Breakout-Session "Data acquisition in animal experiments and parameters influencing scientific results" (Tutors: E. Thein and T. Brill)

Workshop "Data acquisition in animal experiments and parameters influencing scientific results"

Phase 1: divide into four groups
Two animal experiments
A) Mouse: behaviour tests of different tg-mice
B) Rat: surgical intracerebral application of AdV-gene-vector
Each of two working groups design one of these experiments
Phase 2: Plenum: discussion of the four designs
Phase 3: Presentation:
Physiologic Parameter and how they influence scientific results
Demonstration of the ARRIVE Guideline (2010)

Mouse: Behaviour Tests of different tg-mice

You have different knock-out/knock-in mouse strains and you want to compare them with different behavioural tests. You think that the genetic modification will influence signal transduction in motoneurons.

Rat: surgical intracerebral Application of AdV-gene-vector

You have designed a gene-vector coding for a protein lacking in some human inborn brain diseases. You want to inject the CMO-AdV into the ventricle via a surgical procedure and assess time course of protein levels and AE.

Design the experiment and
List the 10 most important parameters of your animal experiment which might influence your results

Mouse: Behaviour Tests of different tg-mice

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List the 10 most important parameters of your animal experiment which might influence your results

Keep an eye on e.g.:

- Housing of the animals
- Selection of animal model
- Legislation
- Anaesthesia/Analgesia
- Readout parameters/endpoints

Presentation part 1:

Physiologic differences and other factors

influencing scientific results

TGN1412

TGN1412 = anti-human-CD28-antibody
intented use: e.g. multiple sclerosis

• Analysis in mice: no problems

• Analysis in two different primate species: no problems

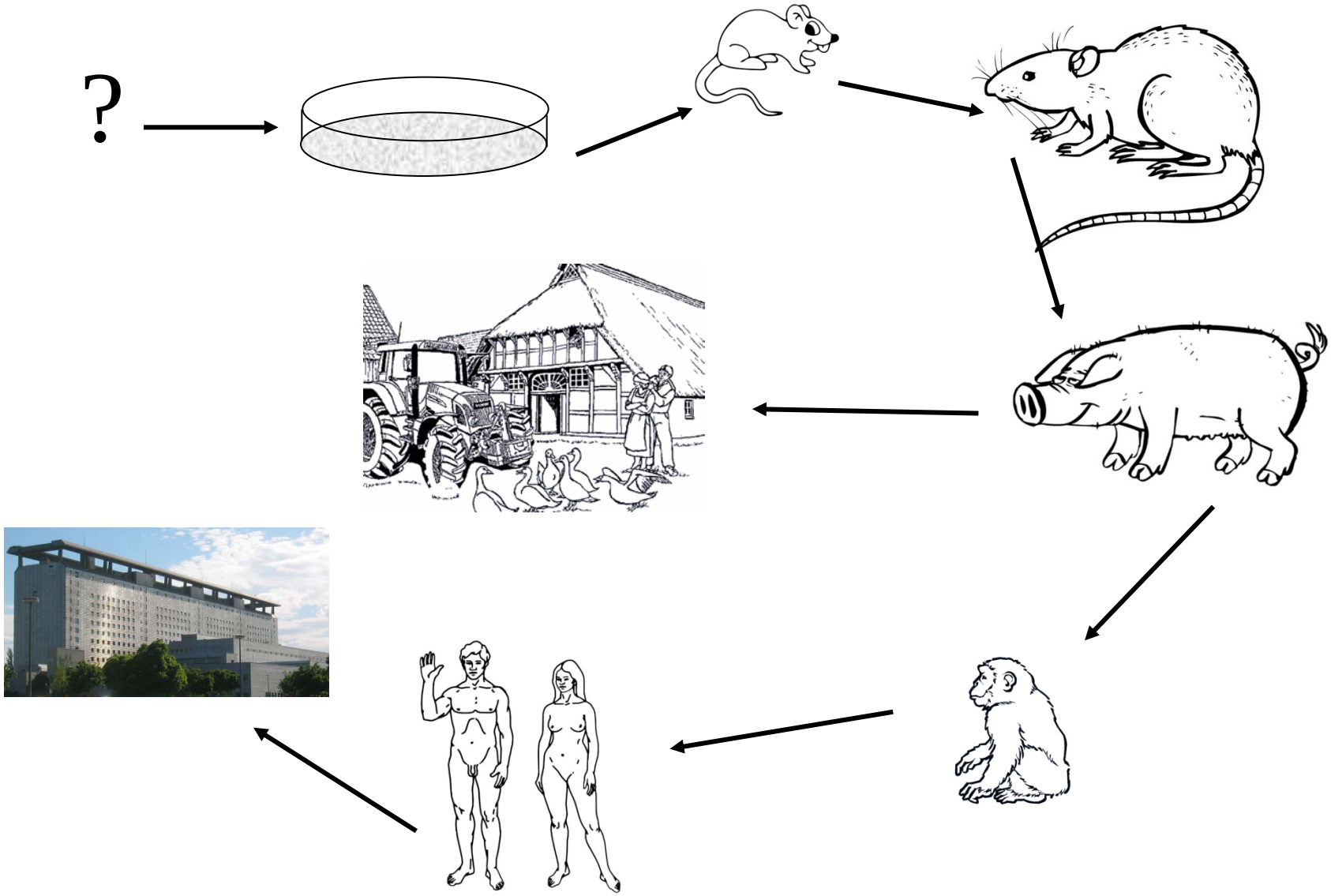
• Analysis for tolerance in six volunteers with 1/160 of the dosage used in primates

