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The biological situation of embryos cultivated in vitro and cryopreserved

In our country, neither the number of cryopreserved embryos nor the conditions in which they were frozen are known precisely. However, the general information provided by the assisted fertilization clinics has made it possible to know some important facts.

1. Normally, assisted reproduction clinics tend to implant embryos that after fertilization show better patterns of cell division. They also try to freeze surplus embryos in their early stages of development, as early freezing of the embryo increases the effectiveness of defrosting and implantation. In the case that one of the embryos does not show the expected activity, it is usual to wait a while to check if the embryo takes up the normal rhythm to proceed then to the freezing, or on the contrary to discard it by considering that it is an embryo Suboptimal. This way of proceeding seems to have been the usual since the beginnings of in vitro fertilization. As a result, some of the alleged 40,000 embryos found cryopreserved in Spain were frozen in the pronuclear phase or bicellular phase, others when they already had about 30 cells, and others even when they had reached some 100 cells.

Three parameters define which morphology corresponds to the degree of intrinsic viability of the in vitro blastocyst; And they refer, as is obvious, to the organization according to the axes designed with the polarization of the zygote and that are maintained in the embryonic development and constitution of the organism (1):

a) Cavitation initiated on Day 4, which originates an eccentric cavity

b) The cavity expands and aligns with the region of the internal cell mass delimited by a layer of trophectoderm

c)The morphology of the internal cell mass presents a single focus. On the contrary, the degree of viability decreases drastically if vacuoles are formed before the expansion and even more if degenerative foci are formed in this zone (2).

The number of unviable embryos is the result of in vitro manipulation of the process and that quantity is always higher than that occurring in the natural process. Chromosome analysis of human embryos cultured in vitro, and early stages, has shown that up to 40% of embryos contain chromosomal abnormalities, including aneuploidies, monosomies or mosaicism (3). Approximately 50% of the pre-implant embryos of 2 or 4 cells grown in vitro do not reach the blastocyst stage (4). Also, of the embryos of 4 cells transferred, only approximately 20% are implanted (5). Three main causes could explain this arrest in development: chromosomal abnormalities, intrinsic defects of the oocyte and preimplantation embryo and, in the case of in vitro cultures, inappropriate cultivation conditions. Technical intervention generates a very high rate of non-viable embryos with genetic defects and developmental alterations.

It is also known (6) that human embryos originated by fertilization of ovules that come from multi ovulation have more difficulty to nest and those that achieve it develop with more malformations than those originated by fertilization of the ovule matured naturally in a cycle; moreover, the mother, due to the effects of the drug used in these cases, contributes a more aggressive microenvironment to the embryo that tries to nest. On the other hand, techniques for preimplantation diagnosis (7), which require taking one or two cells from a three-day embryo, have revealed the astonishing ability to compensate for the damage (8).

In summary, the morphological criteria allow assessing the viability of the embryos with certain certainty. In turn, some anomalies can be detected that are not compatible with the development of the fetus, as in the case of triploids. Now only once thawed can be recognized, seeing if the cells begin to divide properly, those that are not viable because they do not show the pace of growth essential to nest and develop a fetus, and which could, according to their Orderly growth capacity, get to implant and continue development.

2. There is sometimes the need to conserve frozen human embryos in the context of assisted reproduction. On the one hand, there is a tendency to restrict to a maximum of two the number of embryos that are transferred. At the recent annual meeting of the European Society for Human Reproduction and embryology of experts, held in Vienna in June 2002, have warned of the need to reduce multiple pregnancies in women subjected to reproductive techniques Assisted, due to the increased risk of prematurity and higher mortality than there is great evidence. The need not to carry out multiple embryo transfers has led to the legislation of some countries not to allow the fertilization of more ova than the embryos that can be transferred; While others admit to storing embryos that are not transferred to use them in a later attempt to reproduce. On the other hand, sometimes the cycle resulting from ovarian stimulation is not adequate to proceed with the transfer of

the embryo and in this case, it is necessary to resort to the conservation of the embryo.

3. The first studies on the freezing of embryos were carried out 50 years ago, showing that it is possible to freeze, and thaw oocytes fertilized rabbits In The presence of a cryoprotectant (9). The first attempts at cryopreservation of human embryos, followed by the transfer of embryos and births, took place at the beginning of the 1980s (10). Embryo freezing is currently considered a routine protocol and completely validated in the treatment of infertility, with implantation rates approaching those obtained with non-frozen embryos (11). Recently it has been conducted in France an analysis of a long series of embryo transfers (involving thousands of cases) that has allowed concluding that gestation by embryo is lower with embryos cryopreserved (7.3%) than in fresh embryos (9.2%) (12). It has been observed that the damage experienced by the embryos as a result of freezing-thawing is of the order of 30% (13).

