Presidency of the Council of Ministers

NATIONAL BIOETHICS COMMITTEE

BIOETHICAL CONSIDERATIONS ON THE SO-CALLED "OOTIDE"

15th July 2005

1. Premise

Requested to draw up a bioethical opinion on the so-called 'ootides', with particular reference to the ethicality of their freezing during assisted fertilisation procedures, the National Bioethics Committee debated at length on the biological aspects that are today available in scientific literature on human fertilisation which can be obtained with artificial insemination techniques, expressing its opinions in the general description proposed below, which – by the very nature of a document aimed at a wider diffusion – does not highlight a number of details.

However, with regard to the interpretation of the importance of the stages which usually describe fertilisation, contrasting opinions arose which will be mentioned hereinafter.

2. The events

In nature, man's biological cycle – like that of all mammals – begins from germinal reproduction, or that is, from the fusion of two gametes of different sex (fertilisation). This type of reproduction (sexual reproduction) realises the transfer of the genetic features from one generation to the next.

According to the description given by biologists, fertilisation – understood as a process – is characterised by microscopic and submicroscopic-molecular events that are all indispensable and of varying length, which develop continuously, beginning with the close interaction between the spermatozoid and the cellular and acellular linings of the oocyte, continuing with the fusion of the gametes and giving place in a variable time of between 16 and 30 hours to the first cellular division (two-cell embryo).

In vitro fertilisation has made it possible to study the succession of bio-molecular events that intervene in its coming about, at microscopic and submicroscopic resolution level. It is thus possible to establish even a chronological sequence that is summarised in the following table:

Phase	Post insemination time
Penetration of the pellucid zone	Within 30-40 min. p.i. (only standard IVF)
Formation of the PB II	From the 2nd to the 8th hour p.i.
Formation of the PN	From the 3rd to the 12th hour p.i.
Juxtaposition of the PN	From the 5th to the 13th hour p.i.
Replication of chromosomes	From the 8th to the 17th hour p.i.
Disappearance of the PN	From the 15th to the 30th hour p.i.
First cellular division	From the 18th to past the 35th hour p.i.
Total insemination time	From the 16th to past the 33rd hour p.i.

ICSI
Total time of standard IVF From the 18th to past the 35th hour p.i.

Key – IVF: in vitro fertilisation; PB II: second polar globule; PN: male and female pronuclei; ICSI: intracytoplasmatic sperm injection.

In particular:

1. The penetration of the spermatozoid into the pellucid zone of the ovocyte begins as soon as the gametes come into direct contact and takes about 30-40 minutes (only standard: IVF).
2. The fusion of the cellular membranes of the ovocyte and the spermatozoid supposes: the reaching of the perivitelline space by the spermatozoid, the fusion of the plasmatic membranes of the two gametes, the incorporation of the spermatozoid into the ooplasma.
3. The so-called 'cortical reaction', by means of physical-chemical modifications of the mucoprotein layer of the perivitelline space, makes the fertilised ovocyte impenetrable to other spermatozoids.
4. The activation of the oocyte, triggered by the incorporation of the spermatozoid, is manifested by the increased permeability and oscillation of the intracytoplasmatic concentration of the calcium ion (Ca2+) and determines the completion of the meiosis, the emission of the polar globule (PB II), with the consequent haploid configuration of the maternal genetic patrimony which will be formed, together with the haploid genetic patrimony carried by the spermatozoid, the genome of the zygote.
5. The removal of the membrane which surrounds the two male and female nuclei, the decondensation of the respective chromosomes and the formation by means of the use of molecular constituents of maternal origin of a new membranous covering which surround them and limit them clearly constitute an extremely complex process in which the two male and female 'pronuclei' (PN) are formed and become microscopically evident (according to some embryologists this point must be further clarified).
6. Within 3-6 hours of the incorporation of the spermatozoid, starting from the sperm aster, polymerised microtubules are organised in radial array from the male centrosome, which, by means of the transport exercised by suitable cytoplasmatic molecules, permit the approach of the two pronuclei.
7. The two pronuclei, starting from peripheral positions, move centrally and are partly juxtaposed, however remaining separate entities.
8. While the perinuclear membranes dissolve and the centrioles are identified, the chromosomes of the two pronuclei double their own DNA, preparing for the first cellular division (mitosis).
9. When these phases have reached completion, the chromosomes that are free in the cytoplasm but still hooked onto the tubular apparatus of the spindle are lined up on a single plane (metaphysical plate) which contains the diploid chromosomal order resulting from the male and female genetic patrimonies: this the amphimixis phase.
10. The paired chromatids of each chromosome separate and move towards the two poles of the cell beginning the anaphase. The cellular division of this new biological entity, called zygote, begins. At the end of the division two cells are formed, each with its own genome (two-cell embryo).

