Presidenza del Consiglio dei Ministri

NATIONAL BIOETHICS COMMITTEE

CHIMERAS AND HYBRIDS

WITH SPECIFIC ATTENTION TO CYTOPLASMIC HYBRIDS

26th of June 2009

Introduction

The National Bioethics Committee (NBC) has approved the opinion Chimeras and Hybrids. With Specific Attention to Cytoplasmic Hybrids.
The NBC's opinion reflects in depth and accurately the committee members' opinions with regards to bioethical questions raised by the production of hybrids and chimeras, but especially by the production of cytoplasmic hybrids (cybrids), obtained through the technique of transferring the nucleus of a somatic human cell in a denucleated animal egg cell in which animal mitochondria can still be found. This technology has been the object of widespread debate in Great Britain after the authorisation issued by the Human Fertilisation and Embryology Authority (HFEA) to carry out this experiment. This is therefore a current topic that the NBC has deemed important to discuss also because of the prohibition of these practices in the law 40/2004 (Regulations for medically assisted procreation).
There are a variety of important ethical issues involved in these new methods of intervention: some concern the evaluation of scientific research and of the reasons given to defend its practicability; others regard the question of the identity of man and of the human species considering the creation, in a laboratory, of new identities mixing human and animal genetic material. This examination also had to take into account the issue of whether cytoplasmic hybrids are human embryos or whether the presence of mitochondrial DNA of animal origin makes them non-human, an issue that has not yet found a shared response in the scientific world.
Within the Committee there are a variety of opinions, some contrary to this research, others favourable although cautious, all presented in a broad and detailed manner.
Some Committee members have raised bioethical problems with regards to the moment when the created organisms are of uncertain identity, as they lead to overcoming barriers between the human species and animal species. Therefore, those members do not think that it is ethically acceptable to scientifically experiment in this way, as it alters the identity of the human being and of the human species, even if this research is carried out in the name of the possible increase in knowledge it could bring.
Other NBC members, believing that the fact that the experiments are not greatly justified by the scientific evidence they produce does not imply their immorality and not agreeing with the thesis that the embryo in his/her very first stages of development is due absolute protection (the situation in which cybrids are not destined to develop is highlighted), do not condemn their creation. They have however recommended a transparent and rigorous control of these types of experiments, which must be carried out with a cognitive purpose.
The opinion has been coordinated and drawn up by Prof. Assuntina Morresi with the contribution of the members of the working group (Prof. Isabella Coghi, Prof. Roberto Colombo, Prof. Maria Luisa Di Pietro and Prof. Lucetta Scaraffia) and with the written contributions of a variety of Committee members (Prof. Salvatore Amato, Prof. Adriano Bompiani, Prof. Roberto Colombo, Prof. Franceso D'Agostino, Prof. Lorenzo d'Avack, Prof. Carlo Flamigni, Prof. Marianna Gensabella Furnari, Prof. Demetrio Neri, Prof. Andrea Nicolussi, Prof. Laura Palazzani, Prof. Alberto Piazza, Prof. Lucetta Scaraffia, Prof. Monica Tpraldo do Francia and Prof. Grazia Zuffa).
In the plenary meeting of the 26th of June 2009 the document has gained the approval of those present (Prof. Salvatore Amato, Prof. Adriano Bompiani, Prof. Roberto Colombo, Prof. Francesco D'Agostino, Prof. Bruno Dallapiccola, Prof. Antonio Da Re, Prof. Maria Luisa Di Pietro, Prof. Riccardo Di Segni, Prof. Carlo Flamogni, Prof. Marianna Gensabella Furnari, Prof. Laura Guidoni, Prof. Aldo Isidori, Prof. Assuntina Morresi, Prof. Demetrio Neri, Prof. Andrea Nicolussi, Prof. Laura Palazzani, Prof.

Alberto Piazza, Prof. Vittorio Possenti, Prof. Monica Toraldo di Francia, Prof. Giancarlo Umani Ronchi, Prof. Grazia Zuffa), with the contrary vote of two members (Prof. Luisella Battaglia and Prof. Claudia Mancina). Prof. Stefano Canestrari, Prof. Lorenzo d'Avack and Prof. Silvio Garattini, absent from the meeting, expressed their agreement with the document. Prof. Luca Marini communicated his abstention.
To better clarify their reasons with regards to some issues discussed and the different conclusions reached in the opinion, two personal remarks have been added, respectively signed by Prof. Assuntina Morresi and Prof. Vittorio Possenti.

The President
Prof. Francesco Paolo Casavola

Document

1. Interspecies living beings

The issue of interspecies organisms is an important chapter in the ethical debate that for decades has discusses the problem of overcoming the barrier between the different species of living beings1.

This poses first of all the problem of defining the identity of the species: it was the use of recombinant DNA technology to raise, in the 60s and 70s, the debate on the existence of natural barriers between different species, and on the opportunity for scientists to one day overcome them2.

The NBC has tackled some aspects of this argument in two documents. In the first one, "The problems of collecting and treating human seminal liquid for diagnostic purposes" (5th of May 1991), dedicated to the tests carried out for diagnostic purposes, we highlight the problem of hybridisation tests (spermatozoon's penetration in hamster eggs); in the second, "Identity and status of the human embryo" (22nd of June 1996), we unanimously state that among the "morally illicit treatments of human embryos, at any stage of their development" we must list also the "creation of chimeras; the production of human-animal hybrids; the transferral of human embryos in animal uterus or vice versa".

Currently the production of interspecies human/animal embryos is prohibited by Italian legislation (L. 40/2004, art. 13).

The NBC believes that it is important to examine the problem directly, given its relevance in the national and international debate, both the in scientific, ethical and legal field as well as in public opinion.

1.1 The problem of the species

The old definition of species, with the principles fixed by Aristotle in the IV century BC, principles acknowledged and widened by Linneo (1707-1778) in type, order, class and kingdom has undergone – with regards to the fixist interpretation – a profound "revolution" with the Darwinian interpretation of living creatures' evolution. There are in any case (without in the document getting into a discussion on this point) a variety of difficulties if we want to adopt an omni-comprehensive definition of the concept of species on the basis of a range of parameters created in order to characterise it in various orders of living creatures (about twenty according to Scott Robert and Baylis3); the same difficulties are found when we want to investigate the mechanism of species formation (speciation).

That said, the most used criteria to define a species are the following:

typological (based on sharing characteristics),

biological (based on procreative capabilities),

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[1] The NBC document does not discuss the problems regarding the creation of mixed embryos through the manipulation of genetic and/or cellular material belonging to different animal species. We refer in this case not only to the issue of the use of somatic cells, but to the experiments aimed at the artificial production of new species. The NBC proposes to return to this topic with a specific reflection, stressing the importance of this issue, also in relation to the eventual pain of the living beings obtained.

[2] Cf. e.g. S. Krimsky, *Genetic Alchemy: The Social History of the Recombinant DNA Controversy*, MIT Press, Cambridge 1982.

[3] J. Scott Robert, F. Baylis, *Crossing species boundaries,* "Am. J.Bioeth", 2003, 3/3, pp. 1-13.

evolutionistic (based on descent)[4].
A still very widespread “popular taxonomy” allows us to classify – for practical purposes – a certain number of the most common living beings on the basis of shared typological characters.
Among modern criteria of great scientific value, it has certainly had considerable circulation the biological concept introduced by Mayr in 1940[5] and in 1959[6] and by Dobzhansky[7], of species as “reproductive isolation”, or lack of genetic exchange between two populations of individuals. The concept of genetic community has then been further clarified by Simpson[8] and by Wiley[9] (in the Darwinian sense); the last one stresses how a species descends linearly “from an ancestral population of organisms that maintain their identity with regards to any other evolutionary line and that undertake their own evolutionary journey with an individual historical destiny”.
It was the attention to the variations found between individuals grouped according to summary typological criteria, to lead Darwin to his theory of evolution, that is, to descent with modification. This way, the species nature and reality could be realised in genealogical links and the reconstruction of philogenesis could substitute a purely descriptive classification.
In this context, which is still today full of heated debates, have emerged – especially from evolutionist biologists representatives of the post-darwinian “New Synthesis” of the early 1900 - redefinitions of the concept of species that, starting with the refusal of typologically defining species as “classes of objects” on the basis of intrinsic and arbitrarily selected characteristics, intend to stress their exclusively biological sphere, defining them as groups of natural populations, the members of which mate with each other and are reproductively isolated from other similar groups. A species is therefore a reproductive community, an ecological community and a genetic community[10].
In this document we will refer to the biological concept of species so defined: a group of individuals capable of mating with each other and giving life to fertile offspring, that is, able to generate other individuals.
In conclusion, the practical usefulness of adopting each of the abovementioned criteria – which do not exclude each other but can be integrated in a synthetic vision of a living organism and of its natural history – depends on the specific problem for which we need a distinction between species. In the case of the production of cytoplasmic hybrid embryos, or cybrids (the transferral of a nuclear genome of a somatic cell of human origin in a denucleated cytoplasm of animal species), the beginning of a development similar to an early embryonic development is the fruit of a biotechnological intervention and not the outcome of a philogenetic process, so that – lacking the ability to develop further – a certain attribution of species creates problems.
There is however no doubt that the important data characterising such organisms is the co-presence, within the single cytoplasm, of a nuclear genome and of a mitochondrial genome, belonging to two organisms of different species, whatever concept of species we want to adopt.

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[4] J.D. White, *Specie e speciazione,* in “Enciclopedia del Novecento” (Italian Institute for Italian Encyclopaedia); N. Smelser, B. Baltes, *Species and speciation,* (International Encyclopedia of the Social Behavioural Sciences, Elsevir 2001); in “The New Encyclopedia Britannic” (vol XI Micropaedia).
[5] E. Mayr, *Speciation phenomena in birds,* “American Naturalist”, 1940, 74, pp. 249-278.
[6] E. Mayr, *Typological versus population thinking,* in “Evolution and the Diversity of Life” (1959); Id. Cambridge Univ. Press, Cambridge 1976.
[7] T. Dobzhansky, *Mendelian population and their evolution,* “American Naturalist”, 1950, 84, pp. 401-418.
[8] G. Simpson, *Principles of animals taxonomy,* Columbia Univ. Press, New York 1961.
[9] E.O. Wiley, *The evolutionary species concept reconsidered,* “Systematic Zoology”, 1978, 27, pp. 17-26.
[10] E. Mayr, *Speciation phenomena in birds,* cit.

2. Definitions and problems

There are three categories of interspecies living beings:

Chimeras: organisms containing cells with a different genetic inheritance, coming from two or more genetically distinct animals, belonging to the same species or to different species. The most common forms of human chimeras are the *artificial* ones, which originate for example from organ transplants or, provisionally, from blood transfusions, and the *natural* ones, temporary and physiological, which happen during pregnancy, because of the passage of embryo-foetal cells into the mother's stream. Much rarer are the *natural* human chimeras deriving from the fusion of embryos originated from independent conceptions or with mechanisms similar to the beginning of pregnancy.

Transgenic: organisms in which the genetic inheritance contains genes added to the original and mitochondrial nuclear DNA, intact or modified. Transgenic animals having a human gene introduced into the animal germinal line that is transmitted to the descendants' cells, can be used to produce substances of potential therapeutic interest and as a model for the study of human illnesses[11].

Hybrids: organisms in which all the cells share the same genetic inheritance, originated by crossing different species. Generally they are obtained by fertilising animals belonging to different species: the mule is probably the best known hybrid animal in nature. The concept of hybrid however is used in biology also, for example, to indicate a cellular population originated by the fusion of cells of subjects belonging to different species (somatic cells hybrids).

In more recent scientific literature there are a variety of examples of hybrids, the development of which does not go beyond the first cellular divisions:

1. mixed embryos, man/animal;

or possible chimerisms from the introduction of:

2. animal cells transplanted in human embryos or foetuses;
3. human cells transplanted in animal embryos or foetuses.

Case 1. is exemplified by animal oocytes (rabbit), denucleated, fused with a cell from human skin, as indicated by Chen et al.[12]: when the blastocyst state is reached (5-7 days), the cells behaving like stem cells, differentiating in different populations, including neuroblasts and myoblasts, are isolated. These experiments have been discussed by the Scottish Bioethics Committee[13], which has raised doubts about their ethical aspects. Similar experiments have been reported by Illmensee et al.[14], who have fused denucleated cow oocytes with ovary granulose cells or with skin fibroblasts. Some of these fusions have created blastocysts after six days 13, [15].

Case 2. according to Mikkelsen[16] has not yet been found in literature.

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[11] Another definition of transgenic is the following: "an organism's genetic inheritance containing hexogen genes inserted in the nuclear DNA, intact or modified", because genes cannot be "added" to the nuclear DNA and even less to the mitochondrial DNA that, being transmittable only through the maternal line, do not have, in the experiments in which they are used, any interest, either experimental or therapeutic.

[12] Y. Chen et al., *Embryonic stem cells generated by nuclear transfer of human somatic nuclei into rabbit oocytes* "Cell Research", 2003, 13, pp. 251-264.

[13] Scottish Council on Human Bioethics, *Embryonic, foetal and post-natal animal. Human mixtures,* 2005.

[14] K. Illmensee et al., *Evaluation of the embryonic preimplementation potential of human adult somatic cells via an embryo interspecies bioassay using bovine oocytes* "Fertil. Steril.", 2006, 85, suppl. 1, pp. 1248-1260.

[15] P.M. Zavos, *Human reproductive cloning: the time is near* "Reproduc., Biomed., Online", 2003, 6, pp. 397-398.