Cytokine burst ⟹ SIRS ⟹ ICU

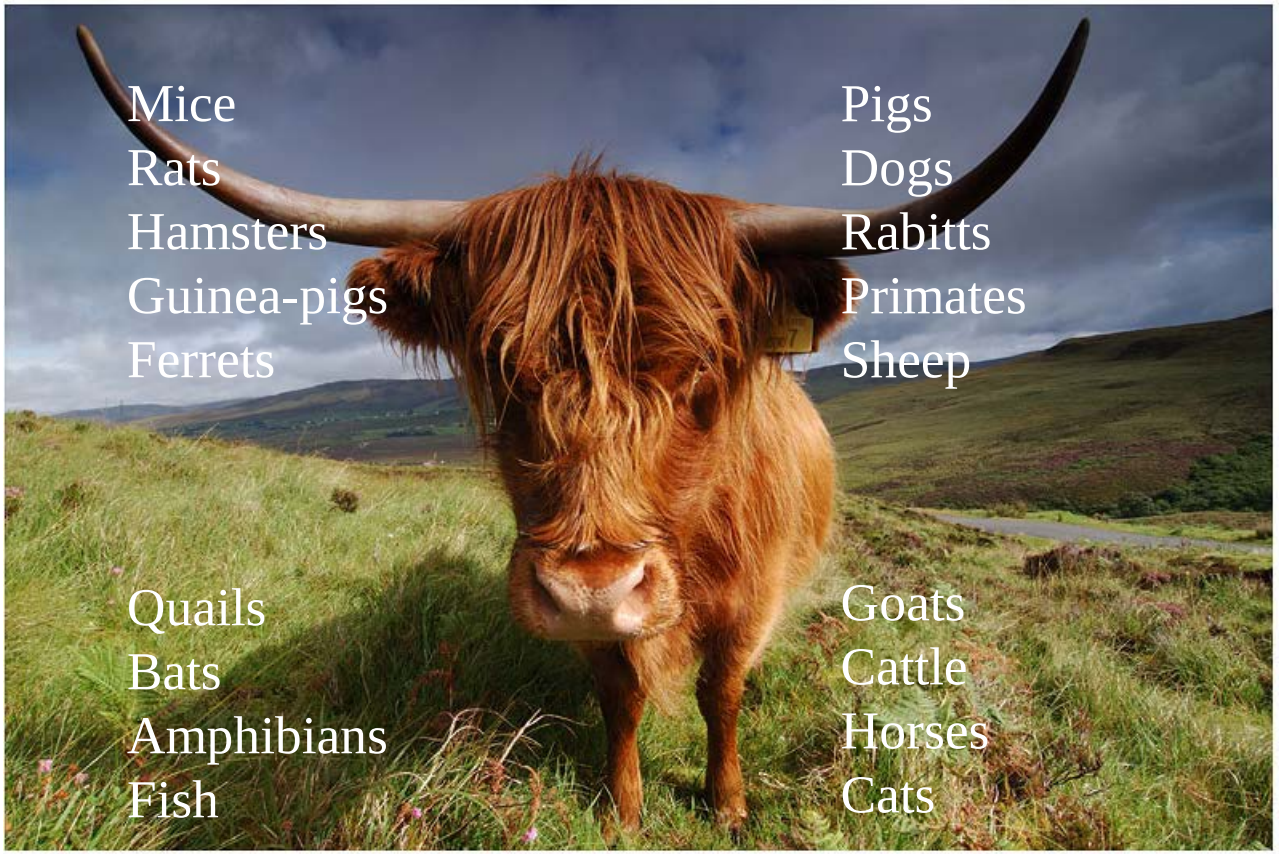
⟹ amputations
chronic diseases

- Reason: <u>marginal</u> difference in the structure of CD28

Development of Drugs



Common Lab-Animal Species



Some Comparisons

Parameter	Mouse	Pig	Baboon	Man
BW	20 – 30 g	300 kg	bis 35 kg	70 kg
Temperature	38 °C	39 °C	37 °C	36,5 °C
Heart rate	600 /min	70 /min	180 /min	70 /min
Blood pressure	110 mmHg	90 mmHg	90 mmHg	80 mmHg
Glucose	240 mg/dl	90 mg/dl	85 mg/dl	100 mg/dl

Some Comparisons

Parameter	Mouse	Pig	Rabbit	Dog
Fibrinogene (mg/dl)	283	172	286	182
Total proteine (g/dl)	4,3	4,9	5,6	6,6
Viscosity (0,7/s) (mPa s)	13.300	24.700	8.300	22.900
MBF (ml/min/g)	5 ±1,0	0,9 ±0,4	2,0 ±1,0	0,9 ±0,3

Animal Models

Aims/prerequisites:

Maximum similarity to intended species

Dosage of Xylazine	
Cattle	0,05 – 0,15 mg/kg
Horse	0,5 - 1,0 mg/kg

Maximum transferability

Maximum standardisation:

- in-bred
- standard food
- reduction of environmental impact
- microbiological surveillance

Dog: Particularities

Ibuprofen

causes gastric ulcer and bleeding even in low dosages

Spleen

high storage capacity ⟹ splenectomy in studies concerning hypovolemic shock

Heart

pronounced arterial collateralisation

⟹ induction of infarcts difficult

Rabbit: Particularities

- Coprophagic: intake of faeces is essential
  therefore: avoid enteral application of antibiotics
- Presence of atropine-esterase in about 30% of rabbits
  atropine has no effects
- Gentamycin is neuro-toxic in rabbits
- Neomycin und Streptomycin are oto-toxic in rabbits
- Bicilline can cause sterile abscesses in rabbits
- False handling + „struggling“ of the rabbit can cause
  fractures of the neck
- Very scary, risk of injuries in case of escape

Kidney of Pig and Man

	Pig	**Man**
Nephrons (n)	1×10^6	1×10^6
Nephr. + Henle. (%)	3	14
Renal BF (ml/min)	420	620
Primary urine (L/d)	140	170
Urine (ml/kg/d)	30	20

Urine of Pig and Man

	Hybrid-pig	**Man**
pH	5 - 8	5,6 - 7
Ca (mmol/d)	7,5	2,5 – 11,5
P (mmol/d)	33	20 - 45
Cl (mmol/d)	200	120 - 240
Na (mmol/d)	13	100 - 150
K (mmol/d)	270	60 – 80
Creatinine (mmol/d)	40	0,8 – 1,7
Protein (mg/l)	-300	-80

Clinical Chemistry

	Mini-pig (20 kg)	**Hybrid-pig (25 kg)**	**Man (70 kg)**
Potassium (mmol/L)	3,4 – 6,7	4,1 – 7,1	3,5 – 5,5
CK (IU/L)	- 1320	- 770	- 170
γ-GT (IU/L)	19 - 45	15 - 45	9 - 65
Albumin (g/dl)	4 – 4,3	3,1 – 4,6	3,4 – 4,8

But:

Molecular Similarity

"Index of Dissimilarity" for human albumin:

Man:	1,00
Gorilla:	1,08
Chimpanzee:	1,14
Orang Uthan:	1,22
Baboon:	2,23
Pig:	> 35,00

Consequences

Clinical liver-xTX

	Man (normal)	**Baboon** (normal)	**Patient** (45 d p. OP)
Albumin (g/l)	40 – 50	20 – 40	19
Total protein (g/l)	60 - 84	40 - 60	40
Cholesterol (mmol/l)	3,1 – 5,7	1,03	1,71
Uric acid (mmol/l)	180 – 420	<30	<30

Starzl et al., The Lancet, 1993

Consequences

Clinical course: haemorrhages

Day p. op.	**Localisation**	**Cause**
24	haemothorax	biopsy
27 – 39	gastrointestinal	esophagitis duodenitis
61	DIC	???
70	subarachnoid	aspergillosis

Starzl et al., The Lancet, 1993

Xylazine: Particularities

Animal	Glucose (ml/dl)
Baboon 1	250
Baboon 2	275
Baboon 3	149
Baboon 4	281
Baboon 5	168
Baboon 6	190
Baboon 7	271
Baboon 8	376
Baboon 9	240
Baboon 10	145
Mean	234.5
SD	±72.3

Anaesthesia for blood sampling:
„Hellabrunner-Mischung“
= Ketamine + Xylacine

Xylacine induces hyperglycaemia
in the baboon

Factors of Influence

- Genetics: in-bred ⟺ out-bred

- Age and gender

- Typ of housing: equipment of cages: enriched ⟺ not enriched
  handling: by hand ⟺ with forceps
  light regimen
  admittance of personal
  different species housed together
  noise, ultra-sound

- Microbiology: direct influence on results of experiments by
  inflammation/replication ⟹ activation of the immunsystem

- Diet: e.g.: ad libitum ⟺ restrictive
  2-year-survival SD 7% ⟺ 72%

Infection and Experiment

Experiment (examples)	Activation of Macrophg.	T-Cells	B-Cells	Endothel.	Complement
Transplantation					
Ischaemia/Reperf.	+	+	(+)	+	(+)
Rejection	+	+	+	+	+
Myocardial infarct	+	+		+	
Cerebral stroke	+	+		+	+
Heailing fractures/wounds	+	+		+	
Arteriosclerosis	+	+	+	+	+
Experimental infection	+	+	+	+	+
Natural infection	+	+	+	+	+

Presentation part 2:
Demonstration of the ARRIVE Guideline (2010)

NC3Rs

National Centre for the Replacement, Refinement and Reduction of Animals in Research

The ARRIVE guidelines

Animal Research: Reporting *In Vivo* Experiments

Carol Kilkenny[1], William J Browne[2], Innes C Cuthill[3], Michael Emerson[4] and Douglas G Altman[5]

[1]The National Centre for the Replacement, Refinement and Reduction of Animals in Research, London, UK , [2]School of Veterinary Science, University of Bristol, Bristol, UK, [3]School of Biological Sciences, University of Bristol, Bristol, UK, [4]National Heart and Lung Institute, Imperial College London, UK, [5]Centre for Statistics in Medicine, University of Oxford, Oxford, UK

The guidelines are intended to:

- Improve reporting of research using animals.
- Guide authors as to the essential information to include in a manuscript, and not be absolutely prescriptive.
- Be flexible to accommodate reporting a wide range of research areas and experimental protocols.
- Promote reproducible, transparent, accurate, comprehensive, concise, logically ordered, well written manuscripts.
- Improve the communication of the research findings to the broader scientific community.