3. Bioethical evaluations

In the light of the above elements, some members of the NBC give the following considerations:

a) Firstly, in literature the uncertain nomenclature is still to be found characterising the description of the first stages of human development, a phenomenon that at times facilitates the use of the same terms to support different interpretational hypotheses or - on the contrary – the use of different terms (often the coining of money is newly coined, but not of terms) to indicate already known phenomena classified under previous names.

This behaviour is not obviously excluded in the subject considered, nor does it seem unproductive if directed at better defining and indicating the stages and processes that the evolution of research has further characterised; However, it becomes particularly sensitive in cases in which – from the simple change of definition – there can derive consequences in the juridical or ethical field which do not correspond either to the reality of the biological facts nor to the substantial modifications of state.

b) In the case in point being examined – by way of example – the use of the words 'conceived' and 'human being' is generally considered appropriate, both having a wide meaning. They do not interfere with other semantic specifications which – within the conceptual field that such terms suggest – can be adopted for the most precise description of biological facts.

Furthermore, the expressions 'conceived' and 'human being' have the advantage of being used and easily understood in current language, as the reference to the generative act of man (by whatever means it is obtained) is transparent.

The expressions 'ovocyte with 2 pronuclei' (2PN) or also 'ootid' – the latter recently adopted only by some authors – on the other hand appear limited to the indication of a stage of fertilisation; nonetheless, this should not create the impression that – in this stage – it is an inert cell, into which the nucleus of another has simply penetrated.

It is considered more appropriate (owing to reasons that will be discussed later on) to speak of zygote[1] or 'unicellular embryo'.

c) Having made these premises, it must be considered that the studies which have been carried out on the first stages of man's life cycle have advanced not only by means of morphological tests, even the most sophisticated ones, which characterise the first steps of the research, but also by means of biochemical and bimolecular contributions which today are essential for an exact understanding of the characteristic phenomena of the first hours of development of the human being. Therefore, many of the descriptions which – above all in text books or publications for public opinion by media and of a high cultural level – are mainly based on the series of 'apparent' morphological modifications with ordinary means of investigations, appear incomplete if not accompanied by the corresponding bimolecular analyses.

d) Owing to a series of reasons which will be presented during this analysis, it must be considered that a 'reply' to the question about how this

[1] In reality one must speak of zygote only when the chromosomes are situated on the same metaphysical plate.

document came to be can be given in strictly scientific terms through the description of the biological events and the rational reflection on the same.

Obviously, in such a way the ethical considerations cannot be avoided, even if they can be examined and usefully discussed once all the elements that a biological analysis can give are at hand. In any case, it is not a question of making every ethical or juridical position depend on scientific evidence, but of recognising that – in the case in point, that is, in the first hours of development of the human being – the conceptus arouses interest by its nature and biological individuality which is an unavoidable factor for any bioethical or juridical judgement that concerns the life and/or health of man.

From these premises it is considered that the following interpretation of fertilisation can be given:

3.1. The interpretation of events

Even though what has been described can be considered as a series of objectively perceivable and ultimately sharable events by any biologist-observer (albeit the description – based on the data available – was carried out only along general lines, given the nature of this document), the interpretation does not seem to be unanimously shared by the National Bioethics Committee of what is understood as the process of fertilisation in its entirety, even though it is in agreement on a 'diachronicity' of the stages, in any case limited to the space of some hours.

For the members of the NBC who propose this interpretation, the event of the meeting-penetration of the spermatozoid inside the cytoplasm of the ovocyte is the event to be considered fundamental, since it is the one that in space and time joins and literally 'fuses' two gametic cells each having a haploid genetic patrimony and makes a biological 'unit' of them that was not there before, given by the genetic molecular structures carrying the necessary information to lead (changing and interacting with the environment) each phase of the successive development.