[16] T.R. Mikkelsen, *Examples of Scientific Articles about Animal-Human Hybrids and Animal-Human Chimeras*, in "Man or Mouse?", The Danish Council of Ethics, Report on Ethical aspects of chimeras research, 2008.

Case 3. is the one most commonly found. According to Mikkelsen 16, transplants of embryonic human stem cells in mouse or other animal blastocysts have not been described. Instead, numerous experiments of stem cells transplant in animal foetuses, including human stem cells obtained from tissue (or in any case more differentiated sources). For example, Ogle et al.[17] introduced human hematopoietic stem cells in pig foetuses and observed both unmodified human cells and human cells fused with pig cells, in a 40:60 proportion. The same phenomenon has also been observed in other chimerical animals[18]. Moutri et al.[19] transplanted human embryonic stem cells into the cerebral ventricles of murine foetuses, in order to study their ability to differentiate in neuronal cells, and observed the formation of synapses between the two neuronal populations, at different levels of cerebral architecture (cortex, hippocampus, thalamus and cerebellum). The overall population of human derivation has been estimated to about 0.1%. Bruestle et al.[20] transplanted cerebral stem cells of human foetuses, 53 and 74 days old, in the ventricles of rat foetuses (17-18 days old). Eight weeks after the transplant, human cells were incorporated in a variety of the guest's cerebral areas (amongst other, in the cortex, in the hippocampus and in the olfactory bulb), where they differentiated in astrocytes, oligodendrocytes and neurons. Zanjani et al. in 1995[21] and in 1996[22] implanted hepatic cells of human foetuses, 12 and 15 weeks old, into sheep foetuses. A few years after the transplant, the 10-20% of hematopoietic cells was of human origin. Almeida-Porada et al. in 1999[23] and in 2005[24] transplanted human neuronal stem cells in ovine foetuses, and at birth (after three months) they observed that transplanted human cells had originated hematopoietic, marrow, hepatic, thymic and spleen cells. The experiment demonstrated the versatility of differentiation in neuronal human cells. Ourednick et al.[25] transplanted neuronal cells taken from a 15-week old human foetus, into a "macaca radiata" (Bonnet Macaque). After 16-17 weeks from the transplant, human cells had reproduced and colonised some distant cerebral areas, although in a reduced number.
The examples cited (not mentioning other experiments involving the transplant of human cells from adult organisms in embryos or in animal foetuses) indicate the possibility of creating man-animal interspecies chimeras, with characteristics of relative stability in time. Each of the two populations deriving from the different species, although they remain isolated, appear to be immunologically tolerated during the observation period. The percentage of human population is in any case very limited.

[17] B.M. Ogle et al., *Spontaneous fusion of cells between species yields transdifferentiation and retroviral transfer in vivo* "Faseb J.", 2004, 18, pp. 548-550.
[18] B.M. Ogle et al., *Biological implications of cell fusion* "Nat. Rev. Mol. Cell. Biol.", 2005, 6, pp. 567-575.
[19] A.R. Moutri et al., *Development of functional human embryonic stem cell-derived neurons in mouse brain,* "Proc. N. Acad. Sci.", 2005, USA 102, pp. 18644-18648.
[20] O. Bruestle et al., *Chimeric brains generated by intraventricular transplantation of fetal human brain cells into embryonic rats,* "Nature Biotechnology", 16, pp.1040-1044.
[21] E.D. Zanjani et al., *Retention and multilineage expression of human haematopoietic stem cells in human-sheep chimeras,* "Stem Cells", 1995, 13, pp. 101-111.
[22] E.D. Zanjani et al., *The human/sheep xenograft model: a large animal model of human haematopoiesis* "Int. J. Hematol.", 1996, 63, pp. 179-192.
[23] G. Almeida-Porada et al., *Transplantation of human neuronal stem cells into fetal sheep give rise to hematopoietic cells in vivo* "Blood", 1999, 94, 129a.
[24] G. Almeida-Porada et al., *In vivo haematopoietic potential of human neural stem cells*, "Brit. J. Haematol", 2005, 130, pp. 276-283.
[25] V. Ourednik et al., *Segregation of human neural stem cells in the developing primate forebrain* Science", 2001, 293, pp. 1820-1824.

Chimeras, transgenic and hybrids can raise ethical problems. At the same time, there is widespread agreement with regards to some typologies of man/animal chimeras[26]: there is agreement, for example, on the fact that the insertion of human cells in animal guinea pigs during scientific experimentations, or the use of organs from genetically modified animals (transgenic animals) or parts of them, for treating the inadequacy of a human organ, are ethically acceptable and belong to the issues of human and/or animal experimentation[27]. With regards to chimeras, for better identifying the critical issues from an ethical point of view, it can be useful to distinguish between those that are man/animal, formed adding cells in an advanced stage of development, and those in which cells are transplanted in embryos in the first stages of development. In the first case, cells do not have the opportunity to develop, differentiate and spread into the organism, the way it can instead happen in the very first stages of development, and the identity of the chimeric organism remains well defined (like, for example, in organ or tissue transplants).
However, some of these experiments can raise ethical problems, in particular when the cells transplanted in adults are neuroblasts or germinal cells. For example in 2005, during a research on Parkinson disease, neuronal stem cells taken from a 13-week old human foetus were transplanted into the cerebral area of an African green monkey, in which dopamine producing neurons had been destroyed. After 7 months, the transplanted cells worked and had partially substituted the ones that had been destroyed[28]. Attempts at cell transplants capable of developing into germinal cells, from man to animal, are also

[26] Some members believe that these experiments are legitimate only when they do not cause useless suffering and harm to animals. In particular, Prof. Luisella Battaglia highlights what follows:
Tackling the issue of transgenic animals means taking seriously the bioethical aspects of science and technology, the consequences in the short, medium and long term, for the health and well-being of the subjects involved and for society overall; finally, questioning the development model we are pursuing and its pitfalls, not only with regards to man but also to the environment intended in its totality. In the bioethical debate on transgenic animal we see the clash between two philosophies that, in extreme synthesis, could be so characterised: one has a vision of animals as instruments and simple means of a scientific-technological process, strictly aimed at human well-being; the other seen in those not human, subjects that have abilities, needs and interests, deserving of careful consideration and worthy of respect and protection.
In the NBC's document, *Ethical and legal considerations on the use of* biotechnologies (30th of November 2001) we see a cautious support of the second perspective. With regards to the patentability of living beings, after recalling that animals with modified genetic characteristics are patentable, if they meet precise objectives of research and biomedical use, the important bioethical principle of "preservation of well-being" is introduced. According to this principle, formulated by the physiologist and philosopher Bernard Rollin, all animal genetically engineered for human use or for environmental usefulness, should not, after the modification of their genetic inheritance, have a worse quality of life than the one they would have had before the intervention or without the intervention.
This is a significant innovation in comparison to previous documents on the topic of animal bioethics, as it is a firmer statement of an ethics of responsibility towards non-humans. In fact, it is not simply a bioethical evaluation of the type of biotechnological intervention, but it requires a precise attitude of care towards animal well-being.
In this regards, it is greatly significant that in the document we read: "For interventions on plants and animals we add the principle of conservation of biological balance based on biodiversity and the consideration of "animal rights" and of the duties and responsibilities towards them. We must critically evaluate genetic engineering interventions also from the point of view of animal well-being and identify appropriate regulations to guarantee that such interventions meet the new emerging ethics, characterised by a growing consideration for animal suffering and by the intent of preventing and alleviating their burden as much as possible. Those working in genetic engineering should therefore seriously respect the social request of reducing the pain, the anxiety and any kind of suffering of the manipulated animals".

[27] President's Council on Bioethics, *Reproduction and responsibility: the regulation of new biotechnologies* USA, 2004.

[28] K.B. Bjugstad et al., *Neural stem cells implanted into MPTP-treated monkeys increase the size of endogenous tyrosine hydroxylase-positive cells found in the striatum: a return to control measure.,* "Cell Transplant", 2005, 14, pp. 183-192.

known, aiming at producing, in animals, human germinal cells. The examples of cell transplants capable of developing in spermatozoons, from human testicles to mouse testicles made immunodeficient[29], or from human ovary to mice[30], are significant. If these experiments had been successful, murine testicles would have produced human and murine spermatozoons, human oocytes would have developed in chimeric mice, and, in theory, human embryos could have been obtained from animals capable of producing human gametes.

The problem of the identity of the new living being always presents itself when the man/animal chimeras are formed in the very first stages of embryo-foetal development – when transplanted cells can develop and spread in the new organism, modifying it substantially – and in all man/animal hybrids. These can be distinguished in two groups: hybrids formed by the fusion of a human gamete with an animal (moreover, as a rule, these are impossible because of the interspecies barriers, which in effect prevent any development subsequent to this cross-fertilisation) and the cybrids, or cytoplasmic hybrids, obtained by transferring the nucleus, inserting the nucleus of a human somatic cell in an animal denucleated egg cell.
In theory, transgenic human embryos can be produced, that is, human embryos in which animal genes are inserted: in this case, very different situations can occur, depending on the inserted genome.
In general, the advent of new reproductive technologies has made us reconsider the definition of human being as the result of natural fertilisation, that is, of the fusion of male and female gametes: the possibility of creating hybrid or cybrid embryos has opened the opportunity of thinking about new life forms, unlike the ones already known.
The possibilities of man/animal combination are therefore different and present a variety of ethical problems; in any case we have the problem of defining what is human. In particular it is necessary to understand if a chimeric, transgenic or hybrid living being, created in a laboratory through the fusion of human and non-human cells, has characteristics that can define it as “human”.
In reality, for some NBC members[31] the most complex problem is not so much the missing of human and animal tissues and cells, but the creation, through the formation of man/animal hybrids and chimeras, of living beings of uncertain identity, in which the boundary between the human and the animal species is no longer identifiable.
Other NBC members[32] however, consider the concept of “uncertain identity” quite obscure, especially because the identity’s “uncertainty” is not substantiated by any reference to any measurement criteria. It seems instead, on the one hand, that such uncertainty – following what has been stated above – cannot depend on the mere combination of biological material; and, on the other hand, that it refers to – as it can be understood by the use of the adjective “visible” – a kind of “intuitive recognition” in deciding whether the new “being” does not belong to the human species. In any case, the biggest perplexity derives from the application of the concept of “identity” to the cybrids, as it is not clear what type of identity we are referring to. If we want to consider only genetic identity, then it would not be uncertain, because it is defined by the procedure used to form the cybrids. It therefore seems that we are considering, at least implicitly, the historical-cultural and psychological-social aspects, in which the concept of identity is

[29] M.M. Reis et al., *Xenogeneic transplantation of human spermatogonia,* “Zygote”, 2008, 8, pp. 97-105.
[30] Y. Aubard, *Ovarian tissue xenografing*, “Eur. J. Obstet. Gynecol. Reprod. Biol.”, 2003, 108, pp. 14-18.
[31] S. Amato, A Bompiani, R. Colombo, A. Da Re, F. D’ Agostino, B. Dallapiccola, M.L. Di Pietro, M. Gensabella, A. Isidori, A. Morresi, A Nicolussi, L. Palazzani, V. Possenti, R. Proietti, L. Scaraffia.
[32] C. Flamigni, S. Garattini, D. Neri, A. Piazza; M. Toraldo di Francia, G. Zuffa.

really rooted. However, the centre of the concept of identity rotates around the subjectivity and the discourse of the subject on itself. Therefore it is evident that the difficulty of applying this concept to the cybrids, destined not to develop, unless we assimilate *tout court* the embryo to the "living human being", so that genetic individuality and human individuality coincide, a thesis that is ethically controversial (and not shared by the abovementioned members).

Bioethical issues connected to the production of interspecies living beings, which include not only the status of the human embryo, but also the question of the species' identity and of the definition of human, are common to all man/animal interspecies living beings described in previous paragraphs. At the same time, each form of interspecies living being implies also specific considerations, relative to the methods with which it was produced, to its development and its purpose.
In this document the NBC examines in detail only one form of interspecies living being, that is, cytoplasmic hybrid embryos, or cybrids, because:

- currently it seems to be one of the possibilities, maybe the only one, in line with the cloning project through the nuclear transplant for therapeutic purposes;
- the protocol followed in creating this hybrids is sufficiently standardised to be reproduced in a laboratory; the organisms derived can be described only from a genetic point of view, although still, for some NBC members[33] remains the problem of their uncertain identity.

The paragraph dedicated to bioethical reflection, refers in particular to the creation of cybrids and, in general, to the creation of interspecies organisms; conclusive bioethical considerations and the legal part (in appendix), for the arguments and the problems tackled, are applied to all man/animal interspecies organisms.

3. Cytoplasmic hybrid embryos

Commonly known with the expression "therapeutic cloning", the SCNT technique (Somatic Cell Nuclear Transfer) allows us, theoretically, to create embryos with the genetic inheritance of an adult individual, using only one gamete. This protocol involves the removal of an egg cell's nucleus, which is then substituted with the nucleus taken from a man adult somatic cell of the same species. Appropriately stimulated – chemically and/or electrically – this new cell can behave as a fertilised oocyte and can divide and differentiate until it originates a new organism, which has the same nuclear genetic inheritance of the donor's adult somatic cell.[34]
The main purpose of this technique is to obtain cellular lines and, consequently, human tissues, compatible to the donor and, theoretically, useful for eventual medical applications and, first of all, for the substitution of tissues damaged by degenerative diseases (like, for example, Parkinson disease), without having any rejection problems.

[33] S. Amato, A. Bompiani, R. Colombo, A. Da Re, F. D'Agostino, B. Dallapiccola, M.L. Di Pietro, M. Gensabella, A. Isidori, A. Morresi, A. Nicolussi, L. Palazzani, V. Possenti, R. Proietti, L. Scaraffia.