Item 1: **TITLE** Provide as accurate and concise a description of the content of the article as possible.

Item 2: **ABSTRACT** Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.

INTRODUCTION

Item 3: **Background**

a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale.
b. Explain how and why the animal species and model being used ("relevant animal model") can address the scientific objectives and, where appropriate, the study's relevance to human biology.

Item 4: **Objectives** Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.

EMA/CPMP: Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products

VIII.B Animal species selection and use of alternative animal models

It is recognised that animal models of disease may not be available for every cellular or gene therapy system. Preclinical pharmacologic and safety testing of these agents should employ the most appropriate, pharmacologically *relevant animal model* available.

A relevant animal species would be one in which the biological response to the therapy would be expected to mimic the human response. For example, a vector expressing a human cytokine would best be tested in an animal species in which that cytokine binds to the corresponding cytokine receptor with affinity comparable to that seen with human receptors, and initiates a pharmacologic response comparable to that expected in humans.

METHODS

Item 5: **Ethical statement** Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal Welfare Act 2013), and national or institutional guidelines for the care and use of animals, that cover the research.

METHODS

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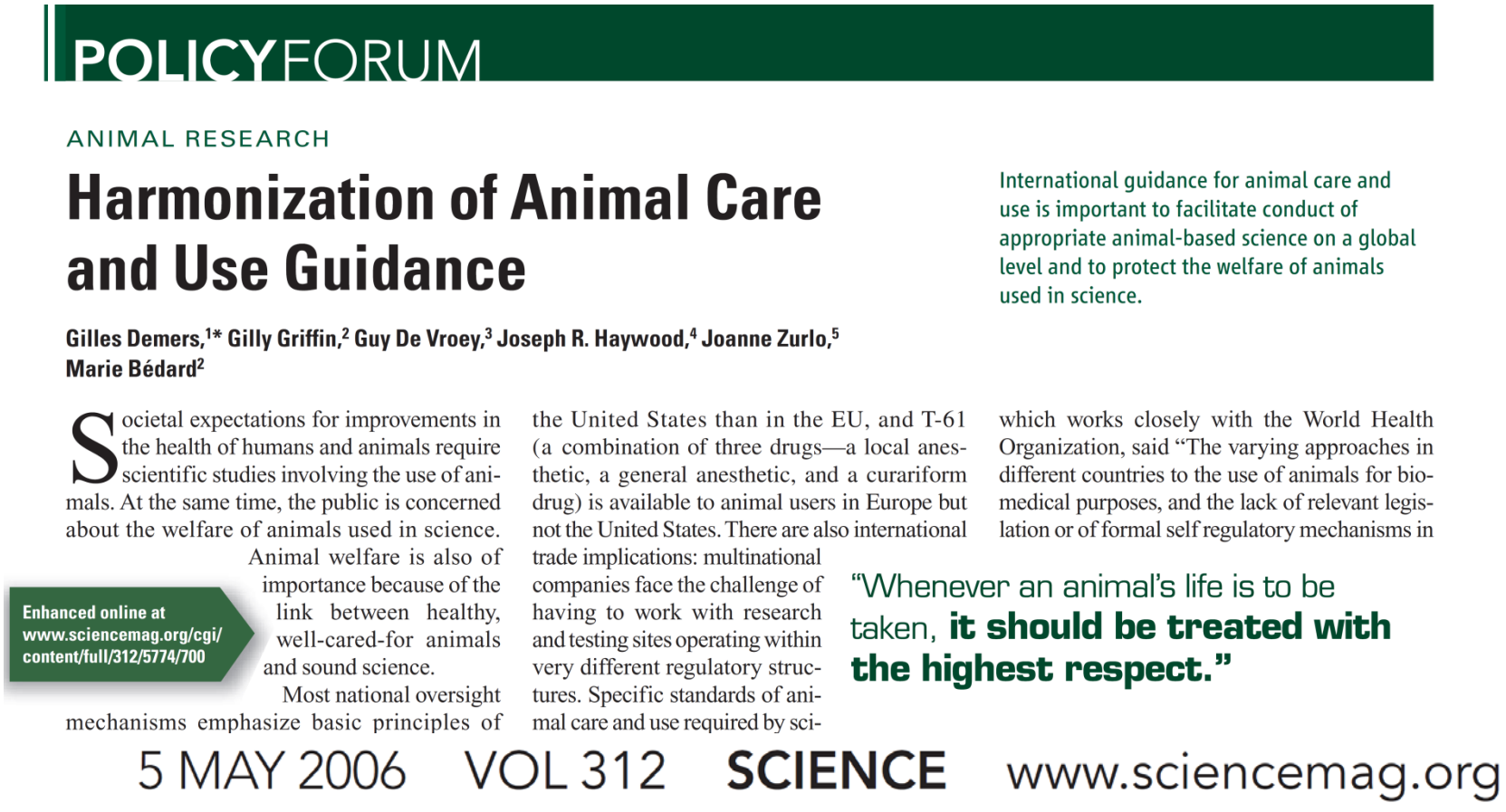