We are currently debating the best time to proceed with the freezing of embryos. Some groups prefer cryopreservation during the pronuclear phase while others favor stages later (14). There is currently an interest in the freezing of blastocysts (15), since the more advanced the development of the embryo later the selection is made, and the best can be frozen so that very few embryos pass this process of selection at this stage Later.

The embryos are frozen in the medium of the culture of tissues that contain, besides, cryoprotectants and sugars to achieve freezing and formation of suitable crystals. In an alternative way, vitrification protocols are used in which the formation of ice crystals is avoided by using high concentrations of cryoprotectants and very fast freezing speeds. Defrosting is done carefully in the presence of adequate concentrations of non-permeable sugars to avoid the overhydration of the cells while the dilution of the cryoprotectant occurs.

Possibly only a percentage, and not very high, of the embryos that have been frozen, are still alive. The effect of the passing of time of cryopreservation is unknown. Although in this state the degrading processes are slowed down, it is predicted that over time they will be altered and that therefore a high percentage of viable embryos have already died. It should be taken into account that the freezing affects more the physical integrity of the living beings that have already low viability, and the cryopreserved are the leftovers that have not been chosen in a first, or second selection of embryos, are those considered less Viable, with possible flaws and less vitality. They also come from the fertilization of unripe ova (product of the induction of multi ovulation) that is known that already of theirs are less likely to develop and to nest. Some also drag the consequences of the genetic alterations of the father since the sperm unable to fertilize were introduced by direct microinjection to the cytoplasm of the ovum, etc.

In any case, there has not been a rigorous analysis of the effects of the freezing,

cryopreservation and thawing of early embryos of mammals. It is serious negligence in human in vitro fertilization practices and the experience of maintaining cell lines cannot be considered extrapolated. It is very different to multiply millions of cells, to freeze them, to thaw some of them after more or less long periods and to grow them again. Many of them do not survive the process, but it is enough to do one to recover the crop. On the contrary, the thawing of an embryo requires that its resuscitation be produced not only by the growth of cells but an organic and differential growth as an organism.

A condition of life in detention, a non-implantable character and a concept of clinical death of the preimplantation embryo.

1. The situation of these human embryos, one or several days old, is of life stopped at the time of freezing. It is common, before proceeding to transfer, to perform an in vitro cultivation of thawed embryos over 24 hours to ensure that development continues, they have to be revived. In the cryopreserved state, the embryo is not simply alive, but paralyzed all its vital processes, waiting for its eventual thawing and resuscitation so that it re-starts its life cycle and can be transferred to the womb of a woman who provides the essential environment for his pregnancy. The fact that these embryos are frozen does not subtract their dignity or integrity as human beings. On the contrary, since its status is even more dependent than that of an embryo in the process of implantation in the maternal womb, these frozen embryos deserve special attention and protection. They have their lives unjustly detained.

2. Some of these human embryos are in fact in a condition that we could call "human embryo not implantable", which refers to those living embryos that cannot be implanted by the absence of a woman who would take them to gestate them and allow them to restart and continue their development. As time goes by, there is a greater chance of being abandoned by their parents and minors of being welcomed by others. Without the hope of being welcomed by a woman these cryopreserved and non-implantable human embryos are not simply alive, but unjustly abandoned to a process necessarily of irreversible deterioration. Cryopreservation only extends a time (unknown how much) the life process of an embryo, one or two or three days, stopped by freezing.

In this situation of the irreversible process of death, it is possible to consider an extraordinary measure of the indefinite maintenance of the cryopreservation. cryopreserved temporary maintenance does not add benefit to the life of the embryo. It only elongates the final phase of life, which is not fair either. Therefore, defrosting them and letting them die is not to kill them actively, but to stop putting an extraordinary medium that only artificially elongates the final phase of life in an irreversible situation.

