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National Health and Medical Research Council



'Human Embryo' – A Biological Definition





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Medical Research Council**

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Discussion Paper

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PREFACE

At the 154th session of the National Health and Medical Research Council (NHMRC) in September 2004, members requested advice from the NHMRC Embryo Research Licensing Committee on the 'biological' definition of 'human embryo'. Professor Jock Findlay, the NHMRC Embryo Research Licensing Committee Chair agreed that the Committee would prepare advice on the scientific definition of 'human embryo' to inform Council and the review of Australia's *Research Involving Human Embryos Act 2002* and *Prohibition of Human Cloning Act 2002*.

To achieve this, the Biological Definition of Embryo Working Party was established, comprising three NHMRC Embryo Research Licensing Committee members and three other Australian experts. The draft report of the Biological Definition of Embryo Working Party was peer reviewed by Australian and international experts. After endorsement of the draft report by the NHMRC Embryo Research Licensing Committee it was discussed at the 159th session of the NHMRC in December 2005. NHMRC members recommended that the report be released as a discussion paper to provide a wider audience with the opportunity to comment on the biological definition of 'human embryo'. Any readers who wish to comment on this discussion paper, being mindful that it is a **biological** definition of 'human embryo' (see part 1.2 'Objectives of this discussion paper'), should provide their comments by email to: embryo.research@nhmrc.gov.au

or in writing to:

NHMRC Licensing Committee Secretariat
MDP 109
GPO Box 9848
Canberra ACT 2601
Australia

I INTRODUCTION

I.1 CURRENT BIOLOGICAL DEFINITIONS OF ‘HUMAN EMBRYO’

There has been a consensus within the scientific literature that a human embryo is an entity in its earliest stages of development that is less than eight weeks gestation (Geller, 2003; Moore and Persaud, 2003; Jones, 1997). After eight weeks it is then considered to be a foetus. However, there is a difference of opinion as to which points of biological development should be covered by the term ‘embryo’.

Two main schools of thought exist:

1. Broad definition: That a conceptus is an embryo from the moment of its creation (eg fertilisation).
2. Restricted definition: That a conceptus should be referred to as an embryo only after gastrulation, at which time the cells that will give rise to the future human being can be distinguished from those that form extraembryonic tissues (Lee and Morgan, 2001).

Broad definition of ‘embryo’ (from fertilisation to the end of the eighth week)

Proponents of the first school of thought define an embryo simply as follows:

- ‘The developing human during its early stages of development. The embryonic period extends to the end of the eighth week (56 days), by which time the beginnings of all major structures are present.’ (Moore and Persaud, 2003); or
- ‘the product of fertilisation of an oocyte. The term is applied to the conceptus from fertilisation until about the tenth week of gestation [*eighth week after fertilisation*] when most of the organs are developed and the embryo becomes a foetus.’ (Reiss, 1998).

Pollard (1994) has described an embryo in similar words: ‘the union of sperm-and-egg-derived genomes (syngamy) can be considered as the end of fertilisation and the beginning of embryonic development.’ However, as discussed below this does not necessarily say that it is an embryo, just that development towards an embryo has begun.

This broad definition of human embryo (ie the human entity developing from fertilisation until the fetal stage) is commonly used by the general public. Misunderstandings do arise between the community using this general definition and scientists using the second, more restricted definition which is described below.

From a scientific perspective, McLaren (1986) states that the practice of using the term ‘embryo’ for the entire product of the fertilised egg, most of which differentiates before the formation of the primitive streak into tissues that will protect and nourish the future embryo, has led to much confusion in the general community. Similarly, Mulkay (1994) has argued that the term ‘embryo’ for the period prior to gastrulation is misleading and has resulted in governments legislating against ‘embryo research’.

However, it may be simpler to use other specific and clearly defined terms to describe early human development than to attempt to inform the general public regarding the meaning of the term ‘embryo’. In support of this proposition, a number of authors of human embryology textbooks use the term ‘embryo’ in a general way but do not include it in their glossary or attempt to give it a biological definition. Instead, they use defined biological terms, such as embryoblast, to discuss specific cell populations and their developmental fate (Sadler, 2004; Larsen, 2001).

Restricted definition of ‘embryo’ (gastrulation to the end of the eighth week)

A number of researchers prefer the second option, whereby the term ‘embryo’ is used in a more specific and restricted fashion. Johnson and Selwood (1996) describe a human embryo as existing only from the time of gastrulation, which is approximately 16 days post-fertilisation. Prior to this time, the developing entity does not have distinct populations of human embryonic cells. McLaren (1986, 1987) had earlier argued the developing entity up to gastrulation should not be termed an ‘embryo’. Additionally, Jones (1997) has described an embryo as the stage of development between implantation through to the eighth week after fertilisation.