POLICYFORUM

ANIMAL RESEARCH

Harmonization of Animal Care and Use Guidance

International guidance for animal care and use is important to facilitate conduct of appropriate animal-based science on a global level and to protect the welfare of animals used in science.

Gilles Demers,[1]* Gilly Griffin,[2] Guy De Vroey,[3] Joseph R. Haywood,[4] Joanne Zurlo,[5] Marie Bédard[2]

Societal expectations for improvements in the health of humans and animals require scientific studies involving the use of animals. At the same time, the public is concerned about the welfare of animals used in science. Animal welfare is also of importance because of the link between healthy, well-cared-for animals and sound science.

Most national oversight mechanisms emphasize basic principles of the United States than in the EU, and T-61 (a combination of three drugs—a local anesthetic, a general anesthetic, and a curariform drug) is available to animal users in Europe but not the United States. There are also international trade implications: multinational companies face the challenge of having to work with research and testing sites operating within very different regulatory structures. Specific standards of animal care and use required by sci- which works closely with the World Health Organization, said "The varying approaches in different countries to the use of animals for biomedical purposes, and the lack of relevant legislation or of formal self regulatory mechanisms in

Enhanced online at www.sciencemag.org/cgi/content/full/312/5774/700

"Whenever an animal's life is to be taken, **it should be treated with the highest respect."**

5 MAY 2006 VOL 312 SCIENCE www.sciencemag.org

Principles for Establishment of Humane End Points

1. There is strong evidence that animals experience pain and distress in situations comparable to those that cause pain and distress for humans.

2. Death or severe pain and distress should be avoided as end points.

3. The earliest possible end point should be used that is consistent with the scientific objectives.

4. Studies should be designed to minimize any pain or distress likely to be experienced by the animals, while meeting the scientific objectives.

5. The duration of studies involving pain and distress should be kept to a minimum.

6. Pilot studies should be encouraged as a means of determining morbidity, time course of effects, and frequency of observations required to set an earlier end point.

7. Before commencing the experiment, agreement should be reached on (i) appropriate end points for the study and (ii) the person or persons to be responsible for making the judgment that the end point has been reached.

8. A team approach should be used, employing the professional judgment of the scientist, veterinarian, animal care staff, and ethics committee to agree on the appropriate end point for the study.

9. Research and animal care staff must be adequately trained and competent in recognition of species-specific behavior and, in particular, species-specific signs of pain, distress, and moribundity.

10. Animals should be monitored by means of behavioral, physiological, and/or clinical signs at an appropriate frequency to permit timely termination of the experiment once the end point has been reached.

BJC OPEN

British Journal of Cancer (2010) 102, 1555 – 1577

www.bjcancer.com

Guidelines

Guidelines for the welfare and use of animals in cancer research

P Workman*[,1], EO Aboagye[2], F Balkwill[3], A Balmain[4], G Bruder[5], DJ Chaplin[6], JA Double[7], J Everitt[8], DAH Farningham[9,18], MJ Glennie[10], LR Kelland[11], V Robinson[12], IJ Stratford[13], GM Tozer[14], S Watson[15], SR Wedge[16], SA Eccles*[,1], An *ad hoc* committee of the National Cancer Research Institute[19],
Observers: **V Navaratnam[17] and S Ryder[17]**

[1]Cancer Research UK Centre for Cancer Therapeutics, The Institute of Cancer Research, Cotswold Road, Sutton, Surrey SM2 5NG, UK; [2]Comprehensive Cancer Imaging Centre, Imperial College London Faculty of Medicine, Hammersmith Hospital Campus, Du Cane Road, London W12 ONN, UK; [3]Centre for Cancer & Inflammation, Barts and The London School of Medicine and Dentistry, John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, UK; [4]Helen Diller Family Comprehensive Cancer Center, University of California San Francisco 1450 3rd Street, San Francisco, CA 94158, USA; [5]Paterson Institute for Cancer Research, University of Manchester, Wilmslow Road, Manchester M20 4BX, UK; [6]OXiGENE Inc., 701 Gateway Boulevard, San Francisco, CA 94080, USA; [7]University of Bradford, Richmond Road, Bradford BD7 1DP, UK; [8]GlaxoSmithkline Pharmaceutical R&D, PO Box 13398, Five Moore Drive, N2.2210.2B, Research Triangle Park, NC 27709-3398, USA; [9]Cancer Research UK, Clare Hall Laboratories, Blanche Lane, South Mimms, Herts EN6 3LD, UK; [10]Tenovus Laboratory, Cancer Sciences Division, Southampton University School of Medicine, General Hospital, Southampton SO16 6YD, UK; [11]Cancer Research Technology Development Laboratories, Wolfson Institute for Biomedical Research, University College London, Gower Street, London WC1E 6BT, UK; [12]National Centre for the Replacement, Refinement and Reduction of Animals in Research 20, Park Crescent, London W1B 1AL, UK; [13]School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, UK; [14]Department of Oncology, K Floor, School of Medicine, University of Sheffield, Beech Hill Road, Sheffield S10 2RX, UK; [15]Division of Pre-Clinical Oncology & PRECOS, D Floor West Block, Queen's Medical Centre, University Hospital, Nottingham NG7 2UH, UK; [16]Cancer Bioscience, AstraZeneca, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK; [17]The Home Office, ASPD (mail point 1B), 1st floor Seacole Building, 2 Marsham Street, London W1P 4DF, UK; [18]Current address: Medical Research Council, 20 Park Crescent London W1B 1AL, UK