[34] However it's not a perfectly identical copy: the oocyte contains mitochondria - small structures responsible, amongst other things, for the cellular energetic cycle – which have their own genetic inheritance, present in the new embryo in different quantities, according to the methods of transferral of the nucleus from the donor to the enucleated oocyte. The term heteroplasmia indicates the presence of mitochondrial DNA with a different genetic make-up, for example the mixture of the egg's and the donor's mitochondrial DNA, because of the transferral of a residue of the adult somatic cell's cytoplasm: in this case, both the mitochondria of the somatic cell's donor and those of the individual who has given the oocyte, can be found in the new embryo.

This is a possible application of regenerative medicine. Another purpose of these experiments is simply of a cognitive nature, that is, aimed at exploring the mechanisms of cellular reprogramming mediated by the egg cell's cytoplasm. The SCNT can also be seen as a technique that allows us to reprogram and therefore "rejuvenate" an adult cell to its embryonic stage, through not yet clear mechanisms that involve the activation of some genes.
Some NBC members35 observe that the use of SCNT to get autologous cells (that is, compatible to the nucleus' donor) to use in regenerative medicine, would have a very limited value in therapy, especially in the case of illnesses (like Parkinson disease) with a strong genetic component. In fact, the cells so obtained contain the genes that caused or contributed to cause the illness, and some scientists36 have been asking themselves for some time if the advantage of the autologous stem cells' compatibility (however obtained) is not strongly put into perspective by the fact that the use of these cells in transplants, in the case of illnesses with a high genetic component, means reintroducing the cause of the illness into the patient. Different is instead – still according to those NBC members – the evaluation of the use of this technique (on human or animal oocytes) in base research, where it can lead to the acquisition of knowledge regarding the processes of normal and pathological development which cannot currently be gained in other ways.

12 years from the cloning of Dolly, SCNT's efficiency seems very limited. The percentages of full term pregnancies for cloned animals is 1-2%, as they are penalised by a high incidence of abortions and still-births37, whilst, according to what we know today, the technique has not produced results on man and, in particular, lines of human embryonic stem cells obtained from cloned human embryos are not available. The Korean vet Hwang Woo Suk38 declared that he had been able to achieve human cloning with this technique, but subsequently his work was revealed to be "the greatest scientific fraud of the century"39.

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[35] C. Flamigni, S. Garattini, D. Neri, A. Piazza; M. Toraldo di Francia, G. Zuffa.
[36] Cf. H.I. Park, *Global gene and cell replacement strategies via stem cells,* "Gene Therapy", 2002, 9, p. 623; E. Snyder, A. Vescovi, *The possibilities/perplexities of stem cells,* "Nature Biotechnology", 18th of August 2000, pp. 927-828.
[37] L. Loi, *Dieci anni di cloni e di fibrillazioni,* "Darwin", 2007, 21, pp. 54-59, and cited references.
[38] In May 2005 the journal "Science" published an article illustrating how the medical team led by Hwang (45 direct collaborators and 183 researchers in total, 26.5 million dollars received as funds in the first six months of 2005, 65 millions dollars in total invested by the Korean government) had obtained 11 embryonic stem lines compatible with some patients affected by different pathologies (diabetes, spinal cord lesions, immunodeficiency). But in the following months it was discovered that the results had been falsified, that no cloning had happened, and that the oocytes used were not 185, donated by volunteers, but more than 2,000, some of which obtained from female researchers within the same Hwang research group, through pressure and payment.
[39] "The Korean stem cell research star Woo Suk Hwang is at the centre of one of the largest investigations of scientific fraud in living memory", in www.nature.com/news/specials/hwang/index.html; "...making it, by number of active fabricators, the biggest case of scientific fraud in history…", Cynthia Fox, in "Fortune", December 22, 2006; " Some analysts are describing his fall from grace as one of the biggest cases of scientific fraud in recent history", http://news.bbc.co.uk/2/hi/asia-pacific/4597416.stm; "Medical researchers say the episode, which has shocked and shamed many South Koreans, is one of the biggest cases of scientific fraud in recent history", ABC news 11.6.2006 in http://www.abc.net.au/news/stories/2006/01/11/1545956.htm; "Ironie de l'histoire, la plus grande fraude scientifique de l'époque moderne masquait…", Michel de Pracontal, in Le Nouvel Observateur, n. 2234, 30 agosto 2007; "Responsible de la plus grande fraude scientifique de ces dernières années sur le clonage, le généticien coréen Hwang Woo-suk…", in l'Express, 19.1.2009, in http://www.lexpress.fr/actualite/sciences/decouverte/ce-soir-on-mange-du-clone_479339.html; "...die viele Experten als größten Forschungsskandal des Jahrhunderts bezeichnen…", "Die Ziet" 16 febbraio 2006, n. .8; even more damning the definition of "La verdad": "El gran fiasco ha sido

Although specialised literature is quite sceptical towards the SCNT, many believe that the lack of results with regards to man is due to the scarce availability of human oocytes. From this derives the hypothesis of taking oocytes from different animal species, available in theoretically unlimited quantity, which could be used without technical and ethical problems40. But the SCNT technique that uses animal oocytes produces biologically new "entities", with a human genetic inheritance that is limited to the nuclear DNA, and animal genetic inheritance with regards to mitochondrial DNA.
Improperly defined by the media as chimeric embryos, or confused with real chimeric embryos, in effect these embryos are cytoplasmic hybrids, or cybrids. The international debate in the last few years has focused on this type of interspecies embryos.
At the moment of drawing up this document, the new nuclear "reprogramming" techniques defined by the Japanese researcher Shinya Yamanaka for the production of induced pluripotent cells (iPS or induction of Pluripotent Stem cell), appear in some ways more promising and efficient in comparison to the SCNT, and have contributed in increasing the doubts about the usefulness, from a therapeutic point of view, of nuclear transfer techniques aimed at obtaining lines of embryonic stem cells41. We must however clarify that it is not completely clear if the iPS are really induced from differentiated cells[42]; what is certain is that they are not cells identical to embryonic stem cells, as demonstrated by the studies of genic expression and of DNA methylation;[43] what is not clear is the system that determines their reprogramming, as some genes originally believed to be essential for this process have subsequently been found to be unnecessary or not relevant[44]; finally, the iPS present safety issues from a therapeutic point of view, as all the factors relevant in the reprogramming are oncogenes and their hyper-expression is associated to tumorigenesis.[45]

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el fraude del coreano Woo Suk Hwang", in http://www.laverdad.es/murcia/prensa/20061227/sociedad/avances-cientificos_20061227.html.

[40] For example, cow oocytes can be obtained directly from slaughtered animals, without any preliminary treatments. Non-human primates, however, need ovary stimulation like humans.

[41] K Takahashi and S. Yamanaka, *Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors,* "Cell", 2006, 126, 663-676; K. Takahashi et al., *Induction of pluripotent stem cells from adult human fibroblasts by defined factors*, "Cell", 2007, 131, 861-872; M. Nakagawa et al., *Generation of induced*
*pluripotent stem cells without myc from mouse and human fibroblasts "*Nat Biotechnol.", 2008, 26, pp. 101-106; K. Okita et al., *Generation of mouse induced pluripotent stem cells without viral vectors "*Science", 2008, 322, pp. 949-953. Currently the three research projects on cybrids that have obtained the HFEA licence, have not been funded by the United Kingdom authorities, which declared that, one of the most promising research, and therefore worthy of funds, is that on induced pluripotent stem cells.: "The Guardian", 13.1.2009 http://www.guardian.co.uk/science/2009/jan/13/hybrid-embryos-stem-cells, "The Independent", 13.1.2009, http://www.independent.co.uk/news/science/funding-halted-for-stem-cell-research-1332000.html; http://www.independent.co.uk/news/science/mps-to-investigate-stem-cell-funding-row-1334254.html.

[42] T. Aoi, et al., *Generation of pluripotent stem cells from adult mouse liver and stomach cells*, "Science"**,** 2008, 321 (5889), pp. 699-702**;** M.F. Pera et al., *Simpler and safer cell reprogramming,* "Nature Biotechnol." 2008, 26, pp. 59-60.

[43] K. Takahaschi, and S. Yamanaka,"Cell", 2006, op. cit. in ref. 37.

[44] S.V. Liu, *iPS cells: a more critical review*, "Stem Cells and development", 2008, 17, pp. 391-397.

[45] S.V. Liu, *iPS cells are man-made cancer cells,* "Logical Biology" 2008, 8, pp. 16-18.

3.1 The state of the art

Only one scientific publication states that the production of cybrids through SCNT can be done. An experiment of this type was published in 2003 by the journal *Cell Research*[46], but no-one yet has been able to reproduce these results, including the authors of the first publication, so that a series of doubts about the scientific validity of the first experiment have emerged.

On the other hand, different experts in the sector explain that the development of these organisms is destined to stop too early[47]. The causes seem to depend on the role of the egg cell in embryonic development, in particular, with regards to the maternal-zygotic transition, that is, the transition of the program of development from the oocyte to the zygote[48] and to the role of mitochondria.[49]

One of the main aims, for the researchers interested in the SCNT problem and in the formation of cybrids, is the study of mitochondrial illnesses, a sought-for sector of the research that investigates in particular the possibility of substituting mitochondria in oocytes of women affected by these illnesses.

With this purpose, the technique of the "cytoplasmic transferral" ("mitochondria donation") from an egg cell to another has been developed. The cycles of in vitro fertilisation that used egg cells in which the mitochondria transferral had taken place, have also created foetuses and new-borns who presented development pathologies and genetic anomalies: this research has been suspended in the USA since July 2001, on FDA request.[50]

The document "Interspecies embryos" of the English Medical Academy, which is in favour of the creation of this type of hybrid embryos, states: "in the context of cytoplasmic hybrid embryos, mitochondria and cytoplasm represent potential retrovirus sources within the

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[46] The foreskin cells of two five year old children, the skin of a 60 year old woman and of two men were fused with rabbit oocytes. Of the 400 embryos created, about 100 arrived to the blastocyst stage, that is, they could give embryonic stem cells, from which cellular lines could be originated, ref. [12].

[47] Robert Lanza (in Andy Coghlan, *Human-animal "cybrids" may not be possible,* "New Sci.", 2007, 2621) of the Advanced Cell Technology, declared that his team worked for a long time to obtain this type of embryos, but without success: arrived at the stage of 16 cells, the one immediately before the blastocyst, the development has always stopped, probably, according to Lanza, for the incompatibility of the genetic inheritance of different species, which would stop "talking to each other". But also C.A. Redi, Scientific Director of the IRCSS Foundation at the Policlinico San Matteo in Pavia, explains "cellular replication is regulated by species-specific enzymes: having joined two different species, the enzymes are different, with the consequence that the reaction after a few days is destined to physiologically stop, for the impossibility of "communicating" "[…] Having been joined, the cytoplasm and the nucleus of two different species and types, this process is destined to stop very soon" ("Repubblica e salute", 5.6.2008).

[48] R.M. Schultz, *The molecular foundations of the maternal to zygotic transition in the preimplantation embryo* "Human Reprod. Update", 2002, 8, pp. 323-331 and cited references; M. Zurita et al. *From the beginning: the basal transcription machinery and onset of transcription in the early animal embryo*, "Cell. Mol. Life Sci.", 2008, 65, pp. 212-227 and cited references.

[49] R. Dumollard et al., *The role of mitochondrial function in the oocyte and embryo,* "Curr. Topics in Develop. Biol.", 2007, 77, pp. 21-49, and rif. cit.; P. May-Panloup et al., *Mitochondrial DNA in the oocyte and the developing embryo* "Curr. Topics in Develop. Biol.", 2007, 77, pp. 51-83, and cited references.; E. A. Shoubridge et al., *Mitochondrial DNA and the mammalian oocyte*, "Curr. Topics in Develop. Biol.", 2007, 77, pp. 87-111and cited references.

[50] "One centre in the USA has performed 33 IVF cycles involving CT since 1996 resulting in the birth of 16 babies. Another fetus was electively reduced (the twin delivered normally later) due to an anomaly of the sex chromosomes called Turner's Syndrome. One early spontaneous miscarriage also occurred and the foetus was diagnosed with the same syndrome. The children born after IVF with cytoplasmic transfer have been evaluated and one 18-month-old child was recently diagnosed with a pervasive development disorder. Two babies have been born in whom mitochondria were derived from the mother as well as from the donor. This research has been suspended in the USA since early July 2001, pending clarification of new requirements suggested by the federal Food and Drug Administration (FDA)." (Hfea, Scientific and Clinic Advanced Group, Mitochondria and Development, 16.7.2005).

animal oocyte. […] The nuclear genome of cows and rabbits contains the endogen retroviral genome. It is therefore possible that the cytoplasm of rabbit or bovine oocytes can contain transcripts (of RNA) or express endogen retrovirus codified by their nuclear genome. These viruses could reintegrate in the transferred human nucleus. This occurrence must be considered highly improbable but not impossible". The same document suggests the preventive assessment of the existence of "expression profiles of endogen retroviruses" (that is, the expression of any retrovirus) before using the oocytes, and it stresses that, for the same reasons, stem cell lines so produced could not in any case be used for clinical treatments. The English Medical Academy also stated that in standard conditions of safety there would be no problem[51]. Different hearings – written and oral – given by the Science and Technology Committee[52] highlighted the same problem, so that the potential use of these cells for in vitro studies emerged, but not for in vivo experimentation.
Some NBC members[53] stress that the eventual clinical application of stem cells derived from hybrids (as with any other type of stem cell) on human beings, will have to undergo the provisions contained in the recent "Regulation (EC) Number 1394/2007 of the European Parliament and of the Council on Advanced therapy medicinal product". Looking out for problems regarding biosecurity involves, therefore, the eventual clinical application of the products, which will not be allowed until the procedures to obtain these products (and the products themselves) are not shown to be free from risks to human health. According to the same NBC members, all this has nothing to do with the experiments as long as they are confined to the laboratory or are used as an instrument to investigate specific biological issues.

