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Ethical questions concerning research on human embryos, embryonic stem cells and, chimeras

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Keywords: Altered nuclear transfer, Human-animal chimera, Human embryonic stem cells, Oocyte assisted reprogramming

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Abstract

Research using human embryos and embryonic stem cells is viewed as important for various reasons. Apart from questions concerning legal regulations, numerous ethical objections are raised pertaining to the use of surplus embryos from reproductive medicine as well as the creation of embryos and stem cells through cloning. In the hopes of avoiding ethical problems, alternatives have been proposed including the extraction of egg cells from "dead" embryos derived from *in vitro* fertilization procedures, the extraction of pluripotent stem cells from blastocysts, technologies such as "altered nuclear transfer" (ANT) and "oocyte assisted reprogramming" (ANT-OAR) as well as parthenogenesis. Initial ethical assessments show that certain questions pertaining to such strategies have remained unanswered.

Furthermore, with the help of new or more differentiated bio-technological procedures it is possible to create chimeras and hybrids in which human and non-human cells are combined. Human-animal-chimeras, in which gametes or embryonic tissue have been mixed with embryonic or adult stem cells, demonstrate a different "quality" and "degree of penetration" from those produced in previous experiments. Not only does this have consequences regarding questions of patentability, this situation also raises fundamental questions concerning the human being's self image, the concept of person, identity and species and the moral rights and duties which are connected with such concepts. There is a need for legal regulation, on the national as well as the international level.

Keywords: Altered nuclear transfer; Human-animal chimera; Human embryonic stem cells; Oocyte assisted reprogramming

1 Ethical questions raised by research using human embryos and embryonic stem cells

In the past few years, the framework as well as the goal of research using human embryos and embryonic stem cells have changed. Initially, embryo research took place within the context of reproductive medicine. For approximately three decades, use of human embryos for reproductive purposes had the "side effect" of bringing about new insights into embryonic development and the interaction of genetic and epigenetic factors. The primary goal was to improve the cultures used for embryos created *in vitro* and to increase the success of embryonic transfer and thus the success rates of assisted reproduction.

Since the late 1990s, in numerous countries, research on human embryos and embryonic stem cells has been conducted to an ever-increasing degree outside the context of reproductive medicine to obtain new clinical-therapeutic applications and to promote pioneer research. Since the cloning of sheep "Dolly", i.e. after a genetically identical organism was created by transferring a somatic cell nucleus into a donor egg cell whose own nucleus had been removed, and the world experienced the first extraction of human embryonic stem cells [1], hopes have been raised that it might become possible to create artificial human tissue. This could open up perspectives for reducing the immune rejection reaction of those receiving such tissue, and for dealing with shortages of transplantable tissue or even entire organs. An additional possibility with clinical relevance is that pharmaceutical substances could be tested on human tissue and that *in vitro* disease models could be constructed on the basis of such tests, which would promote research on pathology and possible therapies. Furthermore, pioneer research efforts aim toward gaining additional insights into human developmental biology and genetic and epigenetic control mechanisms.

1.1 Ethical problems pertaining to the extraction and/or creation of embryonic stem cells

There are currently three ways to extract or produce embryonic stem cells [2], but from an ethical standpoint, none of them are unproblematic.

First of all, it is possible to obtain embryonic stem cells from so-called surplus embryos that come into being as a result of *in vitro* fertilization (IVF) in reproductive medicine. Secondly, embryonic stem cells can be cultivated from aborted fetuses. In recent years, the first method has been favored. As opposed to creating human embryos for the sole purpose of conducting research, many view the

use of "surplus" embryos as unproblematic because they would otherwise be destroyed anyway.[1] However, the use of surplus embryos, which are obtained for the most part from reproductive-medical treatment of women, gives rise to the following objections:

For both methods, the self-purposefulness of the embryos is given due to the reproductive context: they have the status of potential human beings who could have developed an existence. By transferring them to a completely different context, i.e., that of research, the purposefulness becomes alienated because as a form of "consuming embryonic research," embryonic stem cell research uses embryos and stem cells for scientific purposes, destroying them in the process. Moreover, it is quite questionable whether any "free and informed consent" on the part of the biological parents or mothers suffices to put the embryos at the disposal of research, for this would entail a shift away from a general right to protection of human life, to a concept of parentage which would be accompanied by an extensive right of disposal and, ultimately, a moral debasement of evolving human life [3]. Habermas [3] points out that in the past, such a right of disposal could only be applied to things, not to persons. Of course, there are good reasons for granting couples or women the right to make decisions concerning the well-being of a potential child and possible medical interventions to a certain degree in correspondence to their responsibilities towards it. The same holds for a woman's right to be respected as a moral subject in her decisions concerning reproduction, which is to say, her right to resist being compelled to continue a pregnancy, become sterilized or use birth contraception. And yet these rights cannot simply be transferred to the context of research without being challenged, because in this case no efforts to produce a viable human being or to promote his or her future well-being, to respect the bodily integrity of the woman involved or to improve her health through medico-therapeutic action are at issue.

Furthermore, one must argue that the use of "surplus" embryos could inaugurate a practice of willfully creating a surplus. A continuous demand for embryos in research, accompanied by the expectation of a continuous supply of embryos, would influence the clinical practice of artificial fertilization. In light of this scenario, it is unlikely that models designed to avoid the creation of surplus embryos, like those implemented in Austria, would be embraced. It is certainly no coincidence that in Valencia, Spain, for example, a stem cell research center has been established adjacent to a fertility clinic; Spain is a country in which research on surplus embryos deriving from reproductive medicine is legally permissible [4]. Apart from using "surplus" embryos, this clinic requests that their patients donate egg cells. The institutionalized egg-cell donation program enables researchers to conduct cloning experiments, as these call for fresh egg cells (ones extracted from the

[1] The Switzerland's Federal Law on Research with Embryonic Stem Cells from 2003 allows embryonic stem cells to be obtained from "surplus," approximately one-week-old IVF embryos.

ovaries directly) in large quantities. This example clearly shows that the problem of planned surpluses resulting from fertility treatment is inherent to any research that relies solely on "surplus" embryos. If one takes this one step further and addresses the question of possible clinical applications, as does Ron Jones, the managing director of PPL Therapeutics, a private bio-tech firm in the USA, a "supply problem" soon arises [5]. To provide a mass market of patients with replacement tissue, the production depends on the use of embryos, the replenishment of supplies will probably not suffice, even if tens of thousands of embryos were to be available in Great Britain [5]. Jones notes: "Many scientists are satisfied if their experiments are successful two or three times", but as a businessman he sees himself forced to think in terms of production capacities on an industrial scale, for, as he argues, he naturally wants to sell his products to as many persons as possible.

The third way to obtain embryonic stem cells is through cloning, which entails importing the nucleus of a body cell into an egg cell whose own nucleus has been removed. No surplus embryos are used in this process, but rather new ones which have been created solely for the purpose of research. The cell nucleus of a somatic cell, which can be extracted from any human being, is introduced into an egg cell whose own nucleus has been removed; 99% of the genetic material then consists of genetic material from the body cell, with approximately 1% comprising mitochondrial gene material from the "donor" egg cell. Thus, the human embryo that is created in this way is, to a large extent, the genetic clone of the organism from which the body cell originated. Through further cultivation processes embryonic stem cells can be obtained from this genetic clone.

The fact that through cloning, embryos are created for the sole purpose of research raises problems unlike those connected with the use of "surplus" embryos. The cloning process itself – as well as so-called therapeutic cloning which is carried out later to produce immune-compatible replacement tissue – requires many egg cells. These must be extracted from women directly because it is very difficult to cryo-conserve egg cells. Apart from health risks (caused by hormone stimulation, superovulation and operative procedures) and the risks of self-exploitation and commercialization, problems pose themselves in cases where egg cell donation involves a potentially damaging procedure, and potential donors are motivated by financial straits or egg cells are viewed as altruistic donations. It is also problematic to encourage women who subject themselves to IVF treatments due to unwanted childlessness, to simultaneously participate in an egg-cell donation program. For one, hormone stimulation must be increased or repeated in to generate additional egg cells, and this results in greater health risks. Secondly, psychological and ethical studies on human research sufficiently document just how difficult it is to guarantee voluntary and informed consent in the context of medical treatment, because many patients feel emotionally dependent on the

goodwill of the physicians whose care they are in. Furthermore, medically assisted artificial fertilization usually involves additional fees or private financing, which might well encourage those involved to make “barter deals”.

