Modelli animali di Patologie Alimentari

corso di laurea in Biotecnologie per l'Alimentazione
2 anno 1 semestre A.A. 2013-2014

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Modelli animali di:

- Patologie infiammatorie intestinali (Inflammatory Bowel Disease IBD)
  - Morbo di Crohn
  - Colite ulcerosa
- Morbo celiaco
- Ulcera peptica
- Carcinoma del colon –retto
- Diabete
- *Tossinfezioni alimentari*

Laura Cavicchioli

Data/e da definire

Laboratorio di istopatologia

Aula microscopi (Pentagono)

Eric Zinni (modelli di diabete)
Data da definire

1-2 studenti

Presentazione (30 min) di un esempio di modello animale di patologia alimentare tra quelli non trattati (diversa patologia o diverso modello)

Valutazione della presentazione concorre alla formazione del voto finale (50%)

Esame scritto con domande a risposta aperta alla fine del corso

Che cos'è un modello?

Nell'uso scientifico e tecnico, un **modello** è una rappresentazione di un oggetto o di un fenomeno, che corrisponde alla cosa modellata per il fatto di riprodurne (eventualmente alla luce di una certa *interpretazione*) alcune caratteristiche o comportamenti fondamentali; in modo tale che questi aspetti possano essere mostrati, studiati, conosciuti laddove l'oggetto modellato non sia accessibile http://it.wikipedia.org

"A model, whether animal or non-animal, is meant to be a mimic or surrogate and not necessarily identical to the subject being modeled" (National Research Council 1998)

"Il modello animale deve imitare o sostituire il soggetto di interesse, non necessariamente essere identico ad esso" (F. W. Quimby, Animal Models in Biomedical Research, in Laboratory Animal Medicine, 2002 2a edizione)

Modelli animali per lo studio di fenomeni fisiologici o patologici (eziologia, meccanismi patogenetici)

Modelli animali di prevenzione e/o la terapia delle patologie

gli animali possono essere usati come modelli di una malattia della specie umana o di una specie animale per studiare..

cause

progressione

terapia

http://www.awionline.org/ht/d/ContentDetails/i/1559

Esempio negativo: la prima specie animale su cui si testò la penicillina fu il porcellino d'India che morì. Se le ricerche si fossero fermate a quel punto si sarebbe potuto concludere che si trattava di un farmaco pericoloso, invece che fare il primo passo verso il nuovo capitolo degli antibiotici. Venne quindi testato negli essere umani per i quali non è letale e in altre specie animali

Esempi positivi:
diabete mellito tipo I, immunodeficienza grave combinata, ipercolesterolemia, distrofia muscolare Duchenne,
emofilia A e B etc..

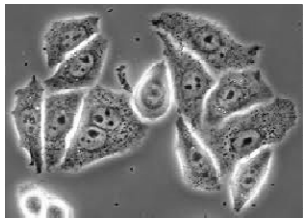
Come studiare una malattia?

In vivo, osservando i pazienti malati
http://www.clinicalstudyresults.org/home/
http://clinicaltrials.gov

Utilizzando dati epidemiologici

http://sh-dreams.blogspot.com/2008/08/dr-house.html

In *vitro*, attraverso linee cellulari o tessuti normali/malati



The NEW ENGLAND JOURNAL of MEDICINE

SPECIAL ARTICLE

The ClinicalTrials.gov Results Database — Update and Key Issues

Deborah A. Zarin, M.D., Tony Tse, Ph.D., Rebecca J. Williams, Pharm.D., M.P.H., Robert M. Califf, M.D., and Nicholas C. Ide, M.S.

Table 2. Summary Objectives and Description of Requirements for the ClinicalTrials.gov Results Database.

Objectives

Satisfy legal requirements

Promote objective, standardized reporting by capturing key trial features in the form of tabular data while minimizing potentially subjective narrative text

Facilitate "good reporting practices," including accommodation of publishing[12] and regulatory[13] guidelines

Provide structured data entry to ensure complete reporting, efficient quality review, and consistent display of both required and voluntary data elements

Support detailed searches with the use of the database structure and other National Library of Medicine functions[14]

Description of scientific modules (in tabular format)

Participant flow: Progress of research participants through each stage of a trial according to group, including the number of participants who dropped out of the clinical trial

Baseline characteristics: Demographic and baseline data for the entire trial population and for each group

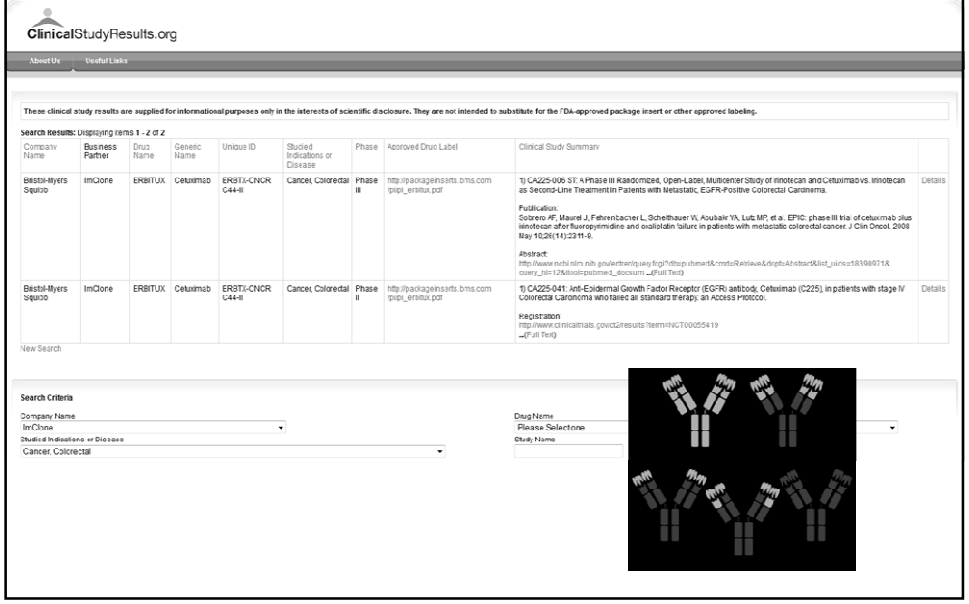
Outcome measures and statistical analyses: Aggregate results data for each primary and secondary outcome measure according to group, statistical analyses as appropriate

Adverse events: List of all serious adverse events; list of other (not including serious) adverse events in each group that exceed a frequency threshold of 5% within any group; both lists include adverse events, whether anticipated or unanticipated, and grouped by organ system

Administrative information

Key dates and contact information

Description of agreements, if any, between the sponsor and the principal investigator that would restrict dissemination of results by the principal investigator



Fase 0
Dosi subterapeutiche farmaco cinetica

Fase I (sicurezza del farmaco)

Valutazione di sicurezza, farmacovigilanza, tollerabilità, farmacocinetica farmacodinamica

Fase II (effetto del trattamento iniziale) IIa IIb
La fase IIa è progettata specificatamente per valutare la quantità di farmaco necessaria.
La fase IIb è progettata specificatamente per studi di efficacia (valutare come il farmaco lavora alla dose prescritta). Vengono anche chiamati con un termine inglese **dose ranging**.
Fase III (valutazione generale)
Quando un farmaco è considerato ragionevolmente efficace e sicuro, viene somministrato a un numero alto di soggetti.

Gli studi in fase III sono trial multicentrici randomizzati e controllati, effettuati su un grande gruppo di pazienti (300-3000 o più, a seconda della malattia o della condizione medica investigata), e vengono utilizzati per effettuare la valutazione definitiva sull'efficacia del farmaco versus il "gold standard" corrente. In virtù della loro grandezza e durata, i trials in fase III sono i più costosi, duraturi e difficili per quanto concerne progettazione e decorso, soprattutto nel caso di malattie croniche.