Bioethical evaluations

NBC members[54] started from two different types of ethical considerations:

I. some concern the evaluation of scientific research and of the reasons given to defend its practicability;
II. others refer, instead, to the issue of the identity of man and of the human species.

I. The ethical value of the experimentations cannot be considered apart from its strictly scientific relevance: from this point of view, any research that is futile, highly and unnecessarily risky or undeservedly costly can be also ethically criticised. In determining the scientific value of research we must assess – as well as its hypothesis' usefulness or interest – also its intrinsic value, in comparison to what we already know.
Starting from this premise, with regards to cytoplasmic hybrid embryos, the following observations have been put forward.

- The scientific literature available indicates that the cytoplasmic hybrids produced do not survive up to a stage that allows us to recover embryonic stem cells.

[51] *Inter Species Embryos,* A report by Academy of Medical Science, June 2007, in http://www.acmedsci.ac.uk/p47prid51.html
[52] Science and Technology Committee, UK Parliament, Fifth Report of session 2006/07; Government proposal for the regulation of hybrid and chimera embryos, 5.4.2007, in http://www.parliament.uk/parliamentary_committees/science_and_technology_committee/science_and_technology_committee_reports_and_publications.cfm
[53] C. Flamigni, S. Garattini, D. Neri, A. Piazza, M. Toraldo di Francia, G. Zuffa.
[54] S. Amato, A. Bompiani, R. Colombo, A. Da Re, F. D'Agostino, B. Dallapiccola, M.L. Di Pietro, M. Gensabella, A. Isidori, A. M

- The high degree of developmental defects and anomalies that characterises the majority of cloned animals, brings into question the quality of stem cells that eventually could be obtained from this type of embryos; on the other hand, cells with genetic anomalies would not be useful or would have limited use, as models for the study of an illness, and could not have any therapeutic application.
- Even hypothesising that embryonic stem cells could be removed from cybrids and that they had no anomalies, they would not have any therapeutic relevance for man, because of their contamination with animal material. In the same way, they would have limited or no relevance in the study of illnesses, because the results would be extremely difficult to interpret, as it would be an unknown cellular model.
- The human derivation of the nuclear genetic information and the animal information of the mitochondrial genome makes the clinical transferral of this type of experimental model problematic, as it also potentially carries the risk of an interspecies transmission of viral agents.
- If the purpose of these studies is an improvement in our knowledge of cybrids, and/or the failure of cellular reprogramming in the egg cell, similar experiments could be designed using biological material of exclusively animal origin, intraspecies or interspecies, although ethical caution and ethical problems would remain, which have to be considered even in animal research. Cloning has a very low efficacy even in animals belonging to the same species: it would therefore be logical to tackle the technical and bioethical problem of cellular reprogramming through the transferral of the nucleus, starting with animal models.

For these NBC members, therefore, the abovementioned reasons make the studies on cytoplasmic hybrids scarcely justifiable at the moment. Also, we stress how the reasons given by researchers[55] in the current debate in support of the justifiability of this practice, are not sufficient for a variety of reasons:

a) the fact that it would produce a hybrid cellular population, the development of which spontaneously stops or, in any case, is interrupted by researchers and is not transferred to the maternal body, is not sufficient to make this practice legitimate: we must in fact face the responsibility of producing these entities. In addition, not interrupting their development would open the possibility of introducing variable percentages of animal genetic material or of liberalising other forms of interspecies fertilisation, even with the possible intention of transferring such hybrid cellular populations in the human or animal body;

b) the fact that researchers produce cybrids because of the low availability of human oocytes, in order to avoid subjecting women to the risks of hormone hyper-stimulation, does not constitute a scientifically and ethically sufficient argument: experimentation not only must not expose women, but also the subject on which the experimentation is carried out, to any risks, and in any case it must answer general scientific and ethical criteria.

c) It must also be highlighted that the objective of this research is to increase the efficacy of cloning, in a perspective of human application. If this aim was achieved, the intention declared by the researchers would be to still continue the research on embryonic cells derived from SCNT, but using human oocytes, as the stem cells eventually obtained from cybrids would not be useful for therapeutic purposes. What would follow, is the paradox of an increase in the demand of human oocytes and, if the technique was

[55] Only the following arguments under a) and b) have been recalled in the HFEA document, *Hybrids and Chimeras Consultation document*, October 2007; House of Commons, Science and technology Committee, Fifth report, Government proposal for regulation of hybrid and chimera embryos, 5 April 2007.

successful, the demand would end up subjecting women to much greater pressures and coercions than the ones they face today to persuade them to give their oocytes.[56]

II. Those same members have also put forward other ethical, more general reasons with regards to illegitimacy of such practices, which can be referred to any type of interspecies hybrid and to some chimeras and man/animal transgenics (see par. 2). These can be articulated in three points.

1. The first ethical reason is given by the protection of the dignity of the human embryo from the beginning of his/her life as well as the integrity of the human species.

Looking into this, the issue of whether cytoplasmic hybrids are *human* embryos or whether the presence of mitochondrial DNA of animal origin makes them *non-human*, has not yet found a shared response.

However, the simple existence of a reasonable doubt about the *status* of cybrids, reopens the question of the dignity of the human embryo. If, in fact, they are human embryos, there are a variety of issues: if it is legitimate, in principle, to create them for research purposes; if it is justifiable to destroy them within a certain date, after having studied them and having eventually taken out their stem cells. This reopens a debate the NBC has already taken part in numerous times, recording divergent opinions[57].

Even if science was able to demonstrate the efficacy or the usefulness of some of these experiments and of their applications, the mixture of even a *quantitatively* low percentage of animal genetic material with human genetic material, would qualitatively damage the identity of the embryo and of the human species, with evident pitfalls with regards to the protection of the dignity of the first and the integrity of the second. The fact that the development of cybrids is destined to stop, is not proof that there is no development, but instead confirms it; the fact that cybrids cannot be implanted or are not implanted, does not ethically justify the experimentation, as such entities are intentionally produced for experimental purposes; the fact that they are "destined to die" does not make them less worthy. In particular, the planned destruction of human/animal mixed embryos, highlights a contradiction in those who state that these are only cellular artefacts: why stop their development on the 14th day of conception, as it happens for human embryos created for research purposes? If one of the objectives is to increase the knowledge that can derive from this type of organisms, and if this is a sufficiently legitimate aim, why not allow their growth and development, as it happens for the creation of genetically modified animal species?

The production of man/animal mixed embryos is not acceptable because it implies a radical manipulation of the human being, so that his nature is uncertain and it prevents us to recognise him as belonging to the human species. The recognition of human supremacy on other living beings is not a speciesist anthropocentric prejudice, but the acknowledgment of the importance of man not only because of his peculiar genetic characteristics, but also because of the potential and natural abilities he has developed, during the history of his evolution, amongst which[58] language, moral intelligence and the

[56] F. Baylis, *Animal eggs for human embryonic stem cell research: A path not worth taking,* "Am. J. Bioethics", 2008, 8, pp. 18-32.

[57] *The Identity and Status of Human Embryos,* 1996; *Opinion on the Therapeutic Use of Stem Cells,* 2000; *Opinion on the Research Using Stem Embryos and Cells,* 2003; *Opinion on the Destiny of Human Embryos Resulting from MAP and not Complying with the Conditions for Implantation,* 2007.

[58] Cf., amongst others, also L. Eisenberg, *The Human nature of the human nature,* "Science", 1972, 176, pp. 123-128.

discrimination between right and wrong[59], free will or cultural elaboration[60], and other characteristics.
The overcoming of the barriers between species, through the use of technologies, alters the natural order and could lead to the possible degradation of the identity of man, violating his intrinsic dignity.
Even those who don't agree with the full protection of the human embryo can however feel that it is necessary to safeguard it as a key value, which completely different from being mere biological material, or from the vegetable or animal world.
The embryo, it is stated, must be surrounded by a duty of respect, as it can be recognised as a subject, in consideration of his human nature (intended in the biological, not ontological sense), and cannot be reduced to mere object.
Recognising the subjectivity of the human embryo means guaranteeing the conditions that in the first instance promote his development and birth and protect him from sacrifices unjustified by scientific interests that are of no clear usefulness, are risky and have an uncertain outcome.
The principle of human dignity allow us to argue the position against the production of man-animal interspecies living beings, even starting from a position of doubt or *tout court* negative about the subjectivity of the human embryo. This principle operates, in fact, in extreme cases like this one, also in defence of the image or concept of the human being as a universal category. Moreover, even if we don't believe in completely protecting the human embryo, but we admit that he has qualities different from the simple animal biological material, it is still possible to distinguish between various ways of use, according to different degrees, intensity and implications. From this point of view, we can well recognise that the creation of human-animal interspecies beings, bringing into question the uniqueness of man and his dignity in comparison to animals, is different from any type of research, destructive to single individuals or not, which however does not bring into question the uniqueness of man. Damaging the principle that distinguishes man and animal has repercussions on the principle of equality that presumes a conception of the human being as a universal category with which each individual can identify.
The idea of being able to remedy this violation of human dignity, prohibiting the transferral into a maternal uterus and imposing in any case the suppression of cybrids on the 14th day in order to avoid their development, confirms – as said above – the violation. Or, in fact, the creation of the cybrid does not damage human dignity, and therefore why arbitrarily establish a legal limit for its development? Or it does damage it, and in that case it is not the duration (14 days) and the prohibition of transferral in the maternal uterus to constitute a cause of justification. On the other hand, it is very difficult to reconcile this practice with the regulations of the Oviedo Convention – which protect the human being in his dignity and identity (art. 1) - , and in particular in articles 18.1 and 18.2. In fact the first establishes that "where the law allows research on embryos, it shall ensure adequate protection of the embryo", whilst the second forbids "the creation of embryos for research purposes".
The European Court of human rights recognises the human dignity of the conceived, however, it does not give an opinion on his subjectivity, because of his development potential. It is difficult to isolate, almost conceptually freezing, the beginning of human life – which in Italy is explicitly protected also by the L. number 194 of 1978 – cancelling his development potential. Forbidding the cybrid's development does not therefore mean cancelling the violation of human dignity, which has already happened with the creation of

[59] Cf. also S. Pines, *Guide for the perplexed: moshe Maimonides,* Chapter 41, Chicago Univ. Press, 1898.
[60] Cf. also D. Loike, M.D. Tendler, *Revisiting the definition of homo sapiens,* "Kennedy Inst. Of Ethics J.", 2002, 12/4, pp. 343-350.

the cybrid for research purposes, but desperately attempting to confine it, leaving open, in this way, a dangerous opportunity for the creation of human-animal interspecies beings, in contrast to that principle of egalitarian universalism on which all post-second world war constitutional democracies are founded[61].

It can be useful to observe at this point that a greater awareness of the unacceptability of such research is found in the law, like the German one, constitutionally inspired to the principle of human dignity, where even those who criticize the overload of human dignity, distance themselves from the possibility of creating human-animal interspecies beings[62]. Italy has a Constitution that, like the German one, is inspired to the human dignity principle and therefore we believe, as a NBC that must inspire its opinions to this principle, that we need to take it into account. With this, we don't want to "judge" the experience of other countries, but only to show how from certain constitutional premises, certain evaluations can be drawn, which instead in other countries' regulations – where the human dignity principle is not recalled with the same insistence – are not accepted. On the other hand, because the European Court of human rights also accepts the reference to the principle of the protection of human dignity in general with regards to the conceived (cf. sentence *Vo contro Francia* 8th of July 2004) the perspective followed here can also be considered the one more in harmony with the principle of the European law in the formal sense.

The issue also lends itself to being considered from the more general point of view of the relationship between law (constitutionally oriented) and technology.

The prohibition of creating mixtures between human gametes and animal eggs, contained in the Italian and German law, beyond any consideration in the matter, can be said to be the expression of a formal principle of guarantee against the claim of technocratic irresponsibility. Limiting to the will of power of those working in the technology field, means in essence preserving the minimal conditions of democratic balance, which presume that the law will not change "from regulating to regulated with regards to the technical capabilities of realising aims without excluding any"[63]. To do this in an extreme case (*Extremfall*), as with regards to the creation of human-animal interspecies beings,

[61] J. Habermas, *The Future of Human Nature. The Risks of Liberal Genetics,* English translation by Hella Beister and William Rehg, Polity Press, Cambridge 2003.

