse whether alterations induced by oestrogen-deficient OP can promote breast cancer cells' invasion to bone. First, through a 2D in vitro co-culture system with rat breast cancer cells and osteoblasts and osteoclasts isolated from healthy and osteoporotic rats, an influence of metastatic cells was detected on the osteoblastic and osteoclastic physiology at different levels: morphology, viability, the release of typical proteins, and proinflammatory cytokines. Second, through advanced ex vivo 3D models of bone metastasis, developed by culturing rat or human breast cancer cells with discarded bone tissue from healthy and osteoporotic animals or humans, respectively, specific and individual differences in tumour growth and colonization between healthy and osteoporotic status were detected. Finally, these 2D and 3D in vitro models were verified by a syngeneic in vivo preclinical model, in which metastatic lesions were induced in healthy and osteoporotic animals. The studies showed an increased metabolism of cancer cells in osteoporotic animals and an activation of specific signal pathways that favour tumour growth, invasion, and progression. This platform of studies highlighted and recognized major differences in tumour growth and colonization between healthy and osteoporotic status, helping researchers and clinicians to have a better comprehension of the importance of the bone microenvironment and its clinical implications for precision medicine in cancer patients.

4.9. DIY: Homemade Enrichment for Rats in Spinal Fusion Model

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Rats are a common model for intervertebral disc (IVD) and spinal research. Here, we present a successful implementation of a homemade enrichment applied to an in vivo spinal fusion rat model in compliance with Section 12 of the PREPARE guidelines. Elderly Wistar Crl/WI(Han) rats underwent spinal tail disc surgery using bone morphogenetic protein (BMP)-coated β-tri-calcium-phosphate (β-TCP) carrier and a customized external ring fixator to allow compression [1]. Immediately after the surgery rats started to chew on the fixator, potentially causing its breakage. We developed a homemade toy by drilling a chewing wood stick, filling it with soft semi-intensive food diet EmerAid Intensive Care Omnivore®, and sealing the holes with pieces of cereal. The rat could then eat the cereal and lick the soft food around the hole, and then it needed to enlarge the hole by gnawing

at it in order to get to the soft food on the bottom. With just one object, we stimulated three senses: smell, taste, and touch. The rats spent hours chewing the piece of wood and did not touch the fixator at all after we implemented it. The homemade enrichment was developed by the animal caretaker staff, thinking out of the box and using material already and easily available in the facility. In this way, we successfully managed to correct a misbehaviour that could compromise the integrity of the implant, the validity of experimental model, and the research outcomes [23].

4.10. Pre-Clinical Models in Oncology: From the Development of New Anti-Cancer Drugs towards Effective Precision Medicine against Cancer

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"Strategic Projects 2019"-POR FESR Lazio 2014–2020 facilitated the establishment of partnerships in effective collaboration for the implementation of research, development, and innovation projects. The project idea and the related investment program arise from the active collaboration of various partners: the Bambino Gesù Paediatric Hospital (OPBG) and the Regina Elena National Cancer Institute (IFO) are research organisations, while Plaisant, Takis, and Menarini Biotech are small, medium, and large companies in the biotechnology/pharmaceutical sector. The joint project called IMMUNO has the aim of carrying out communication and dissemination activities of the results of the four different lines of industrial research and experimental development. Below are the five projects admitted to the benefits of the public notice "Strategic Projects 2019"-POR FESR Lazio 2014–2020:

- Development of an immunotherapy platform for the treatment of patients with sarcoma (CARSA);
- GEneration of new CAR T and BiTE to convert the tumour microenvironment (GEMMA);
- Humanized Mouse for Antibody Discovery (HuMAD);
- Production and scale-up of retroviral vectors in a bioreactor (TraZimAb);
- IMMUNO.

Plaisant participated in the first three studies, which differ in therapeutic typologies and experimental model but involve the use of the same strain of immunodeficient mice, the NOD/SCID/IL2rγnull (NSG), and have as their common aim the implementation of a platform for the development of new therapies in the haematological oncology field, exploiting the application of CAR-T cells and monoclonal antibodies and the possibility of obtaining a possible patent on phenotypically characterized humanized models.

Plaisant joined the partnership as an innovative company in in vivo research, being the first of its kind to introduce a new professional figure in Italy aimed at the care and well-being of laboratory animals. It provided experimental support to biotechnological research, generating mouse models able to mimic the pathologies under study in the three different projects, and processed the experimental data obtained. Thanks to the specialization acquired over the years, Plaisant will develop and publish animal models with experimental evolutions of considerable interest. These humanized models can prove to be a relaunch for Plaisant towards a research and development activity effective for increasing future collaborations with national and international research bodies.