These arguments are supported by Johnson and Everitt (2000) who state that ‘...the first 14-16 days of human development are concerned mainly with the elaboration within the conceptus of various extra-embryonic tissues and their discrete separation from a population of cells, the embryo, that will give rise exclusively to a single foetus. This 14-16 day period is therefore said to be the embryogenic phase of development, ie generating an embryo. Prior to this stage the total product of fertilisation is properly called the conceptus (also called pre-embryo or pro-embryo).’

Clearly, from Johnson and Everitt’s perspective, it would be more appropriate to refer to a human entity from fertilisation till the appearance of the primitive streak as a conceptus. This developmental period is the embryogenic phase as it involves processes that lead to the formation of an embryo. Following the formation of the primitive streak the conceptus consists of embryonic and extra-embryonic parts. This is the embryonic phase during which the embryo is developing. The embryonic phase ends at the end of the eighth week of development, following which the developing entity is referred to as a fetus.

Problems with current biological definitions of 'human embryo'

Two problems remain with the current biological definitions of human embryo.

The first is that they describe a developmental time period during which the term 'human embryo' applies, but do not specifically define the term 'embryo'.

Secondly, definitions of a human embryo normally include those entities created by the fertilisation of a human egg by a human sperm. However, there have been a number of recent technological developments that have made it possible to create embryos by other means, such as somatic cell nuclear transfer (SCNT) and induced parthenogenesis. It is possible that these entities may also be considered as 'human embryos'. Due to these examples and developing technologies, it was considered appropriate to re-visit the biological definition of a 'human embryo'. This may also prove valuable from a legal perspective.

1.2 OBJECTIVES OF DISCUSSION PAPER

The overall objective of this paper is to describe a human embryo from a biological standpoint that takes into account emerging technologies in reproductive science and not in accordance with legal, moral, religious or social views. Therefore, we will focus only on previous 'factual' definitions as described in the scientific literature.

As the definition of a human embryo must reflect the multifactorial processes of development, an approach has been adopted which combines recognition of observed events with potential for further development. This acknowledges that fertilisation and development are not static processes and as such embryo status can only be defined by observation of specific markers. This is analogous to measuring growth and development of humans since birth by age, height or reproductive function.

A definition of the human embryo has been developed in the context of early development and in consideration of the following benchmarks:

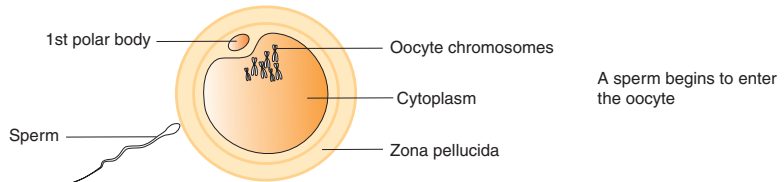
- the entity has an integrated organisation;
- it has a self-directed active disposition to mature to the next stage of development; and
- genetic identity is established from the beginning.

2. KEY EVENTS OF EARLY FERTILISATION AND PREIMPLANTATION DEVELOPMENT

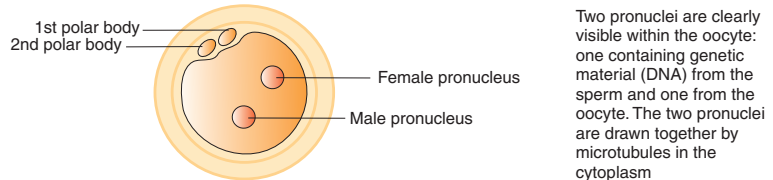
In developing a biological definition of human embryo, it is useful to examine the key events of the naturally occurring mammalian developmental processes (Fig. 1). Some recently developed techniques and horizon technologies do not involve all of the steps that would occur during the naturally occurring developmental process, and will be considered in a later section of this paper on emerging technologies (Table 1).

