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JOCHEN TAUPITZ · MARION WESCHKA
Editors

CHIMBRIDS
Chimeras and Hybrids in Comparative European and International Research

Scientific, Ethical, Philosophical and Legal Aspects

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Edited by
Thomas Hillenkamp, Lothar Kuhlen, Eibe Riedel,
Jochen Taupitz (Geschäftsführender Direktor)

Jochen Taupitz • Marion Weschka
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Editors
Professor Dr. Jochen Taupitz
Institut für Deutsches, Europäisches
und Internationales Medizinrecht,
Gesundheitsrecht und Bioethik
der Universitäten
Heidelberg und Mannheim
Schloss, Westflügel
68131 Mannheim
Germany
taupitz@jura.uni-mannheim.de

Marion Weschka, LL.M., Mag. rer. publ.
Institut für Deutsches, Europäisches
und Internationales Medizinrecht,
Gesundheitsrecht und Bioethik
der Universitäten
Heidelberg und Mannheim
Schloss, Westflügel
68131 Mannheim
Germany
Marion.Weschka@imgb.de

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Preface

The research project "CHIMBRIDS - Chimeras and Hybrids in Comparative European and International research – Scientific, Ethical, Philosophical and Legal Aspects", coordinated by the Institute for German, European and International Medical Law, Public Health Law and Bioethics of the Universities of Heidelberg and Mannheim (IMGB), focuses on the scientific, ethical and legal implications of the creation of and research with human-animal mixed creatures.

National, European and international concepts and strategies concerning the legal and ethical framework of this research are still largely missing, even though they are absolutely necessary in order to use the potential of chimera and hybrid research effectively and efficiently for the benefit of science and society. Accordingly, dynamic development of chimera and hybrid research creates insecurity on the part of decision-makers and society at large. This has been evidenced recently when the approval of the Human Embryology and Fertilisation Bill by the UK House of Commons was controversially debated.

With research activities lasting from 01.10.2005 to 30.11.2007, CHIMBRIDS contributes to a solution for the existing problems, sheds light on the chances and risks of chimera and hybrid research and suggests legal solutions and prospective research practices in order to help decision-makers fulfill their tasks in an informed and efficient manner.

We would like to express our gratitude to all participants of the project for their support of CHIMBRIDS and this publication:

First of all, this applies to all authors of this volume for their scholarship and dedicated involvement on an interdisciplinary level.

Furthermore, the research assistants and the staff of the IMGB provided invaluable support. Dr. Sara Kranz drafted and implemented the financial and organisational framework of the project, defining the structural and topical composition and progression of international workshops and conferences and aligning this development to the parameters required for the interim reports. This task, eventually leading to the publication at hand, was continued by Markus Fuderer. Prof. Dr. Marcus Oehlrich, Heike Malone, and Annette Wedler provided their insight and support with regard to the organisation of the various project milestones. Anne Laspeyres and Katharina Teske assisted the project coordination between the participants. The editing and typographical layout was carefully performed by Christoph Balmert, Beate Braunagel, Johanna Carl, Juliane Geldermann, Fabian Geyer, Kristina Helmer, Lisa Hochhaus, Sebastian Köbler, Gerrit Krämer, Stephanie Lohr, Mayte Richter, and Andrea Sautter. The continuous support of the "Verein zur Förderung des deutschen, europäischen und

internationalen Medizinrechts, Gesundheitsrechts und der Bioethik in Heidelberg und Mannheim e.V." was also valuable during this project.

Tom Finnegan from Durham University was responsible for proofreading and harmonising the language of the multinational contributions into one consistent style for this volume.

Administrative assistance was also provided by the University of Mannheim, especially by Sabine Volz.

Without the substantial financial contribution by the Research Directorate of the Commission of the European Communities in the course of the 6th Framework Programme of the European Union, a work of such scope and detail would not have been possible.

January 2009

Jochen Taupitz

Marion Weschka

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Introduction

Marion Weschka

For two years, the CHIMBRIDS-project, which dealt with the scientific, ethical and legal aspects of the creation of human-animal mixtures has been sponsored by the EU. The final aim of this project was to provide guidance for decision-makers who face the challenge of regulating the difficult area concerning the creation of and research with chimeras and hybrids.

In order to be able to give such advice, first of all, a sound understanding of the scientific background regarding the creation of a multitude of different constellations of chimeras and hybrids was required. As there are so many completely different chimbrids constellations, such as xenotransplantation, interspecies somatic cell nuclear transfer and gamete mixing, to name but a few, the CHIMBRIDS partners preferred to follow a case-centred approach. Consequently, ten different chimbrids cases were chosen in order to help explain the most difficult ethical issues arising in this research area.

As the CHIMBRIDS-partners did not want to impose their personal or the group's ethical views on the decision-makers and on the readers, an ethical framework was built which tried to explain, for each case, the possible solutions according to different philosophical approaches.

From a legal point of view, the different national lawyers of the CHIMBRIDS group explained the views that their national law took on the ten different cases. In a second step, comparative legal case analyses were written. These legal case studies served as background information and revealed the different possibilities of how to regulate chimbrids research. However, they also showed that in many countries, legislation in this field is rather wanting. Apart from the national level, also EU law and international law was taken into account. However, even though there are regulations that might come into play regarding the creation of chimeras and hybrids, there is no explicit direct regulation dealing with this interesting research field in an encompassing way.

CHIMBRIDS was both an international and interdisciplinary project: with partners coming from EU-countries such as Germany, Austria, France, the United Kingdom, Sweden, Spain, the Czech Republic and Hungary and from non-EU-countries such as Switzerland, Israel, the United States, Canada, Japan and China, and comprising of renowned scholars and researchers from the disciplines of science, ethics and law.

The present report presents the outcome of this two-years-cooperation. It has been attempted to structure the individual reports to a certain degree, however, as it is always the case when many great but different personalities come together, a

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complete unification was not aimed for as it would have been impossible to achieve.

As mentioned before, the comparative legal case reports are based on the case reports submitted by the lawyers responsible for their respective countries, and therefore had to be written after the country case reports. Generally, they reflect the current situation at the time of writing. However, as a few country reports have been updated at a very late stage in the course of the project, it has not always been possible to rewrite also the comparative reports.

Therefore, in order to get the full picture, it is, of course, best to read the whole report. However, for those who want to get a good overview in a few pages, a quicker approach is possible: Part C of the report with the title "Summary, Conclusions and Recommendations" is a condensed version of the full report and gives an overview of the scientific, ethical and legal background as well as the group's recommendations, which are the essence of the entire project.

Moreover, part A of the report contains a broad analysis of general research aspects regarding chimeras and hybrids, starting from problems of definitions and the etymological background of chimeras and hybrids and then covering all aspects of science, ethics and law. Part B contains the ten chimbrids cases which are all structured in the same way, also covering science, ethics and law. The main part of the full report concludes with Part C, which is, as mentioned before, the essence of the report with the "Summary, Conclusions and Recommendations".

The Annex to the report contains in part A the full reports of scientists, ethicists and lawyers that had first been submitted at the start of the project in their most current updated versions. These very instructive reports provide very detailed information on all aspects of chimbrids research from all three disciplines. Finally, part B of the Annex contains the country case reports, which served as a basis for the comparative case reports contained in the main part of the report.

A. General research aspects

I. Problems with terminology and definitions

Michael Bader

Terms used in discussions about **chimeras** and **hybrids** are often applied with different meanings, since they are not clearly defined. The distinction between chimeras and hybrids is sometimes not acknowledged, or the terms are used in the wrong context.

Hybrids were originally defined as the result of interbreeding between two animals of different taxa. A chimera is an individual whose body contains different cell populations derived from different zygotes[1]. While these terms have served well in classical animal breeding, modern embryo technology has made their applicability problematic. Indeed, there are cases where both terms may be used. Foster mothers for transgenic or nuclear transfer embryos become microchimeras with circulating hybrid foetal cells due to their passage through the placenta.[2] At first glance, recipients of cells from another species are classical chimeras, but since fusion of foreign cells with host cells has frequently been reported they also have characteristics of hybrids.[3] Furthermore, cloned animals, the product of nuclear transfer from one individual to an enucleated oocyte of the same species, are considered to be hybrids yet this is not covered by the definition of the term hybrid mentioned above.

This terminological problem was the reason for the invention of the word "**chimbrid"** for any mixed creature dealt with in this report.

Another unclear definition concerns the words "**embryo"** and "**foetus"**. While everybody would agree that the product of fertilisation before implantation into an uterus is an embryo, the term foetus is not clearly defined, and both terms are very often used for the same developmental stage in utero. The term "**functional embryo"** for an embryo able to develop to term when implanted into a uterus presents even more problems. This term is particularly important where cloned embryos are concerned. However, the functionality of an embryo cannot be tested, when it is of human origin, without ignoring serious ethical concerns. Thus, it is problematic to apply the term to embryos with human contribution. A comparable problem is created by the terms "**pluripotency"** and "**totipotency"**. A totipotent

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1 Blood DC & Studdert VP 2000 Saunders Comprehensive Veterinary Dictionnary (2nd edition) WB Saunders.

2 Nguyen Huu S, Dubernard G, Aractingi S & Khosrotehrani K. 2006 Feto-maternal cell trafficking: a transfer of pregnancy associated progenitor cells. Stem Cell Rev. 2:111-116.

3 Nygren JM, Jovinge S, Breitbach M, Sawen P, Roll W, Hescheler J, Taneera J, Fleischmann BK & Jacobsen SE. 2004 Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. Nat Med. 10:494-501.

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cell is able to form a whole organism when brought into a suitable environment (e.g., a uterus), while a pluripotent cell is only able to form tissues of all three germ layers but cannot form a complete organism. Again, in the human case, this capacity of a cell can mostly not be tested without violating ethical rules and therefore the terms are also often obsolete.

Equally problematic is the term "**species"**. A species is one of the basic units of biological classification. A species consists of organisms that are very similar in anatomy, physiology and genetics, due to having relatively recent common ancestors. Most textbooks define a species as all the individual organisms of a natural population that generally interbreed at maturity in the wild and whose interbreeding produces fertile offspring.[4] This definition serves quite well when normal breeding mammals are concerned. However, as soon as hybrids or chimeras are considered, the term becomes inadequate. In particular, in the rare cases of fertile inter-species hybrids, and when one asks to which species a mixed embryo belongs, the term is useless.

One of the best defined species is the species **human**. No natural hybrids with other species have been reported. But it is also obviously the most problematic species in ethical terms when mixed creatures, chimbrids, are concerned. For ethical discussions the question of whether a chimbrid belongs to the human species is essential, but can often not be answered by scientists. It becomes even more difficult for scientists to define whether such an entity has human dignity, since this term has no scientific meaning. The level of "humanness" may be higher or lower depending on its state of development and the contribution of the human components to the creature, but there is no clear border or scientific definition of what is really human and what is not.

[4] Blood DC & Studdert VP 2000 Saunders Comprehensive Veterinary Dictionnary (2nd edition) WB Saunders.

II. Etymological background and further clarifying remarks concerning chimeras and hybrids

Josef Kuře

1. Introduction

Biological and medical research involving techniques and entities commonly called chimeras and hybrids raises not only new scientific questions but also questions about ethical, social, and legal implications of this kind of research. The terms chimera and hybrid are used in many different ways, in various disciplines and contexts, denoting diverse entities – even among scientists these terms are not used univocally. The fact that the language surrounding hybrids and chimeras is not clear, and that these terms stand for different entities naturally has a number of ethical, legal and social implications. It seems that, methodologically, the first necessary step is semantic differentiation and clarification i.e.: what do we mean by the term chimera and hybrid? To which entities do we refer, using these terms? In which context do we use these terms? What are deeper concepts behind different notions of chimera or hybrid?

2. Semantic analysis

a. Chimera as an archetype

The original meaning of the term chimera is a compound of different animals or a configuration of human and animal bodies – as it is know from the Greek mythology.[5] The primordial Greek mythological Chimera (Χιμαιρα) is a fire-breathing monstrous creature made of parts of various animals: resembling a lion in the forepart, a goat in the middle, and a snake/dragon at the back.[6] Chimera, the offspring of Typhon and Echidna,[7] was of a grisly nature. Sighting Chimera was a sign of storms, shipwrecks, and (natural) disasters.

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[5] A worthwhile introduction to the Greek mythology can be provided by Robert Graves (Greek Myths. Garden City/N.Y.: Doubleday 1981 [Baltimore: Penguin Books 1955]).

[6] Descriptions of chimera vary; in one description, chimera had the body of a goat, the tail of a snake or dragon and the head of a lion; in another description, it had heads of both the goat and lion, with a snake for a tail (in Hesiod's description, chimera has three heads). Nevertheless, in all descriptions it breathed fire from one or more of its heads.

[7] The origin of Chimera has chimeric features: Typhon was a grisly monster with a hundred dragon's heads; Echidna was another mythological monster: half-woman, half-serpent.

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There are five characteristic features of the Chimera:

1) a mixture of different species (animals)

2) divine origin

3) deterrent nature

4) causing harm (breathing fire, devastating land)

5) removal (Chimera was slain by Bellerophon with the help of another chimeric creature Pegasus – see below).

Ancient Chimera as a mixture (creation) of different animals can be considered a chimerical archetype. In Greek mythology, chimera was not only a goat with a lion's head and a snake's tail. Mythological chimera can be any amalgam of human and animal features but also a mixture of human and god or of animal and god - although the god may look like a human or animal. Greek mythology contains many examples of chimerical mixture of human and animal, e.g., Pan (Παν), god of shepherds, was a man with hindquarters, legs, and horns of a goat.[8] As chimerical amalgams of human and animal, the following examples can be mentioned: a mermaid (an aquatic creature with the head and torso of human female and the tail of a fish: half-human, half-fish), a Centaur (a combination of a man and a horse), or a Minotaur (a combination of a man and a bull). Pegasus (a combination of a horse and a bird) can stand as an example of an intraspecific legendary chimera. One part (e. g. the human one) usually remains substantially unchanged except for occasional quantitative limitation – usually to one half. Imprimis, the whole is restricted quantitatively (a qualitative diminishment is not necessary and usually does not arise). For that matter, there is an abundance of human-animal and animal-animal chimeras in all world mythologies.[9] One could state that the whole of Greek mythology – as well as other mythologies – is grounded on the idea of a chimera.

Despite the fact that ancient chimeras look like humans or like animals, they represent a mixed nature composed of human and animal features or of human and divine features. One nature does not necessarily absorb the other. Rather, they combine and create a new entity which comes out as a combined nature or as an extension of one of them. In other words, one (invisible and empirically not verifi-

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[8] Because Pan's genealogy, having many variations, lies in deep mythic time, probably related to a district of primitive mountain folk of Arcadia – Pan has also analogy in other ancient mythologies (his Roman counterpart is Faunus) – Pan as archaic "pastury god" can be regarded for an archetype of human-animal chimera.

[9] See C. A. P. Ruck, D. Staples, The World of Classical Myth. Durham/NC: Carolina Academic Press 1994; E. M. Thury, M.K. Devinney, Introduction to Mythology: Contemporary Approaches to Classical and World Myths. New York: Oxford University Press 2005; R. Willis (ed.), World Mythology. New York: H. Holt 1993; K. C. Davis, World Myths. New York: Harper Collins Publishers 2005.

able) nature comes to the visible one – so for instance, the nature of a god comes to the animal nature of a bird, and the bird represents a hybrid of animal and god as a bird-god. Is this entity a chimera? If we understand chimera – in a broader sense - as a fusion of (at least two) different natures or substances, then the answer is positive. It seems that people in ancient history, surrounded by (terrestrial and divine) hybrids and chimeras, were not bothered by social, ethical or legal difficulties caused by the fused creations of human and animal or human and divine nature as we are nowadays. Chimeras and hybrids were part of their everyday life. Although they did not call these entities (predominantly gods) chimeras or hybrids (they would be horrified by these terms).[10] However there are some significant differences between ancient and contemporary understanding of chimera: the ancient chimera existed only in the mythological framework while the contemporary chimera is a real entity. Another difference is the notion of chimera: the ancient chimera is a mixture of various species as individuals, the contemporary chimera (and hybrid) is a mixture formed on cellular (respectively on organ) level. Therefore, the precise content of the notion of chimera in present time means something very different from the ancient notion of chimera.

In spite of their chimerical nature, chimeras did not lose their attractiveness but retained its fascinating and archetypical form over the centuries. This is true in our era in particular.[11] Are mythological concepts – in some way – present in contemporary biotechnologies? What are the driving ideas that move research and progress forward? Is the idea of crossing species a very ancient and primordial archetype of a chimerical form? Is the old archetype of chimera used now? Is it used unconsciously? Or is there no connection and we are simply facing the ongoing scientific development and new technological methods which have their own internal dynamics?

Indeed, the fusion of two (or more) entities, extending the possibilities of both the human and the animal by combining their features and qualities, is a wonderful ancient vision realised by modern technology. Only a few decades ago, we would have thought of chimeras exclusively as figments of our imagination. What once was possible in fantasy fiction only, became recently possible thanks to technology. When reproductive barriers were removed, the boundaries between species was crossed, and experimental animals produced (e.g., sheep-goat) in the 1980s, we simply pulled out the idea of chimera which had been in our culture since ancient times. Finally, what once was a chimera (a chimerical/legendary entity) became a reality produced not by "divine stock"[12] but by human technology.

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10 See the meaning of the term ″hybrid″ below (2.d.).

11 Cf. E. Bazopoulou-Kyrkanidou, Chimeric creatures in Greek mythology and reflections in science. In: American Journal of Medical Genetics, 2001, 100(1): 66-80.

12 The Chimera in Homer's Iliad "was of divine stock, not of men".

In this context one should take into account the fact that every human being is constituted of at least two parts – and this two part constitution does not imply any kind of dualism (as we know it from the time of Plato and Platonism). Rather, it is on the genetic level: two sets of 23 genes (maternal and paternal). Biologically and psychologically, no "pure man" or "pure woman" exists but rather each woman and each man is composed of both masculine and feminine "elements" (without dealing in detail with what these masculine and feminine elements reside in; on the genetic level, chromosomes X and Y). In spite of the fact that human beings predominantly understand themselves either as a man or a woman, some people, experiencing difficulties concerning their gender[13] identity, do not place themselves so easily into one of these two gender categories. We can distinguish not only a human and an animal nature but also a male and a female nature. From the biological and psychological point of view, it is rather difficult to draw a precise line between men and women. The purpose of this observation, however, is not to open the gender issue in the context of chimera (e.g., transsexuality as chimerism?), but to merely state that to be a human always means to be composed of (at least two) elements, without any possibility to be "reduced" to one of them only – regardless of the troubles that the synthesis may bring. Before dealing with the issue of the interspecies boundaries, we should take into account the fact that there are other boundaries existing within the species (and they are not subspecific boundaries). We know that these intra-specific boundaries exist and sometimes it is very difficult for us to distinguish them and to mark them.

b. Etymological background

Etymologically the word chimera denotes a variety of meanings. In English, the term chimera is associated with notions like phantasm, phantom, delusion, illusion, apparition, pipe dream, a castle in the air, unreal ambition, a fanciful scheme or unreality. The word chimera, especially in its old meaning, also denotes a Harpy, a monster, a bogey or irrational fear. Chimera in our common understanding means something (especially an idea or hope) that is not really possible and can never exist. Chimera is linked with a fantastic, impracticable plan or desire; thus chimera is any (futile) attempt to present impossible.

In Classic Greek χιμαιρα stands for a mythical non-existent being; the adjective χιμαρικος means chimerical, unreal, fantastic, impossible, utopian.

Etymologically, therefore, there are three main sets of meanings related to chimera:

- *chimera as a monster*
- *chimera as a delusion/phantom/unreality*
- *chimera as an (unrealistic and practically) impossible mixture of two different sources.*

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[13] The term "gender" is here used in a wider sense than "sex" solely, indicating the original meaning of genus.

From an etymological point of view, the term chimera is highly inadequate for the current biotechnological methods and products know as chimeras. Chimeras as biotechnological products are not chimeras at all, they are not utopian or chimerical; they are real, very real entities with clear ontological status. They are not common, frequent, or customary but they are not chimeric. Under a basic semantic assumption that terms (words) have to symbolise things that the words stand for, the use of the word chimera for entities (biotechnological products) such as a pig with human blood or a mouse with human brain does not fit into the common semantic scope of the term "chimera". A pig with human blood is neither chimeric nor horrendous. Similarly a mouse with one per cent of human brain cells is neither a phantom nor a monster. To call these entities chimeras does not, semantically, make sense. Otherwise we have to, in each individual case when we use the term "chimera", clearly explain that, in fact, we do not mean chimera though we are saying "chimera" and that we are using this term for a kind of interspecific combination. This does not seem very helpful in normal communication. The only way to prevent linguistic confusion is not to use the term "chimera" for entities that are, in reality, interspecies mixtures. No suitable term is being proposed hereby. Only that the use of the word "chimera" – out of the mythological framework – is, for semantic reasons, criticised as impropriate and misleading.

Beside the semantic inappropriateness, in general, any human activity related to chimera or to chimerical goals has some ethical connotation which also includes moral relevance (e.g., moral judgement about act/behaviour in the relation to chimera).

c. Chimera as a metaphor and as a terminus technicus

The term chimera, having (historically) been used in many different contexts (in Medieval logic "chimera" was used as the word signifying "nothing"; in Christian art, chimera has been used as a symbol of Satanic forces),[14] has become a metaphor, denoting things that have combined attributes from different sources. This metaphorical use of chimera has been transformed into *terminus technicus* in science, especially in zoology, botany, palaeontology, genetics, biochemistry, and - as far technological procedures are concerned – in biotechnology generally. In a wider sense, a chimera is also an individual, an organ, or a part consisting of tissues of diverse genetic constitution, produced as a result of organ transplant, grafting, or genetic engineering. Most of the uses of the term chimera (chimera as *terminus technicus*) are related to the metaphor (combination of two or more different genetic sources).

So the term chimera is a *terminus technicus* for:

a) single plant organism with genetically distinct cells (botany)

b) single animal organism with genetically distinct cells (zoology).

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[14] Cf. L. N. Roberts, A chimera is a chimera. A medieval tautology. In: Journal of the History of Ideas 1960, 21(2): 273-278.