Once the sperm has penetrated the ovocyte, a *continuum* of events takes place which continues without the need for further genetic impulses external to the unit itself, as seems to be moreover sustainable considering the inclusion of the ovocyte in a thick glycoprotein membrane (called 'pellucid zone') and the rapid realisation of the 'cortical reaction' of the ovocyte itself, which usually stops the penetration of other genetic material carried by sperm into it.

The fact that this process – which today can undoubtedly be described with greater accuracy than in the past owing to the in-depth analyses, not only morphological but submicroscopic and by molecular biology, that have been acquired– begins with the penetration of sperm and then goes on with perfect continuity is substantiated also by that unitary 'functional' index characterising it in its duration, represented by the immediate onset of oscillations in the cellular concentration of the Ca^{2+}, with higher and more frequent waves at the beginning, more infrequent and with less intensity when the metaphysical plate has been formed.

In short, according to this interpretation, as the two gametes are biologically 'predisposed' to this meeting, all the phases following the penetration of the sperm in the first hours of life of the new 'being' have the same 'need' to take place as they are regulated along a line of development

that appears clearly directed, continuous, progressive, and which at least in natural conditions cannot retrogress to already crossed stages (risk of the interruption of the process and the material dissolution of the entity involved).

This interpretation is not hindered but is rather strengthened by:

- the fact that in the very first phases, the 'penetrated' ovocyte uses its own genetic material (RNA and mitochondrions) accumulated for this purpose for as long as they last, to realise the 'conversion' to a new functional structure.

- phenomena taking place for the remodelling of the biochemical structure of the chromatin of the two pronuclei, of the organisation of the cytoplasmic organelles of the oocyte and other submicroscopic phenomena (only some of which were mentioned in the paragraph 'A description of the events').

Furthermore, this interpretation is corroborated by the widely demonstrated 'co-participation' of factors deriving both from the ovocyte and the spermatozoid in the realisation of the development which – in the space of just a few hours – from the fertilisation of the ovocyte leads to an embryo with two blastomeres.

For the ovocyte factors, one can mention as an example, the substitution of the nuclear envelope without the 'pore complexes' of the sperm head with a membrane whose molecular constituents are maternal, which makes it possible to restore the communication channels (pore complexes) necessary for the metabolic exchanges, with the rapid incorporation and substitution of maternal histones with sperm protamines: this is what leads to the so-called 'male pronucleus'.

For the sperm factors one can mention as an example the giving of the 'centrosome' of paternal origin to the new monocellular complex, which is the active one in the human species: the centrosome is the organelle necessary for the organisation of the fibres (microtubules) of the spindle and the successive activation of the molecular 'engines' which will allow the coming together of the two pronuclei and then the migration of the chromosomes during the course of the first mitotic division of the zygote.

In short, the 'principle of continuity' of the development is applied immediately from the spermatic penetration onwards, and clearly goes over the temporal limit of what is didactically called fertilisation, pervading the whole life of the individual, even though changing in time and according to the age in question.

On the other hand, philosophical reflection offers a further argument in support of the principle of continuity in the measure in which it recognises that the beginning of a human being's life constitutes a 'quality leap' (a passage from not being to being) and that, once such passage has taken place, there are only accidental modifications (quantitative) and not substantial transformations (qualitative).

Lastly, this interpretation is not hindered by the fact that the possibility appears of deviations or standstills in development at various stages (now recorded even in IVF); these events must be considered as errors in the working of an extremely delicate (and still in many ways not completely known) balance of molecular actions.

The significant spontaneous 'selectiveness' of human reproduction has never however been denied; the divergences that are recorded in the evaluation and the interpretation of the extent of the phenomenon in nature must be highlighted, which can hardly be compared with what happens in-vitro, owing to the undeniable artificiality of the in-vitro conditions.

3.2 Ethical and juridical consequences

The members of the NBC who agree with these considerations, share the opinion that the counter posing of the biological phenomena in the interpretation that today can be analysed in the first stages of development of the ovocyte, from the 'spermatic penetration' to the formation of two blastomeres cannot be considered a simple theoretical debate (like many others that exist in scientific activity), since the interpretations adopted are given a different ethical meaning, in relation to the operational choices that could be made for the protection of the embryo.

It seems opportune to remind these members of the elements needed to formulate a reasoned opinion on the subject.