1.2 Reproduction and research dimension of the cloning procedure cannot be separated

The distinction between reproductive cloning on the one hand and non-reproductive, research-driven, or therapeutic cloning on the other has become well-established. However, this conceptual differentiation is based on a form of pragmatic decisionism, which is misleading in terms of ethical assessments. What happens to clones depends on decisions made by the researchers in question; if they want a clone to grow, it is implanted into the uterus of a woman, and if its stem cells are to be used for research, it is destroyed. Thus, the terms of common usage refer to the applications for which the technology is to be used, which is to say, the intentions of the researchers involved, and not to the cloned embryo itself, its developmental potential or the issue of ethical need for protection. Other ethical problems are also posed if one artificially reproduces and fosters a living organism whose chromosome set is almost completely identical with that of the original, rather than creating and “consuming” cloned embryos solely for the purposes of research. In any case, terminology that suggests, from the very start, that non-reproductive cloning is less problematic than reproductive cloning must be criticized. Furthermore, the use of such terminology dissolves the connection between reproduction and embryo research on a conceptual level, but the fact is that stem cell research continues to rely on the context of reproductive medicine, for female egg cells are indispensable for procuring stem cell lines and this fact raises problems.

As mentioned previously, the hormonal stimulation and superovulation that this process requires pose short- and long-term health risks for the women involved. Yet neither they nor they children profit from these serious medical risks. Furthermore, the issue of women’s rights and gender must be taken into account [6]. Should women really be morally motivated to contribute in such a way to the common good, the future of national research practices or future patients? In research contexts, which are dominated by men, role-specific empathy and altruism are expected of women who only rarely fulfill decision-making functions in the political and the scientific arena, and who very often put their generative and reproductive capacity at the disposal of their family and society at no cost [7]. Should an entire branch of research be established on this foundation?

A possible alternative might be to pay the donors, as is done in the case of assisted reproduction in many countries. In the past few years, some female researchers have argued in terms of women’s rights, speaking out in favor of regulating egg cell donations on the model of property rights and contract law. The question that poses itself, however, is whether the problem can be solved by

commercializing egg cell donations for use in other branches of research. By abandoning the principle that the human body may not be commodified, or the model of gratuitous donation, the tendency towards exploiting women from low-income groups or impoverished countries would increase[2]. Furthermore, it would only be logical to expect a market for reproductive tissue to establish itself, a market on which egg cells and sperm are treated as replaceable raw materials completely divorced from their pro-creative biological and social significance.

For one, it is questionable whether such practices would stop at non-productive cloning because endeavors are being made to obtain organ-compatible progeny. In the USA, some couples capable of reproducing receive IVF treatment in connection with pre-implantation diagnostics (PID), for the purpose of so-called HLA matching[3] in order to give birth to a child which could act as a bone marrow donator for a diseased sibling [8, 9]. Furthermore, pioneer researchers are interested in the development of the genotype as a whole, the embryo produced in the laboratory and the phenotype, i.e., the living organism which later evolves, not only the early, embryonic stage of development. It goes without saying that these two "slippery slope" arguments, which warn against potential further developments, fulfill the principle of plausibility more than they serve to justify proscriptions in any strict sense of the word. For the cause of the "slippery" chain of events would not lie in the actions taken to perform research cloning itself, but rather in the expansion of conditions that would prove favorable for certain developments, such as altruistic motives or scientific curiosity. At the same time, the analogies that are based on experiences call attention to future kinds of problems and plausible connections between actions.

1.3 The researcher's burden of proof concerning the moral status of human embryos and embryonic stem cells

Embryos, and under certain circumstances embryonic stem cells, possess the potentiality of becoming human beings, i.e., individuals. Even if their moral status is debatable, a consensus exists that in contrast to other cell systems, human embryos and embryonic stem cells constitute an ethical good. But what characterizes this ethical good and what need for protection derives from this status[4] [10, 11]?

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[2] On the free market, egg cell "donations" which cost between 10,000 and 50,000 US-dollars are offered via Internet, for in the context of reproductive medicine, there is a scarcity of egg cell donations. Cf. Blondinen bevorzugt: das Geschäft mit den Eizellen blüht. Spenderagenturen in den USA vermitteln tausende Studentinnen an ungewollt kinderlose Paare, *Nürtinger Zeitung* from August 19, 2006.

[3] HLA matching, i.e., human leukocyte antigen matching: through PID-selection of a suitable sibling bone marrow diseases of already existing children are to be treated through the transfer of healthy stem cells.

[4] For a differentiated line of argumentation concerning the moral status of human embryos see Düwell (1998) and Mieth (2002) as cited.

The potential of a young embryo, and perhaps also that of embryonic stem cells, can be illustrated by the fact that we cannot imagine the process of becoming a human being without imagining ourselves as having been an embryo before nidation occurred, with this organism already constituting our bodiliness, our gender and our hereditary dispositions. An embryo has potential in the sense of the possibility and capacity to become a human being if it develops in accordance with its predisposition, i.e., if no action is taken or omitted which runs contrary to this predisposition. The argument that "nature" does not implant all fertilized egg cells is not admissible here, for nature cannot be conceived of as a responsible subject. The argument that advocates worthiness of protection in regard to embryos is based on their continuity and identity with human beings as persons and on their potential for being able to develop into one themselves. This provides no compelling reason for viewing embryos as carriers of moral rights directly in the way we view, for example, self-determined adults. And yet the moral status of embryos establishes a worthiness of protection that at least prohibits any unspecific authorization of use for research. As concerns medical contexts in which moral rights of others, e. g., women or parents, are directly affected, or research contexts with a potential for developing concrete therapies for patients who already have diseases, the situation is somewhat different.

Within the framework of this investigation, the few remarks which I have made concerning the moral status of embryos and their worthiness of protection will have to suffice. I hope to make it clear that researchers who use human embryos must provide special justification for the procedures they adopt and the goals they pursue. The burden of proof lies on the side of the researchers; they must demonstrate concretely that an individual human right meriting high regard exists, which is opposed compared to the demand for the protection of human embryos against instrumentalization and the levelling of its moral status in the process of becoming a human being. Although worthiness of protection as it pertains to embryos differs from that enjoyed by self-determining adult human beings, it does not follow that embryos do not constitute human beings.[5]

In ethical and legal evaluations, distinctions are sometimes made between human embryos and embryonic stem cells, with the former being designated as totipotent, while the latter are labeled as merely pluripotent [12]. If embryonic stem cells are not capable of developing into human beings, but rather only have the capacity to develop into various types of cells – this being the assumption behind Switzerland's stem cell legislature and the position held by the German National Ethics Council [13] – then, as the implicit conclusion goes, no substantial moral status can be attributed to these cells. However, the notion that embryonic stem cells are merely pluripotent does not reflect

[5] According to Mieth, this would constitute an ethicistic fallacy. Cf. Mieth (2002) as cited pp. 97–98 [11].

any unanimous scientific opinion, as is often claimed. In fact, a consensus is insinuated that, in light of scientific uncertainty, does not hold.

For many years, a juridic dispute regarding the use of the terms “totipotence” and “pluripotence”, as well as ongoing scientific uncertainty as to ways in which embryonic stem cells do indeed evidence totipotence, have contradicted allegedly reliable biological facts. The term “totipotence” designates, on the one hand, the ability to form derivatives from all three germ layers, i.e., to develop into the various cell types which constitute the human body. Some authors use the term “pluripotence” when referring to this ability. On the other hand, the term “totipotence” is used to refer to the ability to develop all the different kinds of cells found in an embryo, and thus the capacity to develop into a viable human being. In this case, if one speaks of the totipotence of a stem cell, what is meant is that it possesses all features of an egg cell necessary to initiate embryonic development. Among other things, this calls for information from the cell plasma. Theoretically, a stem cell which is totipotent in this sense of the word should be able to develop into a fully functioning blastocyst. Whether human embryonic stem cells do in fact fulfill this definition of the term “totipotent” is a question that could only be answered by implanting some of them into a uterus. As concerns embryonic stem cells in mice and several other species of mammals, this has been proven to be the case [14, 15]. Although no one has yet succeeded in creating viable embryos from embryonic stem cells of human beings alone, these can apparently develop into completely viable individuals if they are cultivated together with the nutrient cells (trophoblasts) which usually surround an embryo [16]. Thus, it remains to be seen whether human embryonic stem cells can in, and of, themselves develop into viable human beings. In light of such uncertainties, it would seem premature to view pluripotence as a given fact. Furthermore, ethical clarification concerning the question as to whether the aforementioned notion of totipotence encompasses the fulfillment of certain preconditions, such as the existence of certain nutrient cells that are usually “naturally” given is required. Any opposing assessment made on the basis of a scientifically unclarified matter without any further substantiation remains inconclusive. In fact, there is substantial evidence to the contrary, namely that totipotence in an exhaustive sense of the word is given here, so that any attempts to deproblematize research with embryonic stem cells on the grounds that a lack of inherent potentiality prevents them from developing into human beings would seem to be unacceptable. Instead, an additional problem might pose itself, namely that by conducting research with embryonic stem cells a large number of genetically identical clones are created that have the potential for becoming human beings.