[62] Interesting, from a legal or ethical-legal point of view, the debate on human dignity that has developed in the last decade in Germany, after the coming into effect (on the 1st of January 1991) of the law on the protection of embryos (Gesetz zum Schutz von Embryonen) with particular reference to the authors who raised the problem of the überforderte Menschewürde (overloaded human dignity) in a controversy against an excessive reference to art. 1 of the German constitution (human dignity's "intangibility") in the issues regarding the embryo (U. Neumann, Die Tyrannei Würde, ARSP 1998, 153 s.; B. Schlink, Die überforderte Menschewürde, in Vergewisserungen, Zürich 2005; Biowissenschaften und Biotechnologie - Perspektiven, Dilemmata und Grenzen einer notwendigen rechtlichen Regelung, JZ, 2008; P. Bahr - H.M. Heinig, Menschewürde in der saekularen Verfassungsordnung, Tuebingen 2006). It is significant, in this regard, the opinion that the authors against the overload of human dignity express about the appropriateness of referring to this principle in the case of the creation of man-animal mixed beings. In fact, although they invite us to differentiate the assessment of the different issues without suffocating them – as they say – under the constant reference to the human dignity principle, they cite the eventual creation of man-animal mixed beings as an example of the certain violation of the human dignity principle (U. Neumann, Die Tyrannei der Würde, ARSP 1998, 162; B. Schlink, Die überforderte Menschewürde, in Vergewisserungen, Zürich 2005, 136). And one author stresses, on this point, that in this case the human dignity principle is legitimately invoked even in defence of a conception of man, and not only in defence of a single man against his eventual exploitation. It is – Neumann states – an unacceptable violation of the image of man (Menschenbild), according to a unanimous point of view.

[63] E. Severino in: N. Irti – E. Severino, *Dialogo su diritto e tecnica,* Bari-Roma, 2001.

certainly does not change the freedom of research, but it is useful in questioning researchers as socially responsible subjects.[64]

2. The second ethical reason regarding the illegitimacy of the abovementioned experimentations moves from the possibility (which cannot be in effect excluded) that scientific research – although initially focusing on the destruction of such entities – could "slip" into the temptation of transferring them into the uterus to verify their possible survival. What must be stressed is the ethical importance of the protection of a balanced relationship between living beings, which is an essential condition in maintaining the ecosystem, and the "responsibility" when facing the risks of such experiments and when considering their effects on subject who cannot express their consent, like future generations[65]. The uncertain consequences, in the short and long term, of an interspecies mixture, for society and future generations, should persuade scientists to have a coherent attitude of cautious interruption of any form of this kind of experimentation. This is one of the cases in which it seems our duty to employ the *precaution principle* as guideline, quoted by many in bioethical reflection (on which the NBC already drew up – in 2004 – a specific document titled *Precaution principle: bioethical, philosophical and legal profiles*), a principle that opens up to a tutioristic and responsible attitude.

3. The third ethical reason of illegitimacy refers to a possible instinctive feeling of "repugnance" towards experimentations which endanger the identity of the human species. This is an argument that goes "beyond reason", as it is not rationally articulated and it is based on an immediate reaction, on emotions and feelings, but is not for this devoid of wisdom, good sense and ethical value (think about the repulsion we feel towards incest or cannibalism)[66]. The repugnance does not come from the strangeness or novelty of such experimentation, but from the intuition of rejecting the excesses of human will, which can violate (in this case) the specificity and separateness of the human. In addition, the reproductive connection that characterises living species distinguishing them from any other cataloguing form, has for the human species a peculiar meaning of "relations"[67], becoming a place of mutual recognition. This recognition of the *humanum* in itself and in others does not mean a discrimination of other living species as inferior – speciesism – but awareness of belonging to our species. It is within the species that the normative understanding of self, as beings of a type is realised, and this allows us to respect each other as free human beings, equal in dignity. The prospect of interspecies reproduction makes the recognition confused and uncertain, endangering the possibility of thinking about our identity as human beings. These arguments must be taken into account when establishing to what extent the genetic "manipulation" of the gametes, the application of techniques allowing cross-fertilisation with other animal species, or in any case man/animal hybridising practices, can alter the "notion" of human species itself and its "representation" at the common level.

[64] These reasons are shared also by Stefano Canestrari and Lorenzo d'Avack, who base their agreement with the first three points of the opinion's conclusions on them.
[65] H.Jonas, Das Prinzip Verantwortung (1979).
[66] The argument of "repugnance" is also taken into consideration in the document of the Danish Bioethics Committee "Man or mouse? Ethical aspects of chimera research" (2008), cf. in particular pages 42-44 and pages 54-57. In this document it is believed that such feeling could have a biological value (as a mechanism inherited during the evolution process, in order to protect our species) or an anthropological-cultural and symbolic value (as social taboo, the taboo of interspecies mixture). These are interpretations which attribute to the repugnance feeling the function of preserving our species, in the strong sense (as it is considered unchangeable by culture) or weak (if changeable).
[67] R. Spaemann, *Personen* (1996).

Some NBC members[68] disagree with the previous bioethical evaluations on the basis of some observations expressed during the debate and that they intend to synthesise as follows.

I. General observations.

Despite the title *Chimeras and Hybrids*, the document tackles almost exclusively the ethical problems raised by cytoplasmic hybrid embryos (cybrids), obtained through the technique of transferring the nucleus of a somatic human cell into a denucleated animal egg cell, in which animal mitochondria are still present. This technology has been the object of ample debate in Great Britain, following the request of authorisation to carry out such an experiment, advanced by some researchers of the *Human Fertilisation and Embryology Authority* (HFEA). The experiment has limited scope and has two aims:

a) studying in more depth the biochemical processes through which the nucleus of the somatic cell is reprogrammed by the egg cell, with the purpose of identifying the biochemical determinants of the reprogramming process so that it can be reproduced in vitro, without having to use egg cells anymore (in the experiment in question, they intend to use animal cells because of the insufficient availability of human egg cells);

b) attempting to acquire embryonic stem cells to compare with those obtained by human embryos formed in vitro for research purposes.

With regards to this second objective (which, in the current state of the art, cannot be achieved for sure), we want to stress that, in any case, embryonic stem cells so obtained can be research instruments to investigate specific biological issues, but can never have a clinical use because of their contamination with animal biological material. The regulations regarding the safety of any biological product used in the human field are contained in the EC Regulations number 1394/2007 of the European Parliament and of the Advanced Therapies Council.

In giving the authorisation, the HFEA felt that the projects – despite the presence of animal mitochondria – involved "living human embryos" and therefore subjected the projects to the same strict obligations that are in force with regards to the authorisations to produce human embryos for research. In particular: a) the embryos obtained in this manner cannot be transferred to a woman's uterus; b) must be destroyed by the 14th day. Clearly, the experiment stays confined to the laboratory, it must finish within a fixed term and categorically excludes (according to English law this would be a crime) the transferral of the product of the experimentation in a uterus.

At the moment we don't know if this experiment will be carried out, but in any case it is part of a wider international debate on the problems arising from interspecies organisms and, therefore, on the so-called "overcoming of the species barriers", and, more in general, on the perspectives of scientific research in the field of stem cells and of their possible therapeutic applications. Because of the arguments' novelty, rather than giving normative instructions, it would be appropriate, according to the abovementioned members, to begin a more in depth study of the whole field of research, in order to clarify the ethical and scientific implications for public opinion: in other words, to open, without any preconditioned moral condemnation, the debate on the wide scope of ethical, philosophical-anthropological and scientific issues raised by these recent developments in research and technology.

But, even wanting to focus the attention, for exemplification purposes, on the experiment of cytoplasmic hybrids, it is necessary, to evaluate the moral meaning of these new

[68] C. Flamigni, S. Garattini, D. Neri, A. Piazza, M. Toraldo di Francia, G. Zuffa.

entities' experimental creation, to discuss at least two points: a) the possibility of a "biological" definition of the human embryo; b) The definition of "beginning of personal human life". It is also true that the NBC has already presented an opinion on these issues, in 1995 (Identity and Status of the Human Embryo), but we don't see why we shouldn't, given the opportunity in this new document, return to this topic – as already proposed by those who subscribe to these observations – focusing our attention on the interesting debate taking place internationally, with regards to the possibility of agreeing on a biological definition of human embryo[69].
The current definition of human embryo, in effect, generally includes all the biological entities created following the fertilisation of a human oocyte by a human spermatozoon. Recently however, it has been possible to create similar entities, to which in principle we have to assign the definition of embryos, in other ways, like the introduction of parthenogenesis and the transferral of the nucleus of somatic cells (SCNT) in human oocytes and, as we mention in this document, also animal; in the case of the transferral, also the possibility of "silencing" some genes in order to make any development biologically impossible (we refer to the so-called *Altered nuclear transfer* proposed by W. Hurlbut and discussed by the President's Council on Bioethics[70]). For this reason many biologists have raised the question of whether it is appropriate to "rethink" the biological definition of "human embryo".

In the light of the various emerging technologies and taking into account the development potential and the genetic make-up of the products so obtained, the abovementioned article by Findlay, proceeds to analyse these possibilities (some, at the moment, only theoretical) to illustrate how biotechnologies can produce "living" structures that cannot be implanted or that cannot allow the birth of beings able to live and can lack the genetic contribution of one of the two gametes or, finally, can contain the DNA of two different species.
Findlay's conclusions can be summarised as follows:

1) in order to be defined as "human embryo", a biological entity must be potentially able to form a living being;
2) It is not instead indispensable, for this purpose, that the biological entity is formed following a fertilisation process and a singamic mechanism;
3) The biological definition of human embryo should not exclude, in particular, entities formed with the DNA of two different species;
4) It is necessary to question whether the definition of human embryo must refer to a specific point in time during the development. In the biology of development the definition of human embryo generally includes the reference to a certain moment in the development, but in the context of the potential capability of continuous development. In this sense, the term human embryo cannot be applied before the singamy, that is, the completion of the fertilisation, the moment in which the genome of the new individual is formed starting with his/her parents' genomes.
A definition of human embryo based on singamy, however, excludes reproductive biotechnologies that don't make provisions for the fertilisation of a human oocyte by a human spermatozoon. Some of these technologies are theoretically capable of leading the creation of living human beings, others aren't. It could therefore be more appropriate

[69] Cf. J.K.Findlay, *Human embryo: a biological definition,* "Human Reproduction", 2007, 22, p. 905.
[70] Cf. President's Council on Bioethics, *Alternative sources of human pluripotent stem cells*, Washington, D.C., 2005, pp. 36-49.

to evaluate the potential ability that these entities have of developing up to the appearance of the primitive embryonic line or of going beyond its appearance.
Therefore the definition of human embryo should be separated in two components: the first, relative to the development processes resulting from the fertilisation of a human oocyte by a human spermatozoon, and the second relative to what can derive from other processes.
At this point, Findlay proposes the following definition of embryo: a human embryo is a distinct unity who can originate from:
a) the first mitotic division, at the moment in which the fertilisation of a human oocyte by a human nemasperm is complete;
b) any other process that starts the organised development of a biological entity with a human nuclear genome, or a modified human nuclear genome, who have the potential capability of developing at least to the stage in which the primitive streak appears.
This definition tries to take into account the categories that represent the various stages of development, the structure's potential capabilities and the origin of the DNA, which contributes to the formation of the new individual. Findlay admits that there can be confused and anomalous situations, like the possibility of defining as embryo an entity who has formed by natural fertilisation and then lacks the potential capability of continuing in his development, whilst a similar entity artificially created might not deserve this definition. On the other hand, Findlay observes, there's no doubt that the definition of embryo must be given by rights to a human oocyte fertilised by a human nemasperm, regardless of his potential development capabilities.
On this considerations is based the second argument, relative to the definition of "beginning of personal human life", which seems indispensable for the purpose of establishing whether personal dignity can or cannot be recognised to the various entities to which we intend to attribute that definition. If, for example, we accept the definition of "beginning of personal life" presented by the supporters of relational personalism, which favours the implantation into the uterus as condition of relational subjectivity, the conclusions regarding the level of dignity to be recognised to cytoplasmic hybrids would be very different from those presented by the previous bioethical evaluations.
This hypothesis of a personal life that begins with the implantation of the embryo in the uterus is at the basis of a third form of personalism (the other two are those defined as functionalist personalism and substantialist personalism), which does not attribute to biology or to functional services the person's diriment character. This personalism, defined as "relational", links the dignity of life to the context of relations of which it's part and to the project of life it expresses. At the end of its nesting within the uterus (14^{th} day) the embryo establishes the cellular communications with the maternal organism and at the same time, within it, the beginning of the differentiation between embryonic component and extra-embryonic component takes place. From that moment the embryo is no longer a guest in the uterus, but he/she is intimately connected to the maternal tissues, a bond that is not only biological, but leads to an intense relationship of communication and from which starts a project of life based on that relationship. In other words, the relationship with the mother is defined and the one with the outside world is clarified: the embryo becomes a *being-in-relation.* In this way his/her identity in the relationship becomes a significant element, which the embryo did not have before the nesting.
According to the supporters of this particular form of personalism, the relationships represent a summative and qualifying anthropological trait, where biology is, anthropologically, necessary but insufficient and the functions are important but not

crucial. A further consequence is that the embryo cannot be considered apart from his/her project of life and context: the implantation into the uterus signals the passage from a phase in which the embryos don't have a configuration of relationship that is significantly human and can be used (for example in scientific research) with vigilance and caution, to a phase in which they have an established configuration of relationship and must therefore have a different kind of protection. Beyond the mere biological fact, the recognition of the mother as a protagonist in the making of the new living being and of the relationship as an element that links biology and biography, make this passage a key anthropological threshold also from a theological point of view.
In conclusion, this connection of perspectives opens the way to the possibility of using the embryos, formed but not yet implanted, for scientific research purposes, but it does not intend to be an indiscriminate consent: if human life – because this is what we are talking about – must not become sacred, it also cannot be used at a whim, in the interest of scientific or economical powers.