Come studiare una malattia?

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http://clinicaltrials.gov

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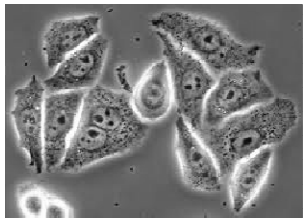

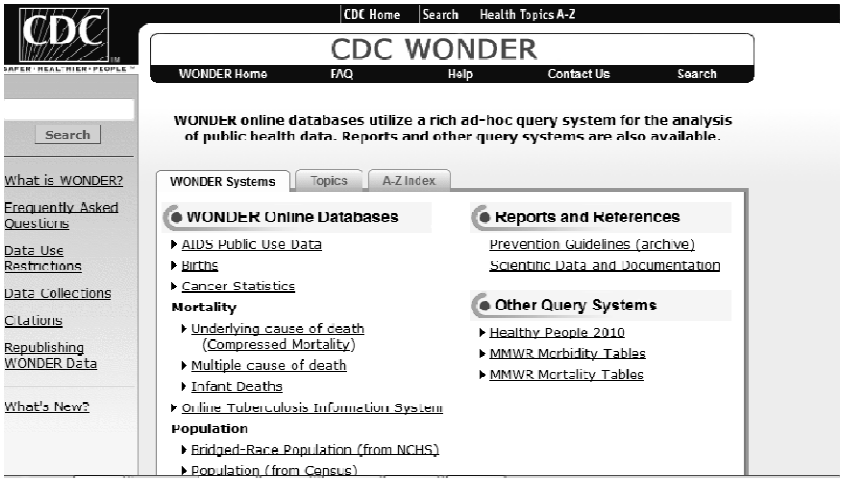

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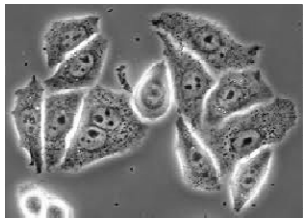
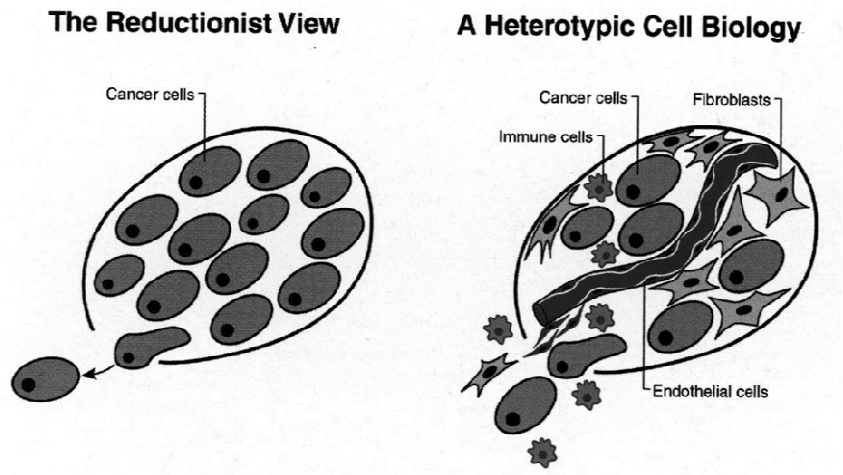


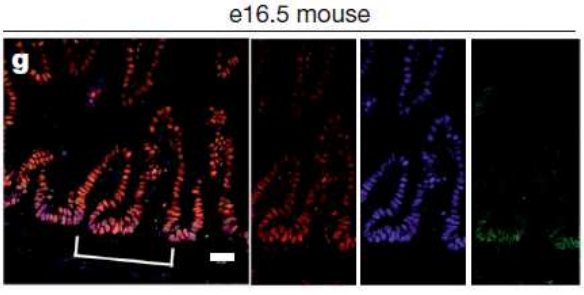


LETTER

doi:10.1038/nature09691

Directed differentiation of human pluripotent stem cells into intestinal tissue *in vitro*

Jason R. Spence[1], Christopher N. Mayhew[1], Scott A. Rankin[1], Matthew F. Kuhar[1], Jefferson E. Vallance[2], Kathryn Tolle[1], Elizabeth E. Hoskins[3], Vladimir V. Kalinichenko[1,4], Susanne I. Wells[3], Aaron M. Zorn[1], Noah F. Shroyer[1,2] & James M. Wells[1]



Osservando animali con una malattia simile all'uomo

Inducendo la malattia in una specie animale suscettibile

Modelli animali

http://news.medill.northwestern.edu/chicago/news.aspx?id=105989

Modelli animali

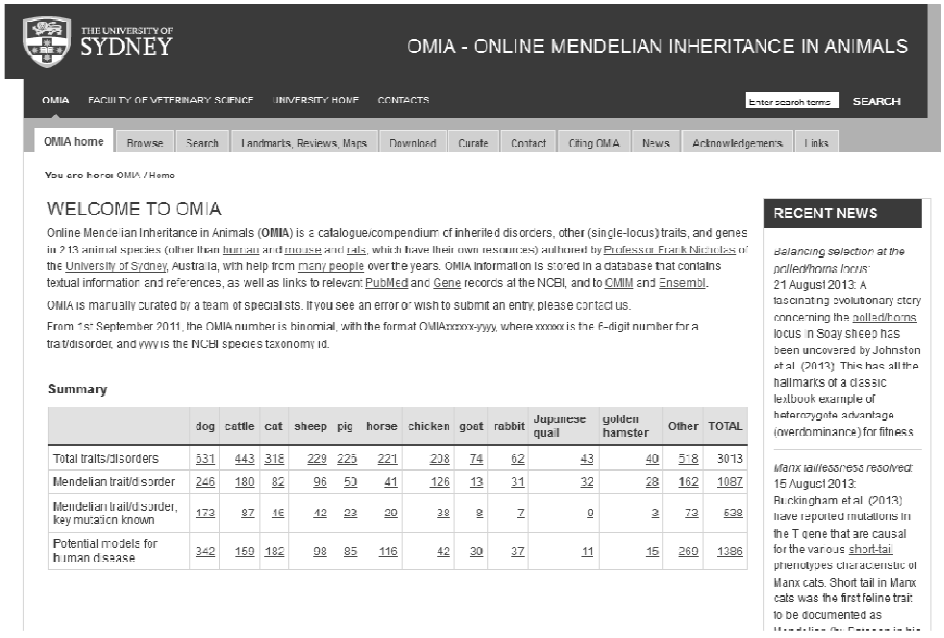
Spontanei: animali normali con meccanismi fisiologici simili a quelli della specie di interesse o individui, appartenenti alla specie modello, anormali a causa di mutazioni spontanee

Indotti: animali normali che vengono sottoposti a manipolazione (chirurgica, chimica, farmacologica, genetica) per indurre uno stato patologico

- I modelli spontanei possono consistere in animali che in condizioni normali hanno processi fisiologici simili a quelli dell'uomo oppure patologie animali che spontaneamente si verificano in una specie