Taking for granted that we are dealing with evaluations concerning the protection to be given to the embryo, those who consider that the entire diachronic process of fertilisation shows a substantial unity in its *telos*, that is the concatenation and articulation of the microscopic, submicroscopic and bimolecular events (which modern technology has made possible to ascertain to a large degree) – such as to lead without solution of continuity to the first mitotic division of the zygote (formation of the first two blastomeres) and then to the segmentation, the differentiation of the cell's destiny and to the successive stages in the continuation of the embryonic development – cannot but accept a unique tutiorist ethic of the human being, as such 'recognised' on the basis of its very 'existence' independently of the stage at which it came about at the moment of observation. And if one accepts that the human being must be recognised and guaranteed dignity and identity – as the Convention on Human Rights and Biomedicine (The Oviedo Convention, 1997) states in article 1 – those who maintain the afore mentioned line of interpretation consider that such conditions are satisfied by the 'penetration' of the ovocyte by the spermatozoid.

The consequent practice deriving from this lies in the fact that any 'manipulation' carried out even during the short diachronic process of fertilisation, which is not directed at the 'good' of the human being on whom it is conducted, exposes the latter to unjustified risks, according to the goals pursued and the rules that any prospective laws, regulations or deontological norms should consider it opportune to apply.

Those who support this line of interpretation consider that such judgement and a precautionary approach – and in the 'tutiorist' case – must be applied, as a rule, to cryoconservation too, the effects of which on the embryo, at least at the moment of the techniques and at the 4-8 blastomere stage, are not without risk and sometimes prove to be harmful.

This does not mean that the data reported in literature should be disregarded, which shows – at present time – the poor 'yield' of the artificial procreation techniques carried out on 'non-penetrated' and cryoconserved ovocytes and – on the contrary – the improved performance (in final terms of 'babes in arms'), should the cryoconservation have been carried out in that

phase defined the ‘two pronuclei stage’, or also by a certain ‘pre-zygote’ or ootide, which showed greater resistance with respect to the ‘non-penetrated’ ovocyte to processes of freezing – thawing.

Nonetheless this notion cannot affect the opinion that every ethical judgement expressed on embryo cryoconservation is also applied to the two pronuclei phase, collocated in the ‘necessitated’ line of the taking place of the natural events without any alteration of continuity.

It must also be considered that – by virtue of the principle of continuity – considering that Act 40/2004 sets down that it is not admissible to produce a ‘reserve’ of morulated embryos (with 4-8 cells, frozen on the 2^{nd} - 3^{rd} day of development from the ‘spermatic penetration’) the fate of which is however uncertain, and in any case represents a strong stimulus for their use in research – the same considerations would be reproposed should a ‘pool’ of frozen ‘2P ovocytes’ be set up, whatever name is given to them.

In conclusion, it must be considered that the solution to these dilemmas has to be dealt with through appropriate and intensified research on the cryoconservation of the ‘non-penetrated’ ovocyte (whether this is ovulated or contained in sections and thin ovary fragments). The cryoconservation of this ovocyte can be granted on plausible medical grounds, moreover in well-defined cases.

It should be remembered that, since the news of a ‘research programme’ had begun to circulate, funded by government contributions to set up a national working group on this issue, and even though the question had not been formally debated in the NBC, the opinions gathered among the members were all positive ones, such as to be defined unanimous as long as ‘non-penetrated’ ovocytes, or ovary sections were concerned.

Today it still remains to encourage the search for this solution within the limits indicated above.

4. Alternative bioethical evaluations

Those members of the NBC come to different bioethical evaluations who consider that, by means of the above described fertilisation process, a ‘generational passage’ is realised which in nature concerns a minority of cases owing to the complexity and delicate nature of the bimolecular interactions and biological events: the fertilizability index in couples of 25-30 is about 25-30% per cycle. The human species has a poor reproductive yield and the formation of a new ‘biological entity’, the embryo created by the parents, foresees a loss of 70-75%. The transition from the gametes to the embryo involves chronologically distinct and successive biological phases which present extensive functional and temporal overlapping which, even though constituting a *continuum*, are not however assimilable at the ontological level.

Scientific knowledge on embryo development has made great progress since the publication of the NBC’s document ‘*Identity and statute of the human embryo*’ (1996), above all owing to the greater resolution of research instruments, but the resulting picture is perhaps more complex and structured, revealing functional networks that are difficult to interpret in which the genomic contribution (the set of genes coming from the parents of the embryo) is progressively and continuously integrated with epigenetic maternal contributions present following conception and at the zygote stage. As could be

foreseen, the interpretation of the biological data updated to 2005 has not however been useful for the formulation of shared ethical options.