FERTILISATION

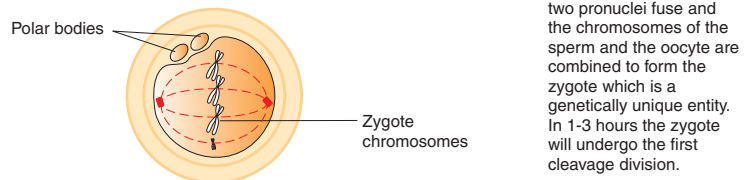
Before fertilisation



During fertilisation



Fertilisation complete



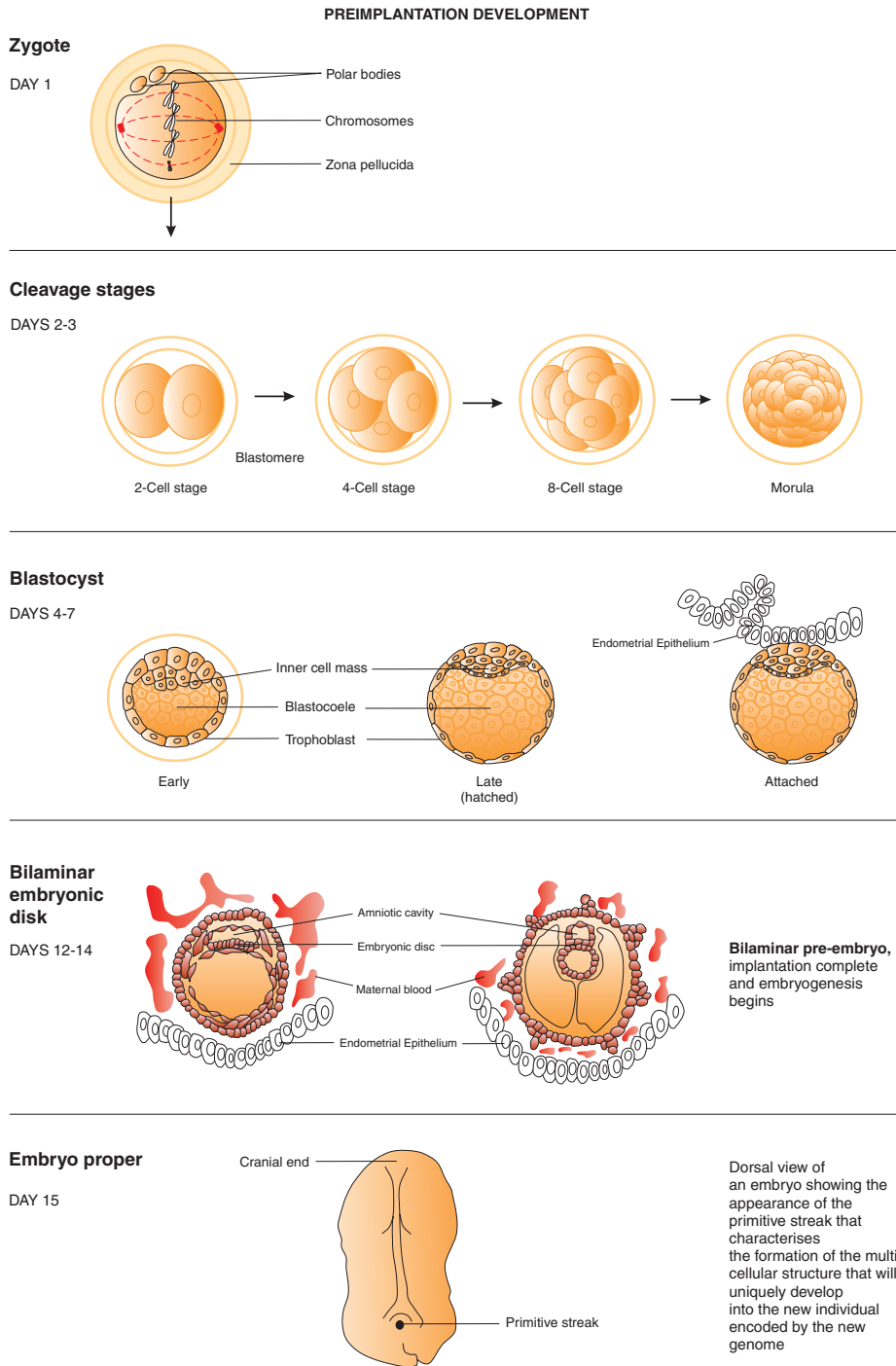


Figure 1. Key events in early fertilisation and preimplantation development of mammals. The stages described in this Figure are based on knowledge of early development in the mouse, and where available, the human. The timescale (in days) refers to human early development. Refer to the text for further details. (Adapted by Sue Panckridge from Dawson, 1993).

2.1 BEFORE FERTILISATION

The sperm cells contain the male genetic contribution to the new genome that will be produced at the completion of fertilisation. Sperm cells are produced during the process of meiosis, which occurs in the testis during spermatogenesis. At the completion of meiosis each sperm contains a haploid genome (one chromatid from each chromosome pair). In order to deliver this genetic material into the oocyte, which contains the female genetic contribution, the sperm must penetrate the zona pellucida and the oocyte membrane. In contrast, the oocyte retains the full maternal chromosome content until ovulation when it initiates meiosis by extruding one chromosome from each chromosome pair to form the first polar body. No conjugation of the two parental haploid genomes has occurred at this stage.

2.2 DURING FERTILISATION

The fertilisation process commences with the sperm initiating the penetration of the zona pellucida. The oocyte then completes the meiotic process and extrudes the second polar body, which contains one chromatid from each chromosome pair. Two pronuclei become visible 9 - 12 hours after the initial contact between the sperm and oocyte. One pronucleus contains the haploid paternally inherited genome while the other contains the haploid maternally inherited genome. Microtubules in the ooplasm draw the two pronuclei together. As long as the pronuclear membranes are intact, the mixing of the genetic material contributed by the sperm and oocyte cannot occur.

