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NEVER ENDING STORY
HUMAN EMBRYONIC STEM CELLS
SCIENCE AND BIOETHICS

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Abstract

The paper presents some of the scientific and ethical problems hampering the use of human embryonic stem cells (hESCs) in biomedical research. A brief review of the real possibilities of employment of hESC or adult stem cells in therapy, as resulting from experts' reports is offered. The substrate of doubts surrounding status of human embryo and therefore legitimacy of research conducted on hESCs is also commented.

Keywords: human embryonic stem cells, adult stem cells, embryo's status

1. Introduction

Use of human embryonic stem cells (hESC) is advertised as a universal cure. "Potential treatments range from restoration of spinal cord after injury to the cure of diabetes" [1]. Applications were envisioned in genomics, developmental biology, cancer study, drug testing and therapy [2-4].

Since the very beginning of the adventure, voices were raised in the scientific community anticipating the moral and scientific challenges of using human embryonic/adult stem cells. Public debate was regarded as "understandable, warranted, and welcome" and the miraculous potential of curing so many serious diseases as a "futuristic agenda" [5].

The paper presents a bioethical evaluation based on bear scientific facts, as well as on elements of Theology as provided by the three Abrahamic religions.

2. Drawbacks of using hESCs in therapy

"An orderly chain of highly regulated processes involving cell proliferation, migration, differentiation, and maturation leads to the production

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and sustenance of most cell lineages in adult organisms. The earliest cell type on this chain has been called a stem cell." [6]

Human embryonic stem cells are usually obtained from the inner mass of the blastocyst, the first developmental stage of the embryo. Obtaining them inevitably implies destruction of the embryo. The outer layer of the embryo is destroyed and the remaining inner cell mass is practically scattered on culture plates [7]. Stem cells can also be derived from embryonic ectoderm and primordial germ cells of the foetal genital ridge [8].

ESCs are usually grown on animal feeder cells layer [9] which arouses concern over the possibility of inter specific transfer of viruses [10]. They usually develop heterogeneous colonies (mixtures of many different types of cells) fact that opens the problem of purification - obtainment of a single type of cell of interest out of such blends [9].

Unimaginable complex interactions are required for normal tissular development in the embryo and the embryo might be the one and only "environment" exhibiting such interactions [9, 11]. The position effect is one of the most intriguing (and practically not reproducible under laboratory conditions) factors that participate to normal tissue generation. An entirely adequate morphophysiological development of a particular tissue requires the cells to occupy a precisely defined area in the organism [9].

"Classical" organ transplantation is blamed because of the rejection potential [12] but the same problem appears when cells are transplanted and also for possibility of infection. Once again, the problem occurs in cell transplantation, too. It has been demonstrated that human embryonic stem cells express high levels of MHC-I (major histocompatibility complex I) proteins and thus may be rejected on transplantation [13].

It is a well-known fact that biological agents such as viruses often trespass species boundaries. As for the increase of the infection risk due to immunosuppressive therapy prior to transplantation, that regards cells transplantation, too, as immunosuppression will also be necessary in order to perform it as in the case of organ transplantation. The rejection/infection problems would be present whenever transplantation is performed regardless the nature of the graft (cells, tissue or organs). Therefore, present approaches are not very convincing, even though enthusiastically presented by some [14].

Cloning has been proposed as an option in order to reduce rejection potential, but its advocates usually do not take into account the fact that mitochondrial genetic information of the oocyte also contributes to generation of antigens. Cells obtained from cloned embryos will also be immunogenic even though at reduced extent because of the non-self information acquired from the participating oocyte [9].

Some authors draw attention on safety, financial and other practical issues (e.g. lack of time in the case of seriously ill people) pending to therapeutic cloning. Even if worry regarding mitochondrial-based immunogenicity will turn out to be exaggerated, other problems remain to hamper this approach [15]. The

objective, irreducible inefficiency of the cloning techniques, on one hand, and ethical concerns on the other, are also listed.

ESCs exhibit genomic instability after long-term growth [10]. They are known to originate tumours in the host organism, which also must somehow be ruled out in order to establish safe therapeutic protocols [2, 16]. Some studies have shown that the same genes that are involved in the maintenance of the pluripotent undifferentiated phenotype are also over-expressed in embryonic carcinoma. [17].