The contrast of the two orientations in the interpretation of the biological phenomena relative to the first phases of development of the ovocyte – from the 'spermatic penetration' to the formation of the first two blastomeres – could seem like a simple academic disagreement if a different ethical approach did not correspond with it in relation to the operational choices that could be adopted for the protection of the embryo.

According to the NBC members in support of this different line of interpretation, the process of embryonic development in its first stages involves a complex network of events that is a lot less consequential than is commonly believed. In their opinion, the biological data show that every phase can entail alternative unforeseeable developments, even reversible in the first phases, with an unequal distribution of the competences and functions of the oocytes and spermatozoids, for which reason it would be really difficult to define the moment in which individual life begins.

Adopting an approach of extreme, even excessive precaution, some could reach the point of not excluding (in a probabilistic sense) that such beginning corresponds to the moment in which the zygote is formed. It however remains somewhat problematic that this coincides with the meeting-penetration of the spermatozoid in the cytoplasm of the ovocyte and that from that moment on it is vital to protect the 'unity' that is formed. Such interpretation, which is obviously legitimate, is founded on the 'need' for the process from that meeting-penetration to continue without stopping, irreversibly and in a preordained way. The fact that an individual of our species is the result of the meeting of a spermatozoid and an ovocyte does not however authorise us to conclude that from such meeting a human being is necessarily born: scientific observation instead teaches us that the probability that this happens is rather low, as already stated, that the process is not even necessary, and that certainly in its initial stages there are a multiplicity of options to which are associated probabilities that we do not know.

It is even more important to highlight how the statement made by authoritative members of the NBC that the conceptus 'is one of us' cannot be acceptable to others: we should in fact agree on what of us we want to be present to 'make us individuals' and embryology could then give us the indications to establish the necessary conditions, but not for this reason sufficient ones. Biology in fact gives a mere description of the phenomena without giving them a hierarchy at the ontological and ethical level.

The data offered by biological research on embryo development are not adequate to clearly and authoritatively define what segment of the entire process can be assumed as crucial for the identification of the moment in which the new individual identity is made. In the NBC's document of 1996, '*Identity and statute of the human embryo*', the possibility was highlighted as controversial of being able to settle at a biological level the question of the beginning of the possession of an individual identity (person) by the embryo, and the *re-identification* criterion was proposed from a philosophical point of view, as considered particularly appropriate to establish the individual identity of the embryo: 'Until when is it possible to retrogress to find the point in which to position this individual identity? According to this criterion, the product of conception is recognised the statute of individual starting from the moment in which the capacity for subdivision into two or more embryos is irreversibly lost.

The identification criterion is one that nonetheless expresses a sufficient but not necessary condition. This means that individual identity could exist even if adequate means of ascertaining it are lacking. Therefore, the ontological interpretation of biological data ends up by being influenced by the moral options of the interpreter, or rather by the way in which he consciously feels he should behave before the embryo right from its fertilisation'[2].

The criteria that are each time indicated as being valid to support a certain assumption (continuity of development, appearance of a new genome, loss of totipotence by the embryo, implantation in the womb, appearance of nervous system etc.) have their legitimacy and rationality but are not a sufficient source to assume the beginning of human life with certainty.

It is useful to stress that such insufficiency is corroborated by the observation that the historical and social context greatly conditions the use of biological knowledge. Until recently, when social control was more strict and knowledge of the 'generational passage' insufficient, the phase considered decisive was that of copulation inside marriage. Today, with the possibility of controlling fertility making it independent of the sexual act and with the increase of knowledge on embryo development, some consider that the decisive phase is the fertilisation, while others believe that this period must be moved into a successive phase, like the amphimixis (moment of the formation of the zygote), the moment in which the embryo is no longer dividable, the beginning of the implant, the 14th day from fertilisation (moment in which the absence of the nervous system is still considered certain), or also successive moments. From the scientific point of view there is no reason to privilege either of the phases and the choice with regard to this depends exclusively on different ethical options, insofar as the various possible 'interpretations' depend on the different value given to the different phases of human development.