II. Specific observations.

The general observations presented above are the background to some specific observations on particular points based on two orders of arguments.
The first refers to the evaluation of scientific research and of the reasons presented in defence of its practicability and concludes with the statement that "the studies on cytoplasmic hybrids at the moment are hardly justified". But the mere fact that the scientific proposition is, at the moment, scarcely justifiable from the point of view of previous scientific evidence, does not imply its immorality, but, at most, its uselessness. The connection between scientific justification and morality of research is important in pharmacological and clinical trials on human beings (who would be subjected to a useless risk if the protocol was hardly scientific) and not in base research. We also want to stress that, in the case of the experiment in question, rather than talking about scarce scientific justification (in the previous bioethical evaluation at the end of paragraph 2, the protocol followed in creating hybrids is described as "sufficiently standardised"), we should talk about scarce evidence with regards to the possibility that this experiment will reach its objectives: but the mere circumstance that, in effect, an innovative proposition cannot demonstrate any scientific evidence that is already established, cannot be a reason to condemn such proposition. From the point of view of the internal criteria governing scientific research, it would be a contradiction to state that a proposition that is not yet referenced by evidence must not, only for this reason, be pursued: this would be the same as saying that no innovative research can ever be undertaken. In 1980 the Nobel Prize Mario Capecchi was refused funding for his research on homologous recombination by the NIH, as they were considered "unworthy of being pursued". Capecchi did now allow this to discourage him and, some years later, granting him the funding, the NIH stated: "We are happy that you did not pay attention to our assessment"[71].
To these evaluations about the scientific value of the research in previous bioethical assessments, we add a critique of the reasons given by researchers in defence of its practicability. In reality, these are ideas taken from the consultation promoted by the HFEA – that cannot directly be attributed to the researchers in question – in the course of which some have voiced the fear (see letter a) that, once this practice is made legitimate, we could give into the temptation of aiming to "liberalise other forms of interspecies fertilisation" and to "transfer such hybrid cellular populations into the human or into the

[71] S. Simple, "Scientist Profile: Mario Capecchi, Ph.D.", Genetic Science Learning Center, University of Utah: www.gslc.genetics.utah.edu/features/capecchi/.

animal body": aims that in addition, if realised, would be a crime according to the current English legislation, as already recalled.
As for the letter b), we observe that the fact of wanting to protect women is an ethically significant caution, to eventually balance against the possible risk for the cybrids, which we actually don't know exactly what it could be and that, in any case, would require a preliminary decision about what type of protection can be assigned to such entities.
Finally, with regards to the letter c, we observe that, should this research open therapeutic perspectives of recognised interest for human health, the eventual increase in the demand for human oocytes – with all the ethical problems that it undoubtedly involves – should be balanced against the importance of such therapeutic perspectives. When we use a consequential argumentative style, we must in fact be ready to examine the positive and negative consequences of the practices under discussion and to attempt to balance them.
The second order of arguments refers to more general ethical reasons, which can be summarised in three points: a) the protection of the dignity of the human embryo and of the integrity of the species; b) the precaution principle, in relation to the protection of the ecosystem and to the consequences for future generations; c) the feeling of repugnance.
With regards to the first point, the value of the argument depends on the answer to the question of whether the cytoplasmic hybrid is a human embryo or if the presence of mitochondrial DNA of animal origin makes it non-human. On this point, we want to recall that such entity is the product of cloning through the transferral of the nucleus of a human cell and that in the most usual definitions of cloning (cf. law 40 "Regulations on medically assisted procreation", art. 12, subsection 7; additional Protocol to the Convention on human rights and biomedicine, art. 1, subsection 2) the nuclear genome is considered essential – for an ethical and legal qualification – not the mitochondrial one.
In any case the meaning of the following arguments about the damage to the "quality of the embryo's identity", the "radical manipulation of the human being" and finally the conclusion to this first point, rests on the attribution of the human character to the cybrid. However, in previous bioethical evaluations we simply state that such attribution "has not yet found a shared response", preferring to fall back on the "uncertainty of identity" that, from what we understand, does not depend on the mere combination of biological material, but on the impossibility of the "intuitive" recognition of the new living being as belonging to the human species. The lack of intuitive recognition – together with the instinctive "feeling of repugnance" (see later) through the overcoming of the so-called "barriers of species" – would be an uncertain proof of the cybrids' identity, which therefore would be a sort of "degraded human beings": from this the charge of damaging human dignity, the species' identity and the meaning of human itself. The link between these statements and the experiment discussed in the whole document is very vague and, in any case, it takes for granted that the circumstances at the centre of the second point of these bioethical evaluations have been realised.
This second point is based on the intentions, attributed to not better identified researchers, aiming at liberalising other forms of interspecies fertilisation, transferring such hybrid cellular populations into the human or into the animal body to verify their possible survival. On this basis, we can imagine a situation in which such entities would be released in the natural and social environment, putting at risk the balanced relationships between living beings and affecting future generations, which would suffer, without being able to express their consent, an "interspecies mixture", also because of the possibility of an "interspecies reproduction", discussed further on, which would threaten the opportunity of thinking about our identity of human beings. There is no way of expressing a logical evaluation of this situation; as stated in the previous bioethical

assessments, the possibility of its occurrence “in fact cannot be excluded”, just like, in fact, nothing can be excluded about the future.

The third point refers to the “wisdom” of the instinctive feeling of repugnance towards experimentations which put in danger the identity of the human species itself. Naturally the entity of this danger is proportional to the plausibility of the situation described above, but the fundamental problem is to understand how much wisdom can be ascribed to the feeling of repugnance. In fact, one thing is to observe that something generates a feeling of disgust or repugnance (we could, at most, believe that this observation is a sort of warning signal), another thing is to state that on this feeling we can found a judgement of immorality about something or, even, to ask for it to be forbidden by law. We cannot in fact forget that, not long ago, that feeling was invoked – as the Danish Bioethics Committee, referred to in the note, recalls – to condemn as immoral homosexuality; and the examples could multiply to show how not always, in past history, that feeling expressed wisdom and good sense, but immoral prejudice instead. In any case, not accepting the recourse to the feeling of repugnance without any criteria of distinction (if not that of the mere passage of time), calling upon it today to condemn this or that practice is only an expression of the personal preferences of those who appeal to it: its content of wisdom or prejudice can only be evaluated by future history.

Synthesis and conclusions

Following the previous considerations, some NBC members[72] arrived to the conclusions synthetically reported here.

- The mixture of human/animal tissues and/or cells and/or genes can raise bioethical problems emerging in particular when the created organisms have an uncertain identity, as they lead to overcoming the barriers between the human species and animal species; this problem presents itself for some types of chimeras and transgenic organisms *and for all hybrids*, in the case of mixtures between the human and non-human species.
- Every scientific experiment that alters the identity of the human being and of the human species is not ethically acceptable, even if carried out in the name of the increase in knowledge that we can derive from it.
- The same NBC members, for the reasons discussed in this document, hope for the suspension of the creation of man-animal hybrids and, only if adequately justified, the use of alternative research techniques, as for example the hybridising between different animal species, which still requires a careful and adequate bioethical evaluation.

Other NBC members[73] instead have reached the following conclusions:
There is no doubt that the problems discussed in the Document – overcoming of the barriers between species, creation of new entities mixing human and animal genetic material in a laboratory – have an enormous relevance and call for great caution in the evaluation and control of these new powers of intervention. However, for those who don’t accept the “slippery slope” argument (especially in the catastrophist form described above), because they believe that the responsibility principle requires, today more than

[72] S. Amato, A. Bompiani, S. Canestrari, R. Colombo, A. Da Re, F. D’Agostino, L. d’Avack, B. Dallapiccola, M.L. Di Pietro, M. Gensabella, A. Isidori, A. Morresi, A. Nicolussi, L. Palazzani, V. Possenti , R. Proietti, L. Scaraffia.
[73] C. Flamigni, S. Garattini, D. Neri, A. Piazza, M. Toraldo di Francia, G. Zuffa.

ever, the recognition of differences, which change things, and do not agree with the ethical thesis of the absolute protection due to the human embryo in the very first stages of development (even when founded on the topic of the uncertainty of identity), the condemnation of the creation of cybrids cannot be shared. We believe, in fact, that the empirical observation that cybrids are not destined to develop makes a difference and that a transparent and rigorous control of this type of experiments – if conducted for cognitive purposes and if they can reproduced in different laboratories – is, even from the bioethical point of view, more acceptable than their a priori condemnation founded on an excessively strict application of the precaution principle.

APPENDIX

Legal discipline

1) The legislation in some countries.

Italian legislation precludes, as well as "interventions of cloning through nucleus transferral", all forms of creation of chimeras and hybrids, punishing with a sanction of up to more than 6 years "the fertilisation of a human gamete with a gamete of a different species and the creation of hybrids or chimeras" (art. 13, subsection 3, c-d of law 2004/40 "with regards to medically assisted procreation"). It's not completely clear, however, to which of the two crime hypotheses we must assign the creation of cybrids, which is this document's topic: the cybrid, in fact, is not created from the fertilisation of a human gamete with a gamete of a different species (letter d), but from the transferral of the nucleus of a human adult cell (letter c), into a denucleated animal oocyte.

We can get other ideas – still not always clear with regards to their legal qualification as hybrids or chimeras – from other countries' legislations concerning the research on embryos, which, in general, move between two extremes: on the one hand, more liberal legislations (for example, some Asian countries like China, Singapore and South Korea); on the other, the rigid ban on using embryos in research (for example some European countries like Austria, Germany, Italy, Poland, Lithuania, Norway, Slovakia)[74]. In between there are countries (for example India, South Africa, Israel, England) that allow the experimentation on embryos (the first two also the nuclear transferral or therapeutic cloning) under the control of specific *Authorities.* A variety of European countries follow a middle way, using only residual embryos below the 14th day of development (Spain, Sweden, Denmark, Finland). Only Germany (*Embryonenschutzgesetz* of the 13th of December 1990) limits the use to the 21st hour from conception. The French parliament, with a measure put in place on the 22nd of January 2002, banned cloning for therapeutic purposes, authorising research on surplus embryos deriving from assisted fertilisation. The Belgian law of the 11th of May 2003 limits research to surplus embryos, but allows cloning (nuclear transferral), when the research objectives require it. Nuclear transferral is, currently, accepted also in Sweden and Finland.

In Australia it is allowed to create embryos for research through SCNT, but it is forbidden to produce hybrids, except than for testing the quality of the sperm[75]. The *Canadian Assisted Human Reproduction (2004*) explicitly forbids the creation of human/animal hybrids and chimeras and their transferral in human or non-human beings. The creation of hybrids for reproductive purposes is also explicitly forbidden. The *USA Draft Human Chimera Prohibition Act (2005)* forbids the creation of human chimeras, the transferral of human embryos in a non-human uterus, and that of a non-human embryo in a human uterus; some types of man/animal hybrids could be defined as chimeras[76]. We must take into account that, in federal legislation, individual American States present a differentiated panorama. Even here, there are States with a more liberal legislation (for example California, Connecticut, Illinois, Maryland, Massachusetts, New Jersey, Missouri, Rhode Island) and others with much more restrictive regulations (for example Florida, Louisiana, Maine, Michigan, Minnesota, North and South Dakota, Pennsylvania).

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[74] M. Fusco, *Embrioni clonati: sì da Londra. Una "fuga in avanti" nella sperimentazione terapeutica* in "Diritto e giustizia", 2004-32, p. 8 and following.

[75] This is the hamster test, in which a human spermatozoon and a hamster oocyte are fused, with the aim of testing the sperm's vitality. Usually what is created, is a zygote that blocks its own development after reaching the bi-cellular stage.

[76] HFEA document.

In Great Britain the revision of the *Human Fertilisation and Embryology Act* (1990) which banned the mixing of human gametes and animal gametes, apart from the concession of explicit authorisations, was extremely complex. The "White book", issued by the English Government in 2006, stressed the opportunity of keeping the prohibition of creating chimeras and hybrids in vitro, foreseeing the possibility of allowing exceptions. Subsequently, the *House of Commons Technology Committee* reached the conclusion that the creation of chimeras and hybrids is necessary for research, but lacks an adequate legislative definition of the threshold that divides the human from the non-human. In 2007 the Department of Hill issued a detail proposal of revision through the *Human Tissue and Embryo (Draft) Bill*[77]. In 2008 the *Human Fertilisation and Embryology Act* was amply revised with regards to the problem of chimeras and hybrids, adopting in point 4, relative to *Prohibitions in connection with genetic material not of human origin*, the expression "human admixed embryo", to which an extremely analytical and detailed series of hypotheses is linked[78]. It is prohibited to implant into a woman's body all these heterogeneous forms of "human admixed embryos", any other non-human embryo, any gamete that is not a human gamete. Nobody can mix human gametes and animal gametes, causing the creation of, preserving and using "human admixed embryos", except when having proper authorisation. It is not possible to authorise keeping or using "human admixed embryos" after the appearance of the primitive streak or after the 14th day from the beginning of the creation process. Even the implantation in an animal cannot be authorised. At the moment of discussing the document, the HFEA *(Human Fertilisation Embryology Authority)*[79] has given three licences as many research groups for the creation of cytoplasmic hybrid embryos, with the condition that they are not transferred to the maternal uterus; that they are destroyed on the 14th day; that research leads to an advancement in knowledge[80].

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[77] In particular art. 17.2 prohibits the implantation of interspecies embryos in a woman's or in an animal's body, describing in detail the various hypothesis: a) embryos created using human and animal gametes; b) embryos created cloning the nucleus of an animal oocyte or a cell derived from an animal embryo with a human cell or the nucleus of a human cell; c) a human embryo altered by the introduction of an animal nuclear or mitochondrial DNA sequence. The authorisation to research cannot allow the preservation or use of an interspecies embryo a) beyond the appearance of the primitive streak; b) beyond the end of the period of 14 days starting from the beginning of the creation process of the interspecies embryo; c) when half of the time of gestation or incubation has passed in which the nuclear or mitochondrial DNA is contained within the embryo.