If we apply the two-source metaphor used in biochemistry (chimera as any substance, created from the proteins or genes of two different species) broadly to medicine, then chimera is any individual who has received a transplant of genetically and immunologically different tissue. That would mean that any patient after any (non-auto) transplantation is a chimera, a constitution of two genetically and immunologically different entities. What about extending such a broad definition of chimera to the whole (technological) biomedicine – moving from an organ approach to a cell approach? Is for instance a regenerated heart a chimera?[15] No wonder that chimera as *terminus technicus* used in various medical settings and in biomedical research induces misunderstanding and confusion.[16] The scientific term chimera has originated from the fascinating and mysterious mixture of animal and human as we understand that notion from history. Apart from non-chimerical polysemy of the term chimera,[17] the distinction between chimera and hybrid should as well be introduced. Chimera can be defined as an entity (a mixture) of two or more genetically different types of cells coming from organisms of the same or different species; hybrid as an entity (a mixture) of two (or more) organisms of the same or of different species (intra-specific or interspecific hybrid).

d. Hybrid

Human–animal hybrids as mixtures of two (or more) organisms of different species were common in Greek mythology. Some examples have already been mentioned (Centaur, Minotaur, mermaid, Pan). However, the Classical Greek did not use the term *hybris* for entities called hybrids today because the word *hybris* has very negative connotations (see below). If we apply the introduced chimera-hybrid distinction, then all creatures mentioned as mythological chimeras were hybrids at the same time. So Greek (and world) mythology is a valuable source of all possible hybrids and chimeras. Anyway, the word hybrid would not sound like a neutral description of a grisly monster for Greek speaking people. The word *hybris* in Greek has a variety of (negative) meanings: vainglory, pride, boast, superciliousness, arrogance, profanation, maltreatment, high-handedness, degrade, abuse; other negative moral connotations of the word *hybris* are: debauchery, revelry, offence, malefaction, crime, injustice. *Hybristes* signifies rapist, criminal. In contemporary Greek, *hybris* means dispraise, invective, dishonour. There are not many Greek words that have such a broad palette of negative meanings as the word *hybris.*

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15 Cf. R. Bolli, Regeneration of the human heart – no chimera? In: The New England Journal of Medicine, 2002, 346(1):55-56.

16 Cf. H. Bok, What's wrong with confusion? In: The American Journal of Bioethics, 2003, 3(3):25-26.

17 Cf. H. T. Greely, Defining chimeras… and chimeric concerns. In: The American Journal of Bioethics, 2003, 3(3):17-20.

The term hybrid in common English signifies “an animal or plant produced from parents of different breeds or types, something that consists of or comes from a mixture of two or more other things“ (Longman Dictionary). This definition does not express the difference between hybrid and chimera as these terms are used in scientific language. It remains a question of language strategy how far we can/should/shall go using the metaphorical language within, otherwise precise, scientific language. In general, the basic question is about the appropriateness of the usage of a metaphor for scientific conduct.

Therewithal, the negative connotations of the word *hybris* in Greek and its (different) common meaning in everyday English, the term hybrid is used in many different disciplines as a *terminus technicus* in a very specific and clearly defined sense – so for instance the term hybrid is used in logic, analytical philosophy, linguistics, technology, chemistry (hybridisation), and biology. In biology, the term hybrid has various meanings:

- the offspring of different species
- the offspring of different genera
- crosses between different species within the same genus (interspecific hybrids)
- crosses between different subspecies within the same species (intraspecific hybrids)
- crosses between different genera (intergeneric hybrids)
- crosses between different populations, breeds or cultivars of a single species (principally in plant breeding)
- in molecular biology, hybrid refers to hybridisation.

Intra-specific and interspecific hybrids are very common in plant and animal breeding. Many agricultural plants grow as hybrids; for plant breeding, hybrids are very effective.

The term hybrid can be used and understood (comparatively) univocally, if used within a clearly defined semantic framework (as biology provides different and clear definitions of the term hybrid).

It seems that neither the term chimera nor the term hybrid properly expresses the scientific reality. Nevertheless, the term hybrid meaning the offspring resulting from cross-breeding of different species is more suitable, because it is more precise; however, by definition, it is limited to cross-breeding of different species or subspecies. No suggestion is being made regarding an appropriate terminology; no alternative terms are being proposed here.

Nevertheless, there are some attempts to use more appropriate terms and to provide a matter-of-fact, correct, neutral, and morally unprejudiced description,

avoiding confusion – for instance the term "creation of novel beings"[18] or "transgenic creatures"[19] (though the appropriateness of these terms is questionable).

e. Some further differentiations

We know what chimera means in Greek mythology. However, we are less sure with respect to the sense(s) in which the term chimera is being used in contemporary biological and biomedical research, including embryonic chimeras.[20] In addition, another issue is the usage of the word chimera by the media. Language concerning chimeras used by media will be not analysed here – although it would be worthy to do so. Another hermeneutical community is the general public.

Since one and the same term (chimera) denotes various entities – that apparently have different moral and ethical relevance – it may be useful to make some clarifications by providing some basic differentiations:

a) plant chimera:

- in laboratory
- in nature (e.g., agriculture)

b) animal chimera (experimental)

c) human-animal chimera

d) plant-animal-human chimera

e) human-nonhuman chimera

f) embryonic chimera:

- animal-animal chimera
- human-animal chimera

g) animal/human embryonic chimera:

- on the genetic level
- on the cellular level
- on the tissue/organ level.

Especially with respect to c) to g), different contexts and various goals have to be taken into account and distinguished (e.g., combining human and animal cells to

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18 Cf. J. S. Robert, Regulating the creation of novel beings. In: Health Law Review, 2002, 11(1):14-19.

19 Cf. L. M. Glenn, When pigs fly? Legal and ethical issues in transgenics and the creation of chimeras. In: Physiologist. 2003, 46(5):251, 253-5 (here 254).

20 Cf. R. A. Ankeny, No real categories, only chimeras and illusions: the interplay between morality and science in debates over embryonic chimeras. In: American Journal of Bioethics, 2003, 3(3):31-33.

study cellular function, transferring a limited number of adult human stem cells into animal embryos in order to learn how they proliferate and grow during the prenatal period, implanting human neurons into the brain of embryonic mice in order to understand better how the brain works, producing human organs,[21] creating human-animal chimeric body or testing whether adding normal cells to an embryo with a genetic defect could make up for that defect[22]).

Another differentiation should be made in chimera usage between basic research and in clinical research. It is obvious that the mentioned examples of chimeras have different moral relevance. We would probably not have many difficulties with moral justification for the second example (to transfer a limited number of adult human stem cells into animal embryos in order to learn how they proliferate and grow during the prenatal period), but we would probably oppose the creation of human-animal chimeric individual. The main concern regarding human-animal chimera is related to the worry whether or not these chimeras will be put to a use that is ethically and medically problematic, risky, or dangerous.

f. Chimera as a strategy

In the debates about human embryonic stem cell research which took place during the last few years, the usage of human embryos for research purposes has been justified by potential benefits (development of new therapies of neurodegenerative and other diseases). The use of embryos for research became very controversial and highly politicised worldwide. Some countries are deeply divided on this issue; researchers in USA and EU are uncertain about public financial support for human embryonic stem cell research. So under the current circumstances, the obvious strategy is to by-pass the (politically and morally) sensitive issue of human embryos by using animal embryos.

Another by-pass strategy, paradoxically, is to avoid usage of animals for research and to use human embryos (especially in countries, where animals have relatively high grade of protection). This means that animals will be not harmed (or killed) through research. In this context, to conduct research on human tissue is less morally sensitive than to conduct research on animals that will be killed afterwards. So this by-pass strategy saves the lives of thousands of laboratory animals. With respect to the other sensitive issue, namely gamete donation, animals could also become very efficient egg donors without the necessity of an informed consent and without the concerns related to potential harm, etc. Another aspect of this by-pass strategy is the use of animal eggs instead of human eggs (advantage of this strat-

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21 See S. P. Westphal, Growing human organs on the farm. In: New Scientist, 2004, 180(2426-2428): 4-5.

22 Such studies (e. g., obtaining eggs from aborted foetuses or to look if some "method is possible") do not have positive social acceptance, provoking horror by their violation of fundamental human values. Cf. - A. Coglan, Studies provoke shock and horror. In: New Scientist, 2003, 179(2403):19.

egy is evident) for the creation of chimeras. Instead of human embryos, animal embryos can be used and consequently human-nonhuman embryonic chimeras can be created, by going around the hot issue of human embryo usage for research.

3. Anthropological clarifications

a. Chimera and the deeper understanding of humanity

At present, the topic of human and human-animal chimeras is discussed on different levels: among scientists, between the scientific community and the public, in the public, between the scientific community and politicians, etc. There is no wide consent on this issue. Even the scientific community is not unified regarding human-nonhuman chimeras.[23] The situation is more complicated and complex than it was in the view of one of the bio-ethics pioneers, Joseph Fletcher when he wrote in 1980s:

"What if an ape had the intelligence and sensibilities of a human, and a human had only the capabilities of an ape? Which would be the human being? The answer is plain; the ape would be the human being. This is no mere play on words. All mammals, man among them, are remarkably close biologically. Modern biology can devise chimeras or combinations of humans and animals, and also,cyborgs or combinations of humans and machines. […] The basic fact is that the body cells of all species will cross-fuse, and the germ cells of many – though not all – will unite sexually.

If a prosthetic device, perhaps an intricate mechanical hand or leg, supplies a person with 50 per cent or more of the function lost in an amputation, that is morally good. An artificial kidney or haemodialysis machine is morally good. This applies equally to heart pacemakers, dacron arteries, metal bones, ceramic hip joints. All such technical contrivances are cyborgs or man-machine hybrids.

Man-animal combinations are in the same ethical class. If a cow's kidney is grown into a patient's thigh to help cleanse his blood, after his own kidney function is gone, that is morally good. If an animal organ or tissue is used to replace something lost by a human (an interspecific transplant) that is good. These are examples of man-animal combinations for medical purposes. […] But what about hybridisation for non-medical reasons? Chimeras or parahumans might legitimately be fashioned to do dangerous or demeaning jobs. As it is now, low grade work is showed off on moronic and retarded individuals, the victims of uncontrolled reproduction. Should we not program such workers thoughtfully instead of accidentally, by means of hybridisation? Cell fusion and putting human cell nuclei into

[23] N. DeWitt, Biologists divided over proposal to create human-mouse embryos. In: Nature, 2002, 420(6913): 255.

animal tissue is possible (such hybrid tissue exists already as a matter of fact). Hybrids could also be designed by sexual reproduction, as between apes and humans. […]

Contrived in order to protect human beings from danger or from disease, a medical reason for creating chimeras and cyborgs would be morally justified. What counts is human need and well-being."[24]

It would be not fair, from our epistemological state of the art, to criticise a text written almost twenty years ago. In general, we would differentiate more precisely today and even the most liberal proponents of chimeras would have difficulties with the idea of programming low grade workers as slaves by means of hybridisation.

Biotechnological developments and achieved scientific advancements challenge our concepts of "humanness" (what it means to be "human"). So before starting the discourse on ethical acceptance of human-animal chimera and before reflecting on ethical and legal guidelines for human-animal chimera research,[25] some fundamental philosophical-anthropological questions should be clarified.

Among these questions are :

What does "human nature" mean?

What does "animal nature" mean?

How do we define human/animal nature? Which criteria do we use for defining?

What is the reciprocal relation between animal and human nature?

What is common for both of them?

Where are the boundaries between these two natures?

How do we define "species"? Should the biological definition rely on the species definition?[26]

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[24] J. Fletcher, The Ethics of Genetic Control. Ending Reproductive Roulette. Buffalo: Prometheus Books 1988, pp. 171-173.

[25] For policy making and legislation, the attempt to find middle ground between prohibitions and self-regulation of biotechnology is of importance. One of most feasible solutions is to extend the existing liberal democratic compromise with respect to equal protection. The compromise also includes banning the monopolisation of certain biotechnologies. Cf. N. A. Adams, Creating clones, kids & chimera: liberal democratic compromise at the crossroads. In: Issues In Law & Medicine, 2004, 20(1):3-69.

[26] L. M. Glenn, When pigs fly? Legal and ethical issues in transgenics and the creation of chimeras. In: Physiologist, 2003, 46(5):254. "A scientist could argue that distinguishing traits between species are manifestations of the genetic material of each species. However, the definition of species is a hotly debated and contentious issue among scientists […] The un-

What is the relation between "human nature" and the biological species *homo sapiens*?

What is the meaning of "human dignity"? Does the human dignity argument justify the prohibition or tolerance of human embryonic chimera?[27]

What constitutes human identity?

What philosophical notions of "human" are used in legislations?[28]

Or in summary, what does it mean to be human? What image of human (*Menschenbild*) do we use?

After these questions have been answered, the issue of moral status, moral options and moral guidelines (and subsequently of legal regulation) concerning humanoid chimeras can be addressed. We need to be prepared to ask: "How can we preserve our human rights and dignity despite the fact that our 'humanness' may no longer be the exclusive possession of Homo Sapiens?"[29]

b. Should we cross boundaries?

Chimeras and hybrids challenge our understanding of human being in general. Similarly, they challenge our understanding of "species" and "nature" (human na-

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comfortable truth is that species differentiation in not as clear-cut as some would like it to be" (ibd.).

27 Although the human dignity argument does not necessarily support and justify prohibition of chimera usage. For instance, the transplantation of adult human neural SC into prenatal non-humans offers a possibility for studying human neural cell development without direct use of human embryos. Such experiments, raising significant ethical concerns especially regarding mixing of human and nonhuman tissues and in development of human-nonhuman chimeras. Some authors argue that human-nonhuman chimeras research does not violate human dignity ipso facto if certain ethical guidelines for conducting such research are observed. – Cf. P. Karpowicz, C.B. Cohen, D. van der Kooy, Developing human-nonhuman chimeras in human stem cell research: ethical issues and boundaries. In: Kennedy Institute of Ethics Journal, 2005, 15(2):107-134; M. Greene, K. Schill, S. Takahashi, A. Bateman-House, T. Beauchamp, H. Bok, D. Cheney, J. Coyle, T. Deacon, D. Dennett, P. Donovan, O. Flanagan, S. Goldman, H. Greely, L. Martin, E. Miller, D. Mueller, A. Siegel, D. Solter, J. Gearhart, G. McKhann, R. Faden, Ethics: Moral issues of human-non-human primate neural grafting. In: Science, 2005, 309(5733):385-386; J. Savulescu, Human-animal transgenesis and chimeras might be an expression of our humanity. In: The American Journal of Bioethics, 2003, 3(3):22-25; T. Seyfer, The ethics of chimeras and hybrids: dignity and original solitude. In: Journal of Medical Ethics, 2004, 29(8):1-4.

28 In law, the term "natural" persons is limited to biological entities that are humans at the same time. Nevertheless, the term "human", being taken for granted, is not defined legislatively. This philosophical-anthropological unclearness concerning "human" (built on the assumption of self-evidence) has serious (not only) legal implications. – Cf. L. M. Glenn, A legal perspective on humanity, personhood and species boundaries. In: The American Journal of Bioethics, 2003, 3(3):27-28; L. M. Glenn, Biotechnology at the margins of personhood: An evolving legal paradigm. In: The Journal of Evolution and Technology, 2003, 13:35–37.

29 L. M. Glenn, When pigs fly? Legal and ethical issues in transgenics and the creation of chimeras. In: Physiologist, 2003, 46(5):254.

ture, animal nature). Is "nature" biologically based on genes, cells, organs or on "species"? What kind of notion of species are we using? What are the boundaries between individual species? Where do they lie? After clarifying these factual biological and scientific questions, we are challenged by fundamental ethical and moral question "Should/can/shall we cross the species boundaries?"[30] Concerning the moral relevance of the species boundaries three main positions can be identified. For some people, species boundaries have no moral relevance;[31] while others view them as having moral relevance;[32] the third group, admitting some moral relevance, would argue that interspecies boundaries can be crossed and such experiments may be conducted ethically – though taking for granted moral relevance of boundaries, including interspecific ones.[33]

From the statement of moral significance of boundaries it does not necessarily follow that crossing boundaries *ipso facto* interferes with moral rightness. Another question is what are the boundaries? Are we allowed to cross them on the vegetal level between plants of various species?[34] Are we allowed to cross them on animal level, creating animal hybrids and chimeras? Should we cross the boundaries separating the plant and animal kingdom? Humans and animals? On genomic, cellular, tissue, organ, embryo/chimera level? Can we discover these interspecific boundaries in a way different from experimental one? How is the biological identity of humans as a species related to personal identity? What does it mean to be "not-fully-human"?[35] What are anthropological and philosophical implications of the biological fact that we are genetically almost identical to apes?

c. The art of slowness

An old wisdom says: it is dangerous to stress the similarities between human and animal, more dangerous is to omit differences between human and animal and the worst thing is to be unaware of both. This old wisdom seems to be of high importance in the contemporary debate about human-nonhuman chimeras. The basic rule for solving problems is: not to solve problems in such a way that creates (new) problems even bigger than the original ones.

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30 Cf. J. S. Baylis, F. Baylis, Crossing species boundaries. In: The American Journal of Bioethics, 2003, 3(3):1-13.

31 See for instance A. W. Siegel, The moral insignificance of crossing species boundaries. In: The American Journal of Bioethics, 2003, 3(3):33-34.

32 As example see R. Streiffer, In defence of the moral relevance of species boundaries. In: The American Journal of Bioethics, 2003, 3(3):37-38.

33 Cf. P. Karpowicz, C. B. Cohen, D. van der Kooy, It is ethical to transplant human stem cells into nonhuman embryos. In: Nature Medicine, 2000, 10(4):331-335.

34 The idea of transgression is a complex one. – See also P. B. Thompson, Crossing species boundaries is even more controversial than you think. In: The American Journal of Bioethics, 2003, 3(3):14-15.

35 S. Franklin, Drawing the line at not-fully-human: what we already know. In: The American Journal of Bioethics, 2003, 3(3):W25-W27.

As we know from experience, the quickest way is not always the best one. First we should learn the virtue of slowness - which is quite incompatible with the rush biotechnological and biomedical development – and to think deeply before we decide to move forward with human-non-human chimeras research. The prospect of creating or redesigning new human life should be held to an ethical standard of appropriate reflection. Since the whole prospect has not yet been defined sufficiently many related fundamental anthropological questions need clarification. A broad public discussion and ethical reflection is needed prior to deciding whether to start with the project of redesigning human life into novel forms (and to extend embryologic research conducted on non-human animals to humans).[36] The whole of society must address the philosophical, ethical, and legal issues of altered organisms. It seems that a thoughtful approach will be appropriate.[37]

[36] Cf. N. J. Jones, W. P. Cheshire, Can artificial techniques supply morally neutral human embryos for research? Creating novel categories of human embryos. In: Ethics and Medicine, 2005, 21(1):29-40. Other authors like Robert and Baylis criticise earlier attempts to forbid crossing species boundaries in the creation of novel beings (J. S. Robert, F. Baylis, Crossing species boundaries. In: The American Journal of Bioethics, 2003, 3[3]:1-13).

[37] Thoughtful approach should be adopted for chimera patent policy as well – despite the pressure on swift and smooth patenting. The keynote "Patent first, ask questions later" is certainly not the best policy. - Cf. M. A. Bagley, Patent first, ask questions later: morality and biotechnology in patent law. In: William Mary Law Review, 2003, 45(2): 469-547.

III. Scientific background

Michael Bader, Regine Schreiner, Eckhard Wolf

1. Introduction

History

An individual whose body contains different cell populations derived from different zygotes is defined as chimera. These different cell populations can be derived from one species resulting in **intra-species chimerism (aa)** or from two different species resulting in **inter-species chimerism (c)**. Hybrids contain equal cells but with mixed genomes of two different taxa. Thus, **inter-species hybrids (c)** are the classical hybrids and **intra-species hybrids (bb)** mean mixtures of subspecies or strains.

Mixed creatures have fascinated people for thousands of years in various societies world-wide. In the mythology of Ancient Middle East and Greece, creatures such as Pegasus (horse/eagle), mermaid (woman/fish), satyr (man/goat), harpy (woman/bird), centaur (man/horse), minotaur (man/bull), griffin (eagle/lion), and basilisk (cock/snake) are occurring. In Ancient Egypt (e.g., the sphinx, a mixture of man and lion) and in the Hindu religion, the majority of deities were hybrids between man and animals and also in East Asian cultures such creatures are known, such as the Qilin (a mixture of several species including dragon, dear, and lion). In the middle ages in Europe, all kinds of mixed creatures were used for the ornamentation of churches in particular serving as gargoyles to scare away bad ghosts. And even nowadays such hybrids have come to a renaissance in fantasy stories such as the Harry Potter series.

Intra-species hybrids are mainly generated in livestock breeding using different strains of animals to exploit the heterosis effect which may increase the strength of wanted characteristics and the fitness in the offspring.[38] Already more than 5000 years ago, subspecies of cattle had been crossbred in Africa to yield the animals used nowadays for farming[39]. Inter-species hybrids have been described as offspring from the breeding of two animal species. However, only closely related species allow the generation of viable hybrid offspring. Anyhow, most of these "natural" hybrids were, indeed, generated by zoologists either by natural mating or artificial insemination. Already in the 19th century, among others, hybrids of lions and tigers (liger or tigon)[40] and zebras and donkeys (zeedonk)[41] were generated in

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38 Burke JM & Arnold ML. 2001 Genetics and the fitness of hybrids. Annu Rev Genet. 35:31-52.

39 Zeder MA, Emshwiller E, Smith BD & Bradley DG. 2006 Documenting domestication: the intersection of genetics and archaeology. Trends Genet. 22:139-155.

40 http://en.wikipedia.org/wiki/LigerHistory.

J. Taupitz and M. Weschka (eds.), *CHIMBRIDS - Chimeras and Hybrids in Comparative European and International Research,*
DOI: 10.1007/978-3-540-93869-9_3,

zoos. Thus, it was only a matter of time until scientists also thought about what might come out from a breeding between man and apes.

The Dutch Hermann Moens[42] and the German Hermann Rohleder[43] independently suggested such experiments in the beginning of the 20th century. Several years later, a Russian team headed by Ilya Ivanov, one of the pioneers of artificial insemination, set about performing such experiments in Africa.[44] However, despite several orang-utans being inseminated with human sperm, a pregnancy was never obtained. Before Ivanov could move his experiments to the Soviet Union, he was arrested for political reasons and died shortly thereafter.