British Journal of Cancer (2010) **102,** 1555 – 1577. doi:10.1038/sj.bjc.6605642 www.bjcancer.com

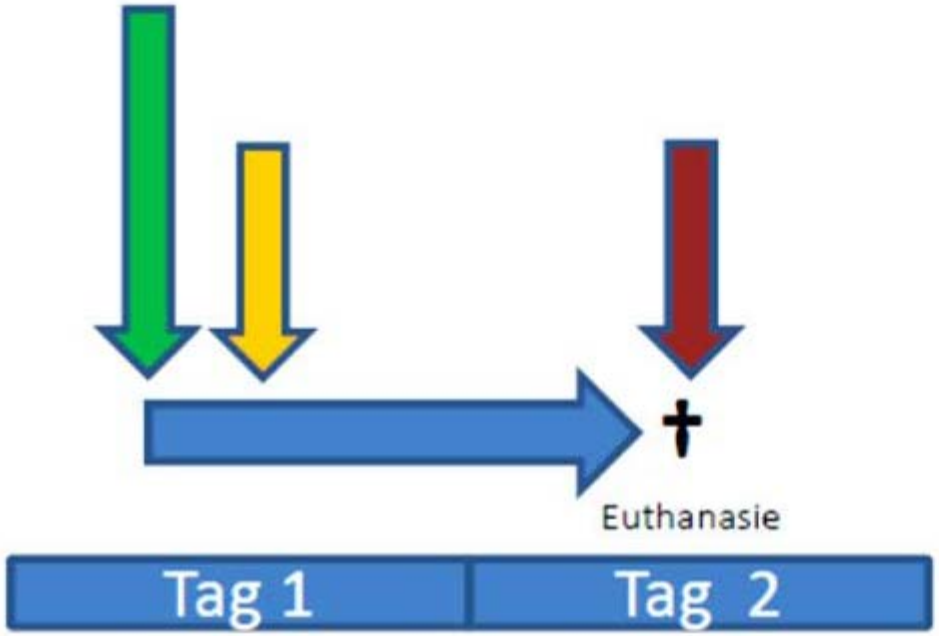
METHODS

Item 6: **Study design**

For each experiment, give brief details of the study design including:

a. The number of experimental and control groups.
b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when).
c. The experimental unit (e.g. a single animal, group or cage of animals).

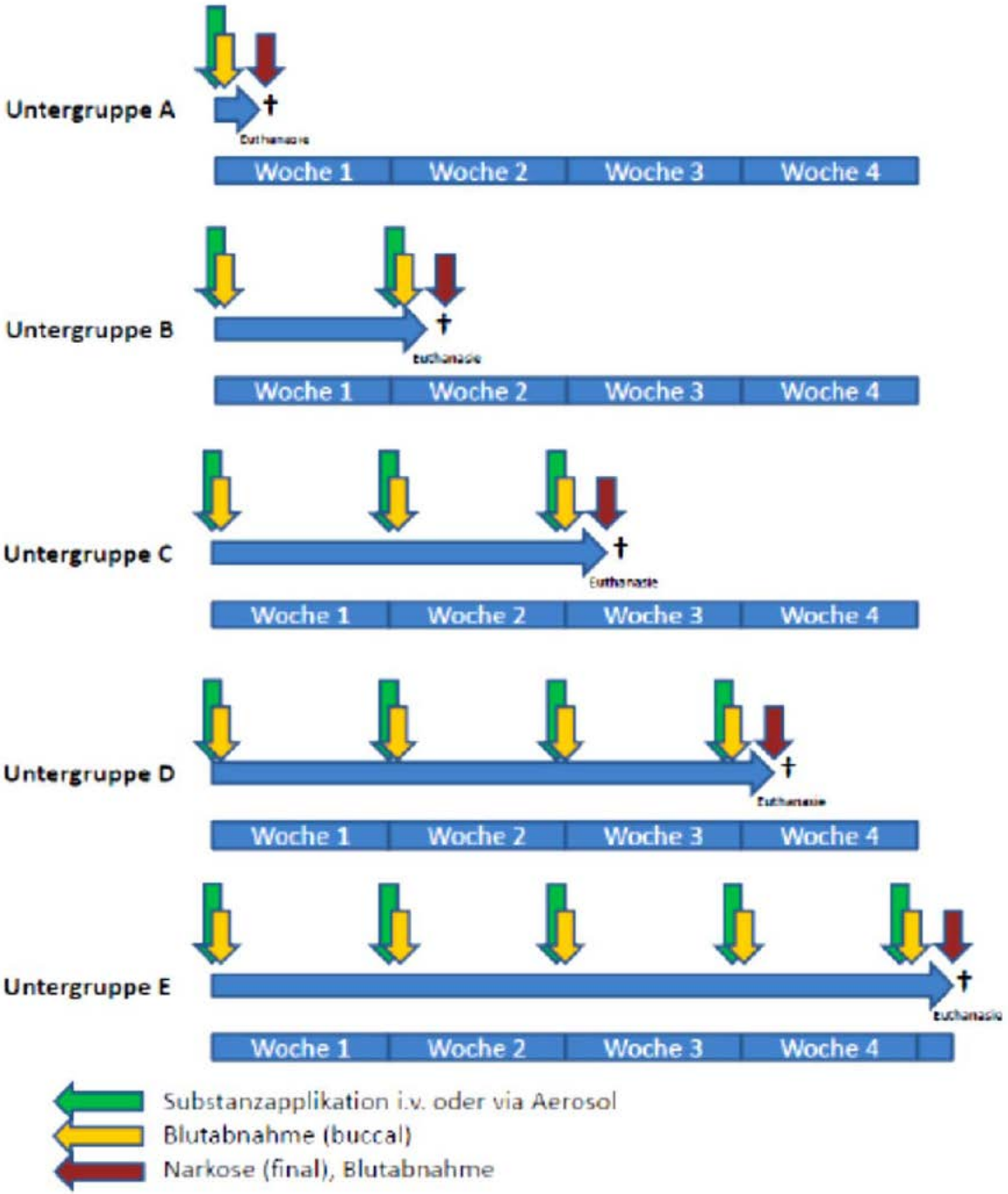
A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.