[78] (a) an embryo created by replacing the nucleus of an animal egg or of an animal cell, or two animal pronuclei, with—
(i) two human pronuclei,
(ii) one nucleus of a human gamete or of any other human cell, or
(iii) one human gamete or other human cell,
(b) any other embryo created by using —
(i) human gametes and animal gametes, or
(ii) one human pronucleus and one animal pronucleus,
(c) a human embryo that has been altered by the introduction of any sequence of nuclear or mitochondrial DNA of an animal into one or more cells of the embryo,
(d) a human embryo that has been altered by the introduction of one or more animal cells, or
(e) any embryo not falling within paragraphs (a) to (d) which contains both nuclear or mitochondrial DNA of a human and nuclear or mitochondrial DNA of an animal ("animal DNA") but in which the animal DNA is not predominant.

[79] HFEA, *Hybrids and Chimeras. A consultation on the ethical and social implications of creating human/animal* embryos in research, April 2007.

[80] The documentation on the projects and licences can be consulted on http://www.hfea.gov.uk/en/1640.html with regards to those given by the University of Newcastle Upon Tyne and by the King's College London, and on http://www.hfea.gov.uk/en/1698.html with regards to those given by the University of Warwick.

2) Aspects of international and European law.

In international and European law we don't find any explicit normative indication in relation to our topic, but a variety of arrangements, regarding aspects relative to the modalities of the reproductive process (legitimacy of cloning), as well as the outcome of this process (patentability of the process or the product), that are linked to each other in a variety of ways by the reference to general clauses of respect of public order (*order public and morality*), of human dignity and of decrease in animal suffering[81]. These express, although in a general way and, as we will see, not easily applicable within the international and European context, the need to leave, in the application of the law, a flexible space, open to scientific development but also to the public perception of the scientific message, to economic pressures, and also to different sensitivities.

In this context, the creation of chimeras and hybrids forces, therefore, a reconstruction of the general lines of legal qualification of the human genome and, within it, the analysis of two specific problems: the legitimacy of the different forms of cloning; the patentability of biotechnical inventions achieved through cloning, including the cloning process itself. In this last case, we would not have a directly repressive intervention, but any possibility of economic incentive would be indirectly affected.

The legal, as well as ethical, relevance of the human genetic make-up is clearly expressed in art. 1 of the *Universal Declaration on the Human Genome and Human Rights* (Unesco 11th of November, 1997) when it states that "the human genome underlies the fundamental unity of all the members of the human family, as well as the recognition of their inherent dignity and diversity. In a symbolic sense, it is the heritage of humanity". In stressing the profound connection between genetic identity and personal identity, the Declaration forces us to respect the "uniqueness and diversity of each individual" (art. 2) and gives very precise limits to research, and to its applications, which can "prevail over respect for the human rights, fundamental freedoms and human dignity of individuals or, where applicable, of groups of people" (art. 10). The collection of values indicated by the Declaration on the genome (dignity, identity, human integrity and the prevalence of human rights on the interests of society and science) characterises also the *Convention on human rights and biomedicine,* issued in Oviedo on the 4th of April 1997 that, in art. 1 number 1 of the additional protocol explicitly tackles, as we will see, the problem of cloning.

In the light of these dispositions two crucial problems remain open: if the recourse to cloning is possible in order to create chimeras and hybrids, and if, in any case, such activity is in contrast with the respect for human dignity and, therefore, with *"public order and morality"*. With regards to cloning we must stress a subtle linguistic divergence between the Declaration on the genome and the Oviedo Convention. Art. 10 of the Declaration forbids "reproductive cloning", whilst art. 1 of the Convention's additional Protocol uses a much more generic expression: "*Est interdite toute intervention ayant pour but de créer un être humain génétiquement identique à un autre être humain vivant ou mort".* Point 2 of the same article clarifies that the expression genetically identical human being means *"un être humain ayant en commun avec un autre l'ensemble des gènes nucléaires".* Can we deduce a general ban, which includes both therapeutic and

[81] The document does not discuss the problems of animal suffering linked to the creation of chimeras and hybrids, reserving the right of tackling this topic in the context of the Document in the course of the elaboration on "Methodologies alternative to animal experimentation". Here will suffice to recall the European convention for the Protection of the Pet Animals (ETS Number 125), which in art.3 (*Basic principles of animal welfare*) states that:

1 *Nobody shall cause a pet animal unnecessary pain, suffering or distress.*

2 Nobody shall abandon a pet animal.

reproductive cloning? In doubt, Holland, at the moment of signing the Protocol, deposited a declaration in which it stated that the term "human being" refers exclusively to the already born and, therefore, it does not include the phases preceding his/her development. Even without discussing the controversy about the various forms of cloning and about embryos' legal status, the Oviedo Convention contains a regulation that is absent from the Unesco text, art. 18 number 2 forbids "the creation of human embryos for research purposes".

We would therefore have a variety of hypotheses:

- hybridizing experiments are in contrast with the ban on any form of cloning (modelled on Italian law and maybe on the Oviedo Convention), even if carried out on animal oocytes;
- independently from the legitimacy of cloning, hybridisations violate the ban on creating human embryos for only experimental purposes (art. 18 Oviedo Convention);
- Although therapeutic cloning is legitimate, hybridisations are, in any case, in contrast with human beings' dignity and uniqueness.

In the last two cases, emerges the problem of the legal qualification of interspecies chimeras and hybrids. They are:

- human embryos, included in the provisions of art. 18 of the Oviedo Convention;
- human genetic material, included in the protection of every human being's dignity and uniqueness
- biological material excluded from any specific form of protection.

We must take into account that these are "entities" obtained without fertilisation, but in many cases through the reprogramming of adult cells for cloning. Scientists themselves present the nature of their research in a different way stressing, in some hypotheses, the importance of knowing the interaction mechanisms between human and animal cells[82] and, in others, the relevance of the study on the possibilities of reprogramming adult cells[83].

Interpreters find themselves facing choices that have profound moral connotations, without having a stable and homogeneous cultural background from which to take inspiration.

For this reason, it is important that every national bioethics Committee fulfils its duty of clarifying the problems and making an effort to highlight the principles and values that should be the foundations of any legal resolutions. Jurists themselves stress the need for a moral integration of their work. The European Commission's Report on *Patenting DNA sequences (polynucleotides) and scope of protection in the European Union: an evaluation*, clearly highlights the need for a moment of ethical reflection: "*the aim of this background study is to give an overview of the various issues involved in the patentability of DNA sequences (polynucleotides) and the scope of protection of such patents. It does not deal with ethical issues, which were excluded from treatment in this study, as they require a different approach, which was outside the scope of the mission of the author and the Expert Group on Biotechnological Inventions"*[84]*.* A similar invitation to reaffirm the centrality of ethics for the democratic legitimacy of the process of integration between

[82] Like in the experiments carried out by the team lead by Hui Zhen Sheng of Shangai's Second Medical University, in which cells of men and women between 5 and 60 years old were transferred into New Zealand rabbit oocytes, developing numerous embryos containing human and rabbit genomes, see ref. [12].

[83] As in the case of the research on cytoplasmic hybrids authorised by the English HFEA in 2007.

[84] *Background study for the European Commission within the framework of the Expert Group on Biotechnological Inventions,* edited by Sven J. R. Bostyn, Directorate-General for Research Food Quality and Safety, 2004 EUR 21122, p. 1.

different European countries, comes form the report of the working Group instituted by the European Commission on *Science and Governance*[85]. Therefore it is necessary not only to identify the individual regulations related to the creation of chimeras and hybrids, but especially to reorganize the problematic issues left open by the reference to morality in the general clauses. A motion towards this was presented by the European assembly: "Embryonic, foetal and post-natal Animal-Human Mixtures"[86], but it has never been questioned. In the draft of the document we read: "The assembly invites the governments of member States to start a wide consultation and reflection with regards to the complex ethical questions due to the creation of animal/human mixed living beings". The aim is to elaborate an additional protocol for the *Convention on Human Rights and Biomedicine*.

3) *Public order* and morality in patenting law.

An important contribution to the models of legal qualification can be gained by the discipline on biotechnological inventions. The 1998 directive of the European Parliament and of the European Council number 44[87], in article 6 subsection 1, excludes the patentability of inventions whose economic exploitation is contrary to public order and morality, however it clarifies that "exploitation shall not be deemed to be so contrary merely because it is prohibited by law or regulation". This clause – already present in many national laws – has always generated many doubts in patenting law's scholars, both with regards to its practical applicability (no patent has even been refused on the basis of this clause), and with regards to its general meaning[88].

It must therefore be taken into account that the concept of *public order and morality* implies a complete overhaul of the widespread feeling of the international community, a feeling that goes beyond the directives of a single country, as recalled by the *Guidelines on biotechnological inventions*, which suggest that *"a fair test to apply is to consider whether it is probable that the public in general would regard the invention as so abhorrent that the grant of patent rights would be inconceivable" (EU Dir. 98/44/EC.).* A trend for the application of the "ethical clause" can be found in article 6 second subsection, which exemplifies a non-exhaustive list of non-patentable inventions: "a) processes for cloning human beings; b) processes for modifying the germ line genetic identity of human beings; c) uses of human embryos for industrial or commercial purposes; d) processes for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefit to man or animal, and also animals resulting from such processes". Also article 27, paragraph 2, of the Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS 2) authorises individual countries to exclude the patentability of inventions that are in contrast with "*ordre public or morality, including to protect human, animal or plant life or health or to avoid serious prejudice to the environment, provided that such exclusion is not made merely because the exploitation is prohibited by their law".*

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[85] *Science and Governance. European Society Taken Seriously.*

[86] Doc. 10716, 11.10.2005 (Mr Wodarg et al.).

[87] The directive has been put into force in Italy by Law 2006/78, which has made more defined and explicit what was more unclear and confusing in the Directive, with regards to the topic of exclusions from patentability. Art. 4 number 1, in fact, in the general part of letter c) reproduces the Directive's generic formula, but in indicating the specific the preclusions contemplates "any proceeding of human cloning, whatever the technique used, the higher stage of development programmed by the cloned organism and the cloning purposes" (point 1), "any use of human embryos, including human embryonic stem cells lines" (point 3) and then it stresses, as a general norm in closing "it is, in any case, precluded from patentability any technical procedure using human embryonic cells" (number 2).

[88] D. Neri, *Etica e brevetti: il caso delle cellule staminali umane,* "Bioetica", 2008, 2, pages 203 and following.

Chimeras and hybrids are not openly mentioned, but "Consideration" number 38 of the EC Directive, clarifying the scope of article 6, explicitly states that the list has a merely indicative character for the purpose of interpreting the reference to public order or morality and significantly adds that "whereas processes, the use of which offend against human dignity, such as processes to produce chimeras from germ cells or totipotent cells of humans and animals, are obviously also excluded from patentability". This principle is stresses by the *Guidelines for Examination in the European Patent Office* (December 2007): *"Also excluded from patentability under Art. 53(a) are processes to produce chimeras from germ cells or totipotent cells of humans and animals (EU Dir. 98/44/EC, rec. 38)".*

We could conclude that the creation of chimeras and hybrids is contrary, beyond the cloning techniques adopted and the embryo's legal status, to the general clause of respect for *ordre public and morality*. However, in the interpretative processes, the boundary between biological sequences easily assimilated to any chemical reaction and human biological sequences which imply protection of dignity profiles, is still uncertain, as recalled in the Commission Report to the European Council and Parliament on the "Developments and implication of patenting laws in the biotechnology sector" (COM/2005-312). Generally, there's no lack of interpretations that assimilate chimeras and hybrids, obtained with any genetic material, to mere "inter-species entity" biological artefacts or "Human/Non-human Interspecifics". An Australian society, Amrad, in 1999 obtained a patent that covered the production of embryos containing human cells and cat, sheep, pig, bovine, goat or fish cells[89]. Edinburgh university had originally obtained a patent (EP number 0 695 351) on the methods of isolation, increment and propagation of transgenic embryonic stem cells. Point 11 clarifies that the term animal cells must be intended in the wider sense "including human cells"[90]. In 2005, the *US Patent and Trademark Office* instead reached the opposite conclusion, rejecting a similar request, because chimeras are too close to human beings to be patentable.

[89] Subsequently, a provider of some of the base materials in the Chinese technological development was sold to an American society, Chimicon international. www.newsmax.com/archives/2003/8/153903.shtml

[90] V. Pignata, *Il contrastato brevetto rilasciato dall'U.B.E. inerente alle cellule staminali animali*, in "Il diritto industriale", 2000-4, pages 313 and following. The patent's owner then modified the request, excluding the possibility of building human stem cells.