Another technical way to generate inter-species chimera is the production of transgenic animals by the introduction of genes of one mammal in another mammal's zygote. This technology was developed in 1981[45] and has since then yielded thousands of hybrid animal models, mostly mice and rats.

Intra-species chimeras also occur in nature. Maybe even every mammal, including each human, is a chimera, since it has been shown that maternal cells circulate in the foetus and can still be found in adult life.[46] However, inter-species chimeras have only been artificially generated for scientific or medical purposes. Probably the first inter-species human chimera was a boy who got a blood infusion from a lamb in 1667 by Jean-Baptiste Denys and who survived this procedure.[47] This experiment was the result of a race between France and England on the primacy of blood transfusion technology in which numerous chimeric animals had already been independently generated before by several researchers. Thereafter, allogenic blood transfusion between humans was established creating numerous intra-species human chimeras. Even much earlier, namely more than 2500 years ago, the famous Indian surgeon Susruta had transplanted skin from one human to another, probably creating the first human (intra-species) chimeras ever artificially generated.[48] Also this technology of solid organ transplantation has gained enor-

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41 Darwin C 1868 The Variation Of Animals And Plants Under Domestication, http://www.esp.org/books/darwin/variation/facsimile/contents.htm.

42 Moens HMB. 1908.Truth: Experimental Researches about the Descent of Man. London: A. Owen.

43 Rohleder HO 1918. Künstliche Zeugung und Anthropogenie, in Monographien über Zeugung beim Menschen, vol. 6. Leipzig: Georg Thieme.

44 Rossiianov K. 2002 Beyond species: Il'ya Ivanov and his experiments on cross-breeding humans and anthropoid apes. Sci Context. 15:277-316.

45 Harbers K, Jähner D & Jaenisch R. 1981 Microinjection of Cloned Retroviral Genomes into Mouse Zygotes: Integration and Expression in the Animal. Nature 293, 540-542.

46 Maloney S, Smith A, Furst DE, Myerson D, Rupert K, Evans PC & Nelson JL. 1999 Microchimerism of maternal origin persists into adult life. J Clin Invest. 104:41-47.

47 Myhre BA. 1990 The first recorded blood transfusions: 1656 to 1668. Transfusion. 30:358-362.

48 Chick LR. 1988 Brief history and biology of skin grafting. Ann Plast Surg. 21:358-365.

mous significance in modern medicine and, thus, has produced a great number of intra-species chimeras in the last centuries. The injection of stem cells into humans is also a medical procedure that creates intra-species chimeras. Bone marrow transplantation is a common procedure to cure leukaemias, which was invented in the 1950s by the later Nobel Prize winner Donnal Thomas.[49] A very special method of creating human inter-species chimeras was invented by Paul Niehans in 1931, the "Frischzellentherapie". He injected foetal or juvenile lamb cells into humans as anti-aging therapy[50].

The first (intra-species) chimeric animals generated by the fusion of two preimplantation embryos were mice in the early 1960s.[51] Only a little more than 10 years later the first inter-species chimeras were produced by this technique between different mouse species,[52] different bovine species,[53] mouse and rat[54] and sheep and goat.[55] The latest development in the technologies to generate chimeras are embryonic stem (ES) cells. These cells were first derived from mouse blastocysts[56] but are now also available from other mammals including humans.[57] They are pluripotent and can be integrated into all tissues when injected into embryos of adult animals creating high expectations for their use in regenerative medicine.

Aims

The first incentive for scientific occupation with the issue of chimeras and hybrids was curiosity. Scientists were just interested to know what such a mixed creature would look like. In the case of human/ape hybrids some politicians might have had the idea of breeding a race of perfect soldiers. But there was no real scientific hypothesis backing up such early projects.

In recent years, however, numerous serious scientific and medical problems have been addressed by the use of chimera and hybrid technologies, in particular when

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49 Thomas ED, Lochte HL, Lu WC & Ferrebee JW. 1957 Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. N Engl J Med 157: 491-496.

50 Niehans P. 1952 [20 Years of cellular therapy.] Med Klin. 47:1-16.

51 Tarkowski AK 1961 Mouse chimeras developed from fused eggs. Nature 190 857-860.

52 Rossant J & Frels WI 1980 Interspecific chimeras in mammals: successful live chimeras between Mus musculus and mus caroli. Science 208 419-421.

53 Williams TJ, Munro RK & Shelton JN 1990 Production of inter-species chimeric calves by aggragation of Bos indicus and Bos Taurus demi-embryos. Reprod Fertil Dev. 2 385-394.

54 Stern MS. 1973 Chimaeras obtained by aggregation of mouse eggs with rat eggs. Nature; 243: 472-473.

55 Fehilly CB, Willadsen SM & Tucker EM 1984 Interspecific chimerism between sheep and goat. Nature.307 634-636.

56 Evans MJ, Kaufman MH. 1981 Establishment in culture of pluripotential cells from mouse embryos. Nature. 292:154-156.

57 Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. 1998 Embryonic stem cell lines derived from human blastocysts. Science. 282:1145-1147.

human components were applied. Gamete mixing ("hamster test") has been used to validate the quality of human sperm for in-vitro fertilisation. Embryo mixtures were generated to study the participation of certain cell types in differentiation processes. The introduction of human cells into animals was performed to study their developmental and functional capacities, and animal cells and organs were transferred into humans to repair damaged tissue ("xenotransplantion"). The transfer of human genes and chromosomes into animals, creating numerous transgenic and transchromosomal animals, respectively, was used to study the function of the transferred human genes and to generate animal models for human diseases as a basis for drug development. Very recently, the transfer of a whole human nucleus into an enucleated animal oocyte has gained greater interest for the generation of embryonic stem cells for regenerative therapies and even as a test system for reproductive cloning. The next chapters will list more comprehensively the aims and scopes of different chimbrid experiment.

2. Intra-species mixtures

a. Intra-species chimeras

Naturally occurring intra-species chimeras

Examples of intra-species mixtures are chimeric cattle. These naturally occurring chimeras are quite common: In cows with twin pregnancy, there is a high incidence of formation of placental anastomoses between the foetal circulatory systems early in gestation. This leads to exchange of haematopoietic stem cells between the two foetuses. The resulting haematopoietic chimeras carry two different populations of all cells derived from haematopoietic stem cells, e.g. peripheral blood cells, Kupffer cells in the liver, lymphocytes and macrophages in lymph nodes and spleen. Clinical significance is seen when one foetus is male and one female: as the female foetus is exposed to male hormones during development, most females from male/female twins (so called freemartins) suffer from malformation of the genital tract and sterility.[58]

Although less common (due to the different placenta types) blood haematopoietic intra-species chimerism does also occur in human dizygotic twins (incidence 8%) and di- or trizygotic triplets (incidence 21%).[59] This phenomenon was first discovered with routine ABO/Rhesus blood grouping, when it was found that some twins had more than one blood group. Those people who were not twins were thought to have received blood cells from a twin that died early in gestation. Haematopoietic

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[58] Rüsse I & Sinowatz F 1998 (2.Auflage) Lehrbuch der Embryologie der Haustiere Parey Buchverlag Berlin, p.443.

[59] Van Dijk B, Boomsma DI & de Man AJM 1996 Blood group Chimerism in human multiple Births is not rare. Am J Med Gen 61 264-268.

chimeras develop a central tolerance resulting in the failure to express the regular antibodies against A or B blood group antigens of the partner twin and in the mutual acceptance of organ grafts.[60] Today, the high incidence of multiple pregnancies following *in vitro* fertilisation has led to an increased number of people with congenital haematopoietic chimerism.[61]

Another intra-species mixture (i.e. haematopoietic microchimerism) can occur naturally after transplacental leakage between mother and foetus. Microchimerism is defined as chimerism which is only measurable by very sensitive techniques like polymerase chain reaction.[62] Foetal microchimerism describes the long-term persistence of foetal cells in parous women, whereas maternal microchimerism concerns persistence of maternal cells in their offspring. Foetal microchimerism is controversially discussed as a potential factor in the pathogenesis of autoimmune diseases, such as systemic sclerosis, in women following their childbearing years.[63]

Spontaneous human chimerism is extremely rare. It implicates the presence of two genetically different cell lines not only in cells of the blood and lymphatic system, but also in solid tissues. Spontaneous human chimerism can result from events like

Aggregation of two non-identical twin embryos in early stage of development;

Suppression of second meiotic division and fertilisation of secondary oocyte and first polar body;

Fertilisation of ovum and first/second polar body;

Fertilisation of first two mitotic products of unfertilised ovum;

Fusion of one mitotic product of fertilised ovum with second polar body;

Suppression of second meiotic division and fertilisation of secondary oocyte.[64]

Most cases of spontaneous chimerism have been detected in people and other mammals carrying one XY and one XX cell population as they often show anatomical evidence of hermaphroditism such as ambiguous external genitalia. Spon-

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60 Billingham RE, Brent L & Medawar PB 1953 Actively aquired tolerance to foreign cells. Nature. 172 603-606.

61 Kühl-Burmeister R, Simeoni E, Weber-Mathiesen K, Milde A Herwartz C Neppert J & Suttorp M 2000 Equal distribution of congenital blood cell chimerism in dizygotic triplets after in-vitro fertilisation. Hum Reprod 15 1200-1204.

62 Sykes M & Sachs D 2001 Mixed Chimerism Phil Trans R Soc Lond B 356 707-726.

63 Lambert NC, Stevens AM, Tylee TA, Erickson AD, Furst DE & Nelson JL 2001 From the simple detection of microchimerism in patients with autoimmune disease to its implications in pathogenesis Ann NY Acad Sci 945 164-171.

64 For references see McLaren A 1976 Mammalian Chimaeras Cambridge University Press p.125 (table 13).

taneous human chimerism can also become obvious in individuals with ancestors of different skin colour - as they can display mottled skin.[65]

Intra-species chimeras in animal breeding and stem cell research

The first artificially generated chimeric animal – a mouse chimera – was produced by aggregation of two early embryos (morula aggregation) in 1961.[66] Since then, the technique of creating chimeric mice has been extended not only to different microsurgical techniques (injection of cells into embryos of blastocyst stage – blastocyst injection),[67] but also to other combinations:[68]

- embryonic cells with teratocarcinoma cells
- embryonic cells with other, slightly asynchronous embryonic cells
- embryonic cells with primordial germ cells
- embryonic cells with embryonic stem cells.

The production of mouse chimeras by injection of gene-targeted embryonic stem cells in blastocysts is the first step in the standard procedure for the generation of genetically modified mouse models in biomedical research.[69] By gene targeting in murine embryonic stem cells, generation of chimeras by blastocyst injection and subsequent breeding of transgenic lines, both "additive gene transfer" and "knockout" of gene function is possible in mice[70]. Searching the Jackson Lab online database on mouse strains with targeted mutations available worldwide, 1436 entries appeared.[71] Mouse chimeras can furthermore be produced by coculture of murine embryonic cells with embryonic stem cells,[72] or by replacing the nucleus in one blastomere of a two cell murine embryo with a genetically different

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65 Zuelzer WW, Beattie KM & Reisman LE 1964 isedGeneralised unbalanced mosaicism attributable to dispermy and probable fertilisation of a polar body. Am J Hum Genet 16 38-51.

66 Tarkowski AK 1961 Mouse chimeras developed from fused eggs. Nature 190 857-860.

67 Gardner RL 1971 Manipulations on the blastocyst. Adv Biosci 6 279-296.

68 Tarkowski AK 1998 Mouse chimeras revisited: recollections and reflections. Int J Dev Biol 42 903-908.

69 Thompson S, Clarke AR, Pow AM, Hooper ML & Melton DW 1989 Germline transmission of a corrected HPRT gene produced by gene-targeting in embryonic stem cells. Cell 56 313-321.

70 Prelle K 2001 Pluripotente Stammzellen – Untersuchungen zur Etablierung und Differenzierung embryonaler Stammzellen in vitro unter entwicklungsbiologischen und gentechnologischen Aspekten. Habilitationsschrift LMU München.

71 www.informatics.jax.org, 5.1.2006 International Mouse Strain Resource (IMSR), unlimited query on targeted mutations.

72 Khillan JS & Bao Y 1997 Preparation of animals with a high degree of chimerism by one-step coculture of embryonic stem cells and preimplantation embryos. Biotechniques 22 544-549.

nucleus from another early stage embryo[73], or by lethal irradiation of individual mice – followed by transplantation of allogenic or xenogeneic bone marrow (so called "radiation chimeras").[74]

Although the mouse is the lead species in experimental mammalian chimeras, intra-species chimeric animals were also produced in rabbits,[75] sheep,[76] cattle,[77] pigs[78] and rats.[79]

Intra-species chimeras in transplantation medicine (allotransplantation)

The recipient of an allograft (i.e. the transplantation or blood transfusion patient) is always chimeric. After solid organ transplantation, the presence of donor cells/DNA has been detected not only within the graft environment but also in the blood: thus haematopoietic microchimerism was established after transplantation of livers, kidneys, hearts and lungs. This holds true not only for recipients of solid organs, but also for the recipients of blood transfusions. Following a blood transfusion, blood cells of the donor prevail in clinically relevant amounts in the recipient for 21 days. After that period, the establishment of haematopoietic microchimerism has been described in patients.[80]

Haematopoietic chimerism, which can be created either by transfusion of blood or transplantation of bone marrow is defined as follows: "microchimerism" is not measurable by flow cytometry (which usually has a detection limit in the range of 0.1-1%), but only by more sensitive techniques like polymerase chain reaction; "mixed chimerism" refers to a state in which donor and host haematopoietic ele-

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73 Kono T, Tsunoda Y, Watanabe T & Nakahara T 1989 Development of chimeric two-cell mouse embryos produced by allogeneic exchange of single nucleus from two- and eight-cell embryos. Gamete Res 24 375-384.

74 Marcus H, David M, Canaan A, Kulova L, Lubin I, Segall H, Denes L, Erlich P, Galun E Gan J, Laster M & Reisner Y 1995 Human/mouse radiation chimera are capable of mounting a human primary humoral response. Blood 86 398-406.

75 Gardner RL & Munro AJ 1974 Succesful construction of chimaeric rabbit. Nature 250 146-147.

76 Tucker EM, Moore RM & Rowson LEA 1974 Tetraparental sheep chimearas induced by blastomere transplantation. Changes in blood type with age. Immunology 26 613-621.

77 Brem G, Tenhumbery H & Krausslich H 1984 Chimerism in cattle through microsurgical aggregation of morulae. Theriogenology 22 609-613.

78 Wheeler MB 1994 Development and validation of swine embryonic stem cells: a review. Reprod Fertil Dev 6 563-568.

79 Mayer JF & Fritz HI 1974 The culture of preimplantation rat embryos and the production of allophenic rats. J Reprod Fertil 39 1-9; Lubin I, Segall H, Ehrlich P, David M, Marcus H & Reisner Y 1993 Conversion of normal rats into SCID-like animals following transplantation of murine SCID bone marrow allows engraftment of human peripheral blood lymphocytes. Blood 82 428.

80 Carter AS, Bunce M, Cerundolo L, Welsh KI, Morris PJ & Fuggle SV 1998 Detection of microchimerism after allogeneic blood transfusion using nested polymerase chain reaction amplification with sequence-specific promers (pcr-ssp): a cautionary tale. Blood 92 683-689.

ments of multiple lineages coexist at levels detectable by flow cytometry; "full chimerism" is established in individuals where essentially all haematopoietic elements are derived from a donor stem cell inoculum (e.g. after myeloablation in cancer patients).[81]

As described previously, the phenomenon of haematopoietic chimerism has been suggested to induce central immunological tolerance in recipients after xenogeneic transplantation (focus on mixed chimerism) and allogenic transplantation (focus on microchimerism).[82]

b. Intra-species hybrids

Intra-species hybrids in animal breeding

Intra-species hybrids are either ubiquitous or practically do not exist depending on the definition of the term hybrid. If the definition is restricted to the offspring of the breeding of two different species, then intra-species hybrids are excluded. If a "hybrid" is an organism carrying a mixture of genetic information of two independent individuals in all cells, then each mammal including all humans are hybrids containing the mixed genes of mother and father. However, the term hybrid is classically used in animal breeding for the result of interbreeding of different inbred strains of domestic mammals and laboratory rodents. In livestock farming and agriculture, intra-species hybrids of animals (and even more importantly of plants) are often generated to mix the characteristics of two strains in order to exploit the heterosis effect which may increase the fitness of the offspring.[83]

3. Inter-species mixtures

a. Inter-species chimeras

In contrast to intra-species chimeras and inter-species hybrids, inter-species chimeras do not, to our knowledge, occur naturally.

Inter-species chimeras in animal breeding and stem cell research

Successful generation of interspecies chimeras developing to adulthood has been noted in *Mus musculus x Mus caroli,*[84] *Bos taurus x Bos indicus*[85] and goat x

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81 Sykes & Sachs D 2001 Mixed Chimerism Phil Trans R Soc Lond B 356 707-726.

82 See section on "mechanisms to overcome immunological barriers".

83 Burke JM & Arnold ML. 2001 Genetics and the fitness of hybrids. Annu Rev Genet. 35:31-52.

84 Rossant J & Frels WI 1980 Interspecific chimeras in mammals: successful live chimeras between Mus musculus and mus caroli. Science 208 419-421.

85 Williams TJ, Munro RK & Shelton JN 1990 Production of inter-species chimeric calves by aggragation of Bos indicus and Bos Taurus demi-embryos. Reprod Fertil Dev. 2 385-394.

sheep[86] combinations. The latter – termed "geep" chimera – resulted from the injection of caprine cells of the inner cell mass into ovine blastocysts and subsequent transfer to ovine recipients.[87] By maintaining the trophoblast integrity of the recipient blastocyst, it was apparently possible to mask the antigens of the foreign foetal cells from the mother's immune system – thereby increasing its chances of survival. Resulting female sheep-goat chimeras demonstrated normal ovulation, normal fertilisation involving oocytes and spermatozoa of one of the component species and the ability to gestate offspring to term.[88]

Another animal biotechnology – somatic cell nuclear transfer (cloning) – has shown to accidentally lead to the formation of animals with microchimerism: maternal microchimerism was detected in cloned cattle. As the phenomenon also appeared in one foetus derived from an *in vitro* fertilisation protocol, the authors suggest malformations of the placenta as underlying cause.[89]

With the generation of human stem cells in 1998,[90] the creation of animal-human chimeras has become feasible. Some scientists claim that such experiments could help to

- test human stem cell pluripotency *in vivo*
- clarify aspects of human stem cell differentiation / regulation of differentiation *in vivo* (with regard to therapeutic cloning)
- create *in vivo* models for drug testing
- create animals carrying organs of human tissue as donor animals for transplantation (so called "humanised organs" as alternative to xenotransplantation).

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86 Fehilly CB, Willadsen SM & Tucker EM 1984 Interspecific chimerism between sheep and goat. Nature.307 634-636.

87 Polzin VJ, Anderson DL, Anderson GB, BonDurant RH, Butler JE, Pashen RL, Penedo MC & Rowe JD 1987 Production of sheep-goat chimeras by inner cell mass transplantation. J Anim Sci 65 325-330.

88 Anderson GB, Ruffing NA, BonDurant RH & Pashen RL 1991 Preliminary observations on reproduction in a femal sheep-goat chimera Vet Rec 129 467-469.

89 Hiendleder S, Bebbere D, Zakhartchenko V, Reichenbach HD, Wenigerkind H, Ledda S & Wolf E 2004 Maternal-foetal transplacental leakage of mitochondrial DNA in bovine nuclear transfer pregnancies: potential iplications for offspring and recipients. Cloning Stem Cells 6 150-156.

90 Thompson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS & Jones JM 1998 Embryonic stem cell lines derived from human blastocysts. Science 282 1145-1147.

Since the first research proposals for differentiation studies of human stem cells involving the creation of animal-human chimeras came up, an ethical debate[91,92,93] and a patent battle[94] on the boundaries of such experiments has risen.

A very controversial publication described the engraftment of human neural stem cells into the brains of old world monkey embryos.[95] This experiment combined a number of critical issues in animal-human chimeras:

The cell populations were transplanted at an early stage of development, resulting in widespread migration and distribution of cells;

The experiment affected one of the two body systems where creation of interspecies chimeras by transplantation of pluripotent human stem cells gives rise to concern (according to National Academy of Sciences of the US): the brain (sensitive and cognitive abilities of resulting chimera might no longer fit known categories) and the germline (resulting chimera might be able to produce human sperm or eggs);[96]

As the two species (human & old world monkey) are closely related, anatomical and physiological barriers to successful and consequence-bearing chimerism are low. For example, it is very unlikely that the structural complexity needed for any significant degree of humanlike mental capacity can be achieved under tight size limitations, e.g. in the skull of a mouse.

Less controversial from the ethical point of view are experiments like the creation of human-animal chimeras by injection of human haematopoietic stem cells (adult

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91 DeWitt N 2002 Scientists devided over proposal to create human-mouse embryos. Nature 420 255.

92 Greene M, Schill K, Takahashi S, Bateman-House A, Beauchamp T, Bok H, Cheney D, Coyle J, Deacon T, Dennett D, Donovan P, Flanagan O, Goldman S, Greely H, Martin L, Miller E, Mueller D, Siegel A, Solter D, Gearhart J, McKhann G & Faden R 2005 Moral Issue of Human-Non-Human Primate Neural Grafting. Science 309 385-386.

93 Karpowicz P, Cohen C & van der Kooy D 2004 It is ethical to transplant human stem cells into nonhuman embryos Nature Med 10 331-335.

94 Check E 2003 Biotech critic tries to sew up research on chimaeras. Nature 421 4.

95 Ourednik V, Ourednik J, Flax JD, Zawada WM, Hutt C, Yang C, Park KI, Kim SU, Sidman RL, Freed CR & Snyder EY 2001 Segregation of human neural stem cells in the developing primate forebrain. Science 293 1820-1824.