Substanzapplikation i.v. oder via Aerosol

Blutabnahme (buccal)

Narkose (final), Bildgebung, Blutabnahme


Untergruppe A
†
Euthanasie
Woche 1
Woche 2
Woche 3
Woche 4
Untergruppe B
†
Euthanasie
Woche 1
Woche 2
Woche 3
Woche 4
Untergruppe C
†
Euthanasie
Woche 1
Woche 2
Woche 3
Woche 4
Untergruppe D
†
Euthanasie
Woche 1
Woche 2
Woche 3
Woche 4
Untergruppe E
†
Euthanasie
Woche 1
Woche 2
Woche 3
Woche 4
Substanzapplikation i.v. oder via Aerosol
Blutabnahme (buccal)
Narkose (final), Blutabnahme

METHODS

Item 7: **Experimental procedures** For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example:

a. *How* (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s).

b. *When* (e.g. time of day).

c. *Where* (e.g. home cage, laboratory, water maze).

d. *Why* (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).

METHODS

Item 8 **Experimental animals**

a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range).
b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.

METHODS

Item 9 **Housing and husbandry** Provide details of:

a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish).
b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment).
c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.

Projekt-Nr.		Maus-Nr.		Vers.Gr.-Nr.			
				Datum			
Kategorie	Verhalten/Symptome			Tag Score	d1 2h	d1 6h	**d2** **24h**
Futter-/Wasser-aufnahme	trinkt und/oder frisst			0			
	trinkt oder frisst nicht			1			
	zugekniffene halbgeschlossene Augen			2			
MGS							
Adspek-tion und Verhalten	Maus ist mobil; Körperhaltung physiolo-gisch; Fell gepflegt; reagiert auf Untersucher			0			
	Maus ist wenig mobil; Körperhaltung nicht ganz physiologisch; Fell nicht ganz gepflegt; Reaktion auf Untersucher ggrd. vermindert			1			
	Maus bewegt sich wenig; leicht gekrümmte Körperhaltung; Fell nicht gepflegt; Reaktion auf Untersucher deutlich vermindert			2			
	Maus ist nicht mobil; deutlich gekrümmte Köperhaltung; Fell ungepflegt; keine Reaktion auf Untersucher			3			
Unterschr.	**PL:**		**Untersucher:**				

METHODS

Item 10 **Sample size**

a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group.
b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used.
c. Indicate the number of independent replications of each experiment, if relevant.

METHODS

Item 11: **Allocating animals to experimental groups**

a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done.
b. Describe the order in which the animals in the different experimental groups were treated and assessed.

Item 12: **Experimental outcomes** Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).