- Non sono spesso anticipabili su base filogenetica

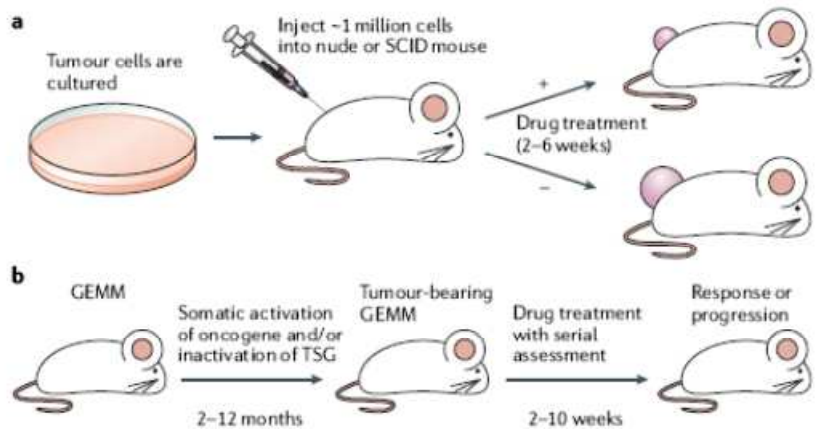
Malattia/patologia	**Cane**	**Gatto**	**Altro animale**
LSD	Si, (20)	Si, (10)	Quaglia giapponese
Epatite B/EpatoK	No	No	Woodchuck
Tumori mammari	Si (misti)	Si (semplici)	Topo, virale
Neoplasie testicolari	Si (20 volte più frequenti dell'uomo)	No	
Ipertensione arteriosa	No	No	Tacchino americano
Cheloide cicatriziale	No	No	Cavallo ("proud flesh")

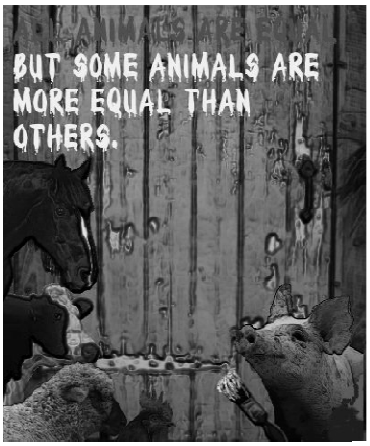


- I modelli indotti sono stati sottoposti a manipolazione chirurgica, chimica, fisica, farmacologica o genetica per creare sperimentalmente la condizione che deve essere studiata

- I modelli negativi sono animali nei quali una malattia non si sviluppa o che non possiedono una reattività ad un determinato stimolo

- La singola categoria di modelli animali più frequentemente utilizzata in biomedicina è quella ottenuta con manipolazioni genetiche (animali transgenici)





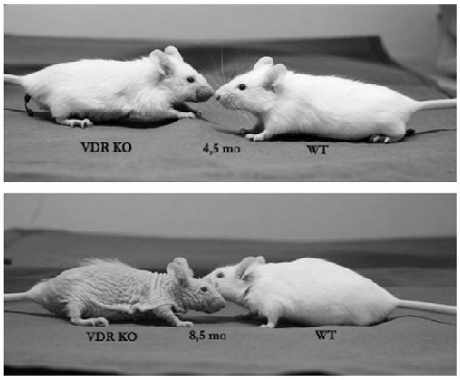
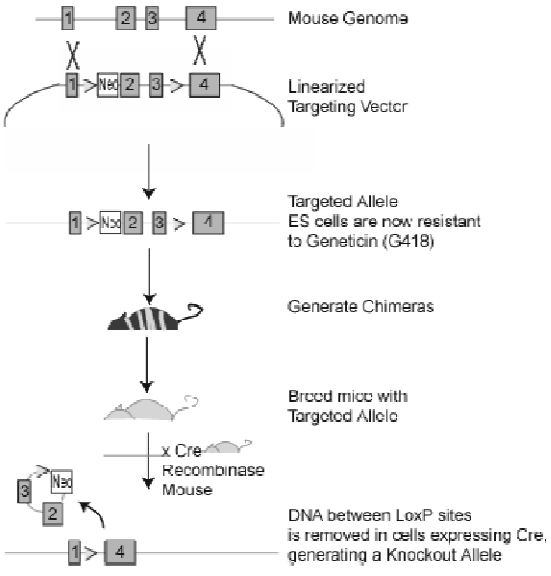


Fig. 2. Phenotype of VDR knockout mouse (KO) compared to wildtype littermate (WT; NMRI background strain) at the age of 4.5 (top) and 8.5 (bottom) months.

Figure 2: Gene Targeting and Conditional Knockouts



Modelli animali ideali
-uguale al processo umano che devono riprodurre
-facilità di manipolazione
-abilità di fornire cucciolate numerose
-economicamente vantaggioso
-facilità nell'eseguire prelievi di sangue e tessuti sequenziali nello stesso individuo
-composizione genetica definita
-facilità nell'esternare la malattia o lo "status" richiesto

Migaki and Capen, 1984, Leader and Padgett 1980, Dodds and Abelseth 1990

Variabili per la selezione di un modello animale appropriato

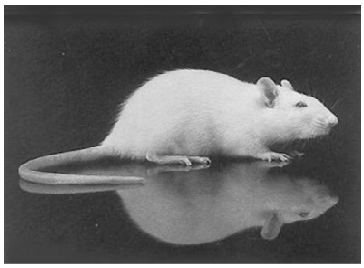
-Specie: mammifero/non mammifero
-Età: immaturo o maturo, velocità di sviluppo
-Dieta: ruminante/monogastrico, carnivoro/erbivoro/onnivoro
-Sesso: maschio/femmina castrato
-Variazione diurna: livelli ormonali etc
-Stagionalità: specie stagionali (es. pecore)
-Temperatura: omeotermi/eterotermi
- Genetica-genomica

Harrison *et al:* Gastrointestinal-tract models and techniques for use in safety pharmacology. Journal of pharmacological and toxicological methods 49(200) 187-199

Selezione di un modello animale

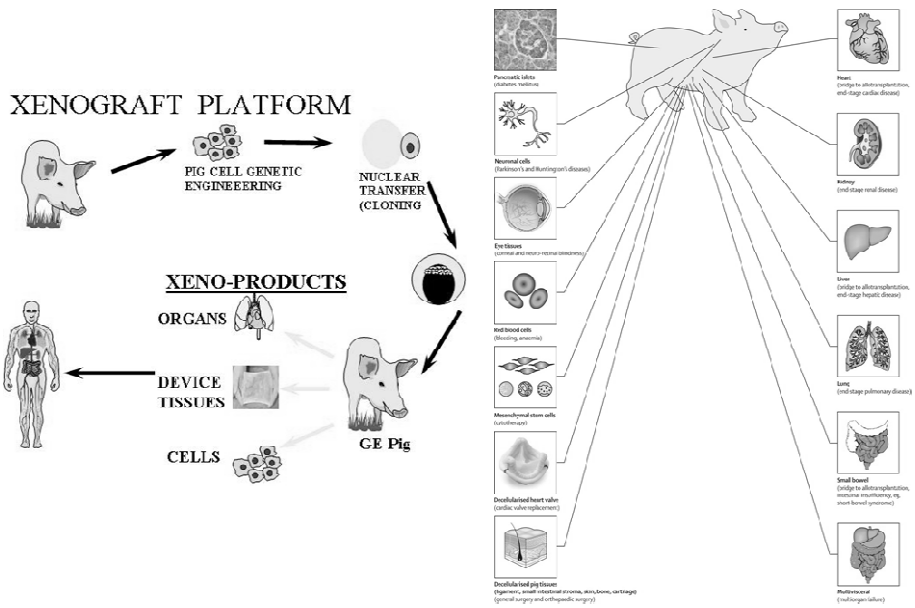
Ratto:
A livello intestinale differisce dall'uomo per 1) assenza di cistifellea, 2) attività notturna
3) flora batterica intestinale; 4) capacità di legare i farmaci a livello plasmatico

Es. ratti di 14 giorni hanno una motilità intestinale limitata, però i ratti allevati con la madre hanno una motilità intestinale spontanea maggiore e sono più sensibili alla stimolazione con analoghi dell'acetilcolina.
La funzione gastrointestinale non è solamente limitata al grado di maturità di un particolare modello animale, ma è correlata allo stress, alla stabulazione, manipolazione