96 Recently, the Guidelines of International Society for Stem Cell Research (ISSCR) have explicitly prohibited the interbreeding of animals likely to harbour human gametes and recommended experiments likely to result in extensive animal-human chimerism of the brain or the germline to be permitted only after strict SCRO review (Stem Cell Research Overview) Daley G, Ahrlund-Richter L, Auerbach J, Benvenisty N, Alta Charo R, Chen G, Deng HK, Goldstein LS, Hudson KL, Hyun I, Junn SC, Love J, Lee EH, McLaren A, Mummery C, Nakatsuji N, Racowsky C, Rooke H, Rossant J, Schöler H, Solbakk H, Taylor P, Trounson A, Weissman I, Wilmut I, Yu J& Zoloth L 2007 The ISSCR Guidelines for Human Embryonic Stem Cell Research. Science 315 603-604.

stem cells) into the foetuses of sheep in the middle of gestation.[97] The authors of the study describe the formation of up to 20% of human hepatocytes in the liver of the resulting sheep-human chimeras. The scientists' original intention aimed to cure unborn children with genetic defects *in utero*.

Meanwhile, the notion of growing "humanised organs" for transplantation to human patients with liver failure, is propagated by the leader of the research group, Esmail Zanjani:[98] This theoretical option – an alternative model to xenotransplantation - would include

- harvest of adult stem cells out of the bone marrow and blood of the patient to treat;
- injection of the obtained human haematopoietic stem cells into a sheep embryo;
- creation of a sheep-human chimera;
- harvest of functional clusters of human liver cells from the chimera;
- transplantation of the liver cells back to the patient.

In addition to monkey-human chimeras and sheep-human chimeras - pig-human chimeras, goat-human chimeras, mouse-human chimeras, rat-human chimeras and chick-human chimeras have been generated (see table 3/supplement of the final report of Prof. Wolf).

Inter-species chimeras in transplantation medicine (xenotransplantation)

The most important feature of the current definition of xenotransplantation is the direction of transplantation, which could roughly be outlined as: "living animal material goes to human organism".[99] Hence, a certain type of human-animal chimera will result. All xenotransplantation research done so far aims at a treated patient being chimeric in the following ways:

Portions of cell populations The animal cell population in the human-animal chimera would represent just a very small portion, compared to the total human cell population.

Differentiation status of transplanted cells Transplanted cell populations, tissues or organs would contain cells in a state of terminal or advanced differentiation. Thus, further uncontrolled differentiation – or worse, malignant dedifferentiation - is not to be expected.

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97 Almeida-Porada G, Porada CD, Chamberlain J, Torabi A & Zanjani E 2004 Formation of human hepatocytes by human hematopoietic stem cells in sheep. Blood 104 2582-2590.

98 Pagan S 2003 "Humanised" organs can be grown in animals. New Sci www.newscientist.com/article.ns?id=dn4492&print=true.

99 See "definition of xenotransplantation" in the first section of the final report of Prof. Wolf.

Developmental status of the patient The development of the human patient would be completed (or at least far along), so that uncontrolled dissemination or migration of animal cells is only expected to fall into the range of microchimerism.

Candidate cell types Animal germline cells or reproductive organs have not been proposed as candidates for xenotransplantation yet. Thus chimerism would not affect the germline.

Taken together, the xenotransplantation patient (a human-animal chimera) is expected to display a kind of chimerism similar to the allotransplantation patient (a human-human chimera). The difference is that the xenotransplantation patient is an inter-species chimera and the allotransplantation patient an intra-species chimera.

b. Inter-species hybrids

Inter-species hybrids by mixing of gametes, in nature and in-vitro

Only closely related species, such as felids, canids, bovids, equids, bears and some monkey species can generate viable mixed offspring, which are normally infertile. If such hybrids were fertile, they would challenge the distinction of the parents as stemming from two different species. Since dogs and wolves produce fertile hybrids they have to be considered as belonging to one species. In areas where closely related species overlap in their distribution such hybrids may occur, such as between the polar bear and the grizzly in Northern Canada.[100]

However, the most well known example of a natural hybrid is the mule, which is generated by breeding of male horse and female donkey (the product of the opposite mating is called a hinny). These animals have been bred for 5000 years and used for carrying heavy loads. Normally, they are infertile but a few cases of offspring from mules mated with horses or donkeys have been reported.[101] There is also one case reported of a viable hybrid between sheep and goat, two species even belonging to different genera.[102] Interestingly, chimeras between the same two species have become one of the few examples of viable offspring from embryo mixing experiments by scientists (see above.)[103]

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[100] http://news.nationalgeographic.com/news/2006/05/polar-bears.html.

[101] Rong R, Chandley AC, Song J, McBeath S, Tan PP, Bai Q & Speed RM. 1988 A fertile mule and hinny in China. Cytogenet Cell Genet. 47:134-139.

[102] Letshwenyo M & Kedikilwe K. 2000 Goat-sheep hybrid born under natural conditions in Botswana. Vet Rec. 146:732-734.

[103] Polzin VJ, Anderson DL, Anderson GB, BonDurant RH, Butler JE, Pashen RL, Penedo MC & Rowe JD 1987 Production of sheep-goat chimeras by inner cell mass transplantation. J Anim Sci 65 325-330.

With the development of embryo culture methods, in vitro fertilisation (IVF) became possible and in 1978 the first IVF baby was born.[104] For IVF technology, it was for some time normal to use hamster eggs to test for the fertilising capacities of human sperm ("hamster test"). [105] But none of these hybrids was ever allowed to develop further. Even before, trials to generate hybrids of human and apes by artificial insemination had failed.[106]

Inter-species hybrids by transfer of genes, chromosomes, or nuclei

In the early 1980s the transfer of single genes from one mammalian genome to another became possible by the use of pronuclear microinjection of DNA constructs into zygotes.[107] Since then thousands of such hybrid animals, mostly mice and rats, have been generated, carrying genes of other mammals (including human). These animals are employed to study the function of the transferred genes and to develop animal models of human diseases in order to develop novel therapeutic regimens. Furthermore, such animals, in particular sheep and goats, have been generated to produce high amounts of therapeutic proteins in their milk.[108] In recent years, the methods for generating such animals have become increasingly various. The genes can also be transferred by viral constructs and can be integrated into certain specified loci in the gene by the use of homologous recombination in ES cells.[109] Such cells were also used to generate mice carrying nearly full-length human chromosome 21 in order to study the pathophysiology of trisomy 21.[110]

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[104] Steptoe PC & Edwards RG 1978 Birth after the reimplantation of a human embryo. Lancet 2: 366.

[105] Yanagimachi R, Yanagimachi H & Rogers BJ 1976 The use of zona-free animal ova as a test system for the assessment of the fertilising capacity of human spermatozoa. Biol.Reprod 15: 471-476.

[106] Rossiianov K. 2002 Beyond species: Il'ya Ivanov and his experiments on cross-breeding humans and anthropoid apes. Sci Context. 15:277-316.

[107] Costantini F & Lacy E. 1981 Introduction of a Rabbit □-Globin Gene into the Mouse Germ Line. Nature 294, 92-94; Harbers K, Jähner D & Jaenisch R. 1981 Microinjection of Cloned Retroviral Genomes into Mouse Zygotes: Integration and Expression in the Animal. Nature 293, 540-542; Wagner TE, Hoppe PC, Jollick JD, Scholl DR, Hodinka RL & Gault JB. 1981 Microinjection of a Rabbit Beta-Globin Gene into Zygotes and Its Subsequent Expression in Adult Mice and Their Offspring. Proc. Natl. Acad. Sci. USA, 78, 6376-6380; Wagner EF, Stewart TA & Mintz, B. 1981 The Human □-Globin Gene and a Functional Viral Thymidine Kinase Gene in Developing Mice. Proc. Natl. Acad. Sci. USA, 78, 5016-5020.

[108] Human antithrombin III ATryn ® produced from transgenic goat (Genzyme Transgenics Corp.). August 2006: European Medicines Agency (EMEA) approved Marketing Authorisationisation Application (MAA).

[109] Capecchi MR. 2005 Gene targeting in mice: functional analysis of the mammalian genome for the twenty-first century. Nat Rev Genet. 6:507-512.

[110] O'Doherty A Ruf S, Mulligan C, Hildreth V, Errington ML, Cooke S, Sesay A, Modino S, Vanes L, Hernandez D, Linehan JM, Sharpe PT, Brandner S, Bliss TV, Henderson DJ, Ni-

The most extreme case of transfer of genetic information from one species to an other is the transfer of nuclei into an enucleated oocyte. By this method a hybrid embryo is created carrying 99% of the genome of the nuclear donor species but still maintaining about 1% of the oocyte donor genome, namely the mitochondrial genes. While such experiments are relatively easy when nuclei from very early preimplantation stage embryos are used, they were thought to be impossible for nuclei from more developed stages or even adult mammals despite some positive reports in the early 1980s.[111] However, in 1997 the first animal was born from a cloned embryo after transfer of an adult somatic nucleus: Dolly the sheep.[112]

Anyhow, these animals and the vast majority of other cloned mammals were not inter-species hybrids since nucleus and oocyte were from the same species. However, there have been cases of transfers of nuclei between different species, mostly to preserve endangered species.[113] Such experiments were also only successful when closely related species were employed. Very recently, human nuclei were also used for transfer into bovine and rabbit oocytes in order to develop ES cells from such embryos or to test the methodology for reproductive cloning.[114] More of such experiments are planned in the UK and have recently been approved by the British Human Fertilisation and Embryology Authority.[115]

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zetic D, Tybulewicz VL & Fisher EM. 2005 An aneuploid mouse strain carrying human chromosome 21 with Down Syndrome phenotypes. Science 309: 2033-2037.

111 Illmensee K & Hoppe PC. 1981 Nuclear transplantation in Mus musculus: developmental potential of nuclei from preimplantation embryos. Cell. 23:9-18; McGrath J & Solter D. 1984 Inability of mouse blastomere nuclei transferred to enucleated zygotes to support development in vitro. Science. 226:1317-1319.

112 Wilmut I, Schnieke AE, McWhir J, Kind AJ & Campbell KH. 1997 Viable offspring derived from foetal and adult mammalian cells. Nature. 385:810-813.

113 Loi P, Galli C & Ptak G. 2007 Cloning of endangered mammalian species: any progress? Trends Biotechnol. 25:195-200.

114 Zavos PM & Illmensee K. 2006 Possible Therapy of Male Infertility By Reproductive Cloning: One Cloned Human 4-Cell Embryo. Arch. Androl. 52: 243-254; Chen Y, He ZX, Liu A, Wang K, Mao WW, Chu JX, Lu Y, Fang ZF, Shi YT, Yang QZ, Chen da Y, Wang MK, Li JS, Huang SL, Kong XY, Shi YZ, Wang ZQ, Xia JH, Long ZG, Xue ZG, Ding WX & Sheng HZ. 2003: Embryonic stem cells generated by nuclear transfer of human somatic nuclei into rabbit oocytes. Cell Research 13: 251-263.

115 http://www.hfea.gov.uk/en/1581.html.

c. Examples of inter-species mixtures
aa. Xenotransplantation*

Marion Weschka

Today, the biggest problem in the treatment of patients with final organ failure is the limited supply of human organs for transplantation. Xenotransplantation could be a solution to this problem. Xenotransplantation in human recipients is defined as any procedure that involves the transplantation or infusion of (1) live cells, tissues or organs from an animal source; (2) human body fluids, cells, tissues or organs that have had ex vivo contact with live animal cells, tissues or organs. This covers the exposure of a person to: (a) human blood or blood constituents that have been in contact with live animal tissues, or (b) human organs, cells and tissues cultured on, or in contact with, live animal cells, or implanted (stored) in animals. The definition does not include the transplantation of animal tissue-derived bioprosthetics, such as e.g. porcine bioartificial heart valves which are commercially available and routinely used for transplantation to human patients, because such animal tissue-derived bioprosthetics are not viable implants anymore.

Heart, kidney and liver are in the focus of research with regard to solid, vascularised organs. As far as transplantations of cell populations are concerned, pancreatic islet cells, neuronal cells and cells of the adrenal medulla are most interesting for diseases such as diabetes, Parkinson's, Alzheimer's, Huntington's, stroke, epilepsy and chronic pain.

Depending on the combination of species, the reactions to xenotransplantation are different: In discordant combinations (e.g. pig to primate), the recipient carries high levels of natural preformed antibodies against the donor, which are not expressed at detectable levels in concordant combinations (e.g. chimpanzee to human). Therefore, after discordant xenotransplantation of vascularised, solid organs, the xenograft is immediately affected by hyperacute rejection. Still, xenotransplantation research is focused on the pig as organ donor for human patients for several logistical, hygienic and cultural reasons: The pig has a short reproduction cycle, it gives birth to several offspring at the same time and the methods for genetic manipulation are established. Moreover, specific- and qualified pathogen-free housing and management of the pigs is possible, pigs are accepted as productive livestock and there are also anatomical and physiological similarities between pigs and humans.

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* This summary written by Marion Weschka is based on the report "Xenotransplantation – Aspects of Chimerism" by Regine Schreiner/Regina Klose/Eckhard Wolf, see below at Annex A.I.2.

Challenges of xenotransplantation

There are several **immunological barriers** creating problems after xenotransplantation: (1) As mentioned above, **hyperacute xenogeneic rejection** occurs within minutes of organ reperfusion after the transplantation of discordant, solid, vascularised organs, but it does not affect non-vascularised small tissue or single cell xenografts. It is mediated by preformed xenoreactive antibodies causing microvascular thrombosis and interstitial haemorrhage, which leads to the destruction of the xenograft. None of the modern immune suppressive drugs used successfully in allotransplantation can prevent hyperacute rejection. (2) **Acute vascular rejection** (acute humoral xenograft rejection/delayed xenograft rejection) occurs within days to weeks after xenotransplantation. Apparently, it is dominated by xenogeneic antibodies that are intensely produced after the recipient's immune response has had first contact with the donor cells and T cell-dependent sensitisation has occurred. (3) **Acute cellular rejection** is frequently associated with acute vascular rejection. It is mediated by T-lymphocytes, macrophages and/or natural killer cells within days after xenotransplantation. However, there is not much data available yet. If it was comparable to acute cellular rejection in allotransplantation, it would be controllable by immunosuppressive drugs. (4) The most common cause for long-term complications and organ failure in allotransplantation is **chronic rejection,** which can occur after weeks, months or years. However, in xenotransplantation there is no significant data available yet. (5) **Thrombotic microangiopathy** leading to graft failure might be a form of immunological reaction although it does not fit into the classical system of xenograft rejection. It might also result from molecular incompatibilities in the coagulation mechanisms between pig and baboon. However, further research is necessary.

There is a lot of ongoing research aimed at discovering **mechanisms to overcome immunological barriers**. For example, to overcome hyperacute rejection, the elimination of preformed xenoreactive antibodies is possible, but antibodies will reappear after a while. With special medication, more permanent antibody reduction is possible. Another option to downregulate hyperacute rejection is the use of transgenic pigs for xenotransplantation. Moreover, strategies against graft rejection by cells of the immune system have to be developed. To some extent, cellular immune response can be controlled by immunosuppressive drugs. However, immune suppression implies a high risk of infection and severe side effects for the transplantation patient.

Further problems of xenotransplantation can be caused by **physiological and anatomical barriers.** As regards pig-to-human xenotransplantation, it must be considered that a fully-grown pig can reach a body weight of more than 300 kilos. Thus, its organs could be too big to fit e.g. into a slim human female. This problem could be solved, however, by using miniature pigs or growth regulation in pigs. Whereas pig-into-baboon heart xenotransplantation seems to be possible, pig-into-human kidney or liver transplantations would be affected by several physiological differences and incompatibilities. Still, in contrast to solid organs,

the transplantation of xenogeneic cells such as porcine islets of Langerhans appear to be promising, because porcine insulin has been used for a long time for the treatment of diabetes in humans and because the blood sugar level of both species is similar.

The risk of zoonosis (the transmission of infectious diseases from pigs to humans) could cause serious problems in an immune suppressed xenotransplantation patient. Whereas the transmission of most infectious agents from pigs to humans by xenotransplantation might be prevented by keeping the pigs in specific pathogen-free conditions, the transmission of porcine endogenous retroviruses (PERVs), which have the potential to infect human cells, being permanently integrated in multiple loci in the pig genome, cannot be overcome so easily. In 2002, it was claimed that individual miniature pigs that did not transmit PERV to human cells in vitro studies had been identified. This might circumvent the need for specific breeding or gene targeting to generate donor pigs for xenotransplantation. Moreover, recent studies suggest that the risk of PERV transmission from porcine cells or tissue to human recipients might not be a very probable scenario.

Chimerism

In medicine, an individual whose body contains different cell populations derived from different zygotes of the same or different species is defined as a chimera, whereas a mosaic is an individual with two genetically distinct types of cells that originate from a single zygote. In contrast to this, a hybrid is an individual composed of a single cell population derived from one zygote created by parents from two different breeding lines, races or species. Nuclear-cytoplasmic hybrids can be generated by intersubspecies or interspecies nuclear transfer, such as nuclear transfer of human somatic nuclei into rabbit eggs.

Forms of chimerism: Although one might expect that chimeras are always artificially created, natural chimerism does occur. For example, chimeric cattle are quite common, but also in humans, **blood haematopoietic chimerism** does occur with an incidence of 8 % in human dizygotic twins and of 21 % in di- or trizygotic triplets. This was discovered when it turned out that some twins had more than one blood group. Also between mother and foetus, **haematopoietic microchimerism** can occur naturally after transplacental leakage. **Spontaneous human chimerism** is extremely seldom. It implicates the presence of two genetically different cell lines not only in cells of the blood and lymphatic system, but also in solid tissues. It can result from events such as e.g. the aggregation of two non-identical twin embryos in an early developmental stage.

Chimeras in animal breeding: In 1961, the first chimeric animal was artificially created. It was a mouse chimera produced by the aggregation of two early embryos. Since then, several microsurgical techniques have been developed as well as other combinations, such as the combination of embryonic cells with teratocarcinoma cells, with other, slightly asynchronous embryonic cells, with primordial germ cells or with embryonic stem cells. For the generation of genetically modi-

fied mouse models in biomedical research, the production of mouse chimeras by injection of gene-targeted embryonic stem cells in blastocysts is the first step. Although most chimeric research is done with mice, chimeric animals were also generated with rabbits, sheep, cattle, pigs and rats. An example of an interspecies chimera that developed until adulthood was a goat-sheep combination, the so-called geep, which was generated by injecting caprine cells of the inner cell mass into ovine blastocysts and the subsequent transfer to ovine recipients.

Chimerism in stem cell research: The generation of human-animal chimeras has become theoretically feasible with the creation of human stem cells in 1998. According to some scientists, such human-animal chimeric experiments could help (1) to test human stem cell pluripotency in vivo, (2) to clarify aspects of human stem cell differentiation in vivo, (3) to create in vivo models for drug testing and (4) to create animals with organs of human tissue (humanised organs) as donor animals for transplantation and as an alternative to xenotransplantation.

An example of a very controversial experiment is the engraftment of human neural stem cells into the brains of old world monkey embryos. There are several critical issues regarding the ethical debate on human-animal chimeras combined in this experiment: Firstly, the cell populations were transplanted at an early stage of development, which resulted in widespread migration and distribution of cells. Secondly, one of the two body systems where the generation of interspecies human-animal chimeras gives rise to concerns, namely the brain, was affected. An involvement of the brain is problematic because the sensitive and cognitive abilities of the chimera might no longer fit the known categories. The second problematic area is the germline, because the chimeras might be able to produce human sperm or eggs and by interbreeding could produce human offspring. Finally, the close relationship between the two species (human and old world monkey) is problematic, because of the low anatomical and physiological barriers to successful and consequence-bearing chimerism. In contrast to this, it is e.g. unlikely that the structural complexity of a human brain that could bring about any significant degree of humanlike mental capacity could develop in the limited size of a mouse skull.

An example for an ethically less controversial experiment is the generation of human-animal chimeras by the injection of human haematopoietic stem cells into the foetuses of sheep in the middle of gestation. In the livers of the resulting sheep-human chimeras, up to 20 % of human hepatocytes were found. Whereas the first aim was to cure unborn children suffering from genetic defects in utero, now the idea of growing humanised organs for transplantation to patients with liver failure has gained ground.

Chimerism in transplantation medicine: According to the definition, a human-to-human organ transplantation patient or a blood transfusion patient would also be chimeric. After solid organ transplantation of livers, kidneys, hearts and lungs, donor cells/donor DNA has been discovered not only in the graft environment but also in the blood of the recipient. After a blood transfusion, blood cells of the donor exist in clinically relevant amounts in the recipient for 21 days. Afterwards,

the establishment of haematopoietic microchimerism has been described in patients.

Chimeras resulting from xenotransplantation: After receiving an animal xenograft, the human patient would be chimeric in the following way: The animal cell population in the human-animal chimera would represent just a very small portion compared to the total human cell population. The transplanted cells, tissues or organs would contain cells in a state of terminal or advanced differentiation. Therefore, further uncontrolled differentiation or malignant dedifferentiation is not to be expected. As the development of the human patient would be completed, uncontrolled dissemination or migration of animal cells is expected to fall only into the range of microchimerism. Consequently, the chimerism displayed by the xenotransplantation patient is expected to be similar to that of an allotransplantation patient. Still, the difference of interspecific chimerism asks for the solution of the questions of how immunological, physiological and anatomical barriers between the different species can be overcome and how the infectious risk can be minimised.

bb. Transgenic animals carrying human genes*

Marion Weschka

Transgenic technology enables the researcher to manipulate a specific gene systematically. Consequently, it makes it possible to gain a multitude of insights into biological processes such as gene regulation, cellular signalling or organismic physiology. Moreover, transgenic animal models of human diseases also help to discover, validate and test new drug targets. This summary will describe the technology for developing transgenic animal modes, list examples of animals with human genes and finally outline some ethical aspects of this research.

A transgenic animal is defined as an animal whose genotype was modified by the introduction of a foreign DNA (called transgene), which is transmitted through the germ line. The term 'transgenic animal' also covers knockout animals, which carry a targeted gene deletion or other modifications in their genome.

Transgenic technology

Currently, there are three different methods used to create transgenic animals: 1. microinjection of DNA into the pronucleus of a fertilised oocyte, 2. retrovirus-mediated gene transfer and 3. gene targeting in embryonic stem cells.