2.3 FERTILISATION COMPLETE

Approximately 20 hours after the sperm has entered the oocyte the pronuclear membranes dissolve, allowing the haploid maternally and paternally inherited genomes to combine. The alignment of the chromosomes from the maternally and paternally inherited genomes on the microtubular spindle occurs at 20-22 hours after sperm entry and is known as syngamy. However, as the pronuclear membranes have dissolved, the occurrence of syngamy cannot be visually confirmed on a live entity until the first mitotic division (cleavage) is initiated, which usually occurs 1 – 3 hours after syngamy. From syngamy until the first cleavage the entity is called the zygote.

2.4 CLEAVAGE STAGES

Individual cells (blastomeres) cleave in two approximately every 18 hours. There is no change in the overall size of the cleaving entity as cell size decreases with every cleavage division. The term ‘cleavage stage’ applies to entities in which it is possible to count individual blastomeres. Generally, by the fourth cleavage, cell compaction is occurring and it is no longer possible to count individual blastomeres. An entity that has reached this stage but has not yet formed the central cavity, or blastocoele, is known as a ‘morula’.

2.5 BLASTOCYST

Blastomeres pump fluid to form a central fluid filled cavity (blastocoele) that progressively increases in size. Differentiation occurs with the first appearance of two different cell types. A single layer of trophoblast cells (trophoblasts) surrounds the blastocoele. The inner cell mass (embryoblast) is a clump of cells which forms at one pole of the blastocyst. As the blastocyst expands it hatches out of the zona pellucida. This is followed by interaction between the blastocyst and the uterine wall as implantation begins.

2.6 BILAMINAR EMBRYONIC DISC

The inner cell mass cells form two layers called the primary ectoderm and primary endoderm. The amniotic cavity forms within the primary ectoderm and tissue differentiation begins.

2.7 EMBRYO PROPER

At the beginning of the third week following initial contact between the two gametes, a faint midline structure, the primitive streak, appears in the primary ectoderm. All subsequent embryonic and fetal tissue will develop from this structure. The appearance of the primitive streak is the point at which the body plan begins to become established and signals the commencement of gastrulation.

From a biological perspective the appearance of the primitive streak is the first developmental time point at which a multi-cellular structure is formed that will uniquely develop into the new individual encoded by the new genome.

3. CONSIDERATION OF NATURALLY - OCCURRING PHENOMENA

There are a number of naturally occurring variations in the processes of human embryonic development following fertilisation. In defining a human embryo, it is instructive to consider these variations and ask whether or not the resulting naturally occurring entities can be considered to be embryos.

NATURALLY OCCURRING VARIATIONS WITHOUT CHROMOSOMAL ERRORS

3.1 IDENTICAL (MONOZYGOTIC) TWINS

Identical twins develop by splitting from a single conceptus into 2 separate entities each with the same genome. These entities undoubtedly have the potential for development into healthy human beings. Clearly, they are each independently considered as an embryo and thus the possession of a unique genome cannot be considered to be an absolute requirement in the biological definition of an embryo.

3.2 CHIMERAS

There are reports of chimeric individuals whose cells display one of two different genotypes (see Table 1). These cases are thought to have arisen when two genetically different embryos fuse to form a single embryo containing a mixture of cells from the two genotypes. This can occur both *in vivo* or *in vitro*. The resulting entity then develops to birth.

3.3 PLACENTAL CELLS

Placental cells have the unique karyotype of the developing individual arising from the recombination of the parental genomes. However, without significant laboratory manipulation (see below for discussion on somatic cells) placental cells do not possess the potential for development into an independent organism.

CONSEQUENCES OF CHROMOSOMAL ERRORS

The following paragraphs outline the consequences of some observed chromosomal anomalies. In this discussion, 'karyotype' refers to the description of the genome with respect to the number and condition of autosomes and sex chromosomes. It can be expressed in an abbreviated way in the form 46,XX where 46 refers to the total number of chromosomes present and XX (or XY etc) describes the number and type of sex chromosomes.

3.4 ANEUPLOIDIES

The term aneuploidy refers to the situation where a chromosome is missing or extra copies of individual chromosomes are present. The phenotypes of aneuploidies are variable but some common features are listed below.

Sex chromosome aneuploidies include 47,XXX; 47,XXY (Klinefelter syndrome); 47,XYY and 45,X (Turner syndrome). Other rare sex chromosome aneuploidies include 48,XXXX; 48,XXXY; 48,XXYY; 49,XXXXX; and 49,XXXXY.

Autosomal aneuploidies include Trisomy 13 (Patau syndrome), Trisomy 18 (Edwards syndrome) and Trisomy 21 (Down syndrome)

Entities with groups of cells with the karyotypes listed above would intuitively be considered human embryos. While most are associated with a higher risk of miscarriage, an embryo with one of these genetic constitutions would have the potential to develop as far as the point of birth.