1.4 Explication, high priority and reachability of therapeutic research goals

In response to the question as to what goals researchers pursue, the first one usually mentioned is a therapeutic goal, namely i.e., to use research on young embryos to improve reproductive medicine, e.g., to increase the success of IVF. One possible improvement concerns the nutrient solution, the aim being to succeed in cultivating embryos for longer periods of time so as to increase the accuracy of pre-implantation diagnostics. Such therapeutic goals require justification, however. At first sight, the possibility of being able to cultivate a human blastocyst for more than 5 days outside the uterus in order to gain more time for molecular-genetic examinations using more than one or two cells is quite appealing. However, if the improvement of genetic pre-implantation diagnostics leads to the rejection of embryos, while at the same time being looked upon as an unchallengeable goal of therapeutic research, what we find ourselves confronted with is a mixture of instrumental and ethical goals. Long-term goals such as the development of an artificial uterus also need to be legitimized in terms of the purposes for which it would be used.

The development of reproductive medicine, for example, illustrates the necessity for explicating and evaluating therapeutic research goals individually, for this field has been characterized by a dual set of objectives ever since it began to establish itself in the early 1970s. Looking back at the development of IVF, Edward Brown, the "father" of the first test-tube baby, attests that the desire to gain new insights into embryonic development and molecular genetics guided researchers' actions to the same degree as did the prospect of developing means of treating infertility [17].

In the meantime, embryonic and stem cell research is used to pursue therapeutic goals in other medical disciplines as well, such as the production of replacement tissue and, under certain conditions, even transplantable organs. In particular, perspectives for developing therapies for diseases that are as yet incurable or hard to treat such as diabetes, Morbus Parkinson and Alzheimer establish a necessity for procuring and utilizing embryonic stem cells. Within the 'scientific community', notions differ concerning the reachability and quality of final therapeutic goals as well as the ability or inability to substitute the means used to reach these goals. For one, the prospect of clinically applicable cell replacement therapies is controversial; one might consider the clinical results of animal models for Morbus Parkinson, for example. Other forms of therapy even seem to offer more promising alternatives for treating this disease, such as electro-stimulation [18].

In attempts to justify research with embryonic stem cells, the argument that it could help to improve therapies and techniques is sometimes used strategically, with patients being instrumentalized by unrealistic promises of curability. The microbiologist and Parkinson patient Hans Zähner reproaches researchers: "We patients feel betrayed and misused. Betrayed, because hopes were

raised by those who could know, who must know, that they cannot be fulfilled. Misused because we are deployed to combat resistance against stem cell research“ [19].

And the politically inspired coinage of new terms such as “therapeutic cloning” helps blur the goals that have been set. In the case of cloning procedures currently in use, it would be more apt to speak of ‘research cloning,’ as the development of any conceivable clinical application remains a thing of the far future. In particular, this concerns the prospect of being able to produce transplantable organs. As yet we have no knowledge of the temporal and spatial degree of coordination that comes into play in the differentiation and growth processes of various cells and tissue within any given organ, nor can we simulate it in any laboratory.

Ethical assessments come to different conclusions in cases where patients who already suffer from diseases have concrete hopes for help provided by new therapies as opposed to cases where researchers can only formulate vague notions concerning possible clinical applications. What is termed therapeutic research must be scrutinized in terms of what realistic goals are pursued to the benefit of which concrete individuals in possession of certain rights and which kind of medical help such individuals are entitled to[6] [20]. Until it is possible to clarify whether such medical performance is really to be viewed as therapeutic – in the sense of providing cures for concrete patients – the argument that research on human embryos will result in an improvement of therapeutic options may not be brought into the equation to an unlimited degree. In the case of an assessment that weighs morally relevant goods and moral rights against each other, one must distinguish between therapeutic research with concrete applicability, i.e., research that offers realistic success for certain individuals, and research whose clinical applicability is questionable or merely anticipated as an option for the far future.

1.5 Prospects for success and lack of alternatives in research using embryos and embryonic stem cells

Concerning the creation of replacement tissue and organs on the basis of cloning, great uncertainty prevails as to whether and when clinically applicable therapies can be developed. Some researchers, such as the brain and stem cell researcher Wiestler, even deem it to be improbable that this will ever happen. As he contends, the genetic programs of all cells obtained in this way evidence defective control mechanisms, arguing that it would be completely inacceptable to implant a cell whose genetic blueprint had been destroyed [21]. This problem fundamentally calls this technology into question, for hundreds of egg cells are required to create a viable mammal clone, and to obtain, in turn, a few intact embryos and ultimately one or two stem cell lines. There are also indications that

[6] As an ethical approach which provides grounds for individual moral rights as well as criteria for weighing conflicting rights cf. Gewirth (1978) as cited [20].

animal experiments motivated by this goal carry a high risk potential in terms of deformities, tumors and accelerated aging.

The hope is that research cloning will lead to the production of immune-compatible organ cells. And yet many questions remain unanswered. How can it be proven that the transfer of cells causes the diseased organ to begin functioning again, rather than resulting in damage of the implanted cells as well? Furthermore, the age of the stem cell deriving from the original organism is apparently transferred via the stem cell lines, as has been shown in animal experiments using the "Dolly method". How can we deal with the problem of cell aging? Has the problem concerning immune defense actually been solved in light of the fact that genetic material stemming from the foreign egg cell whose nucleus has been removed also influences the cloned cell material? Thus, there is complete uncertainty as to how the embryonic stem cells that have differentiated into individual body cells would behave in the human body. In light of this, Wiestler sees the relevance of clone experiments, which according to his assessment can for the most part be conducted on animal cells, as limited to basic research: "Perhaps they help us to understand which factors in the cell sap of an egg cell serve to bring a cell nucleus back to a very early stage of development" [21].

Not only can we ascertain that cloning technology is not necessarily suitable as a point of departure for the clinical therapies that researchers aim to develop, but that other forms of basic research that might prove to be relevant for the treatment of those diseases named in connection with research cloning have not been pursued. Cell replacement therapy would not enable us to cure Diabetes mellitus or Morbus Alzheimer, for example [22]. In the widest sense of the word, diabetes mellitus is an autoimmune disease, and thus replacement cells would presumably be affected in the same way that the body's own cells are. Until we have gained an understanding of the pathological process it is unlikely that much can be done by repeatedly implanting replacement tissue. In addition, problems concerning tissue rejection and tumor formation have not been solved yet either [23]. Alzheimer's disease, which entails damage of the brain caused by protein deposits, is a similar case.

To justify conducting research with embryonic stem cells it does not suffice to raise general hopes of new forms of therapy. Instead, already existing therapies and prospective therapies as well as their future developmental potential must be analyzed. Furthermore, it would be necessary to show whether and which clinical symptoms of a disease are so serious that they warrant resorting to ethically sensitive goods for research purposes under certain conditions.

At present, cell or even organ therapy cannot be looked upon as the remedy for the future, and for this reason, prospects of its future feasibility do not serve as justification for embryonic research. Therapy goals that claim high priority in research must be scrutinized in terms of their realizability

before they can carry any weight as legitimation for the utilization of human embryos. The sciences must provide concrete evidence and also show that no other means and methods exist that are less problematic from an ethical perspective. Moreover, the problem-solving rule must be taken into consideration, which says that new developments should not create problems more serious than the ones they claim to solve. The "problem balance sheet" for somatic nucleus transfer, for example, does not necessary speak in favor of its clinical application.