Harrison *et al:* Gastrointestinal-tract models and techniques for use in safety pharmacology.
Journal of pharmacological and toxicological methods
49(200) 187-199

Suini:
-Anatomicamente e fisiologicamente molto simili all'uomo (monogastrici, onnivori, lunghezza del tratto intestinale, termoneutralità, limitata superficie corporea coperta dal pelo, stato endocrinologico simile alla nascita)
-I suinetti di una settimana mostrano un quadro di motilità intestinale simile a quello di un bambino prematuro di 6 settimane
-Il suino viene utilizzato come modello animale soprattutto per i sistemi cardiovascolare, digestivo e cutaneo.
-la vascolarizzazione coronarica è molto simile e permette studi sull'infarto miocardico Acuto (IMA) e sull'impianto di stent
-sviluppano aterosclerosi quando viene somministrata una dieta ad elevato livello di grassi
-la fisiologia dell'apparato digerente è molto simile
-sono utilizzati come modelli per la guarigione di ferite cutanee e per gli studi di tossicologia cutanea
-trapianti di organi e fonte di organi per xenotrapianto
-modelli chirurgici



Transgenic pigs and virus adaptation

Pigs offer the best hope of providing organs for transplantation to humans. But, in overcoming the problems of tissue rejection, we may be increasing our risk of infection from pig viruses.

Robin A. Weiss

Homer's story of what Circe achieved with an early formulation like cyclosporine has inspired the name of a biotechnology company that provides pig hepatocytes for *ex vivo* treatment of fulminant liver failure, and we named a pig retrovirus in her honour. Down the ages there have been many tales of men being transformed into beasts, and princes into frogs. Marie Darrieussecq's *Truismes*, now available in English translation[1], is a modern parable on the porcine fate of a young woman in a 'massage' parlour who gradually turns plumper and pinker and who begins to grunt and squeal in a way that initially delights her clients. Billed as a feminist version of Kafka's *Metamorphosis*, it probably owes as much in its undertones of fascism to Ionesco's *Rhinoceros*. Conversely, anthropomorphism in animals is an equally popular genre for cautionary tales, ranging from Aesop's *Fables* to the telling climax in Orwell's *Animal Farm* when the pigs totter around on their hind legs. It is this second aspect, the humanization of pigs, that I wish to address in this commentary on xenotransplantation — in which tissue is transferred from one species to another — and infection.

Circe offered Odysseus's men a potion mixed with cheese, honey and wine. And when they had emptied their bowls, they grew pigs' heads and bristles, and they grunted like pigs; but their minds were as human as ever.

Int. J. Biol. Sci. 2007, 3 179

International Journal of Biological Sciences
ISSN 1449-2288 www.biolsci.org 2007 3(3):179-184

Review

Advances in Swine Biomedical Model Genomics

Joan K. Lunney

Table 1. Advantages of Swine as a Biomedical Model

- Human size – particularly miniature pigs
- Physiology similar to humans
- Large litter sizes
- Cloning and transgenic technology well-advanced
- Numerous well defined cell lines
- Similar disease progression
  - metabolic , e.g. obesity and heart disease
  - infectious diseases – numerous organisms cause infections across species
- Ability to deliberately time studies and collect repeated and, at kill, detailed tissue samples
- High sequence and chromosome structure homology with humans
- Improving genomic and proteomic tools

Table 2. Swine Biomedical Models

Model	Current Ref.
• **Heart physiology**	
○ Stent design, tissue engineering of blood vessels	[26, 26]
○ Atherosclerosis	[9, 10]
○ Myocardial infarction	[27, 28]
○ Ex vivo heart model	[29]
○ Emergency procedures	[30, 31]
• **Reproductive function**	
○ Maternal-fetal interactions	[14]
○ Embryo development	[15-17]
○ Sperm	[20, 21]
• **Transplantation**	
○ Cell and organ transplants	[32, 33]
○ Xenotransplantation	[5, 34, 35]
○ Drug therapies and biotherapeutics	
• **Skin physiology**	
○ Percutaneous permeation	[36, 37]
○ Contact dermatitis	[38]
○ Skin equivalent culture model	[39]
○ Melanoma	[40,41]
• **Brain**	
○ Stroke - focal cerebral ischemia	[42]
○ AIDS dementia - Multinucleated giant cell formation	[43]
○ Drug binding sites and interactions	[44]
• **Gut physiology and Nutrition**	
○ Gut structure and intestinal metabolism	[45, 46]
○ Obesity	[47]
○ Probiotics and gut physiology	[48, 49]
○ Biologic and immunological basis of food allergies	[50, 51]
• **Biomechanical models**	
○ Response to injury	[52]
○ Imaging techniques	[53, 54]
○ Bone density analyses - Osteoporosis	[55]
• **Tissue engineering**	
○ Cartilage repair - chondrocytes	[56]
○ Spinal fusion	[57]
○ Organ specific gene delivery	[58]
○ Lens capsule epithelial cells for cataract repairs	[59, 60]
○ Polymer scaffolds	[61, 62]
○ Tooth development - dental enamel	[63]
• **Respiratory function**	
○ Neonatal respiratory distress	[64]
○ Thoracic artificial lung	[65]
○ Disease models and therapies; Asthma	[66,67]
• **Infectious disease models**	
○ Therapeutics, Vaccines, Biotherapeutics, Drug therapies	[68, 69]
○ Developmental interactions	[70, 71]
○ Mucosal tissue responses	[72-75]
○ Genomics of host responses	[76]

Table 3. Lessons learned from Swine Melanoma Studies

- Well established and characterized models
  - Sinclair melanoma
  - Melanoma-bearing Libechov Minipig (MeLiM)
- Large numbers of affected individuals for mapping studies
- Detailed phenotypic information
  - Comparative analyses of normal versus tumor tissues
  - Assessment of factors determining development of pathology
  - Well defined tumor regression
- Access to cancerous tissues
  - Analyses of specific tumor subsets
  - Cell migration and tumor infiltrating lymphocyte analyses
  - Definition of regulatory pathways

Table 4. Utilizing genomics for Swine Models - Issues to Consider

- Genotyping
  - Population Design
  - Mapping or gene/protein expression
  - Increasing number of SNPs available for swine
- Phenotyping
  - Extensiveness of phenotype improves ability to reveal full details of genetic control
  - Importance to sample local tissues, not just peripheral blood or mucosal secretions
  - Additive information provided by in vitro cellular systems
  - Impact of imaging techniques on expanding phenotype
- Candidate genes versus hypothesis independent analyses
  - Experimental effort required to consider numerous candidate genes
  - Potential for unbiased arrays/ comparative maps
  - Datasets – Current limits on number/complexity of samples to compare phenotype with genotype

Data Sheet: DNA Analysis

illumina®

PorcineSNP60 Genotyping BeadChip

More than 62,000 SNPs that deliver the densest coverage available for the porcine genome

Highlights

- **Comprehensive and Uniform Coverage**
  Evenly distributed polymorphic SNPs with a median < 28 kb gap spacing
- **Unrivaled Call Rates and Accuracy**
  > 99% average call rates and > 99.9% reproducibility
- **Simple Workflow**
  PCR- and ligation-free protocol
- **High-Throughput Format**
  Up to 12 samples can be interrogated in parallel