The majority of aneuploidies are not capable of developing to the point of live birth, eg monosomies of autosomal chromosomes are fatal during early embryo development (Larsen, 2001). However some karyotypes may have the capacity for early development beyond the appearance of the primitive streak. Such entities result from the fertilisation of a human oocyte by human sperm and have an organised structure.

If a particular aneuploidy is known to halt development before the appearance of the primitive streak, the question arises as to whether the entity would be considered to be an embryo. The clinical syndrome of 'blighted ovum' or anembryonic pregnancy would appear to fit into this category. Can such entities be considered as embryos in biological terms if they can be demonstrated to have no potential whatsoever for subsequent human development?

3.5 TROPHOBLASTIC DISEASE

This takes three forms: complete hydatidiform mole, partial hydatidiform mole and choriocarcinoma.

Complete mole from fertilisation of an anucleate egg

A complete hydatidiform mole is a pathologic conceptus consisting only of placental tissue. It does not contain observable embryonic or fetal tissue. In the majority of cases, it arises from fertilisation of an anucleate egg by either two sperm or a single sperm which then duplicates its chromosomes. The karyotype is most commonly 46,XX although approximately 10% are 46,XY.

Complete mole from fertilisation of a nucleated oocyte

In rare cases, a complete mole can result from fertilisation of an oocyte by a sperm giving an entity with biparental origin. Although fertilisation has occurred and the resulting conceptus has genes from both parents, maternal imprinting has been lost, giving the functional result of 2 paternal genomes. Recurrent molar pregnancies of this type are familial and appear to be inherited as an autosomal recessive trait.

Partial hydatidiform mole

Discussed below under Triploidy.

Choriocarcinoma

This is a malignant tumour arising from residual cells from a previous pregnancy (or complete mole) which have re-grown and invaded other tissues. Such cells have undoubtedly resulted from fertilisation of a human egg by a human sperm. However they have no organised structure and lack the potential to develop into a viable human being.

3.6 TRIPLOIDY

Triploidy can give rise to two different syndromes (Type I and Type II triploidy) depending on the genetic aetiology.

Partial Hydatidiform mole (Type 1 Triploidy)

A partial hydatidiform mole has a similar morphology to a complete mole but has a different genetic origin. It results from either the fertilisation of an oocyte by two sperm or by duplication of the paternal chromosomes following fertilisation. Fetal tissue is normally present and although a live birth is uncommon, it has been reported. The chromosomal complement is 69,XXX or 69,XXY. As in a complete mole, hyperplastic trophoblastic tissue and swelling of the chorionic villi can occur. Recurrent trophoblastic disease is a possibility, although less common than in the case of a complete mole.

Type II Triploidy

Another form of triploidy involves fertilisation by a single sperm of an oocyte that has not undergone the final reduction division to a haploid (single set) chromosome complement.

In all of these forms of trophoblastic disease, fertilisation, albeit atypical takes place. However, except in the case of the partial mole, no foetus forms and the entities have no potential to develop. Thus, these entities are not generally considered to be embryos.

4. EMERGING TECHNOLOGIES

The last decade has seen the development of reproductive technologies that have raised significant debate in the public and political arena as well as in the scientific community. Among the recent developments, none has gained more attention and generated more debate than SCNT. Dolly the sheep, the first mammal created using SCNT (Wilmut et al., 1997) became an instant star in both the scientific literature and the media.

Other advances have resulted in considerable debate as to whether the entities that can, or could theoretically be generated would fall within current definitions of embryos. Definitions based on a potential for further development might capture entities that might not be covered by definitions that specify a critical early developmental time point (eg completion of fertilisation). For example, since some of the technologies do not involve fertilisation by a male and female gamete, it has been proposed that the entities produced may not be considered to be embryos under some legal definitions (Morgan and Ford, 2004). This has been argued even though in some cases there is the possibility that if placed into the correct uterine environment, a viable individual could theoretically be produced. For instance, to date SCNT has been used to produce cloned human blastocysts from which embryonic stem cell lines were derived but there is no credible evidence of any cloned human beings having been born. However, the fact that in several mammalian species, such as mice, sheep and cows, SCNT has resulted in live births that developed into healthy adult animals would suggest that this may be achieved in humans.

When considering what defines an embryo in the light of recent technological advances it is important that the definition does not become so wide as to encompass human cells or cellular structures that traditionally have not been previously considered to be embryos. For example, it has been argued (Bailey, 2001) that a human somatic cell, the nucleus of which theoretically could become incorporated into a live entity after much manipulation, as demonstrated by the success of SCNT, could be considered a potential embryo. Further, hydatidiform moles which may have derived from an embryo have traditionally not been considered to be embryos.