As soon as researchers stop setting their sights on clinical-therapeutic applications, and focus, instead, on basic scientific insights, ethical goods and goals must be weighed differently. From an ethical perspective, the issue as to whether human embryos and stem cells may be used to achieve far-reaching goals of pioneer research or not is extremely controversial, for in this case it is not possible to argue that there is a lack of alternatives. This argument does not hold for any general gain in knowledge, but rather for research on therapies and the development of techniques and technologies which promise to cure or alleviate diseases, and, one must add, in a justifiable way. Equally inadmissible is the argument that, in the interests of new insights, research should not be constrained in any way. Thus, pioneer research that explicitly defines itself as such must be evaluated separately. The expectations placed in embryonic stem cell research are that, among other things, new insights into fundamental mechanisms of cell programming, the transition from the geno- to the phenotype and embryonic development could be gained through it. This is not objectionable, and yet in pioneer research, means and methods for which a moral consensus exists should be favored – particularly in light of the fact that their outcome is uncertain. Thus, it would be possible, for example, to continue working with animal models, animal embryos and embryonic stem cells from animals as well as human adult stem cells with an aim towards gaining a better understanding of fundamental mechanisms that regulate the programming and reprogramming of somatic stem cells or the immune system. Were research on human embryos and embryonic stem cells to be prohibited, this would not bring pioneer research in the area of molecular genetics and embryology to a halt. Were research funds to be invested in creative, farther-reaching efforts to find ethically unproblematic alternatives, this could help to establish new areas of research.

1.6 Procedural limitations

For several years, procedural limitations at the national and international level have been set to fulfill the demands for protection of embryos. These include notification requirements, justification requirements and licensing through a commission, for example. Legally speaking, if this practice continues to be pursued, this will mean a shift in German, and, to a large extent, European interpretation of law in the direction of the Anglo-Saxon legal system.

Ethically speaking, if decisions concerning important ethical issues are delegated to commissions and procedures, this means that the protection of embryos will no longer hold for the integrity of each individual embryo, thus implicating, in turn, a rejection of what has in the past been deemed a high priority[7] [24]. For regulatory procedures cannot fundamentally change questionable research practices. What lies behind such procedures is, instead, the ethically questionable assumption that a good end justifies poor means if these are used "sparingly and carefully".

2 New alternatives for procuring human embryonic stem cells: initial ethical assessments

In the search for alternative, ethically less problematic procedures for producing and conducting research using human egg cells, embryos and embryonic stem cells, several techniques and research designs have come into focus.[8]

In the USA it is not permissible to fund research projects that aim towards "consuming" embryo research with public resources. For this reason efforts are made here to find methods for obtaining embryonic stem cells which do not entail producing or killing embryos. In a statement made by the President´s Council on Bioethics from May2005, several alternatives were presented and discussed [25].

2.1 Extraction of egg cells from organically dead IVF embryos

The so-called Landry-Zucker proposal entails extracting stem cells from organically dead IVF embryos in the blastocyst stage [26]. As reported, it often happens that the cells of an embryo stop dividing at some point after fertilization has occurred, rendering them no longer suitable for transfer into a uterus. It is argued that the situation is similar to that of transplantation medicine, which involves removing organs after brain death has been ascertained, and thus calls for a criterion to determine what constitutes death. Here, we are told, it is more difficult to define clear parameters, because, as opposed to the case with adults there are no central organs such as heart or brain which control the functions of the organism. If, on the one hand, the relative or complete inability of the blastocyst cells to divide is to be viewed as a criterion for death – using, e.g., an observation period of two 24-h segments – and on the other hand the stem cells which are to be extracted still have to be capable of dividing, the parameters would be a central issue. To develop a practical definition

[7] Cf. Mieht, D., Embryonale Stammzellen, die spezielle Forschungsverantwortung, 2006, unpublished contribution, University of Tübingen, Germany.

[8] I am grateful to Matthias Behrends for his competent literature research on ANT, chimeras and hybrids.

one would have to identify physical or biochemical cell markers in experimental studies which indicate a termination of cell division.

At present it is not possible to implement such a procedure. Not only does it appear difficult to arrive at the necessary "definition of death" in empirical terms; like the criterion for death used in transplantation medicine, such definitions would be oriented towards pragmatic interests. Furthermore, the question is raised as to the ethical legitimacy of looking for embryos suitable for research purposes within therapeutic contexts and approaching couples who are engaged in such therapeutic measures.

2.2 Extraction of pluripotent stem cells from living embryos

Another alternative could consist in extracting pluripotent stem cells from living embryos in the blastomere stage [27]. As is the case in pre-implantation diagnostics, where 1 or 2 cells are extracted during the 6-to-8 cell stage, it might be possible to extract embryonic stem cells from the inner germ layer at a later point in time, perhaps during the 100-cell stage.

Apart from the fact that dysfunctions occur to an increasing degree the longer *in vitro* cultivation of embryos continues, such extraction currently results in the destruction of the trophectoderm (the precursor of the fetal contribution to the placenta) and thus in the death of the embryo. For this reason, the US-Bioethics Commission does not deem this procedure justifiable. As is held, uncertainty prevails as to the point of embryonic development at which the totipotence of the blastomere cells is no longer given. In addition, the danger of damaging embryos that are to develop into human life is so great that the members of the US Bioethics Commission even speak out against exploring this method via experiments on animals.

As regards this issue, an article published by the stem cell researcher Lanza in which he presented his laboratory method as an ethically unproblematic solution called much attention to itself [28, 29]. The researcher who works for an American firm in Massachusetts had initially developed his procedure using mice, and now aims to extract individual cells from fertilized human embryos in the eight-to-ten cell stage for purposes of cultivating stem cells. However, using a total of 16 human embryos in this way, the laboratory only succeeded in developing two stabile stem cell lines. In addition, contrary to what the author claimed in his contribution to "Nature", the viable embryos were destroyed[9] [30–32]. But even if it were possible to extract cells in the early embryonic stage, as in experiments conducted on mice, the danger of damaging the embryo would still pose itself, and the question as to whether the stem cell lines created in this way could be used for farther-reaching purposes would remain unanswered; also, the potential problems concerning "supply and

[9] Cf. numerous critical press releases, among other some publicizing assessments by German stem cell researchers Schwägerl (2006), SPIEGEL online (2006), Stollorz (2006) as cited.

demand" would remain unsolved. Furthermore, to obtain cells from future babies, this method for obtaining stem cells would have to be integrated into the process of artificial fertilization. However, this research procedure would then encroach upon the medico-therapeutic area of reproductive medicine.

2.3 "Altered nuclear transfer" (ANT) and "oocyte assisted reprogramming" (ANT-OAR)

Most of all, the proposal to produce human embryonic stem cells using such methods as "altered nuclear transfer" (ANT) and "oocyte assisted reprogramming" has set off vehement reactions and ignited controversy. ANT and ANT-OAR both depend on the technique of cloning: a somatic cell nucleus is introduced into an egg cell whose nucleus has been removed, but in doing so, certain modifications are made.

In 2004, the human biologist Hurlbut from Stanford University presented the US Bioethics Commission with a paper in which he proposed using ANT[10] [33]. As he reports, the ANT technique relies heavily on gene deletion to prevent "normal" embryonic development. The objection that an already existing human embryo would be damaged "after the fact" for research purposes motivated Hurlbut, in collaboration with other stem cell researchers, jurists and theologians, to propose a special modification of this technique – ANT-OAR – as what they deemed to constitute a morally unproblematic method ([34] also at http://www.eppc.org/publications/pubID.2374/pub_detail). Other stem cell researchers such as Grompe and the law professor George (also a member of the commission), who apparently abandoned his initial reservations against ANT in light of the possibilities which ANT-OAR opened up[11], published a statement following the issuance of the "White Paper" by the US Bioethics Commission [35] addressing the ANT-OAR method and hailing it as the preferred alternative [36].

Rather than destroying human embryos, this method from the very start entails creating biological artefacts that merely possess a similarity to embryos, the goal being to produce an entity which does not constitute a human embryo, but which behaves in such a way as to make it possible to derive stem cells from the cell culture. The aim is to produce an unorganized cell system which could never develop into a viable embryo because of the genetic defect, which is built in "at the start" to prevent this from happening. A factor such as Cdx2, which an experiment with mice conducted by Chawengsaksophak et. al. has shown to be indispensible for embryonic development, might play a crucial role [37–39].

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[10] Hurlbut, W. B., *Altered Nuclear Transfer as a Morally Acceptable Means fort he Procurement of Human Embryonic Stem Cells*, Commissioned Working Paper, discussed at the Counci's December 2004 Meeting, President's Council on Bioethics, Washington D. C.

[11] Cf. „Personal Statement" of R.P. George at the end of the White Paper of The President's Council on Bioethics (2005) as cited [35], pp 79–81.