PERSONAL REMARKS

Supplementary personal remark signed by Prof. Assuntina Morresi

This remark exists because of a pledge that was publicly although personally taken, as the coordinator of the NBC working group on the topic "Chimeras and hybrids".
On the 15th and 16th of October 2007, the Luca Coscioni Association had the initiative of organising some public encounters in Rome, with the participation, as spokesman, of Stephen Minger – the English scientist leading one of the three research groups which obtained the HFEA's (the English authority on fertilisation and human embryo research) authorisation to experiment on cybrids – and Emily Jackson, HFEA official.
I participated to the meeting held on the 16th of October, directed mainly to Italian parliamentarians, and open to the public. The previous day a similar encounter had taken place at Rome's University La Sapienza, in front of students, teachers and authoritative scholars. It would suffice to leaf through the newspapers published in those days, to see how the visit to Italy was preceded by a widespread media campaign in which this type of scientific research was presented as very promising; through interviews and statements, care possibilities for currently incurable, important diseases like Parkinson were offered. The refusal and the perplexity with regards to the opportunity, from an ethical and scientific point of view, of creating man/animal mixed embryos were often attributed to a conservative position, typical of those who refuse to face the new opportunity offered by scientific progress.
Another argument of the media campaign in favour of cybrids was the ways in which public debate was carried out in Great Britain: seeing the doubts and the controversy raised in the public opinion by this topic, the HFEA in fact promoted a citizens consultation, involving them in encounters, discussion days with experts, and producing informative material on it. A positive model of "participative democracy", to imitate, according to our press.
Starting with this experiment, disallowed by the English law in force at the time, some generative commissions began a more in depth study in order to come up with a new bill on embryology, which would update the one currently in force, and would allow, amongst other things, the creation of interspecies embryos.
Having followed the international debate on this topic for a long time, and having examined the scientific literature and the documentation produced by English institutions, I feel that the research hypothesis was scarcely consistent, from a scientific point of view. I was also unconvinced by some passages in the procedures followed by the HFEA. I agreed and still agree with the idea of a public debate on the topic: but a debate that offers a real opportunity of scientific discussion, fully transparent.
During the encounter of the 16th of October 2007 in Rome, I therefore asked some questions to Stephen Minger and Emily Jackson, pledging to make the answers public – whatever they would be – attaching them to the NBC opinion on hybrids and chimeras, on which, in the meantime, I had started working.
The questions are printed at the end of this remark.
The answers arrive in writing after one year.
Stephen Minger wrote on the 14th of December 2008, directing us to read one of his scientific articles (S. Minger, *Interspecies SCNT derived human embryos- a new way forward for regenerative medicine*, "Regenerative Med." 2007, 2, pages 103-106), in which he explained his experiment's ratio.

Emily Jackson's answer, received on the 9th of January 2009, is printed at the end of this remark, after the questions.
In the document "Chimeras and hybrids" approved by the NBC we can find many of the scientific objections which are the object of the abovementioned questions.
The creation of cybrids for the aforesaid purposes, as it is presented, in the current state does not seem to have any chance of success. The objection often posed is that the purpose of any research is to answer unsolved problems, and to increase the level of knowledge. This aspect is more closely looked at in the attached document, and I would not repeat its arguments here. I simply observe that, as any researcher who has asked for founding at least once will know, every project has its ratio, and it is the researcher's task to prove its feasibility. If a line of research has been shown to be impossible to follow, if it has already failed, if it has presented some insurmountable or irresolvable problems, it is unfeasible to continue proposing it in the same terms.
All this – that is, the possibility of creating man/cow cybrids or not – should have been the object of debate within the scientific community, but superficial information has spread the idea that the experiment is valid and concretely feasible, influencing also the political debate, and directing the English one in particular towards the legalisation of such experiments.
According to the writer, Stephen Minger's and Emily Jackson's answers did not clarify the queries posed in the questions, but I believe that it is important to make the questions and answers known, in order to allow everyone a personal evaluation.

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Questions to Stephen Minger:

1. You wish to produce cytoplasmatic hybrid embryos. To do so, you intend to use a technique that at this point is widely considered ineffective and unhealthy for the animals created.
It is well known from the scientific literature that the rate of success of Somatic Cell Nuclear Transfer is 1-2% for the cloning of animals and zero for the cloning of humans. Ten years since the Dolly sheep cloning, this technique has failed to produce any human embryonic stem cell. The only thing SCNT actually produced is Korean veterinary Hwang Woo Suk's well known fraud.
The importance of mitochondria in the establishment of oocyte functional competence and early development was recently reviewed.
Many advanced the idea that SCNT's failure is due to incompatibility between mitochondrial and nuclear DNA among individuals belonging to the same species.
The cytoplasmatic hybrid embryos you would produce would have both animal mitochondrial DNA and human nuclear DNA. That is to say, these embryos would inherit DNA from different species.
Only one published scientific paper reports a SCNT success in producing embryonic stem cells from hybrid embryos (Chen et al. Cell. Res. (2003).
So far, neither the same research group nor any other one managed to successfully repeat that experiment. In the scientific literature we found out that many expressed doubts and criticisms towards that paper.
On the New Scientist issue of 15th September, in an interview with Robert Lanza of Advanced Cell Technology, we read: "his company has made many unsuccessful attempts to produce embryonic stem cells from animal-human hybrids". They grow to the 16-cell stage, then just before going on to become blastocysts, they block," he says.

Lanza thinks this happens because the mitochondrial genome of the animal "stops talking" to the human genome, blocking further growth.
At this point, my question is: what is the scientific foundation of your experiment?
Perhaps it is the expectation to attain better results due to having an unlimited number of available oocytes?
If so, why is that the cloning of cows failed to yield any satisfactory result so far? Is your research project based upon any more solid scientific evidence?
From which argument you infer that success, that is to say to produce hybrid cytoplasmatic embryos, is possible?
Given that the mixing of DNA coming from different individuals belonging to the same species failed, how can it be possible to successfully do the same with individuals from different species?

2. Your aim is to create "embryonic stem cell lines in order to determinate therapies for neurodegenerative illness".
Many neurodegenerative illnesses are due to altered mitochondrial metabolism. Given this, how can you use a model for neurodegenerative illness in which mitochondrial metabolism is altered since its initial formation, and it is altered for causes other than those inducing the illness?

3. In "Inter species embryos. A report by Academy of Medical Science", one reads: "In the context of cytoplasmatic hybrid embryos, the mitochondria and the cytoplasm represent potential sources of retrovirus within the animal oocyte. [...]. The nuclear genomes of cows and rabbits do contain endogenous retroviral genomes. It is therefore possible that rabbit or bovine oocyte cytoplasm may contain RNA transcripts or express endogenous retroviruses encoded by their nuclear genome. Such viruses might conceivably re-integrate into the transferred human nucleus. While this scenario is not impossible, on balance we consider it to be highly unlikely. To ascertain whether such a genuine problem, expression profiles for endogenous retroviruses could be sought for oocytes from potential recipient species".
The authors specify that for these reasons, these cell lines should not be used for clinical treatment purposes. They also state that with standard laboratory procedures there shouldn't be any problem in this kind of research.
The same problem was noted in several others auditions - oral and written - of the Science and Technology Committee.
After the Creutzfeld-Jakob virus alarm, which originated in your country, I will be very surprised if Europeans institution will not ask for specific guarantees about these experiments. I'll be even more surprised if the ecological associations, which were so openly critical at the time of the Creutzfeld-Jakob virus issue, will not express themselves.
How will your laboratories manage security issues for these experiments? How do you intend to control animal oocytes?

Questions to Emily Jackson:

On certain conditions, Hfea can issue licences for human embryo research.
In your legal system, the definition of human embryo implies that its genome must be entirely human, and the embryo itself must be viable – it must have the potential to grow in the uterus.

Newcastle's Dr Armstrong declared that since the animal contribution to cytoplasmatic hybrid embryos is small, it can be ignored, and the genome can be considered entirely human.
However, no geneticist treats genes in numeric terms. Genome has priorities, it has hierarchies, and as we know well, a very small flaw in one gene can cause a devastating disease, and generally have remarkable effects on an individual's development. Therefore the "numeric" argument is weak.
The second condition requires the embryo to be viable, which is able to grow once implanted into the uterus. With cytoplasmatic hybrid embryos, the law forbids you from testing whether the embryo can grow there.
From parliamentary auditions, and from the observations that followed, we saw it's impossible to define how human and how animal a cytoplasmatic hybrid is.
Therefore, we are not yet sure that Hfea holds the authority to issue such licences.

1. In January 2007 Hfea asked for a legal advice regarding the legitimacy of issuing licences for animal/human hybrids and whether cytoplasmatic hybrid embryos are considered human for the purposes of the HFE Act.
HFEA obtained the legal advice they asked for, but they haven't made it public. They say that they are 'probably' allowed to release licences.
Why hasn't the legal advice made public?

2. I now refer to the pamphlet on line in the Hfea site, "Hybrids and Chimeras", which was published to inform people about the public consultation on interspecies embryos.
On page 5, as the pamphlet describes the importance of stem cell research, one reads "a number of team are carrying out research on stem cells - some derived from cloned embryos and some from embryos created through cloning". It seems that this is refers to human embryos. But we know that human embryonic stem cells obtained from cloned embryos either don't exist, or such results have never been published. Can you explain us the meaning of this phrase?
On page 7 the scientific background is described, and cell nuclear replacement technique is referred to as if it were an effective, currently practised procedure. We know that this is false. Such technique has not been successfully practised in humans, and this fact is not explained in the pamphlet. Why? Perhaps you have information we ignore about cell nuclear replacement being successfully done in humans?

Answers from Emily Jackson:

The High Court this week decided that issuing licences for hybrid research is within our legal powers, and that the application for permission to judicially review whether or not we have that power was 'without merit'.
It is not true to say that in our legal system, 'the definition of human embryo implies that it must have the potential to grow in the uterus'. We exercise regulatory powers over all human embryos, including those that are genetically/chromosomally abnormal and hence unable to grow in the uterus.
The admixed embryo contains a full human nuclear genome, falls within the same genus of facts as an embryo created through normal fertilisation and is live. On that basis, and taking into account that parliament intended the regulatory scheme to be comprehensive, 'there was to be no free for all' (Quintavalle v HFEA, House of Lords, 2003), the HFEA

decided that it could consider applications for research licences which satisfy the statutory requirements.

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Personal remark signed by Prof. Vittorio Possenti

The link between freedom of scientific research and bioethical issues is a fundamental and delicate problem: this personal remark discusses some considerations in relation to the document on chimeras, hybrids and cybrids.
I start from two of the document's phrases which mirror the first position: "The ethical value of the experimentations cannot be considered apart from its strictly scientific relevance: from this point of view, any research that is futile, highly and unnecessarily risky or undeservedly costly can be also ethically criticised." (p.15). In addition, we raise bioethical doubts about the studies on hybrids, believed to be "scarcely justifiable at the moment" (p.16). Within the NBC, there is a discrepancy in the evaluations of these points, between those who adopt an attitude of caution and prudence, and those who believe that the criteria of freedom of research and science are of primary importance. We pause on this issue, asking whether intrinsically unjustifiable research from a bioethical point of view exists, or whether the recourse to biotechnologies is without exception a categorical or unconditional imperative.
Supposing that we give a categorical imperative in favour of the recourse to technology, does this mean controlling nature? Researching useful effects? Lowering human suffering? That these purposes are not completely the same is clear when looking at the issues they tackle: that they are generally legitimate comes from the fact that they depend on specific inclinations present in man and rationally justifiable. Is there an unconditional obligation in them or in one of them? No matter how much we reflect on this complex problem, not one of the mentioned purposes has the character of an unconditional objective and therefore of a categorical imperative without exceptions: not even that of lowering human suffering and malum naturae – which is the most plausible -, because its unlimited and therefore unconditional exercise would end up dealing with human dignity's fundamental aspects as subordinate means and instruments. This criterion represents a hindrance even for technology, in the sense that in its practice it is never legitimate to put in danger the person's essence, existence and dignity.
It is possible to foresee these conclusions observing the profound difference between knowing and acting. Knowledge is always good: there is no bad or forbidden knowledge. Different are things with regards to action, in which the division between good and bad prevails, so that there are good and legitimate actions, and bad and forbidden actions. Pure knowledge, scientific or of other nature, sought for in order to widen the scope of our knowledge, it is not only a profoundly human need and nobility, but it is something intrinsically good. To this pure knowledge it is applied, without restrictions, the axiom according to which there is no forbidden knowledge. But when knowledge brings in itself, in an indissoluble way, a technological action and its access into our life (human and non-human), knowledge's good without restriction is not unconditionally valid.
In effect, knowledge can be increased as much using immaterial and "poor" means, and in this way its increase has an unconditional value, as through the recourse to "heavy" and intrusive methods of acquisition. In this case the increase in knowledge can stop being a categorical imperative, and consequently the freedom of research can stop having unconditional value, even when its purpose is care and therapy. The method of

acquisition of knowledge and its technological application to man cannot be beyond ethical guidelines and the limitations imposed by the need of respecting human dignity. The appeal to the scientist's responsibility, to his/her self-limiting ability, always important, is especially so in "technical sciences", that is, in those sciences that, unlike the strictly theoretical ones who simply know, know by changing, transforming, manipulating their object.

It is therefore not only the minimal level of methodological reference of the research on cybrids that advises us against putting it into practice, but the fact that there is not, at the moment, any evidence on the nature of the entity that would be created and on the eventual threat to human dignity. The criterion of freedom of research can and must be intrinsically limited by the principle of human dignity. In recent years the "heuristic of fear" (cf. H. Jonas) and the "wisdom of repugnance" (cf. L. Kass) have also been mentioned as ideas we can call upon to work in defence of dignity, and, in the case of cybrids, to reject overcoming the species' barriers. These are criteria we need to refer to with some caution, but not completely irrational, also because they invite to carefully evaluate not only the benefits but also the damage they bring. The species' barrier must be protected because of both the anthropocentric privilege and the intrinsic value of what is natural.

"Looking into this, the issue of whether cytoplasmic hybrids are *human* embryos or whether the presence of mitochondrial DNA of animal origin makes them *non-human*, has not yet found a shared response." (p.17). The particular difficulty of this problem comes from the fact that cybrids do not exist in nature, they are not the outcome of the evolution process: cybrids are not part of what has naturally developed but of what is technologically produced, being the result of biotechnological intervention, not the outcome of a philogenetic process, as the document stresses. From this point of view the creation of cybrids is not about the acquisition of pure knowledge obtained without any intervention on the object, but is part of that do-act that cannot escape the dichotomy licit-illicit.