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* This summary written by Marion Weschka is based on the report "Transgenic Animals Carrying Human Genes: Methods and Ethical Aspects" by Michael Bader; see below at Annex A.I.1.

The Microinjection technique: For the creation of transgenic animals by pronucleus microinjection of DNA, the exogenous DNA is injected directly into the male pronucleus of a fertilised ovum. Then, the microinjected embryos are transplanted into the oviduct of a pseudopregnant female, where they develop to term. In rats and mice, about 5-30% of the newborns, the so-called founders, have integrated the transgene into their genome. These founders are mated with wild-type animals to establish transgenic lines.

Although the mechanism of transgene integration is not fully understood, it is preferable if the integration occurs during the first zygotic replication before the male and female pronuclei are fused, because then all cells of the embryo, including the germ line cells, will contain the transgene. Mostly, the microinjection technique is applied either to overexpress the gene of interest or to study transcription regulatory elements with a reporter gene. (Transcription is the process through which a DNA sequence is enzymatically copied by an RNA polymerase to produce a complementary RNA, i.e. the transfer of genetic information from DNA to RNA.)

As the insertion of the transgene occurs at random, the integration site is not predictable and the transgene may come under the control of the genetic neighbourhood. Because of this and several other problems, the expression level of the transgene and its pattern of expression is not completely predictable. Therefore, it is necessary to generate more than one founder and accordingly more than one line of animals for each transgene, because only similar changes in the phenotype of different transgenic lines can be attributed to transgene expression. Another difficulty is that sometimes embryonic lethality may occur due to toxic effects of the transgene product. However, there are methods that allow for the tissue-specific or developmental stage-dependent switch-on of a transgene and therefore help to overcome this problem. These methods help to generate animals with a silent transgene, which can be activated at any time in the life of the animals by the application of certain drugs. Despite all these limitations, microinjection is still the most frequently used method to generate transgenic models in different mammalian species for the overexpression of a gene.

Retrovirus-mediated gene transfer: Historically, however, transgenic animals were for the first time successfully created by infection of preimplantation mouse embryos with retroviruses. Here, a single copy of the transgene is incorporated into the genome in a site-unspecific manner by the virus integration machinery. The generation of transgenic animals with oncoretroviruses results in the efficient integration of proviral copies in germ-line cells. However, the number of provirus integration sites in the genome varies substantially from one animal to another ranging from one to more than twenty. Additionally, only low to undetectable levels of transgene expression result. However, recently, a lentiviral technology established for cultured cells proved to be an efficient tool for the generation of transgenic mice and rats, because they can infect non-dividing cells and overcome the problem of epigenetic repression.

Marion Weschka

Gene targeting in embryonic stem cells: Since about 15 years ago, the so-called knockout-technology (gene targeting in embryonic stem cells) allows for the total ablation of genes or the specific exchange of one gene by a modified version. This technology is based on two methods: (1) the permanent culture of embryonic stem cells and (2) the targeted disruption of a gene by homologous recombination.

The generation of gene-targeted mice is based on ES cells that have been isolated from the inner cell mass of mouse blastocysts and remain pluripotent in cell culture. In these ES cells, targeted alterations of a gene can be performed by homologous recombination. With suitable DNA-constructs, segments of genes can be replaced by in-vitro engineered DNA-fragments, and, for example mouse genes can be replaced by human genes. Accordingly, in the knockin mouse, the human gene expresses in a correct tissue and time specific manner.

After the gene alterations have been performed in the ES cells, the cells are retransferred into host blastocysts, which are subsequently transplanted into a foster mother. In the first generation, chimeric animals are born consisting of the targeted ES cells and the cells of the inner cell mass of the host blastocyst. These chimeras are bred with normal mice. If the targeted ES cells have colonised the germ line, animals will be born that carry the genetic alteration in all cells of their body, but only on one homologous chromosome. Consequently, they still have a normal allele of the targeted gene. If these heterozygous animals interbreed, homozygous mice are born which carry only targeted alleles of the gene of interest. However, for reasons which are yet unknown, so far, germline-competent ES-cells can only be established from mice, but not from other species.

Examples of transgenic animals with human genes

Transgenic rodents with human genes are mostly generated for the functional analysis of the relevant gene. Whereas it is not strictly necessary to use human genes for this research purpose, for the development of drugs, it is essential to use human genes. Examples are the generation of (1) double transgenic rats for the human renin angiotensin system, (2) transgenic animals with the human kinin B1 receptor and (3) transgenic mice with a human T-cell receptor.

(1) The human renin-angiotensin system is an important system in cardiovascular regulation and in the pathophysiology of diseases affecting heart, kidney, vessels and brain, such as hypertension, myocardial infarction, heart failure, arteriosclerosis, renal fibrosis and stroke. For most cardiovascular diseases, the renin-angiotensin system is the most efficient drug target. Pharmaceutical companies are interested in developing a new class of drugs affecting this system – inhibitors for the enzyme renin. As human renin is rather different from rodent renin, potential drugs cannot be tested in normal rodents. Therefore, rodents with the human renin gene must be developed. Moreover, it is also necessary to substitute the rodent angiotensinogen by its human equivalent. Thus, double transgenic animals are needed, which have been developed several years ago with the microinjection technique. These double transgenic rats develop severe hypertension with all sub-

sequent damages of heart, kidney and vessel. Therefore, they are ideal models for the testing of human-specific renin inhibitors.

(2) Bradykinin is a potent biological peptide with many functions in the cardiovascular system and during inflammation and pain. It works via two receptors: B1 and B2. The B1 receptor is especially interesting for the pharmaceutical industry as a target for new antialgesic drugs. As human and rodent B1 receptors are very different, humanised rodent models had to be developed which carry transgenic B1 receptors in order to test antagonists for human B1 receptors. Initial results show that in the transgenic animals (rats and mice) human-specific B1 antagonists bring about significant effects, which prove the suitability of this approach.

(3) Due to a lack of animal models mimicking all aspects of multiple sclerosis, only limited and ineffective therapies are available so far. In order to overcome this problem, mice carrying a human T-cell receptor gene from an MS patient have been generated. If these mice are crossed with knockout mice lacking their own T-cell receptors, the resulting mice develop a progressive disease that closely resembles MS. Consequently, these mice are suitable models for the testing of new therapies.

Ethical considerations

Mammals including humans have around 25000 genes with only very few differences in each single gene between the different species. Moreover, there are hardly any genes that can be found only in humans and not in animals. Still, biological humanity must depend on the genome. Accordingly, the question of which genes make a human and how they do it is still unanswered. Most probably, the interaction of a number of genes with slightly different functions between animals and humans, especially those active during embryogenesis, are responsible for the morphological and physiological differences between the species, especially regarding the brain. According to estimations, between some 100 to some 1000 genes are involved in these processes that define the appearance and behaviour of a certain species. It is fairly certain that there is not one single gene that makes a human. Consequently, the addition of one human gene to the genome of a rodent does not create an animal that is more human, because the human genes in the relevant experiments only affect physiological processes that are not fundamentally different between animals and humans, such as blood pressure regulation or the immune system. Therefore, these experiments are no more ethically problematic than transgenic animal research in general.

However, if primates were involved, humanising experiments with considerable ethical problems could be imagined. Chimpanzees are our closest neighbour on the evolutionary tree, with roughly 99% genetic identity. The most obvious differences are the size of the brain and the ability to speak. It is unknown which and how many genes are involved in these characteristics that would have to be changed to bring about human characteristics in a chimp.

Some insight comes from genetic diseases in humans, which affect language, e.g. the disorder called verbal dyspraxia. Verbal dyspraxia patients have problems with the expression and articulation of language due to an orofacial movement disorder. According to genetic studies, a single gene causes the disease. A comparison of this gene between humans and chimpanzees shows that two amino acid changes only occur in humans –quite a big difference compared to the usual homology. If a transgenic chimpanzee was generated carrying this human gene and if it were therefore able to speak and learn a spoken language there would be a considerable ethical dilemma. Further candidates that could potentially create ethical problems are genes involved in the determination of brain size, which are defective in families with microcephaly. There may be many more.

Therefore, one can conclude that all experiments with human transgenes performed in rodents do not really create ethically problematic hybrids, because too many genes would have to be humanised. However, if transgenic technology was used on primates, ethical problems could arise after relatively limited genetic manipulations.

cc. Nuclear transfer research*

Andras Dinnyes, Marion Weschka, Qi Zhou

Nuclear transplantation (NT) is a technology of recombining an enucleated egg with a new diploid nucleus. The result of this procedure may have the potential to go through embryogenesis and even develop into an adult. In intraspecies NT, nuclear donor and recipient egg come from the same species, whereas in interspecies or cross-species NT both come from different species. Intraspecies NT offers the possibility to study the totipotency or pluripotency of differentiated nuclei, while cross-species NT allows research into the interaction between nucleus and cytoplasm involved in development.

Historical Development of NT research with animals

The first reports on NT worldwide date back to 1952, when Briggs and King performed cloning research with frogs. Chinese scientists firstly specialised in interspecies NT with fish in order to explore the contributions of nucleus and egg cytoplasm to vertebrate development. In 1963, the first interspecies cloned fish were produced in China using carp and goldfish. The common carp and goldfish belong

* Summary of the Reports "Interspecies Nuclear Transfer Research in China" by Qi Zhou and "Nuclear transfer, chimeras and hybrids: Activities and legal aspects of research in Hungary" by Andras Dinnyes. The information regarding the "Key elements of NT" is taken from the Dinnyes-Report, everything else from the report by Qi Zhou. See below at Annex A.I.3 and 4.

to different genera. They hardly mate in nature, but can be artificially hybridised. However, the survival ratio of the offspring is very low and the offspring is generally sterile. This research showed that the donor nuclear genome contributes to the nuclear genome of the cloned fish in contrast to the recipient egg. However, most of the donor nuclei could not be completely reprogrammed and only a few NT embryos developed to term. Only about 1 % of the carp-goldfish NT embryos developed to adult stage. The fact that most of the apparent characteristics of the resulting fish resembled those of the nuclear donor, the carp, shows that the carp nuclei directed the development of the cross-genus cloned fish. The NT experiment also works in the opposite direction with goldfish nuclei being transferred into carp oocytes. Apparently, the recombination of nucleus and egg cytoplasm from different species of fish is more feasible than that of other vertebrates – maybe because fish were the first vertebrates to evolve.

As the mammalian egg is less than 0.1 % the volume of an amphibian egg, successful NT technology in mammals required the development of micromanipulation techniques that could handle, enucleate and fuse a very small mammalian egg with a single somatic cell. These techniques were developed in the late 1960s and early 1970s. After initial cloning research in mammals had not been very successful, in 1986, NT of embryonic sheep cells into enucleated sheep eggs resulted in the birth of two healthy cloned animals. Subsequently, NT with embryonic donor cell nuclei was successfully performed in rabbits, pigs, mice, cows and monkeys. In 1997, Campbell and Wilmut used nuclei of cultured adult mammary gland cells for NT, which resulted in the birth of the sheep Dolly.

However, mammalian NT cloning is as inefficient as NT cloning of amphibian. Less than 1% of all nuclear transfers from adult or differentiated cells result in normal offspring and developmental and physiological abnormalities have been observed in a significant number of cases. As many of these abnormalities are not inherited, it seems that they are not caused by deficient chromosome replication, but rather by a failure to reprogram epigenetic characteristics of somatic cells, especially imprinted genes.

Key elements of NT

Nuclear reprogramming and epigenetics: The process of returning a differentiated somatic nucleus to a totipotent stage is called nuclear reprogramming. In this process, genes which were deactivated due to cell differentiation are reactivated. Scientists believe that the inactivation of genes during cell differentiation involves epigenetic modifications of chromatin. Epigenetics is defined as nuclear inheritance which is not based on differences in DNA sequences. As regards nuclear transfer, the somatic nucleus carries the specific epigenetic modifications of its tissue type, which must be erased during nuclear reprogramming. The reprogrammability of donor cells might be affected by the levels of epigenetic modification. The treatment of donor cells with pharmacological agents to remove some epigenetic marks before the nuclear transfer may improve the ability of the donor cells

to be fully reprogrammed by the recipient karyoplast. However, several animal experiments showed that the reprogramming of epigenetic marks by the current nuclear transfer technology is abnormal. The more the processes of epigenetic reprogramming in cloned animals are understood, the more the cloning technology will improve, because it can then take place under ideal conditions for a complete reprogramming of the somatic nucleus.

Genetic imprinting: Usually, the maternal and paternal genomes are essential for normal embryonic development. The different maternal and paternal contributions are mediated by genetic imprinting, an epigenetic mechanism by which the monoallelic expression of these genes depends on whether they are inherited from the mother or the father. Most imprinted genes are involved in foetal growth regulation, and the disturbance of normal imprinting as seen in human patients can result in severe developmental abnormalities. Apparently, nuclear transfer can cause imprinting disruptions, because many developmental defects in cloned animals are similar to experimentally created imprinting disruptions in mice and naturally occurring imprinting diseases in humans.

Choice of the nuclear donor: The first step in cloning is the choice of a nuclear donor. There are two broad categories of cloning depending on the epigenetic status of the donor cells: embryonic cloning and somatic cloning. The success of cloning decreases as donor cells differentiate. Accordingly, embryonic cloning is generally much more efficient than somatic cloning. Although abnormal phenotypes also occur in blastomere cloning (e.g. oversised foetuses and higher perinatal loss), their incidence and severity are greatly reduced. This might be due to the blastomeres retaining an epigenotype, which is more compatible with early embryonic development.

In animals, less than 10% of the 200 morphologically distinguishable cell types have been tested as nuclear donor and many repeatedly failed to generate viable offspring. In the mouse model, live offspring were obtained after nuclear transfer with fibroblasts from various tissues (skin, liver, muscle and gonads), Sertoli and cumulus cells. Most cell types repeatedly failed to develop after implantation, including macrophages, spleen and brain cells. In cattle, live offspring resulted from follicular and oviduct epithelial, mammary gland, skin, liver and muscle fibroblasts and blood leukocytes. The best source of cells for NT for any given somatic cell type is still a matter of debate.

Intact donor genome: A potential prerequisite for successful cloning is an intact donor genome, because donor-derived chromosomal anomalies can considerably affect cloning success. This emphasizes the need for rigorous pre-screening of donor cells before NT. However, it is possible that higher aneuploidy rates can also be introduced by the NT procedure itself. On the other hand, there is no evidence that accumulating mutations during ageing or time in vitro affect cloning efficiencies. Cells after long-term culture or near the end of their replicative life span do not result in significantly reduced cloning efficiencies compared to fresh or short-term culture cells.

Mitochondrial heteroplasmy: However, mitochondria mutate at 10-20 times the rate than nuclear DNA and some components even mutate up to 100 times more rapidly. This may contribute to potential problems with mitochondrial heteroplasmy. Mitochondrial DNA in mammals is exclusively maternal in origin. The sperm only contributes up to 100 functional mitochondria at fertilisation, i.e. less than 0.1 % of the total number present in the egg cytoplasm. Moreover, all paternal mitochondria are selectively destroyed during early cleavage stages. In NT cloning, a variable number of foreign mitochondria are initially introduced into the oocyte during cloning. At later developmental stages, some, but not all cloned animals show low levels of mitochondrial heteroplasmy, which means the presence of more than one type of mitochondrial DNA within a cell. Theoretically, this heteroplasmy could result in pathologies. However, the link between heteroplasmy and cloned offspring-syndrome remains largely speculative.

Telomeres: Telomeres are protective structures that cap the ends of mammalian chromosomes in order to prevent repair mechanisms, which would bring about end-to-end chromosomal fusions. If there is no activity of the enzyme telomerase, the telomeres progressively shorten, which correlates with cellular ageing of human fibroblasts in vitro. However, the correlation between telomere length and ageing is weak and depends on cell type, species and culture conditions. If cellular senescence occurred in vivo and if it caused organismal ageing, cloning from adult cells could result in offspring with shorter telomeres, premature onset of ageing and a reduced lifespan. However, studies with cloned cattle and mice on the one hand and cloned sheep on the other resulted in conflicting data: the first two animal species showed normal telomere length after cloning, whereas the sheep had shorter telomeres. Therefore, potential effects on the overall longevity of cloned animals still need to be determined in long-term studies.

X chromosome inactivation: Another problem of NT cloning might be X chromosome inactivation. In somatic NT cloning, the cloned zygotes receive one active and one inactive X chromosome from the donor cells, whereas in naturally fertilised female zygotes, both X are active. It is unclear whether the inactivated X is reactivated during nuclear reprogramming or whether the X chromosome inactivation pattern is maintained in the cloned animals. Cattle studies showed aberrant X chromosome inactivation in cloned calves that died shortly after birth and placental abnormalities in live and deceased cloned calves. However, reprogramming of the inactivated X chromosome in cloned female animals seems to be different in different species. Even if there were a problem with faulty re-activation and subsequent non-random inactivation, this would not play a role for male cells and those 50% of somatic female donor cells with a paternal inactive X. As studies which compared the cloning-competence of male and female somatic donors found no significant differences in male and female tail-tip fibroblasts, it could be demonstrated that abnormal X chromosome inactivation is not the main limiting factor of nuclear transfer.

Choice of nuclear donor cell cycle stage: Cell cycle coordination between the nuclear donor cell and the enucleated recipient cell can increase cloning efficiency for two reasons: (1) by maintaining normal chromosome constitution (ploidy) in the reconstructed NT embryo and (2) by promoting epigenetic reprogramming of the donor genome. There is ample evidence for the importance of donor-recipient cell cycle coordination to maintain normal ploidy. Somatic donor cells in presumptive G0/G1 and G2/M-phase can result in offspring upon transfer into non-activated cytoplasts, whereas S-phase donors cannot. However, it is as yet unknown whether any of the cell cycle stages compatible with ploidy is better for epigenetic reprogramming and overall cloning efficiency.

Selection and enucleation of recipient oocytes: The oocyte cytoplasm contains factors that reprogramme the donor cell genome after NT. The comparison of oocytes of different developmental stages and maturation treatments for their utility during NT has shown that there is a subpopulation of recipient oocytes, which currently works best for somatic NT procedures: It consists of mature non-activated MII-arrested oocytes derived from developmentally competent follicles of slaughtered adult animals. Fertilised oocytes (zygotes) and early cleavage-stage embryos (2-cell blastomeres) have also been successfully used for early mammalian cloning experiments with embryonic but not with somatic donor cells.

Prior to NT, the genetic material of the recipient cell has to be removed or destroyed. The resulting enucleated recipient is called ‘cytoplast’. Efficient enucleation should completely inactivate the recipient genome without compromising viability and reprogramming potential of the cytoplast. In mammals, the most frequently employed technique is the use of a capillary glass needle to gently aspirate the chromosomes and some surrounding cytoplasm (the ‘karyoplast’) without penetrating the plasma membrane. Another more invasive method is the manual cutting in half of the whole oocyte with subsequent UV-selection and discarding of those halves containing the maternal chromosomes. Afterwards, in order to restore the original cytoplasmic volume, the two half-cytoplasts must be fused again. However, this procedure increases the risk of mitochondrial heteroplasmy. A third method is chemically assisted enucleation. However, this has so far only worked well for mouse metaphase I oocytes.

Nuclear transfer method: The introduction of the donor cell DNA into the recipient cell is called nuclear transfer, although usually not isolated nuclei but whole cells are transferred. Proper nuclear transfer is done by microinjection, whereas whole-cell NT is achieved via fusion. For microinjection, the donor cell membrane is mechanically ruptured within the glass injection pipette and then injected within a few minutes of isolation. The amount of co-injected donor cytoplasmic components (cytoplasm, organelles) and culture medium is small compared to electrical fusion approaches. However, fusion methods are easier to master and the most widespread method of NT by far. During fusion, all cytoplasmic and plasma membrane contents of the donor cell, including organelles,

centrosome and cytosolic factors are introduced into the cytoplast. The consequences of this are not clear.

Artificial activation: Cloning subverts the sperm-mediated fertilisation step that would normally lead to physiological activation of the oocyte. As mammalian donor cells cannot activate the recipient cytoplast, various artificial activation protocols have been employed in order to mimic the sperm-induced cellular events that typically occur during oocyte activation. However, artificial activation and fertilisation are not functionally equivalent and different developmental consequences might be expected resulting from different activation protocols.

In vitro culture of cloned embryos, embryo transfer and subsequent in vivo development: Cloned mammalian embryos are mostly cultured in vitro for various periods of time, usually until the blastocyst stage. For embryo transfer, the best clones are selected. Still, the rate of post-implantation survival is low, as most cloned embryos fail to develop into viable offspring. Cloned embryos are transferred into appropriate surrogate mothers of the same or a closely related species. Amongst somatic clones, a failure of the placenta to develop and function correctly is a common feature, which has been shown in cattle, sheep and mice, but so far not for goats and pigs. This indicates that species-specific problems with clones exist. A big problem are the losses in the second two-thirds of gestation; especially the rate of hydrops in cattle. Moreover, clones often have parturition difficulties because of an increased gestation period and an increased birth weight. Scientists try to overcome the birth weight problems by caesarean section.

Adult clone phenotypes and trans-generational effects: Somatic cell NT is characterised by a number of developmental abnormalities collectively referred to as the 'cloning-syndrome': it encompasses higher rates of pregnancy loss, prolonged gestation, higher birth weight, higher rates of peri- and post-natal mortality and specific adult phenotypes. Only about two-thirds of all clones born survive at least until weaning. Some claim that at least the long-term survivors can be physiologically normal and apparently healthy, showing normal behaviour, growth rates, reproduction and productivity. However, there are also reports of abnormal clone-associated phenotypes, such as higher annual mortality rates in cattle, reduced maximal lifespan and obesity in mice and compromised immune function in both species. The anomaly rate varies according to the species, genotype, donor cell status or specific aspects of the NT protocols, but it is not clear which of these anomalies could be eliminated by technical improvements. As offspring produced by mating males and females cloned from the same cell line is normal, this shows that abnormal clone phenotypes are epigenetic in nature and can be corrected during gametogenesis.

Inter-species NT

As regards inter-species NT, so far, only NT with donor cells and recipient oocytes from closely related species from the same genus (gaur or banteng and bovine; muflon, argali (wild-sheep) and domestic sheep; African wildcat and domes-

tic cat) resulted in pregnancies and progeny. Some of these animals died shortly after birth, maybe because of the incompatibility between the new nuclear material and the recipient oocyte mitochondria. A positive example was the cloning of an African wildcat male which successfully bred with wildcat females. Although the male was heterozygous for wildcat and cat mitochondria, the next generation contained only wildcat specific mitochondria.