Using the special ANT-OAR technique, either the somatic cell nucleus or the egg cell plasma or perhaps even both could be modified before nucleus transfer. Those who advocate this technique refer to results from experiments with animals: this could be achieved through manipulation of genetic or epigenetic factors such as the expression of certain master genes, i.e., key transcription factors which in turn control the expression of many other genes; through such manipulation, the somatic cell nucleus could be brought to develop into a pluripotent cell directly rather than into a totipotent one. The 2003 study designed by the Japanese research group Mitsui and Tokuzawa using animal experiments showed that the transcription factor "Nanog" could not be traced until the morula stage, and that its occurrence became extremely pronounced in the blastocyst stage [40]. A further study conducted by the Japanese team Hatano and Tada in 2005 showed that the removal of "Nanog" did not, in fact, prevent the early stages of embryogenesis, but that it did prevent the formation of epiblasts, which means that blocking the "Nanog" of a mouse embryo causes it to lose its pluripotence [41].

What is recommended is a procedure that combines epigenetic reprogramming of the somatic cell nucleus with forced expression of transcription factors that are characteristic of embryonic stem cells. In this way, "Nanog" or similar factors would be expressed in the somatic cell at high levels before nuclear transfer, and at the same time the messenger RNA (mRNA) for these factors could be imported into the egg cell *before* the transfer of the nucleus occurred. In this way, he argues, one could ensure that the epigenetic condition of the resulting egg cell would immediately differ from that of an embryo and be similar to that of a pluripotent stem cell. As the plan goes, the somatic cell nucleus would be transformed into a pluripotent stem cell nucleus during the very production of the "biological artefact", thus preventing the embryonic stage from taking place.

Since 2004, proposals pertaining to ANT and ANT-OAR have ignited controversial ethical debates. For some of those who have engaged in the debate, the ANT-OAR method in particular seems to constitute a morally unproblematic method because, as they claim, the problem of consuming embryo research is solved by not creating any viable human embryos in the first place, but rather mere cell complexes that allow us to obtain pluripotent stem cells. Debates conducted by Catholic theologians in which advocates and critics put forth differentiated, opposing views on the nature and moral status of such projected artificial entities[12] are an indication that the ethical assessment of ANT-OAR is anything but clear-cut.

The position held by the US Bioethics Commission of 2005 soberly points out that until now all we have are a few results from animal experiments. As is reported, it remains to be seen whether and in

[12] Cf. the series of publications and replies in *Communio. International Catholic Review* with the debates in Winter 2004, Spring 2005, Summer 2005.

what way genetic and epigenetic interventions during the creation of embryos could be implemented effectively or not. Despite ethical reservations they advocate careful, continual pursuit of animal experiments to test these techniques, which, as they say, promise to prevent the creation of viable embryos while at the same time producing embryonic stem cell lines [42]. Not until then could a precise ethical evaluation be made, they claim. To be sure, the commission also raises the critical question as to whether this strategy might not raise more new ethical problems than it solves.

The ethical questions raised by the commission's paper must be expanded upon. Generally speaking, one should not lose sight of the fact that ANT as well as ANT-OAR are based on the technique of cloning, which means that the ethical questions pertaining to this technique are an issue here as well. Furthermore, one must consider that in the majority of mammal embryos and embryonic stem cells, cloning results in genetic defects. The planned genetic or epigenetic manipulations would probably even lead to an increase in this potential for defects. In the case of pioneer research, e.g., research directed towards discovering key factors of embryonic development, such sources of error could also call the reliability of scientific findings into question.

It is surprising that some stem cell researchers have nevertheless become advocates of these modified forms of research cloning which are currently not implementable, claiming, in doing so, that they constitute ethically unproblematical technologies. They receive support from several substantialistically oriented theologists who, like the Bush administration, merely concern themselves with cases in which human embryos are killed without taking into consideration contexts of action [43]. The task of sounding out the potential of such technologies is currently being delegated to study designs using animal experiments. And yet viewed realistically what can be expected are for the most part basic insights into key factors of embryonic development of mammals, not so much genetically intact embryonic stem cell lines.

To be sure, the vivid notion that it might be possible to make built-in gene defects, "stopper genes"[13] [44] or other hindrances – in the case of ANT-OAR "from the very start" – is appealing. However, the controversy as to whether "subsequent" (retroactive) "gene deletions" (ANT) must be assessed differently than in-advance manipulations of egg cells whose nuclei have been removed and/or of the somatic cells which are to be transplanted (ANT-OAR) distract attention from the fact that the cloning technology which is applied in such cases always stays the same: the entire genome of a body cell is introduced into an environment, namely an egg cell without a nucleus, making embryonic development possible. Thus, in this case, the preconditions which define the human

[13] Even the Bioethics Commission of Rhineland-Palatinate devoted a short segment of their most recent report *Fortpflanzungsmedizin und Embryonenschutz* from Dec. 12, 2005, cf. pg. 68, to the idea of "terminator genes", but without elucidating what they actually entail.

embryo, namely potentiality, individuality and continuity, are given, as is the worthiness of protection which these preconditions determine. In light of these prospective new technologies, it has become clear that in the future more intensive debates as to what constitutes human life must be conducted.

To be sure, ANT and ANT-OAR are alternatives that attempt to use human embryos and embryonic stem cells in the research context in such a way as to preclude reproduction. But no one currently knows to what extent intended or introduced genetic or epigenetic defects prevent embryos from continuing to develop or whether they could be eliminated if necessary, i.e., to what degree embryos created in this way would be capable of surviving. At the same it is uncertain whether manipulations carried out on the genetic and epigenetic level during the embryonic stage might bring about viable, but genetically damaged embryos.

Another critical aspect lies in the fact that once again, we find a shift from a therapeutic to a research context; in this case it is defined in terms of manipulations of the human genome. Apparently, it used to be permissible to alter the human genome for treatment purposes within an experimental design for somatic gene therapy. In contrast, use of ANT or ANT-OAR would constitute an abandonment of the therapeutic aim. Instead, egg and body cells would be manipulated genetically for non-therapeutic purposes before a human embryo ever existed or shortly after the fusion that brings about an embryo had taken place. This opens up the experimental field of bio-technological genetic "engineering." It remains unclear whether and to what degree such actions constitute interventions into the human germ line, however. The fact that there is no plan to make these "biological artefacts" viable or to transfer them to a uterus does not make genetic interventions in the embryonic stage, which have been proscribed internationally up to the present day, per se unobjectionable.

2.4 Parthogenesis

In the case of parthogenesis, a human egg cell is stimulated biochemically in such a way as to double the haploid female genome so that the egg cell "thinks it has been fertilized" and it responds by beginning to divide until it reaches the blastocyst stage (50–100 cells) [45]. Stem cells could be extracted at this point. The report of the US Bioethics Commission cites Karl Swann from the University of Wales, who has experimented with human egg cells in two studies [46, 47]. A majority of researchers hold that these parthogenetic cells, which divided for several cycles, do not have the potential for developing into a human being. To be sure, the way to verify whether this assumption is correct would be to implant such a parthenote into a woman. The question concerning

the potentiality and status of parthenote-blastocysts must therefore remain unanswered, just like the question as to whether embryonic stem cells obtained in this way could even be used.

One can add to what the commission's report says by pointing out that the ability of a parthenote to survive cannot be completely ruled out. A clinical pediatric case involving a 1-year-old child is a case in point; this child being a human chimera because it possesses cells from two different genetic sources, part of which are of parthenogenetic origin[14] [48]. Mouse, sheep, pig and rabbit parthenotes have been created experimentally. The period of survival for these parthenotes was between 10 and 29 days [49]. The parthenogenetic mammal embryos died in an early stage after implantation due to a lack of control genes on the father's side. The authors of this survey article do not give any information as to whether and to what degree these results might hold for human egg cells as well.

3 Human embryonic stem cells and the creation of chimeras and hybrids: initial ethical assessments

3.1 New technologies and "profound" changes in organisms

When research is conducted on human embryos and embryonic stem cells, it may be possible that one could also create chimeras and hybrids in which human and non-human cells are combined, especially considering the fact that due to new or more differentiated techniques now used in stem cell research, embryology and molecular biology, manipulations can be made at various organismic and developmental-biological levels. Thus, it is not too surprising to learn that several new forms of chimeras and hybrids that combine humans and animals have been created, although such practices have been proscribed internationally or even been interrupted by researchers themselves. Other such manipulations have taken place in animal experiments, resulting in animal-animal chimeras.

In the following, I want to show that human-animal chimeras and animal-human chimeras, in which gametes or embryonic tissue were combined with stem cells, would possess a different "quality" and "degree of penetration" than that evidenced by some chimera experiments of the 1990s. The distinction I attempt to make, namely that between previous and "new" experiments with chimeras, may not always be totally precise. And yet it can help us to grasp ethical problems that pose themselves more urgently or arise anew, indicating, as the case may be, a need for legal regulation.