Last, but not least, there are many significant differences between well studied animal models (especially murine models) and the behaviour of human stem cells [18] which places under doubt the value of experiments in animals and the perspective of extrapolating results in humans.

Human embryonic stem cells are sought as replacements for destroyed tissues, an example being the cardiac muscle damaged by infarct. Even the most enthusiastic authors admit that in order to really improve heart function, transplanted cells must survive for long periods (quite logical) but the infarcted area of the heart and its surrounding region is not an appropriate environment for the survival of transplanted cells [12]. Then, why bother? This should be a common problem for all types of cells that might be transplanted into infarcted heart, even though it is sometimes presented as hampering only the transplantation of skeletal myoblasts.

Using skeletal myoblasts in the treatment of infarct makes a good lesson of "responsibility" in research. Skeletal myoblasts are cells that end up as building blocks of muscles that are attached to the bones. They differ in some essential respects from those that become cells of the heart muscle, which is of a very special type. Still, attempts have been made to repair heart muscle damage using such inappropriate cells. Cells of the body muscles behave very differently of cardiac muscle cells, it should have been obvious that they do not match and cannot replace each other or work together in the heart. It is somehow like trying to put a wild horse and a donkey to pull a cart together. Differences in their functional pattern logically result in perturbation of the heart beat (arrhythmia). So, even though serious risk of arrhythmia could be anticipated purely on a theoretical basis [19] it was necessary to perform experiments on animals and even humans in order to accept that such procedure would be useless [12].

Immature cardiac myocites seem to be present in the heart [19] encouraging the idea of self-repair possibilities that have been thought to be excluded till recently. Furthermore, the use of adult stem cells such as bone marrow stromal cells has been shown to be able to differentiate into cardiac-like tissue when transplanted and improve heart function.

The hESC are far from being characterized. Despite this, some people already promote most dangerous methods such as transfection of embryonic stem cells with lentiviral vectors (modified lentiviruses), with the aim to genetically modify the host cells [20]. A tip: the HIV virus is a lentivirus. The peril of viruses reactivating makes the recurrent nightmare of immunologists preparing vaccines and taking responsibility for them to be administered to

people. A reactivated lentivirus, HIV's next of kin wandering into human cells may do irreversible damage.

The actual options in case of loss or failure of cardiovascular function include organ transplantation, surgical reconstruction, mechanical or synthetic devices, or the administration of metabolic products, treatments that each has its own difficulties.

Tissue engineering has been proposed as an alternative [21]. It would consist in the obtainment, through special techniques, of replacements consisting in living tissue grown with the help of synthetic support tissue that should be morpho-physiologically as close to the natural one as possible. Problem is that usually the prognostic of such severe conditions is far from being optimistic and there would not be enough time effectively for the cells to develop into a properly organized substitute [12].

3. Do adult stem cells make a viable option?

"For a long time, adult stem cells have been considered to be developmentally committed in such a way that they appear restricted to produce specific cell lineages, namely those from the tissue in which the stem cell resides. This rather deterministic concept (i.e., bone marrow forms blood cells, epithelium forms epithelial cells, etc.) has been recently challenged by several bizarre and unexpected findings." [6].

For some scientists, adult stem cells are more credible therapeutic tools than embryonic ones [22]. A direction might be the treatment of common pancreatic disorders such as diabetes mellitus by obtaining functional islets (insulin secreting structures) from adult stem cells. Stem cells seem to be available even though scarce in adult pancreas [23]. Besides adult islet stem cells existing in the pancreas, some cells derived from the liver and bone marrow might also prove to be able to generate functional insulin producing tissue [24].

Adult mammalian central nervous system contains multipotent cells (in the ventricular zone, the external germinal layer of the cerebellum, the subgranular zone of the dentate gyrus, the ependymal layer of the spinal cord) that may be employed for repair in neurodegenerative and demyelinating diseases [25].