There is a clear difference between the action of destroying a cryopreserved embryo and defrosting it and letting it die. Destroying it, or killing it, would be after

defrosting to revive the embryo cryopreserved, with the life stopped in its day one or three of life, cultivating so that resume or restart its development and then when it has grown enough (up to 9-12 days) undo to obtain the cells of their internal cell mass from which to derive embryonic stem cells. To let it die daylight of the low temperatures is very different both with the same material act, as with the intention. A life stopped by freezing does not go forward, it does not advance, if it is not reactivated after the mere thawing by a crop under very demanding and concrete conditions. This resuscitative culture should not be done for non-implantable cryopreserved. If they cannot be allowed to be welcomed into a woman, it would not make sense to be revived to kill him later.

It rests in this sense the question of the time of permanence cryopreserved. It is a prudential question, with pros and cons regarding the creation and increase of ethical insensitivity. In my opinion, only the possibility of some form of a donation to be gestated would justify long periods after limits established by law. It is not that they "interfere" in the freezers, nor that it is economically expensive the cryopreservation, but that the passage of time allows accumulation in high number, numbs the consciousness of abandonment of their parents, increases the negligence in their care once you have lost interest in them for procreation for which they were formed, etc., all of which further degrades the social perception of the personal character of the embryos in the preimplant state.

3. The concept of clinical death of the cryopreserved embryo must be considered attentively. For the biology of the embryo, it is not obvious the affirmation that it is not distinguished between the death of the embryo and the permanence with the life of some of its cells. The human individual of several days is alive and exists (although its existence is stopped in time by freezing) or is dead. The cells that make up the internal cell mass will give rise to all organs and tissues as long as they are forming part of the organic living unit that is that person, and only then. If the development process has stopped by freezing it will require to restart and continue to live that that embryo is thawed and revived.

If he died without frostbite or died after cryopreservation or because he was not reanimated after defrosting, he is a human corpse. The cells of the embryo of two or four cells, or those of the inner area of the embryo of eight or more cells are only theoretically and potentially totipotent. But the possibility of artificially producing a new embryo does not imply that they are itself an embryo. They would only be (and for now only theoretically) able to restart a new development, as the twin of the first embryo, in very specific conditions of wrapping in the Pellucida zone, special means of cultivation, insertion in another blastocyst to give a chimera, etc. In such cases, a living embryo would not have been destroyed to take its cells, but a twin, or a chimera, would have been artificially produced from the biological material of the donor corpse embryo. There has been an in vitro production process a new embryo by artificial twining or by fusion.

There is no biological ambiguity in the individuality of a preimplantation embryo.

The organization of the embryo has been shown without a doubt since day one of its existence as a vital unit. The same argument that leads to denying the reality "death of the embryo" by being able to artificially maintain any of its cells with the capacity to give rise to a new being, leads to denying its character as an individual from conception. In each of the initial stages of the existence, each embryo requires a medium and specific interaction very precise to develop in a process of embryonic development that is continuous. Without these essential conditions, the embryo dies, when the vital functions that it possesses then have growth and cellular differentiation around precise axes dorsum-ventral and anteroposterior. This vital function of organized differential growth, in body space and time, had its start in the mutual activation of the gametes in the fertilization that originated the zygote. Stopped life by freezing immediately ceases the vital function that is stopped if after defrosting it does not have the required conditions to restart and then continue the vital process of development. There is in embryonic life a central nervous system that coordinates and maintains the vital unit as it occurs in the fetus or the born. But analogous to how the detection of brain activity allows verifying whether it has already occurred, or not, the death of the individual, the factual impossibility of resuming the process of organic development, is, in my opinion, indicative that the death of the embryo has occurred.

Embryo destination and biomedical research

The authorization of research with human embryos should be restricted to those already clinically and naturally dead. Every human being has an absolute value and cannot be used as a means for any purpose, however noble it may be, or however impaired the life of the latter is. The same ethical principles guiding human research are to be applied to human embryos irrespective of their situation.

The law establishes that both dead and non-viable embryos may be used for research purposes. Not so viable human embryos, which could only be implemented. The ethical and legal problem arises with the fate of the living human embryos left over from the practice of IVF and cryopreserved. The current legislation does not permit its use for research, nor does it contemplate the possibility of its destruction. The only exit envisaged is that of its implantation, and however, it is foreseeable that there are no couples that want to receive them, or there is not at least enough to accommodate all. Given the dilemma of what should be the fate of the living human embryos that have been cryopreserved for more than five years, and which are not going to be implanted on the part of the progenitor, clear proposals have emerged.