Table 1 below summarises the developmental potential and genetic constitution of emerging technologies in reproductive science as well as horizon technologies that are based on indications from the literature. For comparison, the naturally occurring reproductive process is also included. Some of the technologies listed have been published as being practically achievable using mammalian species, including humans, whereas others are proposed to be theoretically achievable in humans on the basis of preliminary studies in non-human mammalian species. The table also includes a number of horizon technologies for which there is no support in any experimental system, but which may theoretically be possible if the appropriate methodologies and experimental conditions were developed.

Table 1 – The developmental potential and genetic contribution of entities produced either by natural processes of fertilisation or as a result of emerging technologies in reproductive science

Functional element Reproductive technique	Male gamete	Fertilisation	Syngamy	Cleavage	Morula	Blastocyst	Potential to implant	Gastrulation	Potential to develop into a foetus	Potential for live birth	Genetic contribution	
											Nucleus	Mitochondria
Processes that occur naturally in humans												
Fertilisation – naturally occurring	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Both gamete donors	Oocyte donor
Chimera – embryo fusion ^{1,2}	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Both gamete donors	Oocyte donor
Embryo splitting – monozygotic twins	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Both gamete donors	Oocyte donor
Experimental techniques that have been successfully conducted using human material (shaded boxes indicate theoretical assessments as the entity has not been demonstrated experimentally to proceed to the developmental stage indicated)												
1) Cloning by embryo splitting ^{3,4}	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Both gamete donors	Oocyte donor
2) Somatic cell nuclear transfer (SCNT)– human somatic cell and human oocyte ⁵	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Somatic cell donor	Oocyte donor

Functional element Reproductive technique	Genetic contribution		
	Nucleus	Mitochondria	
3) Pronuclear transplantation – transfer of pronuclei from fertilised human oocyte to enucleated donor human oocyte ⁶	Yes	Yes	Oocyte donor
4) Parthenogenesis – human oocyte activation ⁵	No	Yes	Oocyte donor
5) Chimera – generated by aggregation of individual viable blastomeres obtained from non-viable embryos ⁷	No	Yes	Multiple origin depending on origin of blastomeres
Experimental technique that has been successfully conducted using human and animal material (shaded boxes indicate theoretical assessments as the entity has not been demonstrated experimentally to proceed to the developmental stage indicated)			
6) SCNT – human somatic cell and enucleated animal oocyte ^{8,9}	No	Yes	Human somatic cell donor
	No (enucleated oocyte)	Yes	Animal oocyte donor
	No	Yes	?
	No	Yes	?
	No	Yes	?
	No	Yes	?

Functional element Reproductive technique	Genetic contribution		Potential for live birth	Potential to develop into a foetus	Gastrulation	Potential to implant	Blastocyst	Morula	Cleavage	Syngamy	Fertilisation	Male gamete
	Nucleus	Mitochondria										
Experimental techniques that have been successfully conducted in animal models involving no human material												
7) Gynogenesis – as for pronuclear transplantation but using 2 maternal pronuclei ^{10, 11}	Oocyte donor	Oocyte donor	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
8) Androgenesis – as for pronuclear transplantation but using 2 paternal pronuclei ^{11, 12}	Oocyte donor	Oocyte donor	No	?	?	Yes	Yes	Yes	Yes	Yes	No	Yes
9) SCNT – mouse somatic cell genetically altered to remove implantation potential and enucleated mouse oocyte ¹³	Mouse somatic cell donor	Mouse oocyte donor	No	No	No	No	Yes	Yes	Yes	No	No	No
10) Chimera – injection of mouse blastocyst with mouse embryonic stem (mES) cells	Host embryo or mES cells (but not in same cell)	mES cells and host blastocyst cells (but not in same cell)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Functional element Reproductive technique	Male gamete	Fertilisation	Syngamy	Cleavage	Morula	Blastocyst	Potential to implant	Gastrulation	Potential to develop into a foetus	Potential for live birth	Genetic contribution		
											Nucleus	Mitochondria	
Proposed and theoretically possible experimental techniques (shaded boxes indicate theoretical assessments as the technique has not been published as successfully conducted)													
11) Fertilisation – human gametes generated <i>in vitro</i> from differentiating human embryonic stem (hES) cells ^{14, 15, 16}	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Derived from hES cell used to generate oocyte	Derived from hES cells used to generate sperm
12) Fertilisation – human gametes produced <i>in vitro</i> ¹⁷	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Female human tissue donor	Male and female human tissue donors
13) Human oocytes produced by animals containing human ovarian tissue grafts fertilised with human sperm ¹⁸	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Female human tissue donor	Male and female human tissue donors
14) SCNT – human somatic cell genetically altered to remove implantation potential and enucleated human oocyte (or oocyte generated <i>in vitro</i> from differentiating hES cells)	No	No	No	Yes	Yes	Depends upon genetic alteration	No	No	No	No	No	Human oocyte donor	Human somatic cell donor