There are many citations regarding the capacity of adult stem cells to transdiferentiate and form various types of cells, even belonging to other types of tissues that their own [8]. A rare human stem cell population with haematopoietic and hepatic potential has been detected in adult bone marrow. Those cells differentiated into human hepatocytes in vivo, in mouse models [26]. Adult human nonhaematopoietic mesenchymal stem cells from bone marrow stroma (hMSCs) are able to differentiate into various types of cells of mesenchymal type (osteoblasts, chondrocytes, adipocytes, tenocytes, myotubes, neural cells and haematopoietic supporting stroma) [6, 27].

The ability of stem cells from bone marrow to convert into various types of cells has also been documented in patients that have undergone bone marrow transplants. Differentiated epithelial and endothelial cells derived from adult stem cells in the grafted bone marrow were identified in the lungs of such persons [28].

In adult tissue, cells that build the muscles can originate from the so-called satellite cells that are found at the surface of adult muscle structures (myofibers) and also from other types of cells (e.g. cells from the bone marrow) [18].

Some authors draw attention to the fact that telomeres of the chromosomes of the adult stem cells are significantly shorter than those in embryonic stem cells. That should mean that the proliferative potential of adult haematopoietic stem cells is limited and decreases with age and that they would not make such good candidates for therapy as hESCs [29]. That could have very well been postulated on theoretical basis. It is a perfectly natural process and one cannot expect adult stem cells to be as "fresh" as embryonic ones. They originate through division, from other cells, from a lineage as old as the host organism. Shortening of telomeres occurs in any cell lineage once it has begun to divide and perpetuate.

On brief, adult stem cells might show promise. Still, we might consider keeping measure in enthusiastically evaluating their possibilities, as in the case of hESCs.

4. Attitudes towards hESC research

President Bush stated in 2001: "blastocists have at least potential for life". Only the fifteen cell lines established before 2001 may be used in research, as for them "the life and death decision had already been made" [30]. It is said that those 15 cell lines are difficult to maintain and insufficiently studied with respect to their characteristics [31]. The ban is valid only for research sustained by public funds. In private institutes things may go on and they do. New cell lines are continuously derived.

Two bills (Weldon & Stupak in the House of Representatives, Brownback & Landrieu in the Senate) were introduced in the Congress in 2004 in order to place an interdiction upon obtaining human embryos by a cloning technique (SCNT, somatic cell nuclear transfer) no matter the source of funding, public or private. Such initiatives will become punishable by a 1 million $ fine AND (not OR) 10 years in prison [32].

In Great Britain the gate was widely opened for manipulating human embryos in order to harvest stem cells, from surplus embryos resulting from in vitro fertilization or from embryos especially brought to life (cloned). Such procedures still remain under provisions of the Human Fertilization and Embryology Act and should be allowed or banned following a case-by-case study performed by the corresponding authority (Human Fertilization and Embryology Authority). A stem cell bank has been founded enabling the

Medical Research Council to administer any further developments employing the deposited cell lines. Deposition is mandatory [1].

A sad example of an absurd approach to regulating experiments on human embryos is the eighteenth article in the *European Convention on Human Rights and Biomedicine*: 'In countries that allow in vitro experimentation on human embryos measures have to be taken in order to protect the embryo.' [33]

What measures, except for totally banning such interventions, can be taken in order to protect embryos during experimentation that inevitably leads to their abnormal development or death?

5. The root of all problems- the perspective upon human life

Implantation of the blastocyst in the uterine wall sets the limit for manipulation of human embryo in some lawmakers' opinion, as blastula is the last stage when embryos can split into normally developing twins. In the USA, for example, the Feinstein (Hatch & Feinstein) Bill seeks to ban human reproductive cloning whilst keeping up the possibilities for using human blastocysts in research. Some think that embryos may be destroyed up to their fourteen day of life as they do not have a nervous system yet and cannot be considered sensate [32]. It is a deeply inhuman approach. To correlate value of human life to nervous system's degree of development and/or functional condition is simply outrageous. If we keep the reference and continue in this line of thinking people suffering from various conditions impeding on the functionality of the nervous system result not to be as human as the others.