First of all, it is essential to maintain the protection of the human embryo that currently underlies under national legislation. Any solution to the dilemma should try to maintain the spirit of protection of the human embryo. In this sense, it should be remarked the prohibition in our current legislation and the Oviedo Convention, of any practice involving the instrumentalization of the human embryo and the express creation of human embryos for research purposes. The act of

assisted reproduction must be changed to restrict the practice of embryo freezing to a maximum; The Ethics Committee report recommends not to produce human embryos for research and not to cryopreserved.

When there is the possibility of using oocyte freezing to ensure fertility in future periods, and when there is the technical capacity to reduce the number of embryos per couple without reducing the effectiveness of the techniques, the accumulation of frozen embryos is a practice that is hardly justifiable, neither from a scientific nor ethical point of view. The reduction of the number has been the object of repeated recommendations on the part of the National Commission of Assisted Reproduction. The maintenance of a lack of control over this practice detracts from rigor and away from good medical practice; and in turn encourages the maintenance of scientific, commercial and media pressure on the use of surplus embryos in the future.

The problem of its fate must be considered an exceptional case to be solved only with the certainty that the door is legally closed to the storage of more surplus embryos and that it is still closed by law the possibility of investigating with viable embryos in a way Destructive to the embryo. It is not possible to speak a solution to the problem of frozen embryos until the repetition of the problem is prevented (modifying the Act of Assisted reproduction): the existence of surplus embryos of which the progenitor couples are not responsible.

The report of the Ethics Committee considers it desirable that the donation of embryos cryopreserved be promoted at present to couples who require them for reproduction purposes. The surplus embryos were produced to alleviate infertility and have the right to be conceived by the biological mother, or an adoptive mother. On a similar line, it is proposed to donate them for prenatal adoption, so that everyone has an opportunity to continue vital development. However, for many, the factual possibility of a massive prenatal adoption is scarce and the assisted fertilization clinics practice donating to other couples on those lists and yet the number of surplus embryos continues to grow.

What to do with those embryos hopelessly surplus?

In the current debate, there is enormous pressure on the line to automatically consider as "non-viable" any human embryo that would have been frozen for five years and not have a woman who would like to receive it. The pressure is exerted with the fallacy that the embryonic stem cells present in the blastocyst stage embryo are necessary to cure serious degenerative diseases. From that perspective, the request for authorization is reiterated.

In this sense, the majority opinion of the National Commission of Assisted Reproduction (with several personal votes against) that was collected in its second report (2000), was that it was allowed to use for research purposes the human embryos that They'll have been frozen for over five years. And the ethics

committee has recommended changes in Spanish legislation to "establish an appropriate legal framework for research with stem cells from surplus human embryos". It is recommended to authorize its use for research purposes, always within strict control criteria and directed to research of a medical nature and that cannot be developed by other techniques and leaving it discarded to seek a mere business Biotechnology.

It is now a matter of settling on the desirability of prohibiting the use of embryonic material or accepting donation for research of cells from dead embryos. The first option would be to thaw all human embryos, let them die and bury (or incinerate) their remains without authorizing the use for research purposes to avoid an embryo-consuming investigation and even the subsequent temptation of to consume embryos for different purposes. Indeed, allowing an investigation, as regulated as it was, runs the risk of slippery slopes: What is now a solution to an exceptional case and should not be repeated, can become repeatable and normal.

On the other hand, it is obvious that although some consider important the embryonic stem cell research know causes and possible remedies for serious and hard diseases, this "great importance" is very relative. In biomedicine, all knowledge is often valuable to health. But it is not human embryonic cells, derived from living human embryos, the only starting point for achieving such knowledge.

Just as the value of an incipient human being is not ponderable concerning any other value and cannot be used only as an instrument, the donation of their cells once their life is finished naturally is, in my opinion, a valid option. Such donations by their parents must meet the same requirements for the donation of body organs. And its use in a perfectly regulated investigation (16).

Regulated research and protected embryos

The regulation of the type and purpose of the investigation that could be carried out with the cells of the non-implantable embryos after defrosting requires as a prerequisite the precise catalog of the cryopreserved embryos; To know its origin and decision of its parents concerning the destiny, the situation in which they were frozen and protocol of the same one, incidences during the period of cryopreservation, etc.