[14] The proportion of human parthenotes in the general population is unknown, but this phenomenon occurs more frequently than many think.

3.2 Initial definition of terms: “chimera” and “hybrid”

In part, the terms “chimera” and “hybrid” are used and defined differently in biology and medicine. Legal regulations also refer to various understandings of these terms, depending on the realm in which they are used [50]. In this article the terms are used as follows: from a biological perspective in the widest sense of the word, chimeras constitute “organisms (individuals) whose body cells do not all originate from a single fertilized egg cell. In other words, various body cells and organs exhibit differing genetic make-ups. One can differentiate further and distinguish between chimeras with genomes deriving from one species and chimeras whose genomes derive from various species“ [51]. Thus, in the case of chimeras, the embryo consists of two cell types with differing genomes. In contrast, hybrids are zygotes in which both genomes are united. This can occur “naturally” between related species such as horses and donkeys, but the mules that result from such crossbreeding are not able to reproduce. To create hybrids experimentally, an egg cell and sperm from two different organisms (which may even be from two different species) are taken und fused in some form in the “fertilized” egg cell.

Apparently hybrids and chimeras differ depending on the approach used for experimental manipulation and the result affected at the zygote or genome level of the cells, which later differentiate. If we adopt the distinction made in this way, one can say that cloning, a technique in which the cell nucleus of an organism is introduced into the egg cell of another organism whose nucleus has been removed, produces “hybrids.” According to this definition, naturally occurring human-human chimeras would fall into this category[15] [52], as would human-human hybrids. In the following the focus is on chimeras and hybrids made up of differing species, however.[16]

3.3 Animal-animal chimeras, animal-human chimeras and transgenic animals previously produced in experiments

In the 1990s animal-animal chimeras were produced experimentally; for example laboratory rats were injected with previously marked adult bone marrow stem cells of mice. Some animal-human chimeras were also created during that time. The aim was to create animal models similar to the human model in order to investigate susceptibility to diseases, to develop therapies for such diseases and to use animals for the production of pharmaceutical substances [53]. For example, some animal-human chimeras were created by implanting adult animals such as mice and chickens

[15] Concerning a clinical example of a naturally created human-human chimera, cf. the genetic analysis of a one-year-old child in Great Britain Strain et al. (1995) as cited [52].

[16] The Canadian Reproduction Law of 2004 defines its terms in this more limited sense, with its understanding of chimeras and hybrids being formulated in reference to various species (cf. Chap. 2: Definitions), published in *Canada Gazette*, June 4, 2004. In this law, only a part of conceivable chimera and hybrid formations is regulated, however. Cf. some critical remarks later on in this text.

with human somatic cells or adult (neuronal) stem cells. In one case, neuronal stem cells of a 10-week-old human fetus were implanted into the brain of an adult mouse. The human stem cells survived and populated various regions of the mouse's brain [54] In some cancer research laboratories, human cancerous tissue was implanted into mice who were immunologically "naked" (inert), i.e., who did not reject foreign tissue. In this way one hoped to be able to test the effectiveness of cancer medication designed to treat certain types of tumors in humans. In a strict sense of the word, such a mouse is a mouse-human chimera [55].

Through recombinant gene technology, mice were created which produced human proteins [56]. The incorporation of a human gene into female mice, for example, produced the protein tissue plasminogen activator (tPA) in the mice's milk, which was then processed to produce medication against blot clots and vascular obliteration. Through the use of recombinant gene technology it was also possible to produce mice with features of the human immune system that could then be used for research human immune functions. Furthermore, human-animal chimeras have been produced using xenotransplantation in which an animal organ (a porcine heart or a bovine liver) was implanted into an adult human being.[17]

How can such procedures be characterized? In part, a mixture of cells or their genomes occurs on the somatic level, albeit in a locally limited manner in an adult animal or human. As regards the scope of such practices this procedure can be compared more or less with "somatic gene therapy," in which cell and gene transfers are performed on the somatic level.

Animal experiments that occur at the embryonic level and in which interventions in the germ line sometimes occur are to be distinguished from such procedures, however. In regard to animals, interventions into the germ line have already occurred experimentally, e.g., for producing transgenic laboratory and production animals. In the past, primarily two methods were used to produce such transgenic animals [57].

For a method that is characterized by micro-injection, fertilized egg cells are needed. Using a micro-pipette, a cloned gene construction is injected into the fertilized egg cell – into one of the pre-nuclei – before nuclear fusion takes place. Such organisms, which come into existence as the result of the injection of foreign genetic information into a fertilized egg cell, and who pass on this foreign genetic information to the next generation, are referred to as "transgenic" laboratory and production animals. Insofar as the genetic transfer succeeds, all the cells of the organism created carry the genetic alterations. As a rule, the success of the gene transfer is tested after the birth of the animal by examining tissue samples. Disadvantages of this method are the low level of efficiency (under

[17] Regarding experiments and an ethical analysis cf.: Schicktanz, S., *Organlieferant Tier?: Medizin- und tierethische Probleme der Xenotransplantation*, Campus-Verl., 2002.

1%) and the high mutation rate (approx. 5–25%), which can, for example, result in tumor growth. Furthermore, due to such mutations many of these animals are incapable of surviving.

Another procedure used to produce transgenic animals requires embryoblasts and embryonic stem cell lines are required, i.e., cells isolated from previous embryos and cultivated in a laboratory. The following method has proved successful in mice: Specific artificially produced gene fragments are transfected into the cultivated embryonic stem cells. A few gene constructs attach themselves to the corresponding gene segments in the target genome and replace each other through homologous recombination. In most cells the gene construct does not integrate at all or it does so only in random places. Markers are used to find the cells which evidence successful gene transfer; these are then implanted into mouse blastocysts. The mice created in this way evidence two types of cells, genetically modified ones, and genetically unmodified ones. Such mice are thus chimeras. If offspring of the genetically modified cells of the mouse chimera are found among the germ cells of these mice and they are used for further breeding, the second generation constitutes transgenic mice that are no longer chimeras, because they now only contain cells with the same gene sets.

How can the two procedures just described be characterized? They constitute interventions into the germ line in the form of changes made at a certain gene location through selective gene-technological modification. Embryonic stem cells are the standard starting-point used for germ line manipulation, at least in experiments with mice. To monitor the success of the experimental manipulation, the mice had to be carried to term. In part such genetic modifications extended to their offspring. Due to the recombination techniques used, the procedures were not always precise; many embryos were malformed and incapable of surviving.

3.4 Sophisticated bio-technologies and new forms of chimeras and hybrids between human and animal

Most recent progress in biotechnology far surpasses previously used transplantation techniques and genetic recombination between humans and animals, and has expanded the potential for mixing species accordingly. In the following the innovative character of such experiments is illustrated by citing several experimental examples.

3.4.1 Fusion of stem cells and embryos for the purpose of creating chimeras

In 2002, some researchers proposed injecting human embryonic stem cells into mouse embryos to test their pluripotence and the potential for developing into various types of tissue [58]. The scientists postulated that the stem cells would survive and prove to be pluripotent. Furthermore, they conjectured that the human cells would participate in the formation of all types of tissue in the

embryonic chimeras, including primordial germ cells. Developmental biologists are engaged in a controversy as to whether it is justifiable to introduce human embryonic stem cells into animal embryos; the debate was occasioned by the fact that an Israeli research team had imported human embryonic stem cells into early-stage chicken embryos [59]. Furthermore, there are reports of an experiment conducted in Korea which involved incorporating human embryonic stem cells into a mouse embryo [60].

In 2004 an experiment conducted in the USA elicited unexpected effects after human stem cells were injected into 40-day-old pig embryos [61]. The developing pigs contained human cells because approximately 60% of the human stem cells that originated from adult bone marrow donators had been incorporated. Thus, this constituted a case of a human-animal chimera. The experimenters were very surprised to find that some of the cells had spontaneously fused, which meant that they contained genetic material (DNA) from humans as well as the pig in question. In other words, hybrid cells had been produced.

As regards the last-mentioned experiment, one can ascertain that it is apparently possible for spontaneous, uncontrolled gene fusions between species to occur in embryonal tissue. One can assume that the hybrid cells constituted different types of cells, i.e., body cells, embryonic stem cells, and perhaps also germ cells were involved. Thus, this experiment is illuminating, for one, in terms of comparing chimeras and hybrids from an ethical perspective. Chimeras that are created in connection with human embryonic stem cells are often characterized as originating in embryos that consist of two genetically differing cell populations of two or more species. In defining chimeras in this way, the assumption is made that such cell populations do not get mixed with each other at the genetic level in such a way as to create new germ cells – this being what distinguishes them from hybrids. And yet as the experiment conducted by Ogle et. al. shows, one cannot rule out the possibility that genome mixture could occur when chimeras are created.