4.11. Genetically Modified Animals' Legal Documents and Their Positive Impact on Model Integrity, Reproducibility, and Animal Welfare: A Swiss Perspective

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GMO (genetically modified organism) passports are tools to secure the quality of the model and, thus, of the scientific data [24]. Here, we present a review of how legal Swiss documents on GMOs, namely the Data Sheet for Genetically Modified Lines and the Form M–Announcement of Strained Animal Lines, can synergically act as comprehensive passports, contributing to track model integrity, enhancing reproducibility, and promoting animal welfare by accurately identifying constraint and mitigation measures,

in compliance with Sections 3 and 10 of the PREPARE Guidelines [1]. Model integrity is enhanced by the accuracy of official nomenclature in accordance with international guidelines and standards. Extended nomenclature must be provided also for in-house-generated strains. To achieve this goal, the use of a specific database is crucial [24]. The official recording and publication of newly generated mutant alleles must be promoted together with the creation of institutions, facilities, and lab group codes, to highlight and track the line retainers and substrains. The genetic background must be accurately provided too, promoting a better understanding of background effects on mutation expressions and phenotypes. Reproducibility is improved by accurate details on model creation, source, generations, zygosities, and hygiene status, enhancing the understanding of the model and the consistency of husbandry and experimental conditions, thus also enhancing data reproducibility [25]. Finally, a positive impact on animal welfare also derives from accurate GMO documents. For new or still under assessment models, perspective considerations are required. Known or expected model characteristics are assessed as part of the project review process. Knowledge on strain-specific phenotypes, mitigation measures, and criteria for withdrawal contribute to reduce the burden and, thus, potentially the degree of severity of the project. When a constraint is recorded or expected, one further Form (M) is required to highlight specific observation plans, breeding strategies, and harm–benefit analysis. This large and well-organized dataset, together with the acceptance of documents in English for an application's review by the Swiss Cantonal Authorities, fosters the quick repurposing of Swiss legal documents into strain passports, which are ready to use for inter-institutional animal exchange. Such documents should be also considered as supplemental materials when publishing results involving any GMO, further enhancing transparency.

4.12. First Observations following Wild Capture of a New Experimental Species: Toward Captive Management Protocols of Coypu

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The Coypu (*Myocastor coypus*) is an herbivorous rodent native to South America, which, after escapes and releases from fur farms, spread widely across Europe. It is considered as an invasive alien species by the European Union, for which eradication or numerical control is planned. The University of Udine was authorized to capture and keep in captivity some specimens to carry out a research project aimed at reducing fertility. Since it is a new species for scientific experimentation, we aimed to define the protocols of entry into the enclosure and management during the acclimatization period, as well as its maintenance. After 3 trap-nights, 14 animals were captured in the wild using trap-cages. At the entrance into the enclosure, individuals were microchipped and registered, with the determination of weight, sex (n = 8 females, n = 4 males, n = 2 NA), and age class (n = 6, yearlings; n = 7, 1 year; n = 1, NA). Biometric measurements and biological samples were collected, and an anthelmintic treatment was carried out. Each specimen was housed in a single cage (80 × 64 × 106 cm) with a nest box and an open side for a period of sanitary control; the first feeding was ad libitum and based on fresh fruits and vegetables. At the end of the period, the animals were given access to the water tank, and the pellet feed was introduced. During the housing period, environmental enrichments were implemented to best fit the animal needs, i.e., consuming teeth and maintaining a suitable behavioural display (closed nest box and free access to the water tank). Individuals were observed for 189 days to assess the degree of well-being through the compilation of individual sheets, considering specific physical (body condition and fur) and behavioural parameters (aggression, appetite, posture of body parts, and self-grooming). Overall, 331 observations were taken, including n = 6 of injured specimens and n = 2 of aggressive attitude.

4.13. Rat Liver Perfusion and Isolation of Primary Hepatocytes: An Old Procedure Crucial for New Cutting-Edge In Vitro Tools

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3D organoids represent a developing in vitro tool to recapitulate tissues in a dish and a potential bridge toward personalized medicine [26,27]. They are usually obtained from stem/progenitor cells isolated from rat, murine, and human liver. Recently, two different groups [28,29] obtained long-term cultures of 3D liver organoids from adult primary hepatocytes. The crucial procedure introduced by Seglen [30] in the 1970s and subsequently improved by Shen [31] is the two-step collagenase perfusion to isolate hepatic cells. However, it is still easy to encounter the failure of the surgical procedure, low hepatocytes viability, or high bacterial contamination, which lengthen the downstream experiments and increase the number of required animals.

Our work aims to describe in an exhaustive, detailed protocol the crucial steps of the procedure in the rat model. During the perfusion, we focused on the transient inferior vena cava clamping, resulting in liver swelling. This passage significantly increased the digestion efficiency and cell recovery. We added a washing step of the isolated liver in PBS +3% antibiotics to avoid contamination. Lastly, we paid attention to the visual description of the procedure to help replicate the method with a high success rate. According to the PREPARE guidelines checklist [1], special consideration was given to comparing our results with previous works and contacting the authors (topic A1). Since organoids can be maintained in culture for up to 7 months, this tool is itself an important way to reduce the number of animals used (topic A3). Through dialogue with the animal facility staff (topic B5), we involved an experienced technician to assist with the procedure (topic B7). An appropriate peristaltic pump necessary to conduct liver perfusion was purchased (topic B6). By showing how to address critical stages, this protocol optimizes the procedure for primary rat hepatocyte isolation to speed 3D organoids generation.

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