In conclusion some members of the NBC consider that the interpretative line of events, maintained in paragraph 3 by those for whom the protection of the embryo must be absolute from conception and who share the ethical option of the non-admissibility of the cryoconservation of the 2PN ovocytes, derives from convictions and not only from biological reasons. The same members of the NBC, considering vice versa the rational impossibility of univocally establishing the beginning of human life, maintain that the cryoconservation of such ovocytes cannot be considered morally illicit.

Thus, these members hope for a modification of Act No. 40/2004 for the purposes of allowing the cryoconservation of at least 2PN ovocytes. Furthermore they also stress that the very formulation of the law lends itself to interpretations that could allow such practice even today. The 'conceptus' according to art. 1 is in fact protected by means of specific norms present in various points of the act where there is explicit reference to the 'embryo', or to an undoubtedly post-zygotic phase.

It must be added – even though it may be obvious – that personal use and not commercial use of the cryo-conserved 2PN ovocytes must be guaranteed, as defined by clear rules in the juridical and ethical criteria attaining to the balancing of the interests and rights of the subjects involved, the mother's being foremost.

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[2] "Identity and statute of the embryo" (1996), pp.17.

5. Conclusions

Following extensive debate in working groups and during the plenary sessions, the Committee unanimously agreed on the *factual description of the events* concerning human fertilisation contained in this document in paragraph 1. On the other hand, despite great efforts made in this direction, no agreement was reached on the issue regarding the *ethical interpretation of those same events*, the description of which, as mentioned before, registered no dissent within the Committee. The second line of interpreation, summarised in paragraph 4, obtained the consensus of professors:

Barni,
Battaglia,
Coghi,
d'Avack,
Flamigni,
Gaddini,
Guidoni,
Neri,
Piazza,
Rescigno,
Schiavone,
Umani Ronchi.

The first of the two interpretations, illustrated above in paragraph 3, gained a greater consensus than the other, with the approval of professors:
Amato,

Belardinelli,
Binetti,
Bompiani,
Borgia,
Casini,
D'Agostino,
Dallapiccola,
De Carli,
Di Pietro,
Eusebi,
Federspil,
Ferrari,
Fiori,
Iadecola,
Isidori,
Manni,
Marini,
Palazzani,
Pistella.
Possenti,
Ricci Sindoni,
Santori,
Scarpelli,

Sgreccia,
Silvestrini.

Appendix

Glossary

ALLELE Alternative form of a gene present in one or the other homologous chromosome.

AMPHIMIXIS Reconstitution of a diploid chromosome complement.

HAPLOID Referred to cell or individual in which the number of chromosomes characteristic of the species is halved, as one and one only couple of each chromosome is present. This condition is typical of the sex cells, egg cell and spermatozoid, and is indicated by n. The double structure, 2n, is typical of the somatic cells.

EGG CELL Mature haploid female sex cell.

HOMOLOGOUS CHROMOSOME Single component of a pair of chromosomes, one of paternal origin and the other of maternal origin, generally with the same gene structure.

CROSSING-OVER Exchange of segments of homologous chromosomes and consequently of genes (more precisely 'alleles' – see).

OOPHORUS CUMULUS Set of cells surrounding the egg cell.

POLAR GLOBULE A minute atrophic cell which is formed at the first and second meiotic division during the egg cell maturation. It contains a haploid nucleus.

GONOCORIC Referred to an individual that produces only one type of gametes, male or female. The term is used also to define a sexed reproduction system with separate sexes.

GENETIC OR GENOMIC IMPRINTING Selective activation or non-activation of paternal or maternal genes that takes place during the maturation of the gametes or the first stages of development and is maintained in the somatic cells. For the genes involved only the allele transmitted by one of the parents is expressed, with consequences in the development and the manifestation of a number of hereditary diseases.

OOCYTE Female sex cell in the various stages of maturation to the formation of the egg cell, which joining the spermatozoid at fertilisation gives rise to the embryo.

OOTIDE oocyte with two pronuclei.

PERIVITELLINE SPACE Space between the cytoplasmic membrane of the egg cells and the pellucid zone.

ZYGOTE is the cell that is formed during amphimixis and possesses a chromosomal patrimony in metaphase.

PELLUCID ZONE Membrane surrounding the oocyte and covering the embryo in the first stages of development.