This knowledge is essential to evaluate an important aspect from the ethics of scientific research: the necessary correspondence between the knowledge that is sought and the quality of the starting material. In general terms, it can be said that the cells of a frozen embryo in the early stages of development (2, 8, 16 cells) can be useful for basic research on fertilization and early stages of development. The cultivation of blastomeres extracted from the embryo of 16 or 32 cells could perhaps be differentiated and organized, such as embryonic stem cells from the internal cell mass, to "embryoids bodies" and to give embryonic cell lines. If the embryo was frozen in more advanced stages of development as an incipient

blastocyst, it may be available directly from embryonic stem cells for research. On the other hand, one cannot forget the fact that these cells come from surplus embryos that were produced from the gametes of parents with sterility problems. The influence of possible anomalies of such gametes in a genetic endowment or embryo development may mask results, so rigorous research would require revalidation of results.

It is considered the usefulness of human stem cells to know the development and to understand the basic mechanisms of differentiation and proliferation. It is interesting to note that both in degenerative diseases and in cancer there is decontrol of the processes of proliferation, programmed cell death, closely related. This knowledge is of great importance in biomedicine; now a good part of the data is or can be taken from research with laboratory animals. And it should not be ignored or forgotten that there are stem cells in all tissues of the organism, with a different state of equilibrium between proliferation and differentiation, with different immaturity and ability to grow and great plasticity to become the Different cell types of the organism and to revert to less mature cells, and even to merge with others. Therefore, it is obvious that most of that research, biomedical that required human stem cells, would be carried out in fact with the reserve stem cells of the adult organism. The limitations of obtaining human embryonic stem cells neither stop nor enlighten biomedical research.

Three areas of applied research have been suggested in which, after animal experimentation, it may be of interest to have human embryonic stem cells:

for the development of new therapies based on stem cells. In the last two or three years, it has become clear that a rational regenerative therapy will always tend to provide the organism with what it requires to enhance its regenerative capacities. And in the case of requiring transfer or implantation of differentiated cells, to supply the destroyed by the disease, these must be derived from those of adults. Therapies based on the use of embryonic stem cells are not possible or clinically suitable for their characteristics; The clinical potential of adult stem cells and their availability has displaced embryonic stem cells. Therefore, a scientific policy without ideological prejudices, or economic pressures, should encourage therapies based on the adult organism's stem cell-based auto regeneration.

b) To generate human cell lines that can be used in the preclinical development of new drugs and is toxicology studies.

c) For use as a genetic therapy vehicle.

The stem cells of the adult organism have already obtained cell lines that can be used as embryonic in these studies pharmacological, toxicological and genetic therapy.

In summary, at present, it is not known if there is any biomedical research that

requires the use of human embryonic stem cells. There may be some. In this case, it would be prudent and rational to go to a system that as parthenogenesis allows us to achieve cells of the type of the internal mass of the blastocyst without generating a true embryo. It makes no sense to create biotechnology that splits and develops around the human embryo if you are determined not to start the so-called embryo-producing and consuming medical science.

The problem of the fate of the surplus embryos has no good solution. Legislation should not tolerate further production. To get a legal framework in which the least bad solutions for these cryopreserved embryos can be applied, it will take a while. It will require a lot of prudence and sensitivity for life. And you will need, I think, for someone to be very careful not to destroy the precious good of those lives unjustly detained in the cold. The knowledge that we have today of the first steps of life after conception marks us the way to respect the life of every living embryo, however, cryopreserved and abandoned, as it corresponds to the absolute value of every human being. And at the same time not deny the "usable" cells for that biomedical research, undoubtedly valuable, that cannot be done in any other way. It is a scientific challenge, not political, to engage in biomedical research without agreeing comfortably with a destructive investigation of human embryos. Perhaps, shortly, it is not enough with a simple committee to evaluate the projects and to ensure that the recommendations are on the right track, but that we need a figure with a factual capacity to protect those members of the human family so harshly treated In this society of ours.

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I share the opinions that the Chairman of the Committee, Professor Cesar Nombela, expressed in the presentation of the document (see ABC March 7, 2003). The only option for embryos that are not implantable is to let them die with dignity.

I think that there does not seem to be any more "need" to destroy them than to stop "getting in the way" in the freezers where they occupy a place. The Committee believes that "only when the alternative is destruction would it be acceptable to use their living cells, for investigations that can answer several fundamental questions for the advancement of medical science". This consideration is motivated by the conviction, demanding the rights of the embryo, that the indefinite conservation of those that cannot be implanted, with no possible way out, would be to keep them alive through extraordinary procedures. When they have stopped living, in parallel with how the organs of a corpse are donated, they can donate for research. This statement is not explicitly found in the consensus recommendations of the Committee among its components. And if it is wanted to affirm that it is implicitly it would very possibly have to force the text.