The only clear standard we have is of a cultural extraction. Human life is regarded as a sacred gift all over the world. A major problem for a bioethician is how to convince beyond doubt that some practices are wrong even though they are in current use (e.g. in vitro fertilization) or advertised as full of promises (e.g. gene therapy). There is a need of strong references regarding human life.

Orthodox, Catholic and Protestant Christianity promote the same strong principles regarding human person and the sanctity of life. A similar attitude is found in Judaism and also in Islam.

When talking about Christianity, Islam and Judaism sharing their views on human life, we talk about the attitude towards human life, in general, not about the attitude towards the embryo. All Abrahamic religions hold human life to be sacred. As for the attitude towards the embryo, things are a little more complicated that it would seem, at first glance.

Judaism states that the embryo becomes a human person only after 40 days from conception. "Beneficial research" on "therapeutic" cloning and stem cells derivation can be allowed to proceed. There is no potential of the fertilized egg to initiate pregnancy and develop to birth unless there is a parental decision to do so, therefore, "within the framework of IVF treatments, it will be permissible to donate supernumerary embryos (…) for the purpose of therapeutic research" [34].

Still, some specialists in Halachic law restrict manipulation of the embryo [35], yet allowing therapeutic cloning as a method with potential of saving human lives: "The creation of any embryo for such research purposes is prohibited. Nevertheless, the creation of in vitro pre-implantation embryos for research should be allowed if it is probable that this research will help to save human life. This includes creating embryos by the cloning technology."

Let's take a look to the Christian perspective upon embryos' status. In the first century, Didache states: 'you shall not murder a child by abortion nor kill that which is born' [36]. In the fourth century, St. Basil the Great stated in opposition to the antique philosophy that the embryo must be treated as a human person, no matter the stage of development. There were other great theologians that underscored that human persons the existence of the body implies the presence of the soul from its beginnings, namely since conception [37]. Since the very beginning of his/her life, the developing child is a bearer of God's image, fully entitled with the rights to life and respect deriving from the special condition of man on Earth. Under these conditions, no destructive intervention on human embryo is justifiable, regardless the aim.

Hard penitence was established for killing unborn babies, by St. Basil the Great in his canons [38] and also by the Councils of Elvira, Ancyra or Lerida [39]. On the contrary in modern times there is no legislative uniformity with respect to the embryo. Whether it is or not a human person still remains puzzling for our secular world. The right to life seems to be inherent only to born humans. The unborn have no definite status in court.

In Islam, the teaching of the Prophet says that "Each of you is constituted in your mother's womb for forty days as a *nutfah*, then it becomes an *'alaqah* for an equal period, then a *mudghah* for another equal period, then the angel is sent and he breathes the soul into it." Hanafi scholars (and also many Shafi'i and Hanbali) taught that abortion was permitted till ensoulment, but only with solid motivation, whilst most Maliki jurists held abortion to be forbidden, no matter the stage of development, as after an ovum has been formed it was not to be disturbed by any means [40].

Some Islamic theologians and bioethicians think there is nothing wrong with stem cells research, as it implies destruction of an embryo which is not yet a person, but that we must observe carefully any abuse, among which producing of embryos with the very aim to use them in such research instead of using the spare embryos from IVF. If stem cells research will show significant potential in helping people then it becomes not only allowed but also mandatory to pursue such research [41]. Still, in present, distinction between the pre-ensoulment and post-ensoulment stages does not mean that one can perform any kind of intervention upon the embryo, as the majority of the Shi`i and some Sunni legists regard eradication of the embryo as sinful, even in the pre-ensoulment stages, simply because it is alive [42]. Even in the case of IVF, Islam has reserves over the fact that in IVF supplemental embryos are discarded [43].

In the middle of the agitation regarding use of human embryonic stem cells Landry and Zucker propose precise criteria for establishing embryonic death in early stages of differentiation [7].

This is intended to clarify/simplify the issue and provide basis for harvesting stem cells under the same conditions as of organ harvesting from cadavers with the aim to be transplanted. In America, for example, death of born humans is pronounced when brain activity ceases completely (according to the Uniform Determination of Death Act of 1981, criteria re-evaluated and reiterated by the American Academy of neurology in 1995). According to the Omnibus Reconciliation Act of 1986 after brain death it is legal and morally licit to harvest organs with the consent of next of kin.