According to unpublished research, Korean scientists generated a cloned wolf by transferring wolf donor cells into dog oocytes. The transfer of cow, sheep, pig, monkey, rat and human cell nuclei into bovine oocyte cytoplasm led to the early development of NT-units. In China, the transfer of somatic cell nuclei of giant pandas into rabbit oocytes was successful up to the blastocyst stage. In order to test whether the panda-rabbit cloned embryos can implant in the uterus of an animal other than the panda, about 2300 panda-rabbit cloned embryos were transferred into 100 synchronised rabbit recipients, none of which became pregnant. However, other research showed that panda-rabbit cloned embryos can implant in the uterus of a third species, the domestic cat.

In other interspecies cloned embryos (sheep-cow, pig-cow, monkey-cow, mouse-rabbit, cat-rabbit), the timing of development was donor-specific and similar to that of IVF-embryos of the donor species. However, it appears that the timing of development for giant panda embryos resembles that of rabbit embryos.

The mitochondrial DNA

The results of mammalian cloning studies regarding mitochondrial DNA of the resulting animal clones are controversial. Whereas the mitochondrial DNA of the first somatic cloned mammals and cross-species cloned mammals was homoplasmic, containing only recipient cytoplasm-derived mtDNA, some cloned mammals were mtDNA heteroplasmic, containing mtDNA of donor cells and recipient eggs. In early panda-rabbit blasocysts, mitochondria from panda somatic cells and rabbit ooplasm coexisted, but rabbit ooplasm mitochondria decreased and panda mitochondria dominated in early foetuses after implantation. However, results from different research suggest that maternal mtDNA replicates after the morula stage.

Fish interspecies NT research shows that the goldfish-derived mtDNA can exist in cloned embryos until the blood-circulation stage, after which it fades away. Accordingly, the mtDNA heteroplasmy in cloned embryos converted to mtDNA homoplasmy over the course of development. The resulting cloned fish is therefore a nucleo-cytoplasmic hybrid fish that contains a combination of common carp-derived nuclear genome and goldfish-derived mitochondrial genome.

Usually, most cloned animals are identical to their nuclear donor species in phenotype. As regards the carp-goldfish clones, accordingly, most developmental characteristics were the same as those of the nuclear donor carp. However, the analysis of somite development and vertebral numbers led to a surprising result: the vertebral development resembled that of the cytoplasmic recipient. The vertebral num-

bers, which have been considered an important element in taxonomic study, were in most cloned fish the same as those of the egg-donating goldfish and different from that of the nuclear-donor carp. Vertebral patterning is a result of somite patterning during embryogenesis, and the vertebral number varies greatly among different fish, but is relatively stable within a given species. Whereas the vertebral number of carp is 33-36, that of goldfish is 26-28. This research suggests that the somite number or segmentation clock of fish is determined in early embryogenesis under the regulation of egg cytoplasmic components during the formation of presomitic mesoderm. However, scientists still lack a comprehensive understanding of the molecular mechanism that controls the somite number and vertebral number.

To sum up, the present study shows that goldfish enucleated eggs receiving common carp nuclei cannot only support the development of the cross-genus nuclear transplants, but also have an evident impact on certain developmental characteristics, especially the somite development and vertebral number of the nuclear transplants.

Interspecies cloning in fish could be a useful method of preserving endangered fish species through cross-species cloning by transplanting the nucleus of the endangered species into the enucleated eggs of another well-populated species.

Future perspectives of NT research and obstacles

What results from NT research so far is that the genome is conserved during cell differentiation and that the cell cytoplasm has the potential to reprogram gene activity and redirect cell differentiation.

Reproductive cloning could be of potential value for animal husbandry, for the preservation of rare genetic stocks and maybe for the production of genetically identical stocks for research. Due to the many postnatal defects observed in cloned mammals, reproductive cloning is no option for human beings.

Another purpose of NT could be therapeutic cloning. As therapeutic cloning could provide donor cells of the same genetic constitution as the recipient, thereby potentially making immunosuppression after transplantation obsolete, it could have many potential benefits if applied to humans. The main ethical objection against therapeutic cloning is that a potential human being is created during the cloning process, which should not be used as a source for spare parts. On the other hand, in the absence of implantation, a reconstituted embryo has no possibility of becoming a human being. Moreover, seriously defective nuclear transplant embryos that cannot survive could still be a useful source for replacement cells.

However, the development of therapeutic cloning still has to overcome many obstacles, such as the limited supply of human eggs. According to some scientists, this limitation could be overcome by interspecies-cloning. As rabbit oocyte cytoplasm can reprogram human somatic cell nuclei, which then possess the properties and phenotypes of conventional human ES cells, some scientists believe that this

might be a potential future method to create human ES cells for any given human patient. However, the fate of the mitochondria of this human-rabbit clone remains unknown. Moreover, as nucleo-cytoplasmic combinations between species do not develop beyond the blastocyst stage, one might doubt that the use of non-human eggs is likely to be a viable alternative.

dd. Transplantation of human embryonic stem cells into animal brain*

Marion Weschka

Another interesting field of chimeric research concerns the transplantation of human embryonic stem cells (hESC) into the brains of animals, as in the Muotri case discussed below. This case is an example of basic research, which is undertaken in order to develop an in-vivo animal model to study the neuronal differentiation of human cells.

In the Muotri case, undifferentiated human embryonic stem cells from an already established stem cell line were transplanted into the brain of mouse embryos at embryonic day 14 using the following procedure: the mouse embryos were removed with the placenta from the anaesthetised pregnant mice and injected with hESC in the lateral ventricle. Subsequently, the mouse embryos were retransferred into the mice where they remained until birth. Delivery was normal without caesarean section. At different ages (2 and 18 months), the chimeric mice were killed and their brains analysed. The results showed that the undifferentiated human embryonic stem cells migrated and integrated to a large extent in the mouse brain, e.g. in the cortex, the hippocampus, the thalamus and in the striatum. A small number of cells integrated in the host tissue individually or in small clusters and adopted the size and shape of the host cells. This means that the human embryonic stem cells developed into mature cells of the neuronal and glial lineages. Neither immune rejection nor tumour formation was observed.

The results of this experiment could help to develop new animal models for neuronal diseases in the future. Moreover, new screening possibilities for therapeutic drugs, in particular combined with genetically altered human embryonic stem cells or a genetically altered host brain might arise. However, one must also distinguish this experiment from other possible experiments, such as the attempts of Irving Weissman to create a 100% human brain in mice or to use primates instead of mice as research animals. In theory, there are cases imaginable where from an ethical point of view the red line might be overstepped and where a quantitative alteration of the animal brain might lead to a qualitative change of its characteris-

* This summary is based on the scientific part of the case study regarding case 1 written by Gisela Badura-Lotter, see below at B.I.1.

tics. This would be problematic if human features could be demonstrated in the animal. In general, the chimeric alteration of an animal with human cells in the brain, which is morally and anthropologically the most important organ, raises a lot of questions regarding moral status, identity, capacities, etc. A preliminary ethical question – and in some countries also a legal question – which is not directly a question regarding chimeras, concerns the use of human embryonic stem cells, because human embryos have to be destroyed for the creation of the stem cell lines. Finally, one might also raise questions with regard to conceptual problems, such as whether the transfer of human embryonic stem cells into the mouse brain makes a valuable model for normal human neural development, etc.

To sum up, this is a case which has a lot of potential and is not, as such, overly problematic from an ethical point of view. However, similar experiments need to be monitored to avoid crossing the ethical red line.

ee. Hamster test: Testing human sperm capacity with hamster ova*

Marion Weschka

An example of the creation of a human-animal hybrid is the hamster egg penetration test. In this test, a hamster ovum is used for testing the fertilisation capacity of human sperm. The aim is to develop an animal-in-vitro-testing-system as a substitute for human ova in order to reduce the wasting of human oocytes and costly IVF-cycles. As this is an artificial combination of very distant species, it is expected that the resulting entities will not lead to viable embryos.

In detail, the following procedure took place: Mature unfertilised ova were collected from the oviduct of superovulated female golden hamsters. 16 to 18 hours after the induced ovulation, the hamsters were killed, their oviducts excised and the oocytes collected. In vitro, the zona pellucida of the oocytes was removed. Moreover, fresh sperm was collected from adult male donors. The sperm was processed, incubated at 37°C and kept at room temperature to capacitate for 16 to 18 hours. Afterwards, in vitro insemination was performed: Sperms were added to the oocytes and both were co-incubated at 37°C for the following 3 hours. Then, the oocytes were placed on microscopic slides in order to observe sperm head decondensation as a parameter for sperm penetration capacity. The activated oocytes were not allowed to proceed beyond this stage. As a result, one can conclude that zona-free hamster ova can be used as a substitute for human ova for the assessment of the capacitation and acrosome reaction of human sperm.

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* This summary is based on the scientific part of the case study regarding case 8 written by Hans-Peter Bernhard, see below at B.VIII.1.

However, one has to consider that the positive results of the in vitro sperm testing do not necessarily mean that the relevant spermatozoa are also fertile under normal in vivo conditions. Still, normal human ova are better subjects for human fertility testing. However, if human ova are not available, the hamster test can be used instead. The use of the hamster egg penetration test as a clinical screening test for patients before their actual IVF cycle remains controversial due to the poor predictive value for the final outcome of IVF.

As no viable hybrid embryos are created by the hamster egg penetration test and as the activated oocytes are not developed further anyway, this test is rather unproblematic from an ethical and in most countries also from a legal point of view. Even in Germany with its restrictive Embryo Protection Act that prohibits the creation of hybrids by the fertilisation of an animal ovum with human sperm and vice versa, this test is legal because no embryo with the capacity to develop is created.

ff. Mixing of embryos*

Marion Weschka

A different possibility of creating human-animal chimeras would be the mixing of human and animal embryos. As this would be ethically highly problematic, so far no such experiments have been undertaken or, at least, no such experiments have been published. Therefore, the case is described using the animal-animal example of the sheep-goat chimera. One aim of this research was to study the factors that influence embryo-uterus interaction in different species.

In this case, pregnant goats and sheep were anaesthetised in order to surgically remove the embryos from their oviducts. Then, the embryos obtained from the two different species were fused. For example, one 8-cell sheep embryo was fused with three 8-cell goat embryos and the resulting combined embryo was transferred into the oviduct of a recipient goat, which carried the embryos to term. In total, the results of this experiment were eight live-born and fully viable sheep-goat chimeras. Their chimeric nature was visible by the wool and the horns.

A similar experiment on the human-animal level, carried out, for example, with human and chimpanzee embryos, would quite likely result in viable offspring. However, such an experiment would raise grave ethical concerns, because of the unclear status of the resulting chimera. Would it be a human being or an animal, or belong to a third, yet unknown, category?

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* This summary is based on the scientific part of the case study regarding case 9 written by Michael Bader, see below at B.IX.1.

gg. Interspecies gestation*

Marion Weschka

Also with regard to our last example, there is no case known where an experiment involving interspecies gestation has been carried out either with human embryos or with a human foster mother. Therefore, an example is described where rat embryos were transferred into mouse uteri. The aim of this research was to study the factors involved in normal pregnancy and pregnancy failures. Other cases are imaginable, where the aim could be to save endangered species.

The procedural setting was the following: Superovulated rat females were mated and killed four days later. The rat embryos in the blastocyst stage were removed and transferred into the uteri of pseudopregnant mice. On day 9 of pregnancy, the mice were killed, their uteri isolated and the embryos analysed.

Interspecies pregnancy works between closely related species, such as mice/rat, sheep/goat and different feline and bovine species. It might also work for different primate species including human, but this has not yet been tested and no obvious scientific reason exists to perform such an experiment including a human foetus or a human foster mother.

It depends on the definition whether the case of interspecies gestation can be labelled as a chimbrids case. However, it is clear that during pregnancy the foetus and the mother exchange cells and therefore, both become microchimeras which carry several cells of the other in the circulation. Accordingly, if human foster mothers or embryos were used in such an experiment, they would become microchimeras.

From an ethical point of view, the microchimerism as such would not be overtly problematic because it would not be likely to bring about a change of status. The problem arises if the pregnancy would be carried to term and if a human baby were born from an animal mother or vice versa: What status would it have? Would it suffer from the knowledge that is was born from an animal mother? What would be the physical influence of the microchimerism? Could it cause disease?

* This summary is based on the scientific part of the case study regarding case 10 written by Michael Bader, see below at B.X.1.

hh. Other mixtures

Matrixes

As shown by the matrixes below at pp. 57, there are many more animal-human or human-animal combinations imaginable.

Remarks on the matrix "Chimera and Hybrids"
Hans-Peter Bernhard

Attempts to classify hybrids and chimeras have been made by various organisations and authorities. Classification was performed based on donor and recipient considering the combinations of specific cell types, tissues and organs[116] on procedures[117] or on the resulting products.[118]

In order to provide an overview for ethical assessments and regulatory proposals the following matrix places major emphasis on procedures and resulting organisms. The matrix also contains information about the developmental stage and properties of donor- and recipient organisms. This is of some relevance because the listed procedures in many cases use biological entities recovered from persons, human foetuses or embryos being in a reproductive context and therefore subject to particular ethical evaluation and specific legal regulations.

The given compilation is within the scope of the Chimbrids project, and is restricted to interspecies animal-human and to reciprocal human-animal combinations. As an exception, recent experimental human-human combinations such as human somatic cell nuclear transfer into human oocytes are referred to only in the context of interspecies nuclear transfer experiments, which prepared the ground for experiments leading to therapeutic and (eventually) human reproductive cloning. Animal-animal combinations are not covered, although the experience gained and the results obtained with mouse models and others are essential prerequisites, and give early signals for future applications involving human entities.

The listed experimental procedures and results reflect the present "state of the art". Starting with "historical" basic and clinical research reports, the attempt was to demonstrate the development of concepts and techniques (for example, in the field of xenotransplantation). More recent experiments are included if they point to novel avenues and applications using new techniques and elements such as the derivation of embryonic stem cells from various sources, the transfer of somatic cell nuclei into enucleated oocytes and the cultivation and use of the resulting cy-

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116 Scottish Council on Human Bioethics, Edinburgh, 2006.
117 Beyleveld D. et. al., Chimbrid Report, Mannheim, 2006.
118 Shimoda M., Chimbrid Report, Mannheim, 2006.

toplasmic hybrid embryos (microchimeras) for research and therapeutic applications.

A retrospective compilation cannot provide an overview of what will or may be done in the long run. An extrapolation of developments in the field of basic research remains questionable due to the intrinsic quality of these necessarily explorative activities. As a consequence, the table preferentially refers to published key scientific experiments in order to provide a working tool for selecting a number of relevant and exemplary case studies to be evaluated by ethical and legal experts. Examples of procedures with already established applications are given, as well as descriptions of intended potential applications, because the table should present a scientific overview for foresighted ethical assessments and proposals regulating research and applications in the area of chimeras and hybrids. More remote and rather theoretical options and applications render the task rather more difficult. We chose to qualify these topics as being of "no apparent scientific interest". It seems important to consider such theoretical experiments, as one cannot exclude the possibility that they are undertaken for other reasons and may represent the type of intentional misuse most relevant for regulatory action.

Table A.1, Matrix Chimeras and Hybrids: Human-Animal mixtures (Chimbrids)

-	Human-Animal				Animal-Human			
Pro-cedure	Donor (Source)	Recipient (Stage)	Result (Organism)	Application (Examples)	Donor (Source)	Recipient (Stage)	Result (Organism)	Application (Examples)
Stem cell (SC) trans-plantation	Human: embryonic stem cell, adult stem cell	Animal: Blastocyst, Post-gastrulation embryo	Chimeric or-ganism do-nor/recipient ratio depend-ing on devel-opmental stage and evolution-ary relation-ship	Research on human devel-opment and differentia-tion [119]	Animal: Embryonic stem cell, Adult stem cell	Human: Blastocyst, Post-gastrulation embryo	Chimeric or-ganism do-nor/recipient ratio depend-ing on devel-opmental stage and evolutionary relationship	No apparent scientific in-terest
Cell, tissue organ trans-plantation	Human: Cells, Tissues Organs	Animal: Em-bryo, Postna-tal stages	Animal chi-mera	Studies of the human im-mune system [120]; oncology	Animal: Cells, Tis-sues, Organs	Human: Em-bryo, Postna-tal stages	Human chi-mera	Xeno-grafts for medical treat-ments [121]
Somatic cell nuclear transfer (SCNT)	Human so-matic nucleus	Enucleated animal oocyte	Human-animal cytoplasmic hybrid embryo [122]	Source of human em-bryonic stem cells for ther-apy, [123] Bioassay for reproductive cloning [124]	Animal so-matic nu-cleus	Enucleated human oocyte	Animal-human cyto-plasmic hy-brid embryo	No apparent scientific in-terest
Chromo-some trans-fer	Human so-matic cell	Animal em-bryonic stem cell	Animal with human chro-mosome(s)	Studies of human chro-mosome ex-pression[125]	Animal so-matic cell	Human em-bryonic stem cell	Human em-bryonic stem cell with animal chro-mosome(s)	No apparent scientific in-terest

[119] Muotri et al. (2005) Development of functional human embryonic stem cell-derived neurons in mouse brain. Proc Natl Acad Sci USA 102:18644-8; Lee et al. (2007) Stem cells act through multiple mechanisms to benefit mice with neurodegenerative metabolic disease. Nat Med, 13:439-447.

[120] McCune et al. (1988) The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. Science 241:1632-9.

[121] Fink et al. (2000). Porcine xenografts in Parkinson's disease and Huntington's disease patients: preliminary results. Cell Transplantation 9: 273-278; Bailey et al. (1985) Baboon-to-Human Cardiac Xenotransplantation in a Neonate. JAMA. 254: 3321-3329.

[122] St. John and Lovell-Badge (2007) Human-animal cytoplasmic hybrid embryos, mitochondria, and an energetic debate. Nature 9: 988-992.

[123] Chen et al. (2003) Embryonic stem cells generated by nuclear transfer of human somatic nuclei into rabbit oocytes. Cell Res 13: 251-263.

[124] Illmensee K. (2007) Mammalian Cloning and its Discussion on Applications in Medicine. J Reproduktionsmed Endokrinol. 1/2007: 6-16.

[125] O'Doherty et al. (2005) An aneuploid mouse strain carrying human chromosome 21 with Down Syndrome phenotypes. Science 309: 2033-2037.

Table A.1 (continued)

-	Human-Animal				Animal-Human			
Procedure	Donor (Source)	Recipient (Stage)	Result (Organism)	Application (Examples)	Donor (Source)	Recipient (Stage)	Result (Organism)	Application (Examples)
Gene transfer	Human cDNA library	Animal: Fertilised oocyte, Embryonic stem cell,	Animal with additional human gene(s)	Production of human proteins in animals[126] Animal models for gene and drug testing	Animal cDNA library	Human: fertilised oocyte, Embryonic stem cell	Humans with additional animal gene(s)	No apparent scientific interest
Embryo transfer[127]	Human embryo	Animal foster mother	Parent and offspring exhibiting microchimerism	no apparent scientific interest	Animal embryo	Human foster mother	Parent and offspring exhibiting microchimerism	No apparent scientific interest
Embryo mixing[128]	Human embryo	Animal embryo	Chimera	no apparent scientific interest	id	id	id	id
Gamete fusion	Human sperm	Animal oocyte	Activated animal oocyte	Clinical fertility testing[129]; historic[130]: Human /ape hybrid generation	Animal sperm	Human oocyte	Hybrid embryo	No apparent scientific interest

126 Ebert K. M. et al. (1991) Transgenic Production of a Variant of Human Tissue-Type Plasminogen Activator in Goat Milk: Generation of Transgenic Goats and Analysis of Expression. Bio/Technology. 9: 835-838.

127 Nan et al. (2007) Increased Th1/Th2 (IFN-gamma/IL-4) Cytokine mRNA Ratio of Rat Embryos in the Pregnant Mouse Uterus. J Reprod. Dev. 53:219-228.

128 Fehilly et al. (1984) Interspecific chimerism between sheep and goat. Nature 307: 634-636.

129 Yanagimachi et al. (1976) The use of zona-free animal ova as a test system for the assessment of the fertilising capacity of human spermatozoa. Biol Reprod 15: 471-476.

130 Rossiianov (2002) Beyond species: Il'ya Ivanov and his experiments on cross-breeding humans and anthropoid apes. *Sci Context.* 15:277-316.

Table A.2, Encompassing matrix

Oocyte/ Germ Cell	Activated Oocyte	Preimplantation				Postimplantation				Adult	
		Embryo				Embyo/Foetus					
		Germ (Line) Cell	Stem Cell	Somatic Cell	Embryo	Germ (Line) Cell	Stem Cell	Somatic Cell	Embryo	Germ(Line) Cell	Stem Cell
Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	No	No	Yes
Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	No	No	Yes
Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No	No	Yes
Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	No	No	Yes
Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	No	No	Yes
Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	No	No	Yes
Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No	No	Yes
Yes	/	No	Yes	Yes	No	No	Yes	Yes	No	No	Yes
Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	No	No	Yes
Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	No	No	Yes
Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No	No	Yes
Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No	Yes	Yes
No	No	Yes	Yes	No	No	No	Yes	No	No	No	Yes
Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	No	No	Yes
Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	No	No	Yes
Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No	No	Yes
Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No	No	Yes
Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Yes
No	No	No	No	No	Yes	No	No	No	Yes	No	No
No	No	No	No	No	Yes	No	No	No	Yes	No	No

Matrixes

IV. Ethics

Ethical Issues Raised by Chimeras and Hybrids – An Overview

Autumn Fiester, Marcus Düwell

1. Introduction

Ethical discussions about the development and use of chimeras and hybrids (hereafter: "chimbrids") are faced with a series of weighty problems. 1) First, not enough is known about either the research aims or the technical, political and social implications of this kind of research to evaluate the benefits, risks and moral implications of the research in the short- or long-term. 2) Second, the debate about chimbrids touches on a variety of important, but very diverse bioethical debates that need to be factored into a discussion of the moral permissibility of this work (e.g., xenotransplantation, animal ethics, human subjects' research, embryo research, abortion, etc.). 3) Third, there is no general, accepted normative framework in place for the evaluation of these novel technologies. And finally, 4) the conventional standards for moral evaluation of work involving human beings and animals presuppose a significant moral distinction between the status of animals and human beings; this distinction is infused throughout public moral discourse and legal regulation, as well as in normative ethical theories, regardless of the particular substantive moral claims defended by any one camp. But with chimbrid research, it is this very distinction between human beings and animals that is at stake.