PERSONAL REMARKS

Personal remark signed by Prof. Carlo Flamigni

At least seven different theories[3] exist on the beginning of human life, all formulated by people belonging to the Roman Catholic Church and all very topical. The fact that one of these might be given priority by the Magisterium does not make the others less important: they are all in fact based on the same conceptual elaboration, which foresees a hypothesis of a philosophical type and a confirmation (or indication) of a biological nature. This is an inevitable convention in the presence of truths that are beyond our reach, which makes it necessary for each specific hypothesis to be given the same amount of verisimilitude. From my point of view it is above all common sense (in other cases it can be an act of faith, a philosophical lucubration or a personal interest) which indicates that of all the moments mentioned the one of the formation of a single genome should be preferred, according to the conclusions made by the embryologists whose names are to be found in the attachment.

I find not so much the fact that different opinions may exist rather peculiar, but the way in which their formulation came about. With regard to this I would like to stress that:

1) In many official documents, the Catholic Magisterium has stated that the moment of the beginning of human life is in the formation of the zygote. By way of example, I quote the document entitled 'Instructions on the respect for unborn life and the dignity of procreation: Replies to certain questions of the day', at the point in which it says: 'Thus the fruit of human generation from the first moment of its existence, that is that is to say from the moment the zygote has formed, demands the unconditional respect …'.

And again: 'This teaching remains valid and is further confirmed, if confirmation were needed, by recent findings of human biological science which recognize that in the zygote* resulting from fertilization the biological identity of a new human individual is already constituted'.

2) On page 568 of the above mentioned Instructions there is a footnote that defines the term 'zygote': 'The zygote is the cell produced when the nuclei of the two gametes have fused'. This is not the definitive version of the document (in the Latin version, which is the one that counts, this footnote changes, as we shall see), but it is however very important to understand the meaning of this note. In reality the definition is wrong, since in the human species there is no fusion of the pronuclei (they disappear), but the reference to amphimixis is more than evident. Having realised their error (a fact of which a number of details are known) the drafters remedied it by substituting the explanatory footnote with a new definition: 'The zygote is the cell produced when the nuclei of the two gametes have fused'. In all the debates on this question, that definition, even in its vagueness (*fusio duorum gametum*

[3] The theory of the activated oocyte; the theory of the formation of a single genome; the hypothesis of the activation of the embryonic genome; the theory of the loss of cell totipotence and the capacity to form twins; the theory of the implant; the theory of the primitive embryonic line; the theory of the appearance of nerve cells; the hylomorphic hypothesis.

indicates a rather lengthy biological process, with no further details) it was considered useful to redefine amphimixis correctly this time.

3) In the documents on the ontological statute of the embryo that were presented in the past to the National Bioethics Committee, it was once again the word ‘zygote’ that was chosen to indicate the beginning of human life.

4) In order to avoid misunderstandings on the meaning of the word ‘zygote’, I quote the definition given by Adriano Bompiani: ‘With regard to the beginning of the new being generically defined as ‘conceptus’, widespread opinion among biologists places this event in the fertilisation of the ovocyte, a process that can be divided into various stages, but which takes place over a relatively short period of time and which however gives place to an event: the possession, in the entity that has been formed, of unique and unrepeatable information. To exactly fix the culminating moment within this process, mainstream opinion identifies the beginning of the new entity or being at this stage called zygote in the phase called amphimixis (or syngamy) ’. (A.Bompiani: *Assisted Fertilisation and the ontological statute of the embryo*. In: *Fecondazione assistita - Una proposta di legge da discutere*. Edited by F.D. Busnelli A.R. Genazzani, E. Ripepe - CIC Edizioni Internazionali, Rome, 1997 - 19 - 32).

5) The new theory proposed by some members of the NBC presents, moreover , a number of biological incongruities. For example, the characteristics of this presumed new ‘individual’ adapt to the phase of conception that the embryologists define as that of the ‘activated oocyte’, in which the egg cell becomes impenetrable to the entry of nemasperms. In this case one can say that, continuing the fertilisation process, the genome that will establish itself cannot be different from the one produced by the fusion of the two protagonist gametes. In this phase the female gamete has not however reached the ‘penetrated oocyte’ stage (for which reason it is not yet one single cell, but adjacent and still separate cells) and has not yet expelled the 2nd polar globule.

6) It is evident that to abandon this theory in order to embrace a totally different one requires considerable effort. Hence the use – in my opinion extremely debatable – of ad hoc neologisms like ‘bipronuclear zygote’.