Figure 1: PorcineSNP60 BeadChip

Legislazioni nazionali sull'utilizzo degli animali nella ricerca

UK animals Act 1986

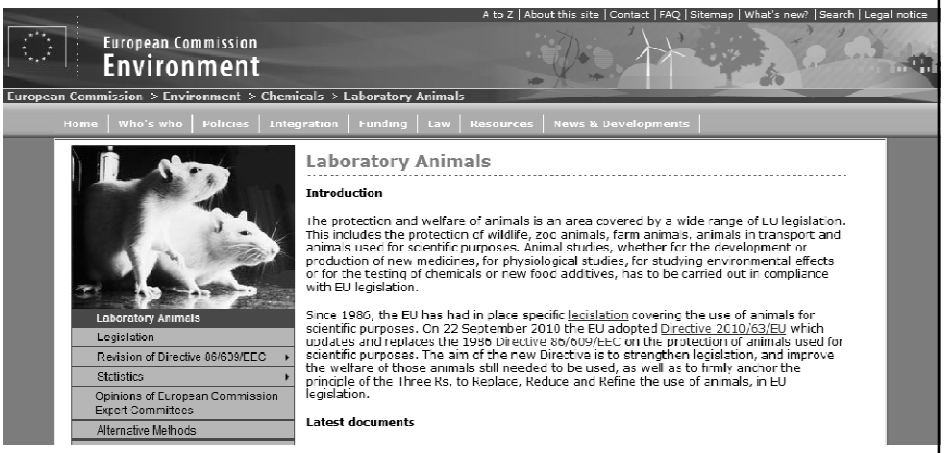
USA Institute for Laboratory Animal Research (ILAR): Giude for the Care and Use Of Laboratory Animals (http://dels.nas.edu/Laboratory

EU webpage of Laboratory animals (http://ec.europa.eu/environment/chemicals/lab_animals/home_en.htm)

Public Health Service Policy on humane care and use of laboratory animals (Office of Laboratory Animal Welfare, NIH 2002)

Direttiva del Consiglio europeo del 24/11/1986 **(86/609/CEE)** sulla protezione degli animali utilizzati ai fini sperimentali o per altri fini scientifici recepita in Italia con il Decreto Legislativo **27/01/1992 n° 116**

Directive 2010/63/EU revising Directive 86/609/EEC
Legge 6 agosto 2013, n. 96, articolo 13



Horizontal legislation on the protection of animals used for scientific purposes

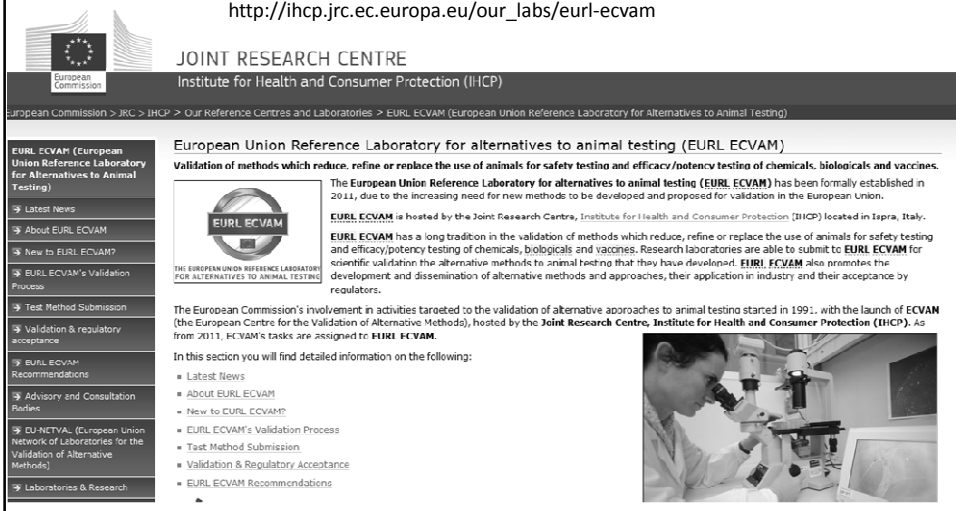
Directive 2010/63/EU

Directive 2010/63/EU revising Directive 86/609/EEC on the protection of animals used for scientific purposes was adopted on 22 September 2010. The Directive is firmly based on the principle of the Three Rs, to replace, reduce and refine the use of animals used for scientific purposes. The scope is now wider and includes cyclostomes and foetuses in their last trimester of development as well as animals used for the purposes of basic research, higher education and training. It lays down minimum standards for housing and care, regulates the use of animals through a systematic project evaluation requiring inter alia assessment of pain, suffering distress and lasting harm caused to the animals. It requires regular risk-based inspections and improves transparency through measures such as publication of non-technical project summaries and retrospective assessment. The development, validation and implementation of alternative methods is promoted through measures such as establishment of a Union reference laboratory for the validation of alternative methods supported by laboratories within Member States and requiring Member States to promote alternative methods at national level. The Directive will take full effect from 1 January 2013.

Concetto delle "3R", proposto da Russell e Burch nel 1959 (“The principle of humane experimental technique” London, Meuthen): Refinement, Reduction and Replacement.
Replacement: rimpiazzare gli animali con metodi alternativi
Reduction: del numero degli animali per ottenere gli obiettivi prefissati
Refinement: utilizzare metodiche che minimizzano la sofferenza degli animali

Il termine "metodi alternativi" è stato introdotto solo più tardi, nel 1978 da Smyth, come sintesi del modello delle 3R, generando tuttavia non poche aspettative e resistenze su tutti i fronti.

http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam



Scopi

Come definito nella comunicazione della
Commissione Europea al consiglio europeo e al parlamento europeo (Ottobre 1991):

1. Coordinare la validazione di metodi alternativi a livello dell'Unione Europea
2. Centralizzare lo scambio di informazioni per lo sviluppo di metodi alternativi
3. Creare, mantenere e gestire un database con le procedure alternative
4. Promuovere il dialogo tra legislatori, industrie, ricercatori, consumatori, organizzazioni e gruppi animalisti con lo scopo di sviluppare, validare e far riconoscere a livello internazionale le procedure dei metodi alternativi

Horizontal legislation on the protection of animals used for scientific purposes

Directive 2010/63/EU

Directive 2010/63/EU revising Directive 86/609/EEC on the protection of animals used for scientific purposes was adopted on 22 September 2010. The Directive is firmly based on the principle of the Three Rs, to replace, reduce and refine the use of animals used for scientific purposes. The scope is now wider and includes cyclostomes and foetuses in their last trimester of development as well as animals used for the purposes of basic research, higher education and training. It lays down minimum standards for housing and care, regulates the use of animals through a systematic project evaluation requiring inter alia assessment of pain, suffering distress and lasting harm caused to the animals. It requires regular risk-based inspections and improves transparency through measures such as publication of non-technical project summaries and retrospective assessment. The development, validation and implementation of alternative methods is promoted through measures such as establishment of a Union reference laboratory for the validation of alternative methods supported by laboratories within Member States and requiring Member States to promote alternative methods at national level. The Directive will take full effect from 1 January 2013.

20.10.2010 EN Official Journal of the European Union L 276/33

DIRECTIVES

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

(Text with EEA relevance)

Article 1

Subject matter and scope

1. This Directive establishes measures for the protection of animals used for scientific or educational purposes.

To that end, it lays down rules on the following:

(a) the replacement and reduction of the use of animals in procedures and the refinement of the breeding, accommodation, care and use of animals in procedures;

(b) the origin, breeding, marking, care and accommodation and killing of animals;

(c) the operations of breeders, suppliers and users;

(d) the evaluation and authorisation of projects involving the use of animals in procedures.