Functional element Reproductive technique	Genetic contribution		Potential for live birth	Potential to develop into a foetus	Gastrulation	Potential to implant	Blastocyst	Morula	Cleavage	Syngamy	Fertilisation	Male gamete
	Nucleus	Mitochondria										
15) SCNT – human somatic cell genetically altered to remove implantation potential and enucleated animal oocyte	Human somatic cell donor	Animal oocyte donor	No	No	No	No	Depends upon genetic alteration	Yes	Yes	No	No	No
16) Chimera – injection of hES cells into animal blastocyst	Host embryo or hES cells (but not in same cell)	hES cells and host blastocyst (but not in same cell)	?	?	?	?	Yes	Yes	Yes	Yes	Yes	Yes
17) Chimera – injection of animal ES cells into human blastocyst	Host embryo or animal ES cells (but not in same cell)	Animal ES cells and host blastocyst (but not in same cell)	?	?	?	?	Yes	Yes	Yes	Yes	Yes	Yes

¹ Strain et al., 1998. ² Yu et al., 2002. ³ Footnote 25 in Daar and Sheremeta, 2002. ⁴ Chan et al., 2000. ⁵ Cibelli et al., 2001. ⁶ Zhang et al., 2003. ⁷ Alikani and Willadsen, 2002. ⁸ Chen et al., 2003. ⁹ Chang et al., 2004. ¹⁰ Kono et al., 2004. ¹¹ Surani, 1986. ¹² Barton et al., 1984. ¹³ Meissner and Jaenisch, 2006. ¹⁴ Hubner et al., 2003. ¹⁵ Toyooka et al., 2003. ¹⁶ Geijsen et al., 2004. ¹⁷ Bukovsky et al., 2005. ¹⁸ Gook et al., 2003.

Table 1 allows the identification of a number of key differences between the emerging technologies described and the naturally occurring reproductive processes. From Table 1 it can be inferred that the emerging technologies could produce embryos that:

- have no potential to implant or result in a live birth; and/or
- do not have a contribution of genetic information from a sperm and an oocyte; and/or
- may contain DNA from two different species.

It may be instructive to examine these key differences between entities produced by the naturally occurring reproductive processes and the emerging technologies in order to determine whether they should be defined as a human embryo.

4.1 SHOULD THE POTENTIAL TO PRODUCE A LIVE BIRTH FORM PART OF THE BIOLOGICAL DEFINITION OF HUMAN EMBRYO?

SCNT (reproductive techniques 2 and 6) involves the removal of the nucleus from a donor oocyte and its replacement with the nucleus from a somatic cell. The resulting oocyte is then encouraged to initiate development by processes similar to those that initiate parthenogenetic development. The source of the donor nucleus could be a fully differentiated somatic cell, a pluripotent (eg embryonic) or multipotent (eg adult) stem cell, an embryonic germ cell derived from gonadal ridges, a totipotent cell derived from cleavage stage embryos or a tumour cell (Hochedlinger et al., 2004). Oocytes for SCNT could be derived from a human donor or from an animal species such as rabbit (Chen et al., 2003) or cow (Chang et al., 2004). Oocytes could also be produced *in vitro* as demonstrated by techniques such as differentiation of embryonic stem cells (Hubner et al., 2003, Lacham-Kaplan et al., 2005) or ovarian surface epithelium cells (Bukovsky et al., 2005).

Animal models have demonstrated that SCNT blastocysts have the potential to implant and develop to a live birth (Wilmut et al., 1997). It is therefore reasonable to assume that human SCNT blastocysts also have the potential to develop into a viable individual if placed within the correct environment. However, with the current state of the art it appears that a SCNT blastocyst is likely to have a significantly lower probability of successful development than one created by gamete fertilisation. It should be noted, however, that in the medical research community the key interest in SCNT is not for the purpose of producing cloned human beings but to generate SCNT blastocysts for the derivation of human embryonic stem cells.

Patients undergoing assisted reproductive technology treatments, such as *in vitro* fertilisation and intracytoplasmic sperm injection, often produce pre-implantation embryos that arrest before developing to the blastocyst stage. In these cases the pre-implantation embryo itself is considered to be non-viable. However, one or more of the blastomeres may in fact be viable. It has been demonstrated that transferring viable blastomeres from non-viable pre-implantation embryos into an empty zona pellucida produces an aggregate pre-implantation structure that can develop to the blastocyst stage, from which human embryonic stem cells can be derived (Alikani and Willadsen, 2002). Although it remains to be tested whether such aggregate blastocysts (reproductive technique 5) can implant and form a viable pregnancy, it is theoretically feasible.

In the mouse model significant progress has been made in the generation of gametes from embryonic stem cells (Geijsen et al., 2004, Toyooka et al., 2003, Hubner et al., 2003, Lacham-Kaplan et al., 2005), although the generation of fully functional gametes from embryonic stem cells *ex vivo* has not been demonstrated to be practically successful at this stage. Another approach has been to derive human oocytes *in vitro* from ovarian surface epithelial cells (Bukovsky et al. 2005). It is yet to be demonstrated whether human oocytes produced using these strategies (reproductive techniques 11 and 12) are able to be fertilised and develop to form viable pregnancies. The use of such gametes in fertilisation may result in the development of blastocysts that theoretically have the potential for implantation and forming a viable pregnancy.