7) The indifference is also peculiar with which well-known biologists and experts of the subject pass from one theory to another, often even forgetting that they have signed ‘compromising’ documents.

8) And lastly the recent tendency to involve biology is rather strange, after official documents had excluded it from the debate as being ‘incapable’. It should be remembered that starting with Claude Bernard a great number of scientists ruled out that biology can intervene in the definition of the ‘notion of life’ and the beginning of human life[3]

CONCLUSIVE DOCUMENT

(Conference: From the oocyte to the blastocyst: the generational passage in man - Rome, 28 September 2004)

The transition of the oocyte in the very first phases of a new human being is a non-instantaneous event which is associated with considerable fundamental biological changes within the oocyte.

Act No. 40 of *19 February 2004* grants the cryoconservation of this cell but expressly prohibits that of the embryo, with the exception of some particular circumstances that come under indispensable conditions. There is no reference, either in the law or in the guidelines (Ministerial Decree 21 of July 2004, Official Gazette No. 191 of 16 August 2004), to the other biological realities that follow one another during the course of this passage and therefore it is not clear if and when the oocyte that began it can be frozen. For the purposes of a better application of the present regulations, the biology experts of human reproduction, lecturers in the Italian faculties of medicine, committed to the training of future doctors and other healthcare workers, gave a technical contribution to the debate by means of a rigorous analysis of the knowledge on the oocyte-to-embryo transition in man, which are summarised as follows:

The oocyte-to-embryo transition in man

The biological cycle of man is characterised by gonocoric sexual reproduction with *fertilisation* being the fundamental process by which the oocyte-to-embryo transition is realised and with it that generational passage which from ‘parents’ leads to ‘children’.

Fertilisation is not an ‘instantaneous’ event and even considering it such only from strictly the cellular point of view, it consists in a process which:

• is triggered off by interactions at short intervals and of various nature between the spermatozoid and the cellular and acellular linings of the egg;

• continues with the fusion of the two gametes each coming from two parents of different sex, the woman supplying the ‘oocyte’ and the man supplying the ‘spermatozoid’;

• terminates, over a time varying from 16 to 30 hours, with the formation of the zygote.

In the dynamics of the process a succession of imbricate events interacting among each other can be seen which, starting with the oocyte, lead to the successive appearance of different biological entities, all having features at the morphological, metabolic and genomic levels.

Among these entities appears the *oocyte with two pronuclei*, defined as ‘*ootide*’ by some authors.

When the expulsion of the second polar globule has taken place, the new genomic structure can be identified in the two haploid genomes, even though these genomes must be still essentially considered genomes of the two parents even if contained in one single cytoplasm. Differently from what happens in other animal species, the pronuclei do not fuse but proceed separately in their

functions and particularly in duplicating their DNA and in dissolving their shell to put their chromosomal structures in common on the same metaphysical plate.

This cell in metaphase is the *zygote*. Only in this phase in fact are the maternal and paternal haploid chromosomal structures joined and coming together form the diploid chromosomal structure typical of the human species.

The creation of the new diploid genome represents the conclusive event of the fertilisation process. This precedes by a very short interval of time the beginning of the development that marks the taking place of the generational passage. This phase is followed by the first segmentation division with the appearance of a bi-cellular entity which is the first of a new unique and unrepeatable genome.

It must be stressed that this beginning does not activate the 'molecular personality' of the new being: the development continues for many hours still, in fact using a programme based on expression molecules of the genome of the two parents even if the maternal one is prevalent.

Lastly, it must be highlighted that, even in man, fertilisation as such is not indispensable for the generational passage and the activation of the development programme. *Parthenogenesis* can in fact take place in which the genome of the new human being derives completely from maternal chromosomes, and *androgenesis* where the genome is only paternal, although these processes do not lead to the birth of a new individual.

***IN CONCLUSION*, the oocyte-to-embryo transition is the result of a succession of events that follow each other in time with broad functional and temporal overlapping. During this transition a peculiar event on which to base the criticality of the generational passage and therefore the beginning of a new human being, is represented by the constitution of the new diploid chromosomal structure and the successive start of the segmentation.**

As I have already stated, in reality this is also my personal conclusion even if my opinion on the beginning of human life is completely different.

If for practical reasons one is forced to state the point of greatest importance in the generational passage of man, this must certainly be indicated in the amphimixis and in the formation of the zygote.