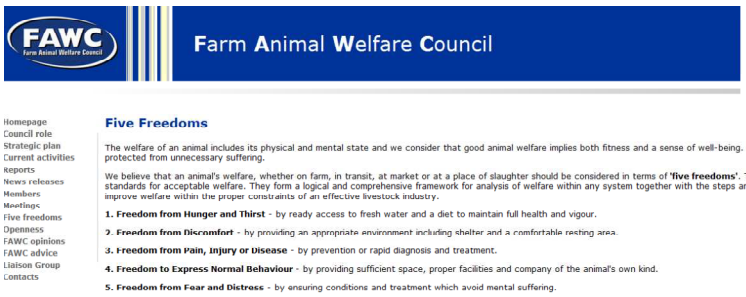
(6) New scientific knowledge is available in respect of factors influencing animal welfare as well as the capacity of animals to sense and express pain, suffering, distress and lasting harm. It is therefore necessary to improve the welfare of animals used in scientific procedures by raising the minimum standards for their protection in line with the latest scientific developments.

Gli animali soffrono?

La sofferenza è una condizione di dolore, che può riguardare il corpo e/o il vissuto emotivo del soggetto. Essa può derivare direttamente da un trauma, fisico o emotivo, oppure può essere espressione di una afflizione interiore più profonda, di cui può essere difficile o impossibile individuare un fondamento oggettivo (…). http://it.wikipedia.org/wiki/Sofferenza

Animal welfare is the physical and psychological well-being of non-human animals.[1] The term animal welfare can also mean human concern for animal welfare or a position in a debate on animal ethics and animal rights.[2]

Systematic concern for animal welfare can be based on awareness that non-human animals are sentient and that consideration should be given to their well-being, especially when they are used by humans.[3] These concerns can include how animals are killed for food, how they are used for scientific research, how they are kept as pets, and how human activities affect the survival of endangered species. http://en.wikipedia.org/wiki/Animal_welfare



1. Freedom from Hunger and Thirst – by ready access to fresh water and a diet to maintain full health and vigour.
2. Freedom from Discomfort – by providing an appropriate environment including shelter and a comfortable resting area.
3. Freedom from Pain, Injury or Disease – by prevention or rapid diagnosis and treatment.
4. Freedom to Express Normal Behaviour - by providing sufficient space, proper facilities and company of the animal's own kind.
5. Freedom from Fear and Distress – by ensuring conditions and treatment which avoid mental suffering.

ELSEVIER

Physiology & Behavior 92 (2007) 422–428

PHYSIOLOGY & BEHAVIOR

Review

A new animal welfare concept based on allostasis

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5. Freedom from Fear and Distress – by ensuring conditions and treatment which avoid mental suffering.

First, freedom from fear and distress is a typical anthropocentric construct. Fear is an emotion produced by the perceptions of impending danger and is normal in appropriate situations. It is a vital evolutionary legacy that leads an organism to avoid threat. Without fear, few vertebrates in the wild would survive long enough to reproduce. Thus, fear has fitness value. However, this does not mean that in the absence of threats animals should feel fear.

Korte et al. 2007

5. Freedom from Fear and Distress – by ensuring conditions and treatment which avoid mental suffering.

sibilities under the Animal Welfare Act [9]. They came up with a working definition of distress: "a state in which an animal cannot escape from or adapt to the internal or external stressors or conditions it experiences, resulting in negative effects on its well-being". The Federation of American Societies for Experimental Biology objected that this definition is "vague and could lead to widely varying, highly subjective interpretations", and "there are no simple physiological or behavioral criteria to mark the point where an animal that experiences stress becomes distressed" [10]. Previously, it has been concluded that the term (dis)stress has so many different meanings that it becomes counterproductive by inhibiting a proper application and critical interpretation of experimental results [11]. Distress has mostly been associated with negative events and consequences. There is, however, no justification for the assumption that the expression of stress responses always compromise health or welfare. Indeed, the functional aspects of stress have often been neglected [12]. The paradox of stress lies in the simultaneity of its adaptive nature and its possible maladaptive consequences [13,14]. The best known

Korte et al. 2007

Second, one might expect that natural selection will shape a body for maximum health and longevity. Unfortunately, this is not always true. Health is not the outcome of natural selection, maximal reproduction is. If a mutation causes a disease, but yields a net increased reproductive success, it will be selected for [19]. Here is exactly where fitness and animal welfare depart: something can benefit reproductive success but involve negative experiences for the individual, causing poor animal welfare.

Korte et al. 2007

3. Freedom from Pain, Injury or Disease – by prevention or rapid diagnosis and treatment.

Third, freedom from pain, injury or disease is a utopia. For example, pain is a natural defense mechanism that helps to protect organisms from potential threats and dangerous substances. Pain, nausea, fever, vomiting and diarrhea are products of natural selection. Although they produce suffering, they are defense mechanisms that protect organisms [20].

Korte et al. 2007

4. Freedom to Express Normal Behaviour - by providing sufficient space, proper facilities and company of the animal's own kind.

Fourth, intuitively it is appealing to improve animal welfare by respecting the nature of the animals. However, one has to realize that due to natural selection, nature is by no way a paradise. For instance, mice from some laboratory lines can survive as long as three years, while free-living wild mice are likely to die much earlier from disease, competition, or predators [21]. Male mice will tolerate their own offspring, but will kill offspring born to females that belong to other demes [22]. Wild animals try to increase their genes in a population. In contrast, many farm and laboratory animals are docile, due to artificial selection on the calmest animals. Consequently, behavior, temperament and associated physiology of these animals may have been modified during domestication. It is important to realize that wild, farm and experimental animals differ in the way genetic (natural or artificial) selection takes place with different consequences for ethics and animal welfare (see Table 1).

Korte et al. 2007

1. Freedom from Hunger and Thirst – by ready access to fresh water and a diet to maintain full health and vigour.

Fifth, freedom of hunger or ad libitum food availability in farm animals and zoo animals also produces problems [23].

Freedom from hunger together with an impoverished environment may disturb mental health as reflected by stereotypic and compulsive behaviors in zoo, circus and farm animals. Quantitively this is the world's largest animal welfare problem. In addition, mammals that are fed a restricted calorie diet live longer. Thus, longevity and hunger are part of a healthy mammal's life.

Korte et al. 2007

affected farmers but also society on a large scale. In European law, animals now are defined as "sentient" creatures, indicating that they are considered as conscious feeling animals, no longer just as agricultural products, and they have a value of their own. This is an understandable change, because conscious feeling animals are crucial to animal welfare, but without scientific background it opens the door for anthropocentric (public) thinking of how animals ought to be handled. This increases the risk that subjectivity, cultural and non-scientific opinions will largely affect legislation on how to keep and treat animals. This creates a considerable obstacle for progress on animal welfare on a more global scale.

However, this anthropocentric thinking of how animals ought to be handled should be rejected because the latest developments in neurobiology and behavioral physiology make it possible to objectively investigate the relationships between emotional individual beings and their environment to understand and improve animal welfare where needed. To understand animal welfare in conscious feeling animals it is crucial to investigate both brain and periphery states in relation to the environmental challenges that have led to these states. Accord-

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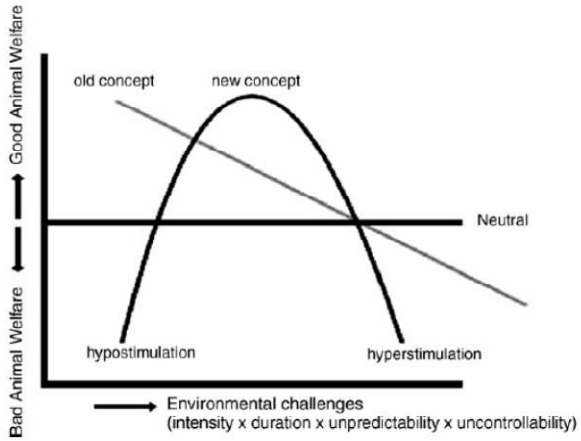
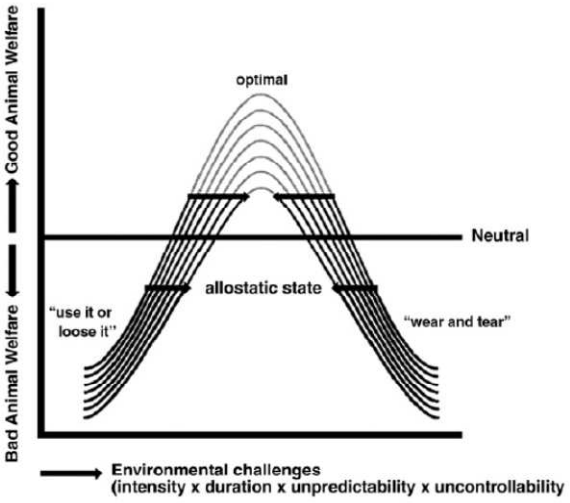