3.4.2 Creation of human-animal hybrids through cloning

Hybrids come about when an egg cell and a sperm from different, but related species form a single zygote. This process can be activated in the laboratory using cloning and other gamete manipulation techniques.

Private research enterprises in the USA fused human body cells with egg cells of cows whose nucleus had been removed. Only one embryo with 99% human DNA and 1% bovine mitochondria survived the 16-cell stage [62]. In an experiment conducted in China in 2003, the nucleus of a rabbit egg cell was removed and replaced by DNA from human body cells. The cloned animal-human egg cells were destroyed after being cultivated for several days [63]. The researchers had aimed to breed

human embryonic stem cells in this way. Animals were to serve as “host cells”, whose task was to replace human egg cells, for example.

In the case of such human-animal chimeras and hybrids certain ethical questions are raised or lent greater urgency because what is involved is “highly potent” tissue, i.e., consisting of embryonic tissue and stem cells, be they adult or embryonic. One can presume that when egg cells, embryonic stem cells or other embryonic tissue is involved, there is a particularly high possibility that gene fusion or gene transfer can lead to uncontrollable effects on various levels, i.e., at the level of body cells, stem cells or even the primordial germ cells. In the past, interventions into the human germ line have been proscribed internationally, and no developments have been made in this direction. But experiments with chimeras and hybrids could open up technological possibilities for new kinds of fusions between humans and animals and cause undesirable effects on the human germ line. It is uncertain whether such creatures are capable of surviving and reproducing; the prognosis is that this is improbable. But apparently the possibility that new types of entities could be carried to term cannot be ruled out, and in this case severe deformities and other damages would have to be expected.

3.5 Possible scientific insights and therapeutic applications made possible by human-animal chimeras and hybrids

Why is there such a pronounced interest of late in conducting cross-species experiments with chimeras rather than limiting efforts to experiments that exclusively involve human embryonic stem cells? Information on possible scientific insights and therapeutic applications remain vague.

One argument pertains to the prospect of overcoming immunological barriers, this being a relevant factor for transplantation medicine. Possible improvement in the area of xenotransplantation is also cited, the idea being that organs with human components could be bred via host animals, the effect being a reduction of rejection responses. As Ogle et. al. argues in the aforementioned article on the incorporation of human stem cells into porcine fetuses, if human and non-human cells fuse to form hybrid cells, then experiments with such hybrid formations could give us insights into the ways in which diseases are transferred from animals to human beings via endogenic retroviruses [64].

Several developmental biologists are of the opinion that chimera embryos such as ones created from mice and human beings are necessary in order to test the pluripotence of existing human stem cell lines. If we understand “pluripotence” to mean the capacity to subdivide into additional cells with differing functions, this feature proves to be important for gauging the potential clinical usefulness of embryonic stem cells. Chimera-embryos produced in this way would be implanted into female mice and allowed to develop [65]. If we look at the designs of some experiments on embryonic

development which have already been conducted, we discover an apparent interest in allowing human embryos or animal-human chimeras to grow in the uterus of an animal up to a certain stage of development. In general debates on embryonic stem cell research, novel animal models (with human components) are proposed with an aim towards eliciting "human-like" reactions when testing the effects of medicaments [66, 67].

Hopes for potential future therapeutic benefits in a far-off future must be met with certain reservations: "classic" xenotransplantation, as pursued for some time, i.e., the practice which involves transferring porcine and bovine organs such as hearts and livers to human beings, posed severe risks, the result being that hopes for clinical application in the short- or medium term were abandoned: problems concerning tissue rejection, the development of new viruses which might prove dangerous for human beings and tumor formation[18] [68]. Assuming that "human-like" organ cells or tissue could be produced using animal-human chimeras as hosts, the problems just mentioned would presumably remain: the risk of rejection, albeit to a reduced degree, the equally serious risk of transmitting endogenic or recombinant viruses and the risk of cancer, due to the fact that undifferentiated stem cells have a particularly pronounced carcinogenic potential. It would probably also not be particularly easy to ensure the physiological integration and functioning of the cells in the receiver organism.

3.6 New ethical problems resulting from more "profound penetration" and "scope"

Concerning the "depth of penetration" and "scope" of the consequences, experiments with chimeras involving various species can be distinguished in accordance with the aforementioned characteristics, with one category constituting experiments with adult human beings and animals as well as locally incorporated tissue, and the other constituting experiments with "highly potent" tissue such as embryonic stem cells or embryonic tissue. Procedures such as xenotransplantation, "tissue engineering" and localized gene manipulation (limited to body cells) evidence a smaller depth of penetration and scope than do the experimental procedures elucidated thereafter. Although they pose ethical problems as well, these cannot be explored within the framework of this inquiry. In contrast, the chimera experiments conducted at the gamete and/or embryonic level are much less controllable and much less predictable as regards possible effects. These can extend from alterations in somatic cells to changes in germ cells.

Chimera experiments can take two directions: the majority of the "highly potent" cell tissue can originate from the animal or the human being involved. First of all, experiments in both directions, i.e., the introduction of species-foreign cells during the non-human or human gamete and

[18] For an overview on the risks of xenotransplantation and "tissue engineering" cf. Bobbert, Brückner, Lilie (2004) as cited.

embryonic stage is to be viewed as problematic. For in light of the potential and genetic capacity of gametes, zygotes, embryos and stem cells for fusion (or the capacity of bio-technical means to achieve this), the number of cells involved is not the decisive factor. Qualitative changes play a more crucial role here. At the same time, it will be necessary to ascertain whether there could be experimental designs that would involve transfer amounts of human cells into "highly potent" non-human tissue that were almost negligible, so that the effects might be controllable or whether other factors might guarantee controllability of possible effects.

From an ethical perspective, an important question concerns the organisms' ability to survive and reproduce. Although biological definitions use the genomes of specialized cells and primordial germ cells as their framework of reference when distinguishing between chimeras and hybrids, experimental findings indicate that experiments involving chimeras can also lead to changes at the germ line level. Moreover, one cannot rule out the possibility that chimeric or hybrid embryos might survive. Experiments designed to produce such chimeras and hybrids would be extremely problematic from an ethical standpoint.

For chimeras, the question which would pose itself from the start, at the embryonic development stage, as well as later, when a creature had continued to develop which might well be brought to life, would concern the proportion of human genes and cells necessary to view the organism in question as a human being.[19] This issue would have fundamental consequences for the self-understanding of humankind, the concept of the person, identity and genus and the moral rights and duties which are connected with such concepts. Superficially speaking, this question already poses itself within the experimental framework, as the issue of patentability is relevant from a legal as well as a financial perspective [69].

One field of application for animal-human chimera might consist in using animals as hosts, be it as "living uteri", be it as means for producing human embryonic stem cells or organs. As yet no artificial uterus has been designed, and nutrient solutions have limitations when it comes to cultivating animal or human embryos over any longer period of time. However, the transfer of human embryos or the incorporation of human embryonic stem cells into animal embryos could produce a human-like entity like no other previously existing ones, and one that might even be viable.

In part, proposals for regulations advocate preventing human-animal chimeras from coming to life, i.e., preventing them from developing into complete individuals. But skepticism is called for in regard to such a proscription, for so-called "stopper genes", which are allegedly built-in, but which

[19] For a list of such questions, cf. for ex. Cohen, C. B., Creating human-nonhuman chimeras: of mice and men. *Am J Bioeth* 2003, *3*, W3-W5.

can also be removed again [70], have a greater symbolic force than they do a factual one.[20] Moreover, laboratory application is not thoroughly appealing, for as concerns molecular-genetic techniques, researchers are (among other things) interested in discovering what the phenotype of a chimera might look like, whether it would be capable of surviving, whether it could be bred in the future as an animal model and used for research purposes, and whether it might even become patentable.

One must not underestimate the interest there is in patenting chimeras and hybrids as non-human inventions, e.g., for use as models for diseases or medicament testing purposes. Within the framework of research with human embryonic stem cells, chimeric study designs and the incorporation of human cells into animal embryos are anticipatable. Efforts could perhaps be directed toward limiting the proportion of human cells involved in such procedures as much as possible, quantitatively speaking. Nevertheless, the considerations regarding scope, depth of penetration and focus of chimera production brought forth at the beginning of this segment should not be ignored in favor of purely quantitative ones. It would also be important to lose no time in setting up legal regulations that would check any grand hopes that human-animal chimeras might become patentable.