In the authors' vision embryonic death should be pronounced when the embryo is no longer capable of integrated cellular division, growth and differentiation, which must be carefully observed. It seems that such embryos often conserve stem cells that are still alive and usable. Reasonable enough. The only problem would be to establish beyond doubt a set of recognizable signs of irreversible loss of proliferative capacity. The proposed approach consists in observing as many dying embryos as necessary in order to establish the criteria. Somehow, those embryos must become available for unveiling the mystery of their dying moments to the researcher.

They are, in fact, available, as thousands of supplementary embryos produced via in vitro fertilization that silently expect to be "discarded" after a few years of cryopreservation [7, 9]. "Organismically dead" embryos might then be used for harvesting of stem cells. Yes, but toxic effect of cryoprotectants and chilling injury that surplus embryos are subjected to might anytime result in genetic abnormalities, not all of them easily detectable [44].

That should solve each and every ethical dilemma, as "it would not seem to significantly encourage the practice of IVF for infertility, a practice in which death is undesired rather than the primary objective". In other words, given the fact that we have to manage the unpleasant details represented by surplus embryos, let's make them useful for mankind, the same mankind that had no right to provoke their life and likewise to provoke their death.

"In sum, application of the ethical framework for essential organ donation to harvesting of human embryonic cells from dead embryos could provide a common ground in which the imperative to safeguard human dignity and the drive for biomedical research are not in conflict." [7]

What about the very fact of their condemnation to death in the frame of the IVF procedure? It seems that we forget the primary (im)moral orientation of the whole issue.

A modern core concept in Bioethics is the precautionary principle. Despite the fact that it is sometimes misused tending to transform from an instrument of the common sense into some sort of a universal weapon that tends to be used against almost any kind of research in molecular biology the precautionary principle is still a valuable acquisition of present-day Bioethics.

The precautionary principle states that when a procedure is considered, on reasonable grounds, by one or more specialists, as potentially dangerous for humans and/or the environment, that procedure must not be applied until further data on its potential outcome is accumulate. Theologians are among specialists that are usually called to issue opinions in Bioethics and they often draw us attention on the dangers hidden beyond apparently inoffensive procedures.

If Christians are right and we are humans since the very beginning of our life then each and every procedure inflicting damage on the embryo becomes a crime.

Sometimes the danger is foreseeable for everyone such as abuses that might occur if euthanasia gets to be legalized. In other cases we need to look deeper for answers. The same procedure might get new significations when dual nature of man, body and an immortal soul is considered.

Of course, atheists might say that they do not admit the existence of the soul thus such viewpoints are useless to their Bioethics. According to the precautionary principle to which I propose a new reading, theologians might then ask *and what if we are right about divine origin of life and about people being human persons since conception?*

"One of the most dangerous trends in this debate is that of offering religious opinions cloaked in the language and veneer of science (e.g. using systems theory to justify the belief that life begins at conception)" [32].

I agree. For some good reasons:

1. Religion, the field of revealed Truth does not at all need to mix that truth with scientific opinions which's invalidity might break out sometime in the future.

2. Science was not able up till now at least to put together a widely accepted definition of life, this is for exemplifying how "full of knowledge" we really are. We have a huge, continuously expanding field, named "life sciences" and we have not agreed, at the universal, philosophical level on *what* does it study.

3. Theological arguments on human life, its beginning and purpose are logical, beautifully intricate and convincing, of course, for those that do not *a priori* reject religion and the possibility that it might say some very interesting things.

4. Science does not possess convincing, well-built arguments with respect to the same issues.

6. Conclusions

Some people say that stem cell research "should go forward because we simply will not know the answers unless we do the research. The desire to know is absolutely intrinsic to humans and has a survival value as well as a moral one." [32]

As a biologist and a bioethician I want to provoke the reader to think about two main aspects. First, the use of hESCs has many objective, scientific

problems, as we could see. Second, we must acknowledge the fact that the moral cost of survival is more important than survival itself. That is valid in most cultures, not only in Christianity.

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