Allostasi, l'evoluzione del concetto di omeostasi

Korte et al. 2007



Allostasi, l'evoluzione del concetto di omeostasi

Korte et al. 2007

Table 2
The different mediators of allostasis, associated allostatic state and allostatic load due to hypostimulation or hyperstimulation, respectively

	Mediators of allostasis	Assessment of allostatic state	Measures of allostatic load	
			*Hypo*stimulation	*Hyper*stimulation
Central nervous system	Glucocorticoids, Amino acids, Cytokines, Serotonin, Dopamine, Norepinephrine, Neuropeptides like CRF, etc.	–change in central MR/GR balance	–violence – impulse control disorders	–cognitive impairment – anxiety disorders
		– altered hippocampal CA3 dendritic tree atrophy	– atypical depression	– melancholic depression
		– altered DG cell turnover	– hypersomnia	– insomnia
		– expression and function of 5-HT_{1A} /5-HT_{2C}	– chronic fatigue	– psychotic states
Cardio-vascular system	Catecholamines: e.g. adrenaline	–elevated levels of overnight urinary catecholamines	–ventricular arrhythmia's	–hypertension
		– decreased vagal activity	– sudden death	– ventricular heart hypertrophy
		– increased clotting factors		–increased blood clotting
Immune system	Glucocorticoids Cytokines: e.g. IL-1,-4,-6,-10, TNF-α, TNF-γ, etc.	–decreased plasma cortisol levels and decreased mobility of white cells	–infection – impaired wound healing	–inflammation – autoimmunity
		– increased levels of inflammatory cytokines	– retarded immunization	
Metabolic system	Glucocorticoids	–elevated and flattened diurnal urinary cortisol	–weight loss	–abdominal fat – atheriosclerosis – muscle wasting
		– increased insulin and glucose levels		– bone thinning – diabetis

Abbreviations: CRF — Corticotropin releasing factor; MR — mineralocorticoid receptor; GR — glucocorticoid receptor; DG — Dentate Gyrus; 5-HT — 5 hydroxytryptophan; IL — interleukine; TNF — tumor necrosis factor.

(13) The choice of methods and the species to be used have a direct impact on both the numbers of animals used and their welfare. The choice of methods should therefore ensure the selection of the method that is able to provide the most satisfactory results and is likely to cause the minimum pain, suffering or distress. The methods selected should use the minimum number of animals that would provide reliable results and require the use of species with the lowest capacity to experience pain, suffering, distress or lasting harm that are optimal for extrapolation into target species.

(14) The methods selected should avoid, as far as possible, death as an end-point due to the severe suffering experienced during the period before death. Where possible, it should be substituted by more humane end-points using clinical signs that determine the impending death, thereby allowing the animal to be killed without any further suffering.

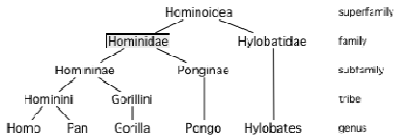
(15) The use of inappropriate methods for killing an animal can cause significant pain, distress and suffering to the animal. The level of competence of the person carrying out this operation is equally important. Animals should therefore be killed only by a competent person using a method that is appropriate to the species.

(16) It is necessary to ensure that the use of animals in procedures does not pose a threat to biodiversity. Therefore, the use of endangered species in procedures should be limited to a strict minimum.

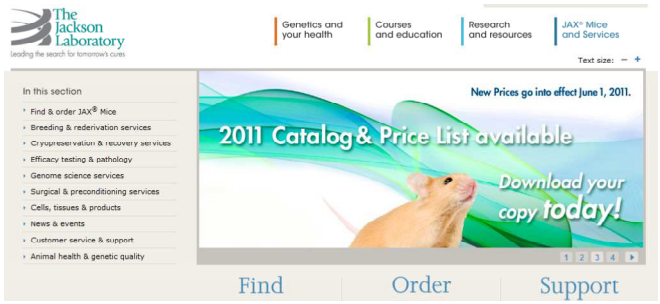
(17) Having regard to the present state of scientific knowledge, the use of non-human primates in scientific procedures is still necessary in biomedical research. Due to their genetic proximity to human beings and to their highly developed social skills, the use of non-human primates in scientific procedures raises specific ethical and practical problems in terms of meeting their behavioural, environmental and social needs in a laboratory environment. Furthermore, the use of non-human primates is of the greatest concern to the public. Therefore the use of non-human primates should be permitted only in those biomedical areas essential for the benefit of human beings, for which no other alternative replacement methods are yet available. Their use should be permitted only for basic research, the preservation of the respective non-human primate species or when the work, including xenotransplantation, is carried out in relation to potentially life-threatening conditions in humans or in relation to cases having a substantial impact on a person's day-to-day functioning, i.e. debilitating conditions.

(18) The use of great apes, as the closest species to human beings with the most advanced social and behavioural skills, should be permitted only for the purposes of research aimed at the preservation of those species and where action in relation to a life-threatening, debilitating condition endangering human beings is warranted, and no other species or alternative method would suffice in order to achieve the aims of the procedure. The Member State claiming such a need should provide information necessary for the Commission to take a decision.

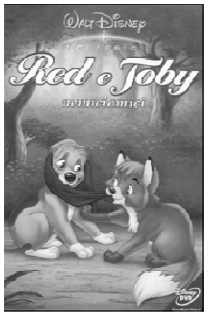


(20) There is a need for certain species of vertebrate animals used in procedures to be bred specifically for that purpose so that their genetic, biological and behavioural background is well-known to persons undertaking the procedures. Such knowledge both increases the scientific quality and reliability of the results and decreases the variability, ultimately resulting in fewer procedures and reduced animal use. Furthermore, for reasons of animal welfare and conservation, the use of animals taken from the wild in procedures should be limited to cases where the purpose of the procedures cannot be achieved using animals bred specifically for use in procedures.



(26) At the end of the procedure, the most appropriate decision should be taken as regards the future of the animal on the basis of animal welfare and potential risks to the environment. The animals whose welfare would be compromised should be killed. In some cases, animals should be returned to a suitable habitat or husbandry system or animals such as dogs and cats should be allowed to be rehomed in families as there is a high level of public concern as to the fate of such animals. Should Member States allow rehoming, it is essential that the breeder, supplier or user has a scheme in place to provide appropriate socialisation to those animals in order to ensure successful rehoming as well as to avoid unnecessary distress to the animals and to guarantee public safety.



(46) The availability of alternative methods is highly dependent on the progress of the research into the development of alternatives. The Community Framework Programmes for Research and Technological Development provided increasing funding for projects which aim to replace, reduce and refine the use of animals in procedures. In order to increase competitiveness of research and industry in the Union and to replace, reduce and refine the use of animals in procedures, the Commission and the Member States should contribute through research and by other means to the development and validation of alternative approaches.