The ethics report of the Chimbrids-Project will try to systematise two different levels of ethical evaluation of this research; it will then concretise this theoretical approach by applying the ethical analysis to specific case studies that focus on particular chimbrid projects.

To systematise the ethical analysis, the ethics group has laid out two different levels of evaluation: 1) first, a fundamental discussion about the moral status of human beings and animals and the criteria of moral status for different entities; and, 2) second, a more concrete discussion about specific moral considerations that apply to chimbrids research regarding, for example, the protection of animals, the evaluation of risks and benefits of this research, etc.

J. Taupitz and M. Weschka (eds.), *CHIMBRIDS - Chimeras and Hybrids in Comparative European and International Research,*
DOI: 10.1007/978-3-540-93869-9_4,

2. The Fundamental Debates on Moral Status: What Makes Entities Morally Significant?

a. Preliminary Remarks

Before outlining key positions in the moral status debate, it must, however, be mentioned that the "moral status" debate itself is in dispute in the field of moral philosophy. Several authors in philosophical hermeneutics or phenomenology (e.g. in the work of Emmanuel Lévinas) argue that we should forego theoretical debates about moral status since it is impossible to assess which relevant features determine the status of entities and why they matter. On this view, to judge that some features of entities are morally relevant and others aren't would require a commitment to a problematic and specious (Cartesian) ontology. Similar criticism is made by proponents of the school known as the "Ethics of Care." Certain traditions in political philosophy also critique the moral status debates arguing that it is the task of the procedures of political deliberation to make such determinations. When this critique is applied to biomedical research, these approaches presuppose that it is the role of political deliberation alone that has to decide moral permissibility of various research projects. And in the field of biomedical ethics, the very famous principlism-approach of Beauchamp and Childress criticizes the idea that ethical debates can be answered by referring to one or more decisive features that can ground moral status; they argue that a commitment to any such feature would imply a preference for or a bias towards one particular normative ethical theory, and they want to avoid prioritising one approach over another.

But although these critiques are leveled against the moral status debates, we argue that it is impossible to genuinely avoid making decisions about the moral status of beings. For example, an "ethics of care"-approach does make assumptions about the creatures or entities about whom we are obliged to care; they do *not* remain agnostic on this question. The procedural approaches of political philosophy that refer to deliberative democracy also already are making presuppositions concerning the status of those who are capable of participation in the deliberation in comparison to the status of sentient, but non-reflective, beings. And in the concrete application of the principlism-approach, it is assumed that beings that are able of autonomy have a greater moral status then other beings. Therefore, it is our view that decisions regarding the relevant features that ground moral status cannot truly be avoided and need to be justified and made explicit. That does not mean, however, that the debate about moral status constitutes the only relevant set of considerations in assessing permissibility of this research; this is worth mentioning because some philosophers hold that the moral permissibility of embryo research, or the creation of chimbrids, depends *solely* on the answers given in the moral status debate.

Here we will discuss three types of relevant features that have been used by various camps to secure moral status: a) sentience, b) rationality and autonomy, c) species-membership. Following that exposition, we will supplement this discussion with some reflections on the application of these features.

b. Moral Status Based on Sentience (i.e., on the ability to feel pain or suffer)

One candidate for the criterion of moral significance is the ability to feel pain or suffer, which for utilitarians is the criterion of central moral importance. If an entity is capable of suffering or of feeling pain, we have to take its interest into account. That means that only those entities are morally relevant which can feel pain, but the interests of those entities always have moral importance. In general, utilitarians hold the view that all equivalent interests (e.g., physical pain) have equivalent moral importance, though some theorists (e.g. P. Singer) claim that the specific ways in which rational beings (persons) perceive their future change the structure – and hence the moral value – of their interests. That means since rational beings are able to anticipate a future, their interests/preferences change significantly and that gives the preferences of rational being more weight (e.g., it gives us the prohibition against harming of sentient beings, but the prohibition against killing of rational beings). The question whether or not a being is a member of the human species is not at all relevant for proponents of this camp. The kind of protection that follows from this criterion of moral status differs significantly from other approaches, e.g. Kantian approaches, since utilitarianism does not have a concept like moral worth that elevates the status of human persons.

From this perspective, the production of chimbrids causes some specific concerns, but in many respects generates no new issues regarding moral status. Since it is not very likely that early embryos feel pain, there will be no objections against the production of chimeric-modified *embryos*. Similarly, in the production of chimeric-modified *animals*, there will be no other relevant aspects of moral status than the general regulations concerning the non-harming of animals. Since the species-boundaries are not relevant to moral status on this view, there are no new problems created by the production of cross-species entities or entirely new species, as long as there are no additional harms generated for these new entities. For utilitarians like Singer, however, it would be as well relevant if chimbrids would develop some kind of rationality and self-consciousness.

Three relevant objections against chimbrids, however, can be formulated:

a) The animal experiment *as such* – with its harming or potential harming of animals – would be morally questionable. But crossing species borders is not in itself morally relevant, unless we have reason to assume that the introduction of human cells into an animal would create specific harm to it. This can be called:

The Animal Suffering-Argument.

b) If chimbrid-organisms would proliferate uncontrolled in nature or the ecosystem, a possible negative impact on the environment could create on long-term harming effects. This can be called: *The Ecological-Risk Argument*.

c) The creation of a human being with significant animal characteristics or the creation of an animal which shows self-consciousness, rationality or agency would

be morally problematic on this view, if we had reason to assume that this being would suffer under this condition (and there are good reasons to assume this). This can be called: *The Human-Suffering Argument.*

c. Moral Status Based on Agency, Self-Consciousness or Rationality

Liberal, contractarian and Kantian approaches, as well as natural law theories, start with the idea that self-conscious, rational agents are specifically important morally. For views that see rational and self-conscious beings as the center of the moral universe, this status can be grounded in the idea of the dignity and rights of an autonomous agent (Kant, Gewirth); or, in communicative skills of human beings that makes them capable of deliberation (Habermas, Scanlon). Even the *imago dei*-tradition refers to those rational capacities that demonstrate that we are in God's image and likeness.

However, the reasons why those particular capacities (i.e., agency, rationality or self-consciousness) are assumed to be morally relevant vary significantly. In the contractarian (Hobbesian) tradition, the agent has to be taken into account since s/he is the partner in the social contract. Since we are potentially a threat to each other, the social contract is a form of mutual protection; therefore, only those beings have to be taken into account that are capable of mounting a – morally addressable – threat against others; a capacity that only agents have. In the Kantian tradition (and in a more modern version, in the work of Gewirth/Beyleveld), agency is a moral criterion because of the fundamental importance of agency for all kinds of moral and practical considerations. Kant sees the rational being as a being with inherent worth – at least insofar as rationality is the basis of autonomy or moral self-legislation.

It seems to be clear that insofar as chimbrid experiments create beings with these characteristics (agency, rationality or self-consciousness); questions of moral status are directly relevant. If animals are used for these chimera experiments, that would matter less in this tradition in general, and would not raise any special concerns (though it must be said that there are several *in*direct arguments for the extension of moral protection to animals, especially those animals where it might be possible or even probably that they have rational capacities at least to some extent).

But these chimbrid-specific moral restrictions only arise in cases where a direct intervention in the basic capacities of an agent is at stake. Chimbrid experiments may result in medical or scientific options that may in the long run become threats to the inherent worth of agents, and that may lead to unequal or unjust life conditions or the use of human agents as a pure means. Ethical approaches, however, will vary significantly concerning the question, to what extent do we have to take the long-term impact on human beings and their life-conditions into account in assessing the moral permissibility of this research?

d. Moral Status Based on the Significance of the Human Species

Another possible criterion for moral status that could be of some relevance for the evaluation of chimbrids is membership in the human species. To articulate this position, one could say: Being a member of the species "homo sapiens" is the feature that gives moral status to a being. Insofar as one alters the species, those interventions are morally in need of justification. This criterion can, however, be understood in two different ways. The first way of understanding this criterion would be the following: if we are of the opinion that human beings are morally relevant *because* they are members of a species that normally develops the morally relevant features of agency or rationality, for example, (and possibly demand moral protection for embryos, for example, because of their potential to become a being with these characteristics), then the underlying morally relevant feature is *not* membership in the species "homo sapiens," but rather the feature of agency, rationality etc. (see above). In other words, "membership in the species" is doing no real work. Relevant is only the species *insofar* the members of the species are normally having the feature like rationality etc.

The second way of understanding this criterion can be articulated like this: only if we argue that the membership in the species "homo sapiens" is morally significant *independent* of an internal connection to the above-mentioned features (rationality, agency) would this be a criterion *sui generis*. In general, however, in the philosophical tradition, it has *not* been the membership in the biological species *as such* that has been seen as grounding moral worth, but the membership of the species depending on the specific features of human beings as rational, self-conscious agents. This understanding enables "membership in the species" to do real work, but it is unclear why biological membership would be at all morally important. It is difficult to find a specific ethical argument that supports this criterion for moral status; therefore, this position seems to entail a kind of "decisionistic" position (what we find, for example, in a "divine-command" theory).

For the chimbrids debate, it is not clear what would follow if we view biological membership in the human species as the ground for the moral status. The question would be: how do we define the biological criteria in order to know whether or not a being is member of the species? One could either argue that chimbrids are by definition excluded from membership in the species and have no moral worth (since the sources used for their production are mixed), or one could argue that

such entities are members, and thus have moral status, and therefore the use of human material for the production of chimbrids is morally wrong in and of itself.

e. Moral Status Based on the Intrinsic Value of Animals

Especially in the context of an ethical framework that grants moral status due to our rational capacities and the self-consciousness of autonomous beings, the question arises: to what extent do sensitive, but not self-conscious, beings have to be taken into account morally? Some ethicists argue that we should take the perspective of such creatures into account in proportion to their human capacities. If we have strong moral obligations towards agents, then we should take the interests of animals into account to the degree that they have capacities of agency. If there are doubts to what extent creatures have those capacities, there would be precautionary reasons to treat them as if they were agents. That would mean that, at least for some apes (Menschenaffen), for example, there would be reasons to give them similar moral protection to humans.

In many of these ethical debates, the moral status of animals is described with the term "intrinsic value." That term emphasizes that animals are not only instrumentally important for human agents (i.e., a mere means to an end), but we must value them as intrinsically important, e.g. beings that are not self-conscious but have some kind of consciousness. They are not "things" that we can use simply for our purposes. On the other hand, this "intrinsic value" is not the same as "moral worth" and "dignity" in the Kantian tradition. So we don't owe animals the respect we owe to persons, but at the same time they are not pure means to human ends. One could say that the term is intended to emphasise that the dichotomous distinction between things and persons is not complete; there are not only things (means) and persons (ends), but there is a third category. It would therefore be morally permissible to use animals to some degree for the human purposes, if there are very fundamental moral rights at stake for human beings, but there is a strong need of justification for this use.

The term "intrinsic value" is, however, not used in a clear and consistent way. The notion "intrinsic value" can be used to describe in general all entities that we value in a not instrumental way (landscapes, works of art, exceptionally good wines and so on). The question is how that notion relates to the notion of "moral worth." In the animal ethics debate it's normally assumed that the animal has some value that is inherent to him, so independent from the fact that we find it pleasant or the like. But using the notion of "intrinsic value" does not tell us why we in a morally relevant sense *should* value animals. One can raise doubts about whether the notion of "intrinsic worth" clearly describes and justifies the moral relevance of animals. The concept will need some kind of justification to explain why the fact that those things have intrinsic value for us is a reason to grant moral obligations towards them. Of course, one can argue that these moral obligations are constituted by the value those entities have for human beings. In that case, there would be a moral obligation towards others to protect the things they value. But that would mean that the obligations towards animals would depend on the fact that there are hu-

mans that value them in a specific way, not on the actual value of the animals in and of themselves.

aa. Applications of Moral Status: Potentiality, Precaution and Symbolic Extensions of Status

The use of symbolic, precautionary or potentiality arguments is, in general, related to one or more of the above-mentioned morally relevant features that are the basis for assigning moral status.

In any case, precautionary and potentiality arguments can only be used in relation to the morally relevant features articulated by various camps in the moral status debate. Before we use precautionary and potentiality arguments, we have to answer the question, what kind of morally relevant considerations will possibly, or probably, be violated by this research? Only if we have answered this question can we say that there are precautionary reasons to avoid certain actions that may lead to a violation of these moral considerations.

bb. Applications of Moral Status: Potentiality

Concerning *potentiality*. This argument can only be used after answering the following question: "Potential to become 'what'?" The term "potentiality" is used in the context of many kinds of arguments (potentiality-, continuity-, and identity-arguments) in which we give a specific moral status to entities that actually do not have any morally relevant features, but under normal circumstances will develop into beings that will have them. These arguments are used to ground the moral significance of human embryos, foetuses or newborns – thus, all human beings not yet having the morally significant features, but who will. Sometimes the arguments are used in a non-gradualistic manner (i.e., granted full moral status from conception onwards); some authors, however, distinguish the moral status of persons versus potential persons.

The use of those arguments only makes sense under very specific ethical frameworks. For example, utilitarianism offers a way of weighing suffering and benefits between different individuals. But we need to ask: why should potential-suffering be aggregated in this concept? The same is true for a Hobbesian kind of a contract theory or rational choice theories: if this type of prudential social contract is asking us to take the interests of others into account because they are a potential threat for us, why *should* we take the interests of potential aggressors into account? A Kantian type of ethical theory, however, grants inherent worth to agents/persons/rational beings. If the rational being has inherent worth, then it makes sense to protect beings that have the potential to develop into such a being. It's worth mentioning that as well "intrinsic value"-theories that grant some moral status to animals could see the potential of an animal to develop such a status as a

reason for special protection. That argument however is not established in the debate.

Special considerations are important for gradualistic positions that hold, that an actual capacity of an embryo (e.g. brain activity, sensitivity, capacity to live outside the mother, birth) is crucial for its moral status, and think, that from that actual capacity follows the status as a person. It has to be mentioned that there are some gradualistic positions that are only referring to actual capacities for granting a moral status, but most of the gradualistic positions that are under discussions (e.g. those who making a distinction in moral status after day 14, or after the development of some brain functions) are in need of assumptions from some kind of potentiality argument. Why e.g. the brain activity or sensitivity could be morally relevant if we not assuming that it's the brain activity of a being that is developing to a person? The brain activity as such would be hardly a sufficient reason to grant a moral status.

cc. Applications of Moral Status: Precaution

Concerning *precaution*: If it is morally relevant to avoid the suffering of beings, then there are precautionary reasons to avoid situations in which it is probable that a sensitive being will suffer. If it is morally relevant to protect the inherent worth of a rational being, then there are precautionary reasons to avoid situations in which it is probable that the inherent worth of a rational being will be affected. But there are differences between these two views. In utilitarianism, the suffering of one being can be weighed against potential benefits of others. In the Kantian tradition, the precautionary reasons may have more weight if a severe violation of the inherent worth of some beings is at stake.

dd. Applications of Moral Status: Symbolic Meanings

Besides the impact of biological research for our health and security (e.g., the risks and benefits), there are other dimensions that may be morally relevant. Biology is making research with elements we fundamentally value in our life. Reproduction, sexual life, birth and so on are moments of human life that are full of symbolic meaning. The traditional religions, as well secular world-views and the arts are full of symbolic references to these dimensions of human life. For our self-interpretation, self-perception and what philosophers call our "being in the world," these symbolic dimensions of life and death are very important. For many people, those symbolic dimensions are of vital importance for their possibilities for human flourishing and their life-perspectives. Individuals who come from a religious tradition will perhaps formulate these symbolic dimensions in terms of a religious framework; other people will articulate it in terms of other frameworks.

This symbolic dimension is not necessarily formulated as a kind of taboo or universal prohibition in relation to biotechnology, but it may well have central impor-

tance for both a Kantian and a utilitarian moral framework. A rational agent could argue that elements of the natural world have symbolic importance for interpreting oneself as a free rational being, and therefore should not be used for arbitrary purposes. One could argue, for example, that some access to the experience of beautiful landscapes is necessary for one's development as a free and autonomous agent.

Thus, there are reasons to see the symbolic meaning we give to the natural world not only as relicts of a pre-modern, non- enlightened, or religious world-view, but to give these meanings importance in a modern concept of morality as well. Nevertheless, it seems to be difficult to determine precisely what importance this argument should have. It seems to constitute a very fundamental criticism of modern biological research in general, though not an outright prohibition. Concerning chimbrids: if one says that the distinction between animals and humans is fundamentally important for our world-view, then one could argue that it would be an attack on our symbolic order if the species-boarder is crossed in certain types of research. The incorporation of animal material into humans may lead to alterations that affect the appearance, the behaviour, emotions etc. As well due to the incorporation of human material into animals it may be possible that animals are developing capacities of a person. This would raise serious moral objections. For a person having features that he would experience as non-human and that would be attributed by others as non-human could be reason for serious identity problems. Due to the respect we owe to persons it is morally seriously problematic to make experiments that can lead to such identity problems. Furthermore it is important for humans to live in a life-world and a symbolic order that make it possible to recognise humans as humans and animals as animals. In general agents are able to perceiving another person spontaneously as a person and are able to act accordingly. The pre-reflexive orientation in the life world is from importance for our ability to develop a culture guided by moral recognition. If chimbrid experiments would develop humans with animal appearances, behaviour etc. that would have severe influence on this ability. Even if there are without technical interventions cases (freaks etc.) where persons are born with the appearance of an animal, it is morally problematic to make technological experiments that could lead to situations like that. These aspects could be from moral importance for different kinds of ethical theories.

But this kind of argument would be a fundamental criticism of all kinds of research in the chimbrids-arena. It would not be a criticism only of particular experiments. If we see the "symbolic argument" as an articulation of a world-view that relies on the central moral importance of autonomous, rational beings, it would be strange (to say the least) if this argument was used to restrict the exercise of our rational capacities to such an extent. On the other hand, this argument can at least show that the "symbolic dimension" of our lives has some fundamental importance that needs to be considered. The precise role of this argument in evaluating biotechnology is, however, difficult to formulate.

f. The Moral Status of Human Embryos and Chimbrids

The above considerations about the morally relevant features that determine moral status form the background for distinctions concerning the debates about the production of chimeric embryos.

a) For those ethical positions that give moral status only to entities that *actually* have interests, needs, etc., it doesn't matter whether or not the embryo is animal or human: If it doesn't feel pain or doesn't have interests, needs or preferences, it shouldn't be protected.

b) For those positions that give moral status to human embryos from conception onwards, it would make a significant difference whether or not the embryo is a human or an animal embryo. That would, in the light of the earlier discussion, presuppose either "decisionistic" position (e.g. divine-command-theory), or a strong use of the potentiality argument (or a combination of identity and continuity of development arguments).

c) For positions that evaluate the moral status of embryos depending on their potential to develop into a human being or a being that has morally relevant characteristics, their status would depend on the question of whether or not the chimbrid embryo is able to develop into a human being or a being with morally relevant characteristics, respectively.

d) For those ethical positions that make a fundamental distinction between pre-embryos and embryos, the Chen-case, for example, would be acceptable as long as no attempt would be made to develop the hybrid embryo beyond the stage of the 14-day.

3. Specific Moral Considerations in Evaluating Chimbrids: An Ethical Matrix

Having laid out both the various stances one can take on the moral status of human beings, animals and chimbrids and the criterion one would use to assess their moral significance, we now turn to a discussion of the specific considerations one should employ to decide the moral permissibility of any particular project in chimbrids research, once the fundamental question of moral status has been decided. In other words, from each of the various philosophical positions one can take on what makes entities morally significant, there is a set of concrete moral considerations we suggest one should use to assess the merits of individual research projects. These concrete ethical considerations fall into two broad categories: moral considerations in the field of research ethics; and, moral considerations in the field of animal ethics.

a. Preliminary Remarks

Before turning to the first set of concrete moral considerations, there is one residual issue remaining from the moral status discussion, namely, when does enough alteration in the chimbrid entity take place such that it begins to exhibit the morally relevant features that grant it moral status on the various philosophical positions? This "moment" – though of course it is more likely a continuum – might be referred to as a "Decisional Criterion for Status." This concept would refer to the point at which the modifications begin to raise concerns that the morally relevant features criterial for status were beginning to emerge (sentience, or the ability to feel pain; rationality, agency, etc.). The potential for how much cognition or of what type on a Kantian view, for example, makes a chimbrid project impermissible? The above discussion treats the criteria for moral status as discrete categories, but chimbrid research by its nature collapses those firm boundaries.

b. Moral Considerations in Research Ethics

There are six concrete moral considerations in the area of research ethics that are relevant to chimbrid projects: 1) freedom of research; 2) risks or costs to persons; 3) risks or costs in social or environmental context; 4) the type and importance of the benefits of the research; 5) the probability for success; 6) issues in informed consent; and, 7) additional patient protections needed. Each of these will be weighed differently not only by the proponents of different camps in the moral status debate, but also by various proponents inside any one camp. It is also possible that some moral considerations will not be deemed morally important by proponents of certain positions on moral status. This will be noted in the discussion as we proceed.

1) The first set of moral considerations in the area of research ethics concerns the freedom of research. Generally research is seen as a moral good because the broadening of our knowledge as such is seen as intrinsically valuable and because it can lead to morally valuable (esp. medical) technologies. It must, however, be mentioned that the freedom of research is not unlimited. Where exactly the boundaries need to be drawn will be judged different from different moral perspectives. Furthermore, is it often difficult to judge in advance whether or not specific research approaches will be useful for medical purposes, for example.