Until now, the points of transition were relatively clear – as long as combinations between animals and humans were limited to the "somatic level", i.e., remained within strict boundaries, and as long as interventions in the embryonic stage and into the germ line only occurred in cases of animal-animal chimeras. Through the "new", sophisticated techniques, in particular those applied in connection with "highly potent" human embryonic gametes, stem cells, zygotes and embryos, mixtures are becoming more comprehensive, and points of transition more and more blurred. Areas of research which have been taboo until now will have to be scrutinized more extensively, and in a more differentiated fashion, than was possible in this investigation. The task will be to characterize and evaluate all conceivable experimental designs and their consequences from an ethical perspective, identifying what they have in common and wherein the differences lie.

3.7 Legal regulations and the necessity for clear definitions of chimeras and hybrids

For the sake of ethical reflection, but even more so in the interest of putting legal regulations into place, it is crucial to clarify how chimeras and hybrids are to be defined. In biology and medicine these terms are as myriad as are the regulations for biotechnical experiments to which they apply. Varying definitions confuse and blur the differences which are relevant for ethical evaluation. How can legislature deal with this problem? As a rule, previous laws made to regulated various areas of

[20] Cf. the remarks concerning so-called "terminator genes" in Chap. 2 of this contribution to ANT and ANT-OAR.

biotechnology have contained quite general prohibitions, for example in regard to artificial modifications of the human germ line, human cloning and the creation of human-animal organisms.[21]

If research with human embryonic stem cells is made legally permissible (under certain conditions), it must be made clear that further regulations will be required to regulate the issue of chimeras and hybrids. The jurist Kopinski has pointed out a gap in US legislature. She shows that the question concerning the extent to which human-animal chimeras and hybrids are subject to patent law is not legally clarified and that it is therefore necessary to establish a specific regulation to cover this problem [71].

Which aspects must be taken into consideration when it comes to establishing a legal regulation? A critical assessment of two recent laws which address the problems concerning chimeras show where the crucial issues lie.

Switzerland's "Federal Law on Research with Embryonic Stem Cells" [12], for instance, fails to define the concepts clone, chimera and hybrid. As far as these phenomena are concerned, several general proscriptions are formulated; the law prohibits the creation of embryos for research purposes, interventions in the genetic material of germ line cells and the creation of clones, chimeras and hybrids [72]. What such proscriptions ultimately refer to remains unclear, however. Strictly speaking, on the basis of a sweeping biological notion of chimeras and hybrids, extremely diverse experimental designs which entail "mixing" human and non-human cells in an organism could be forbidden. On the other hand, one could argue that in light of the fact that the law addresses the question of research with human embryonic stem cells, the only practice the law forbids is the incorporation of human embryonic stem cells into any non-human organism, but not, for example, the incorporation of adult stem cells into embryonic animal tissue. And yet, as was shown above, this could also have ethically problematical consequences.

Thus, it is apparently necessary to incorporate experimental designs and bio-technological methods into legislative texts. In doing so one must observe what is meant when texts state that human cells "cannot" develop into human beings, as Switzerland's law on stem cell research from 2005 does [12]. On no account should this be misconstruable in the sense that the developmental capacity of an organism depends on any actual incorporation into a nutrient solution or a uterus. For if this were so, the regulation would not only fail to cover cases of extra-corporal totipotent cells; how the normative question was answered (is a cell completely capable of development and thus worthy of protection?) would depend solely on what is actually done or not done in the laboratory. German

[21] The German Embryo Protection Law (*Embryonenschutzgesetz [EschG])* from Dec. 13, 1990 constitutes an exception. Cf. remarks made below.

law addresses this problem in its Embryo Protection Law by treating a cell as totipotent which is "capable of dividing and developing into an individual if all further preconditions which this process depends upon are given" [73]. In light of new procedures and techniques it might prove useful to formulate more precisely what is meant by "further preconditions."

Because hardly any legal regulations exist worldwide which address the creation of chimeras, the Law on Reproduction passed in Canada in 2004 and the definitions which it contains are often used as a frame of reference [74]. And yet it would be a mistake to attempt to grasp the general problem on the basis of the definition of chimera provided in this piece of legislature, for it is too limited – having been arrived at from the perspective of a law which regulates reproduction practices. The definition only pertains to such cases as follow: a human embryo in which the cell of a non-human form of life has been transfected or an embryo or fetus which consists of cells from more than one human being, i.e., a human-human chimera or a human-human hybrid.[22] This definition fails to take into account all cases in which human embryonic stem cells, embryos and adult stem cells are incorporated into non-human embryos.

Apparently efforts to establish legal regulations must not only address the issue of definitions, but also identify existing experimental designs and procedures and decide which actions and results of such experiments and procedures are desirable or reprehensible from an ethical perspective. This even holds true for the German Embryo Protection Law (EschG) of 1990, for although this legislation contains quite precise definitions and regulations on cloning and the creation of chimeras and hybrids [75], these only pertain to partial areas; in light of new procedures such as nucleus transplantation using animal material, they require greater specification [76, 77].

Furthermore, in accordance with the results of the investigation at hand, legal regulations should not only provide a basic definition as a frame of reference but ultimately make methodological stipulations. The definition of chimeras and hybrids used at the beginning of this inquiry was quite general, but in the course of my deliberations I have shown that there are what one might call "previous" and "new" forms of experimental chimeras and hybrids and that the new bio-technical possibilities raise "more profound" ethical problems than the old ones did. It is apparently imperative to describe exactly which experimental methods are involved and what must be scrutinized, and, as the case may be, what must be avoided in connection with the creation of human-non-human chimeras and hybrids.

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[22] The fusion of embryonic cells of two different people also raises ethical problems and should therefore be regulated. Apparently such an experiment on the human-human level has already been conducted: Norbert Gleicher created a so-called hermaphrodite (hermaphrodite: with two sexes). At the meeting of the European Society of Human Reproduction and Embryology (ESHRE), reproductive medicine specialists Gleicher from the Center for Human Reproduction in Chicago and New York reported that he had mixed the cell of a male embryo with that of a female embryo and obtained a hermaphroditic hybrid which he destroyed after cultivating it for 6 days in the laboratory. Cf. Kopinksi, (2004) see ref 351, as cited [56].

Ethically relevant aspects of experiments involving chimeras and hybrids which initially present themselves are named below, albeit not in any exhaustive sense:

(1) Two or more species are involved, one of which is the human species (for the sake of simplicity, the special problem concerning human-human chimeras will be neglected; this case could be regulated in a separate step);

(2) The genome of one or both species is not modified in one targeted place, as is the case with gene recombination techniques (cf. for example knock-out experiments and gene transfers), but rather pluripotent (and under some circumstances even totipotent) stem cells and their genomes are brought together during embryonic development, with this process not necessarily always resulting in an embryo which consists of two cell populations, each with a different genome, for it is possible that untargeted gene transfers and genome mixtures occur to a relatively large degree, involving the embryonic stem cells and various embryonic cells in the course of further differentiation.

(3) Thus it is possible for an uncontrolled exchange of genes to occur within a "highly potent" tissue which can lead to the creation of a chimeric species of animal-human if this gene exchange takes place in the primordial germ cell tissue. The formation of mixed germ cells in human-animal chimeras should be absolutely prevented, which is to say, experiment designs must completely rule out this possibility.

(4) It is very improbable that chimeras and hybrids could survive, but it is not absolutely impossible. No animal should be allowed to develop which has any noteworthy degree of human genetic characteristics. Furthermore, animals with human genetic features should be prevented from developing the capacity to reproduce. The same holds true for humans who possess (genetic) features of animals.

(5) In the case of formulations which address the question of the capacity of produced organisms to survive, the distinction should be made between the capacity to development into a creature and the actual provision of environmental conditions such as nutrient solutions, transfer into a uterus etc.

(6) Regulations concerning chimeras whose aim is to prevent new species capable of reproduction from being created must comprise all transfer techniques which are applied during the stage of embryonic development and which at the same time utilize totipotent or pluripotent stem cells from the one or the other species. The stages ***before*** embryonic development should be included as well, for it is even possible to fuse different species

genetically by modification of egg cells (intact ones or egg cells whose nucleus has been removed) or sperms or of fertilized egg cells during the pre-nuclear stage.[23]

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