Art. 13

Criteri di delega al Governo per il recepimento della direttiva 2010/63/UE del Parlamento europeo e del Consiglio, del 22 settembre 2010, sulla protezione degli animali utilizzati a fini scientifici

1. Nell'esercizio della delega per l'attuazione della direttiva 2010/63/UE del Parlamento europeo e del Consiglio, del 22 settembre 2010, sulla protezione degli animali utilizzati a fini scientifici, il Governo e' tenuto a seguire, oltre ai principi e criteri direttivi di cui all'articolo 1, comma 1, anche i seguenti principi e criteri direttivi specifici:

a) orientare la ricerca all'impiego di metodi alternativi;

b) vietare l'utilizzo di primati, cani, gatti ed esemplari di specie in via d'estinzione a meno che non si tratti di ricerche finalizzate alla salute dell'uomo o delle specie coinvolte, condotte in conformita' ai principi della direttiva 2010/63/UE, previa autorizzazione del Ministero della salute, sentito il Consiglio superiore di sanita';

c) considerare la necessita' di sottoporre ad altre sperimentazioni un animale che sia gia' stato utilizzato in una procedura, fino a quelle in cui l'effettiva gravita' delle procedure precedenti era classificata come «moderata» e quella successiva appartenga allo stesso livello di dolore o sia classificata come «lieve» o «non risveglio», ai sensi dell'articolo 16 della direttiva 2010/63/UE;

d) vietare gli esperimenti e le procedure che non prevedono anestesia o analgesia, qualora esse comportino dolore all'animale, ad eccezione dei casi di sperimentazione di anestetici o di analgesici;

e) stabilire che la generazione di ceppi di animali geneticamente modificati deve tener conto della valutazione del rapporto tra danno e beneficio, dell'effettiva necessita' della manipolazione e del possibile impatto che potrebbe avere sul benessere degli animali, valutando i potenziali rischi per la salute umana e animale e per l'ambiente;

f) vietare l'utilizzo di animali per gli esperimenti bellici, per gli xenotrapianti e per le ricerche su sostanze d'abuso, negli ambiti sperimentali e di esercitazioni didattiche ad eccezione della formazione universitaria in medicina veterinaria e dell'alta formazione dei medici e dei veterinari;

g) vietare l'allevamento nel territorio nazionale di cani, gatti e primati non umani destinati alla sperimentazione;

h) definire un quadro sanzionatorio appropriato e tale da risultare effettivo, proporzionato e dissuasivo, anche tenendo conto del titolo IX-bis del libro II del codice penale;

i) sviluppare approcci alternativi idonei a fornire lo stesso livello o un livello superiore di informazioni rispetto a quello ottenuto nelle procedure che usano animali, ma che non prevedono l'uso di animali o utilizzano un numero minore di animali o comportano procedure meno dolorose, nel limite delle risorse finanziarie derivanti dall'applicazione del criterio di cui alla lettera h), accertate e iscritte in bilancio;

l) destinare annualmente una quota nell'ambito di fondi nazionali ed europei finalizzati alla ricerca per lo sviluppo e la convalida di metodi sostitutivi, compatibilmente con gli impegni gia' assunti a legislazione vigente, a corsi periodici di formazione e aggiornamento per gli operatori degli stabilimenti autorizzati, nonche' adottare tutte le misure ritenute opportune e al fine di incoraggiare la ricerca in questo settore con l'obbligo per l'autorita' competente di comunicare, tramite la banca dei dati nazionali, il recepimento dei metodi alternativi e sostitutivi.

2. Nell'applicazione dei principi e criteri direttivi di cui al comma 1, il Governo e' tenuto a rispettare gli obblighi che derivano da legislazioni o farmacopee nazionali, europee o internazionali.

3. Dall'attuazione della delega di cui al presente articolo non devono derivare nuovi o maggiori oneri per la finanza pubblica.

La presente legge, munita del sigillo dello Stato, sara' inserita nella Raccolta ufficiale degli atti normativi della Repubblica italiana. E' fatto obbligo a chiunque spetti di osservarla e di farla osservare come legge dello Stato.

Data a Roma, addi' 6 agosto 2013

NAPOLITANO

Letta, Presidente del Consiglio dei ministri
Moavero Milanesi, Ministro per gli affari europei
Visto, il Guardasigilli: Cancellieri

Metodi alternativi

Modello matematico:	viene comunque elaborato da un essere umano ed è legato a ciò che si conosce fino a quel momento della malattia
Studi in vitro	il limite è dato dal fatto che è una porzione di tessuto rimosso che non funziona esattamente come quando è in rapporto con il sangue, il sistema immunitario, il sistema nervoso, i tessuti adiacenti etc…
Volontari	Malattie concomitanti Il normale decorso può essere molto lungo Problemi etici

Organizzazioni

ILAR Institute for Laboratory Animal Research:
-Animal models and genetic stocks (AMGS) information program
-International laboratory code registry

National institute of health (NIH)
/science/models
Office for Protection from Research Risks

-Animal welfare institute (AWIC)
http://www.awionline.org/index.php?ht=d/sp/i/214/pid/214

American college of laboratory animal medicine (ACLAM)

Armed force Institute of Pathology (AFIP)

Armed Forces Institute of Pathology

United States
National Agricultural Library

American College of
Laboratory Animal Medicine

Riviste

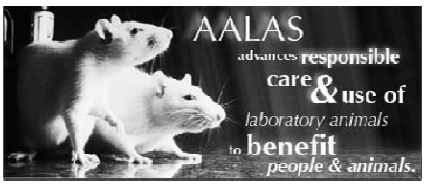
ILAR journal

Contemporary topics in laboratory science and comparative medicine

Lab animal

Scandinavian journal of laboratory animal science

…



ATLA
(Alternatives To
Laboratory
Animals)

Cosa dicono gli oppositori dei modelli animali?

tutte le specie animali sono diverse, un modello animale non potrà mai mimare una condizione presente nella specie umana. I modelli animali sono analoghi di condizioni umane con le quali condividono alcune caratteristiche

i modelli animali sono utilizzati per 2 scopi: pronosticare la risposta di un essere umano agli stimoli (infettivi, traumatici, tossici, farmacologici etc…) ed offrire nuove strade per spiegare l'anatomia, la fisiologia e la patologia umana (H. LaFollette N. Shanks)

-LaFollette H, Shanks N. Animal models in biomedical research: some epistemiological worries. *Pubblic affairs quarterly* 1992;7(2):113-130
-LaFollette H, Shanks N. The intact systems argument: problems with the standard defense of animal experimentation. *Southern journal of philosophy* 1993;31_323-333
-LaFollette H, Shanks N. Animal modelling in psychopharmacological contenxts. Behav Br Sc Dec 1993

Un dato modello animale assomiglia ad una condizione umana in alcune caratteristiche es A, B e C, quindi sarebbe necessario asserire che un'ulteriore caratteristica, D, presente nel modello animale (es una particolare funzione fisiologica o risposta ad una terapia) si debba riscontrare anche nell'essere umano.
La Follette e Shanks dicono che tale affermazione è logica solo se la caratteristica D è causalmente correlata a A B e C sia nel modello animale che in quello umano

Esempio
2 cani abbaiano, amano mangiare le ossa e scodinzolano quando i loro proprietari arrivano a casa, siccome i 2 cani hanno un comportamento simile, ci si aspetta che appartengano alla stessa razza. Quindi se conosciamo la razza del 1 cane possiamo predire la razza del secondo
La RAZZA però non è causalmente correlata alle altre caratteristiche che i 2 cani hanno in comune
Se conosciamo la razza del primo cane e sappiamo che il secondo cane ha gli stessi genitori del primo allora possiamo dire anche la razza del secondo cane anche se i 2 cani non sono identici per colore del mantello o comportamento.