2) The potential risks posed to or costs incurred by persons who are directly or indirectly affected by a chimbrid project have also to be taken into account. The categories of persons include: the patients in a therapeutic setting or human research subjects in a clinical trial that utilizes chimbrids; persons who are indirectly related to the research or therapy; and unrelated third parties, who may ultimately be affected. For patients or human subjects, the risks include both the possible physical and psychological affects of the research or therapy, both in the short- and long-term. Concerns about the assault to an individual's dignity by receiving non-human cells or tissues, for example, would constitute a possible psychological risk. There may be unforeseen adverse physical affects that persist long after the chimbric experiment or clinical trial has ended. By "related persons," we mean

both donors of human tissue and family members of those involved in chimbric research or therapy, and their risks may also be physical or psychological. Individuals who donate gametes or foetal material that will be used in chimbric research, for example, may face particular emotional or psychological risks above those raised by non-chimbric research. A clear example of a risk to third parties would be unknown pathogens spread by xenotransplantation.

3) A second area of potential risk and cost relates not to human persons, but to the social or environmental context. By social risks, we mean financial burdens caused by chimbric research, or costs to the image, respect or social support for the scientific enterprise, etc. If chimbric research does not secure wide public support, but proceeds nevertheless, this could have serious costs to science, in terms of funding, acceptance of future therapies, etc. Environmental risks include any possible threat to the ecosystem, for example.

4) The type of benefit and the importance of that benefit for the human good constitute a third area of moral consideration for chimbrid research. Given the unknown costs and risks of this research to persons and their social context, the possible benefit to the human good needs to be very high to mitigate or outweigh those unknown and potential risks. A series of questions follows. What is at stake in this research? What is the research trying to achieve? How will the success of this research improve human life or well-being? How important is the addressed problem to scientific knowledge, to clinical applications, etc.? How will scientific progress be impeded if this project does not move forward?

5) But even if a chimbric project has a potentially high benefit to scientific knowledge or clinical medicine, the predicted probability for success also constitutes a possible moral consideration. A chimbrid project might have extraordinary benefit if it succeeded, but it may also have a low probability for success, which may deem it unjustifiable or impermissible on some views. Solid organ xenotransplantation may be an example of this type of project. Given the immense organ shortage world-wide, the possibility of safe xenotransplantation would be of tremendous benefit in clinical medicine; but the serious, and possibly insurmountable, problems in xenotransplantation research make its short-term success highly unlikely. Do we want to allow more leeway or licence for projects with a high probability for success, as opposed to experiments that are very preliminary? If so, then a high likelihood that the research will achieve its desired or intended outcome will weigh heavily in its favor in the moral assessment of the project.

6) A sixth moral consideration within research ethics is the amplification of the traditional concept of informed consent, now burdened with an even higher standard for appreciation of and assent to possible known and unforeseen risks. Can a patient fully comprehend the possible risks in therapies of this type? For example, how does one weigh the unknown risks of becoming infected with a zoonotic pathogen when deciding to consent to a xenograft?

7) Finally, the sixth moral consideration. Closely connected with a concern about a fully informed consent are concerns about the additional patient protections that may need to be in place in order for clinical trials involving chimbrids to move forward. Perhaps a patient advocate, for example, needs to be provided in order to ensure that the patient's or subject's interests are protected.

c. Moral Considerations in Animal Ethics

As in the category of research ethics, we find six moral considerations in the category of animal ethics: 1) pain and suffering; 2) substitutability; 3) animal quality of life; 4) the instrumentalisation of life; 5) species integrity; and 6) debasement or adulteration of life. Although the first four concerns are raised by conventional animal research, they may be intensified by projects involving chimbrids. The last two considerations are raised specifically by chimbric work.

1) As in all research involving animals, the most pressing moral consideration is the animal pain and suffering involved in the research. Although seemingly a concern that requires little explanation or elaboration, it is worth noting that animal pain and suffering in chimbric research may extend in ways that have not been seen in conventional animal research. The creation of new entities may inadvertently create new modes of suffering (for example, suffering that may be "psychological").

2) The second moral consideration under the rubric of animal ethics is a question of the possible substitutability of non-animal or non-chimbric models in the proposed chimbrid research project. Given the high moral stakes involved in this research for human beings and animals, it seems imperative that there be no other means by which researchers could investigate a particular question or therapy. This moral consideration comes directly from conventional animal ethics, but the concern is intensified here because of the potential for much broader implications of the work, beyond, for example, merely pain and suffering of sentient life to concerns about animal integrity, debasement, etc.

3) The quality of life of the animals used in chimbric research constitutes a fourth moral consideration. Like animal pain and suffering, this moral consideration preexists chimbric research, but research that uses or creates chimbrids may potentially exacerbate the already serious issue of the quality of life of these creatures.

4) The fourth moral consideration under animal ethics is a more general concern about what might be called the "instrumentalisation" of life, conceiving of sentient creatures as merely a means to human ends without being due any respect for their species-life that is independent of human intervention or creation. This concern in again not limited to chimbric work, though the creation of new genetic types by human hands may, for some, suggest a new level of objectification of animal life.

5) Unlike the first four moral considerations in this category, the fifth concern is specifically raised by chimbrid research. With the crossing of species boundaries – the defining feature of chimbrid work – there is a new concern about protecting

species integrity. If we define the concept as the "wholeness or intactness" of the animal, and we consider that wholeness or intactness as valuable and important, then many chimbrid projects threaten the integrity of animal life.

6) Related to, though not captured by, a concern about species integrity is a final moral consideration: the debasement or adulteration of animal life. Here the concern is the downgrading of an animal's function, rather than merely a concern about an alteration of the animal's form. The concern with debasement is that human beings by their actions are participating in the "degeneration" of sentient life that has value apart from the instrumental value for human beings. With debasement, the concern is not merely that the animal is altered significantly, but that animal's capacities, functions, or faculties have been reduced.

Table A.3a, Ethics Matrix Part 1

Ethical Domain	Moral Consideration	Application of Moral Status
Fundamental Questions of Moral Status	Interests based on Sentience ability to feel pain or suffer (Utilitarian) Explanation/Questions Is this a sentient creature? What is the set of interests of this entity? (Freedom from pain; ability to perceive future suffering, etc.) How are these interests to be weighed against the interests of other parties?	Precaution
		Symbolic Meaning
	Value of the Human Species Explanation/Questions Is this a member of the biological category: homo sapiens?	-
	Agency, Self-Consciousness, Rationality, Dignity (Kantian) Explanation/Questions Is the entity in question one with these important human characteristics? Is this an entity that can be said to possess human dignity? Will human dignity be compromised or affronted by this work?	Potentiality Moral status of human and human-chimeric embryos Explanation/Questions Is there a potential for assault to human dignity in the future, due to this work? What type of moral consideration is due to embryonic life? Is there special consideration due here because of the potential to develop into a human being? Three understandings of the moral status of the embryo (relative to actual): Potential = Actual Potential < Actual Pre-Embryos < Embryos
		Precaution
		Symbolic Meaning
-	Intrinsic Value of Animals	Symbolic Meaning
Decisional Criteria for Status	Turning point from quantity to quality Explanation/Questions When does a quantitative alteration turn into to a qualitative change at which point the considerations of dignity, agency, etc come into play?	-

Table A.3b, Ethics Matrix Part 2

Ethical Domain	Moral Consideration	Explanation/Questions
Medical/Clinical/ Scientific Research ethics	Freedom of Research	- instrumental value of freedom of research for technology, health care and other areas of human life, which can't flourish without it - intrinsic value that follows from the nature of free and autonomous beings
	Risks and Costs to PERSONS: To patients To related persons (donors, family members) To third parties (Physical/Psychological; Present/Future)	Actual and persisting risks for current and future patients Risk for third persons (new infectious diseases; embryo shortage) Costs to person's dignity
	Risks and Costs in CONTEXT Social Risks/Costs Environmental Risks/Costs	Perturbations in society/image of science … Risks to the ecosystem Financial burdens on society
	Status of scientific approach: importance of the project, possible benefits	What is at stake in this research? What is the research trying to achieve? How will success improve human life or well-being? How important is the addressed problem? For example: Insights into biological / pathological processes **…;**Clinical benefit (e.g., future treatments, new transplantable cells/organs); Progress of scientific knowledge and technical possibilities**…**
	Probability for "success"	How likely is it that the research will achieve its desired or intended outcome? Do we want to allow more leeway for projects with a high probability for success, versus experiments that are very preliminary?
	Informed Consent	Is the patient fully informed about the potential risks of this novel therapy/experiment?
	Additional Patient Protections	Is there a patient advocate who provides additional protections for the patient? Will there be compensation for injury in the case of an adverse event?

Table A.3c, Ethics Matrix Part 3

Ethical Domain	Moral Consideration	Explanation/Questions
Animal ethics	Suffering	Physical pain Killing procedures …
	Status of scientific approach: Substitutability	Can a non-animal model be used? Do we need to use human materials?
	Quality of life	Living conditions appropriate for the species; is the species-life of the animal protected…
	Instrumentalisation of Life "Treatment of animals 'pure means' rather than also as 'ends'"	Concern that we treat other beings as purely a means to our happiness; "instrumentalisation" of life; reinforcing the view of life as pure product Animals may be used for human benefit if they are also treated as entities with their own set of species-specific interests
	Species integrity	"wholeness and intactness of the animal and its species-specific balance" (Bovenkerk, Brom & Van Den Berg)
	Debasement/Adulteration "Degeneration of other living beings"	Is the function of the animal downgraded? Creating ridiculous creatures just to show that it is possible 'Dignity' of human action

V. Law

1. Legal tools and strategies for the regulation of chimbrids

Elisabeth Rynning

a. Introduction

When society is faced with emerging new phenomena or technologies, not least in the area of biomedicine and research, issues of regulation sooner or later arise. Some policy makers and stake holders will call for protective legislation, restricting the area of lawful activities and providing public control, whereas others will advocate extensive freedom, often in the interest of scientific and economic development. The overall picture is rarely simple, since conflicting and coinciding private and public interests form an irregular and complex pattern, to be investigated and contemplated by the responsible regulatory body. Openness, control and certain appropriate restrictions may be necessary to preserve public trust, actually facilitating a more liberal policy.[131]

One of the more important functions of legal norms is precisely to provide a just means for the balancing and protection of important interests in society. This can be done in many different ways and at various levels. Sometimes, legal norms are laid down at the international or supranational level, sometimes in domestic constitutional law or ordinary law enacted by parliament, and sometimes they are found in government ordinances or regulations issued by lower public agencies. Judge made law may also be part of the system, especially in common law countries.

When the need for new legal regulation is considered, the process must also include the assessment of alternative solutions, as well as the applicability of already existing legislation. Before setting out to develop a whole new system of regulations and decision-making bodies, tailor-made to handle a specific emerging activity or phenomenon, there must be an investigation and evaluation of what is already covered, at various regulatory levels, and what is actually unregulated or inappropriately regulated. There may be both advantages and disadvantages to an extended application of already existing regulation and procedures. This discussion is well known from the area of genetics, where the justification of so-called

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[131] Cf Nielsen, Linda. From Bioethics to Biolaw. In: A legal Framework for Bioethics (ed. Mazzoni, Cosimo Marco), Kluwer Law International 1998, pp. 39-52, at p. 43.

J. Taupitz and M. Weschka (eds.), *CHIMBRIDS - Chimeras and Hybrids in Comparative European and International Research,*
DOI: 10.1007/978-3-540-93869-9_5,

genetic exceptionalism has been debated.[132] At all events, any new regulation should be consistent with established basic legal principles and form a coherent part of the regulatory system in question.

As already mentioned, however, it may of course be argued that not all areas of biotechnology are suited to legal regulation. One reason often brought forward is the need for flexibility in areas of rapidly developing science and technology.[133] Other steering modes could be used to influence the behaviour of the public and various parties concerned, for example information and public debate, aimed at raising the level of knowledge and general awareness in a certain field, thus paving the way for responsible choices and self-imposed restrictions, also in the form of self-regulation.[134] Financial incentives can play an important role, for example in the way of funding policies or administrative fees and taxes. There may thus be situations where other alternatives are found more appropriate or effective than legal regulation. A combination of different approaches is often believed to provide the best results.

b. Determining the aims of the regulatory project

In any regulatory project, a primary task is thus to establish the overall *aims* and objectives. What is the perceived need for new or revised regulation in this particular area? This stage is about identifying the different interests at stake and the degree of protection that is needed and justified, and then deciding on the required scope of the regulation and what norms to be applied, based on a careful balancing of the interests concerned.

Identifying the interests concerned and the appropriate level of protection is perhaps primarily a task for philosophers, ethicists and experts in political science, but also for lawyers, when it comes to requirements of respect for human rights and other fundamental legal principles. Interests generally protected by law may include for example the improvement of scientific knowledge, public health and safety, sustainable environment and biological diversity, animal welfare, privacy and autonomy of individuals, human dignity and the genetic heritage of future generations.

Defining the appropriate scope of the regulation involves decisions on what kind of measures, activities or results should be covered by the rules. It must then be decided whether a more or less complete prohibition is called for, or if an appropriate level of protection can be reached by the introduction of certain restrictions,

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132 McNally, Eryl; Cambon-Thomsen, Anne et al. 25 recommendations on the ethical, legal, and social implications of genetic testing. European Commission, Science and Society 2004, p. 8-9.

133 Supra note 131, p. 39.

134 Eijlander, Philip. Possibilities and constraints in the use of self-regulation and co-regulation in legislative policy: Experiences in the Netherlands – Lessons to be learned for the EU? Electronic Journal of Comparative Law, vol. 9.1 (January 2005) http://www.ejcl.org.

conditions and controls. There is also the question of whether or not the norms can be sufficiently generalised. Even though a certain interest may generally be given precedence over other interests, there is often a need for allowing exemptions from the main rule. Such exemptions may entail a need for more careful consideration of the specific circumstances of the individual case. The final balancing of the interests involved may thus be delegated to a body or agency other than the one that has laid down the prerequisites for the operation. The wider the scope of discretion allowed this body, the more important are of course its qualifications for performing the balancing.

c. Identifying the appropriate regulator

As already mentioned, regulation could be introduced at different levels of society. Some issues are of such global interest that they warrant regulation in public international law and some fall within the remit of supra-national organisations such as the European Union, whereas others are considered to be primarily a question of national policy and regulation, maybe even state/provincial or local regulation. The appropriate level of the regulation is a question of legal competency, but also of suitability and principles of subsidiarity.

To a certain extent, the level at which different norms can be found may illustrate the perceived importance and universality of the interests they are aimed at protecting, for example in the area of human rights or environment. However, in some areas of society, pluralism of cultural and religious values, as well as legal traditions, can make it difficult to reach international or even regional consensus. Also highly important issues may thus be subject to binding regulation only at the domestic level, or even left unregulated.

d. The choice of regulatory instruments

At each of these levels, there is also a variety of regulatory instruments to choose from. One distinction often made is the one between formally binding hard law regulation and various types of so-called soft law, in the form of codes of conduct, declarations, recommendations, guidelines, policy documents etc. Even though soft law regulation is normally classified as rules that are not legally binding as such, the boundary between hard law and soft law is not distinct and the two types of regulation often interact in different ways. Soft law instruments may thus have certain – indirect – legal effects, and are aimed at and may produce practical effects.[135] Sometimes soft law can be viewed as a transitional mode of regulation, a precursor to binding legal instruments, but it may also be used as an independent, alternative steering mode, conveying power to actors that have only limited influ-

[135] Senden, Linda. Soft law, self-regulation and co-regulation in European law: Where do they meet? Electronic Journal of Comparative Law, vol. 9.1 (January 2005) p. 23 http://www.ejcl.org.

ence in traditional regulatory processes.[136] Soft law regulation can also be introduced in areas where "the legal competence [of the regulatory body] is weak or nonexistent",[137] and is thus a tool available to non-governmental organisations[138] as well as other actors with limited regulatory powers, for example the OECD. The status of soft law documents will thus be dependent on context as well as time. Guidelines issued for example by a ministry or by a competent public authority are likely to have a formally stronger standing than a code of conduct issued by a professional organisation. Nevertheless, the latter type of guideline may be indirectly binding, if considered to express the professional standard required by law, i.e. "good practice", or if it is explicitly referred to in binding regulation.

Although questions may well be raised concerning the democratic legitimacy of soft law,[139] it is argued to have certain advantages. Soft law is thus considered to leave more room for flexibility and rapid reactions, and is believed to be particularly useful "when dealing with complex and diverse problems that are characterised by uncertainty".[140] This type of regulation is therefore often used in areas of human rights and the environment. However, since soft law in itself does not provide any legal sanctions, such regulation alone would often seem insufficient where important interests must be guaranteed appropriate judicial protection.

Another distinction can be made between regulatory instruments with detailed substantive provisions (in the interest of predictability) and framework regulations that leave considerable scope for discretion and decision-making on a case-by-case basis (thereby meeting the need for flexibility).[141] Both hard law and soft law regulation can thus be specific and detailed or general and vague. This means that a need for flexible rules could be met also within the area of legally binding regulations, at least to a certain extent. Regardless of whether the rules are legally binding or not, however, decisions based on vague and flexible framework regulation will always be less predictable than those based on more specific and detailed rules. Finding the right balance between the need for flexibility and a sufficient

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136 Mörth, Ulrika. Conclusions. In: Soft Law in Governance and Regulation: an interdisciplinary analysis (ed. Mörth, Ulrika), E. Elgar Pub 2004, pp. 191-193, at p. 198.

137 Frykman, Henrik & Mörth, Ulrika. Soft Law and Three Notions of Democracy: The Case of the EU. In: Soft Law in Governance and Regulation: an interdisciplinary analysis (ed. Mörth, Ulrika), E. Elgar Pub 2004, pp. 155-179, at p. 163.

138 Consider for example the impact of the World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects (adopted by the WMA General Assembly in 1964, revised in 1975, 1983, 1989, 1996 and 2000). http://www.wma.net/e/policy/b3.htm.

139 Supra note 137, p. 155-170.

140 Mörth, Ulrika. Introduction. In: Soft Law in Governance and Regulation: an interdisciplinary analysis (ed. Mörth, Ulrika), E. Elgar Pub 2004, p. 3.

141 Tala, Jyrki. Lagstiftningsforskning – ett nödvändigt perspektiv inom rättsvetenskapen. In: Interaktiv rättsvetenskap (eds. Gräns, Minna & Westerlund, Staffan), Uppsala University 2006, pp. 139-162, at pp. 145-146.

degree of predictability is thus a challenge that must be addressed. One way of increasing the flexibility of a regulation could be to avoid focussing the rules on certain techniques or methods, and primarily identify results and risks to be achieved or avoided.[142] At the same time, this will of course be more problematic in areas where the potential risks and benefits, as well as more complex long term consequences in society, are difficult to foresee.

In many countries, the rules traditionally regulating animal research and biomedical research involving humans consist of framework legislation and/or guidelines to be applied at case-by-case assessment. More detailed hard law can be found in certain areas perceived to involve particular risks, such as clinical trials of medicinal products and, to a growing extent, research related to assisted human reproduction or the use of human gametes.

Legal regulation can take the form of material provisions, e.g. allowing or prohibiting certain activities or results, or stipulating the conditions under which they may be lawful. However, there are normally complementing general or specific procedural norms, dealing with the composition and remit of the decision-making bodies, as well as their management of cases. One way of achieving flexibility could apparently be to focus more on procedures and less on fixed material rules. Even so, there is still the problem that a generous margin of appreciation and delegation of powers to lower decision-making bodies could be considered inappropriate in areas where important values may be at risk. Such referred decision-making means less control for the democratically elected legislature, and could be in conflict with the basic principles related to the rule of law. It may also become difficult to develop a reasonably uniform application of the regulation if case-by-case assessment takes place at provincial or local level, unless there is an effective system for appeal. It is clear that case-by-case assessment with a wide margin of appreciation must be coupled with strict requirements regarding the decision-making bodies.

With regard to both animal research and research involving humans, as well as certain issues related to protection of environment, there is a clear tradition of ex ante review of planned projects and interventions. As for research involving humans, prior ethics review of research projects was required already in the 1964 WMA Declaration of Helsinki,[143] a precondition that has later been codified also in legally binding international instruments such as the 1997 Council of Europe

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[142] Cf Roscam Abbing, Henriette. The Convention on Human Rights and Biomedicine – An Appraisal of the Council of Europe Convention. European Journal of Health Law 5: 377-387, 1998, at p. 384.

[143] Supra note 138.

Convention on Human Rights and Biomedicine[144] and Directive 2001/20/EC on the implementation of good clinical practice in the conduct of clinical trials.[145]

e. Decision-making in individual cases

The bodies or agencies making decisions in individual cases may again be of very different types and function.[146] They may operate at an international, national, regional or local level and may be set up to deal with highly specific issues (such as measures with human embryos) or have broader, more general tasks (as for instance many courts). They may be expected to review individual cases or projects ex ante (granting approval, authorisation or licences) or ex post (checking the lawfulness of measures already carried out). The composition of the decision-making bodies may vary with regard to expertise, representatives of particular interest groups, lay members etc. They may be more or less independent in relation to different stake holders and the government. Their powers and the allowed room for discretion may be wide or limited, their decisions legally binding or advisory. Some of these bodies may publicise their decisions and motivations openly, whereas others will restrict their openness to a minimum. In some areas the competency of different decision-making bodies may overlap, causing duplication of work or rivalry and uncertainty regarding the correct forum, which may also leave certain issues "orphaned" in a situation where every agency potentially concerned considers them the responsibility of another.

f. Monitoring and sanctions

Finally, an effective regulatory system requires some kind of monitoring, supervision and sanctions to be applied in cases where stipulated requirements are not met or prohibitions are violated. The monitoring may include internal control functions aimed at facilitating quality assurance of the research or application in question, follow-up and reporting of adverse events. In addition, external supervisory agencies are normally needed for unbiased monitoring and follow-up, based on reports and notifications as well as inspections. Just as the decision-making bodies described above, agencies or bodies entrusted with this kind of supervisory tasks may be of many different types, with a remit varying from the very broad to the highly specialised. Private organisations and associations may have a role also in this area, for example professional organisations or ethics committees.

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144 Convention for the protection on Human Rights and dignity of the human being with regard to the application of biology and medicine: Convention on Human Rights and Biomedicine (1997, ETS no. 164).

145 Directive 2001/20/EC of the European Parliament and the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use, OJ L 121/34, 1.5.2001.

146 See for example Research Ethics Committees, Data Protection and Medical Research in European Countries (eds. Beyleveld, Deryck; Townend, David & Wright, Jessica), Ashgate 2005.