METHODS

Item 13: **Statistical methods**

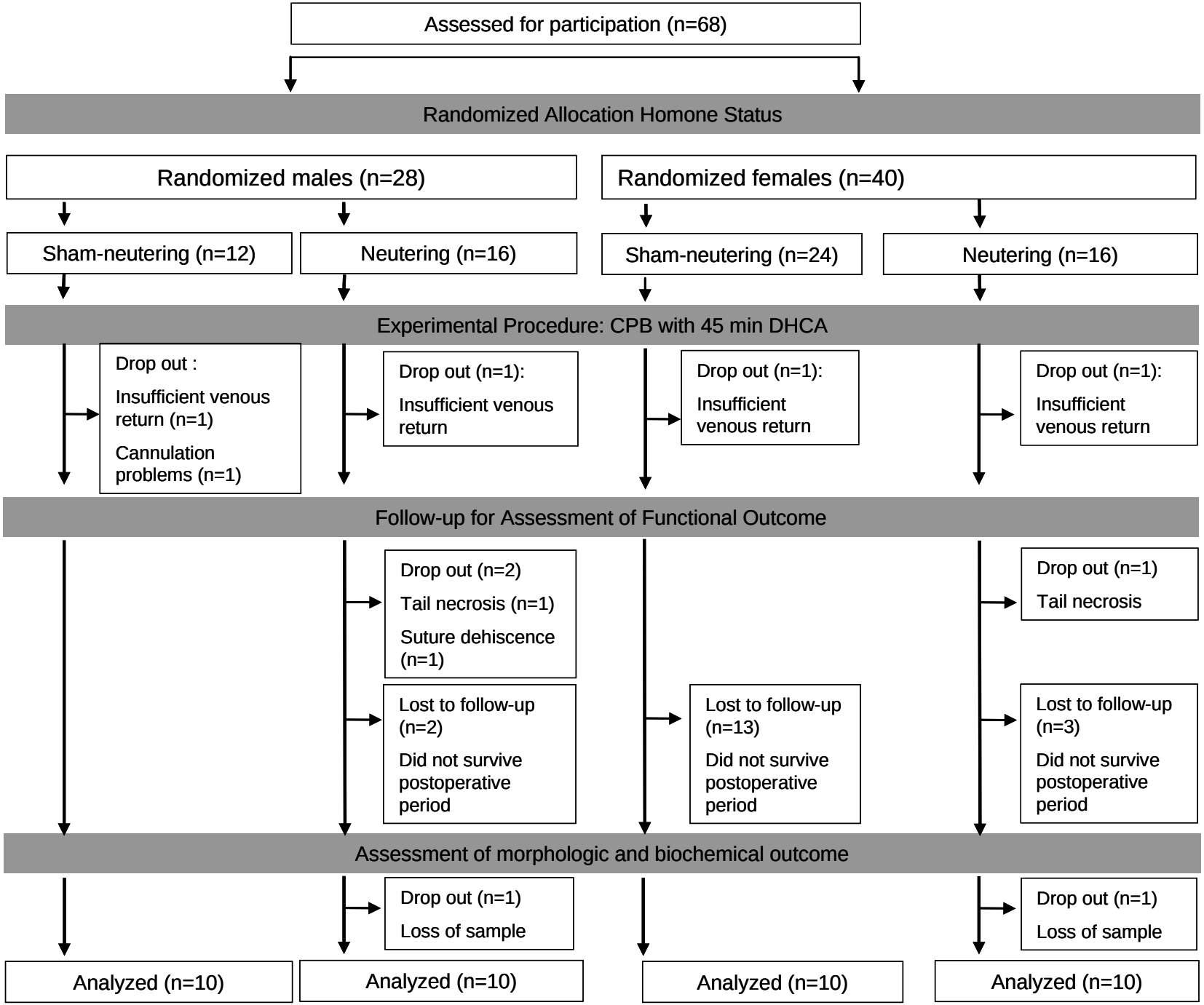
a. Provide details of the statistical methods used for each analysis.
b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron).
c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.

RESULTS

Item 14: **Baseline data** For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing. (This information can often be tabulated).

Item 15: **Numbers analysed**

a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%).
b. If any animals or data were not included in the analysis, explain why.


Assessed for participation (n=68)
Randomized Allocation Homone Status
Randomized males (n=28)
Randomized females (n=40)
Sham-neutering (n=12)
Neutering (n=16)
Sham-neutering (n=24)
Neutering (n=16)
Experimental Procedure: CPB with 45 min DHCA
Drop out :
Insufficient venous return (n=1)
Cannulation problems (n=1)
Drop out (n=1):
Insufficient venous return
Drop out (n=1):
Insufficient venous return
Drop out (n=1):
Insufficient venous return
Follow-up for Assessment of Functional Outcome
Drop out (n=2)
Tail necrosis (n=1)
Suture dehiscence (n=1)
Lost to follow-up (n=2)
Did not survive postoperative period
Lost to follow-up (n=13)
Did not survive postoperative period
Drop out (n=1)
Tail necrosis
Lost to follow-up (n=3)
Did not survive postoperative period
Assessment of morphologic and biochemical outcome
Drop out (n=1)
Loss of sample
Drop out (n=1)
Loss of sample
Analyzed (n=10)
Analyzed (n=10)
Analyzed (n=10)
Analyzed (n=10)

RESULTS

Item 16: **Outcomes and estimation** Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).

Item 17 **Adverse events**

a. Give details of all important adverse events in each experimental group.
b. Describe any modifications to the experimental protocols made to reduce adverse events.

DISCUSSION

Item 18: **Interpretation/scientific implications**

a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature.
b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results.
c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research.

Item 19: **Generalisability/ translation** Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.

Item 20: **Funding** List all funding sources (including grant number) and the role of the funder(s) in the study.