Another option being pursued by researchers is the generation of animals that produce human gametes. To date, it has been demonstrated that mice containing a human ovarian xenotransplant can produce human oocytes (reproductive technique 13; Gook et al. 2003). Human gametes could in theory also be made by chimaeric animals produced by injecting human embryonic stem cells into animal blastocysts (reproductive technique 17). The use of gametes produced by grafted or chimaeric animals in fertilisation theoretically could result in blastocysts that are capable of implantation and forming a viable pregnancy.

There have been proposals to genetically alter the nucleus of the somatic cell before transfer into an enucleated donor oocyte in a manner that would remove the implantation potential of any resulting human embryo clones (reproductive techniques 14 and 15). This technique has recently been demonstrated in the mouse model (Meissner and Jaenisch, 2006). Briefly, the donor cells were genetically altered to disrupt the expression of a gene that is essential for the formation of a functional trophoblast. The resultant entities formed inner cell masses from which embryonic stem cells could be derived but were unable to implant into the uterus. It has been argued that this technique, otherwise known as altered nuclear transfer, circumvents the ethical objections to using SCNT for the generation of human embryonic stem cells (Melton et al., 2004, Hurlbut, 2005b, Pacholczyk and Hurlbut, 2005, Hurlbut, 2005a).

Androgenetic and gynogenetic pre-implantation embryos have only a paternal or a maternal genetic contribution, respectively (reproductive techniques 7 and 8). Such uniparental pre-implantation embryos can be created by pro-nuclear transplantation. Androgenetic pre-implantation embryos can also occur without experimental manipulation and result in what is known as a partial or complete hydatidiform mole depending upon morphology and genetic origin. Pathologically indistinguishable hydatidiform moles can also be biparental and are associated with a rare autosomal recessive condition which may result from mutation of a gene, or genes involved in laying down genomic imprints during oogenesis (El-Maarri et al., 2003, Fisher et al., 2004, El-Maarri et al., 2005). Although androgenetic and gynogenetic pre-implantation embryos may be able to develop to form blastocysts and implant, they are not able to establish viable pregnancies.

Parthenogenetic pre-implantation embryos (reproductive technique 4) are generated by the activation of an unfertilised oocyte. These pre-implantation embryos are also uniparental as they have only a maternal genetic contribution. Although they can implant they have limited subsequent developmental potential. In mice parthenogenetic embryos with potential to develop into a viable individual can be produced, but only after a substantial amount of genetic manipulation (Kono et al., 2004). In mice (Mann et al., 1990, Allen et al., 1994) and macaques (Vrana et al., 2003) it has been demonstrated that parthenogenetic pre-implantation embryos can develop to the blastocyst stage and are amenable to the generation of embryonic stem cells. In humans, however, parthenotes are unlikely to develop beyond the first few divisions as the centrioles contributed by the human sperm are required for the formation of a functional centrosome (Pickering et al., 1988).

Most of the emerging technologies summarised in Table 1 produce blastocysts that have the potential to implant and form a viable pregnancy. Indeed, it is likely but not proven that should they be allowed to develop to term, live births would result. It is therefore reasonable to conclude that if these techniques are conducted using human material only, they could produce a human being.

Some of the emerging technologies discussed above produce blastocysts with no potential to form a viable pregnancy. From a purely biological perspective, conducting these techniques using only human material would produce a human blastocyst but not a viable pregnancy or live birth. If the potential to produce a live birth is to be a key element of a definition of human embryo, then androgenesis and gynogenesis (reproductive techniques 7 and 8) would not be considered to be techniques that can produce a human being, even if only human material is used.

The above discussion suggests that the potential to form a new living being may indeed be a useful component of a definition of ‘human embryo’ as it allows the differentiation between emerging technologies that may lead to live births from those that do not.

4.2 SHOULD FERTILISATION AND/OR SYNGAMY FORM PART OF THE BIOLOGICAL DEFINITION OF HUMAN EMBRYO?

A number of the emerging technologies summarised in Table 1 do not involve the contribution of chromosomal DNA by both a sperm and an oocyte or the completion of syngamy (reproductive techniques 2, 4 – 9 and 14 – 15). Some of these techniques (reproductive techniques 2 and 5) might have the potential to produce a live birth. In theory, if these techniques were to be conducted using human materials only, a new human entity could be produced. Given this, it would be expected that these techniques would at some point involve the creation of a human embryo. The use of fertilisation and syngamy in a definition of ‘human embryo’, would eliminate emerging technologies that have the potential (even if theoretical at present) to produce a new human being. Therefore, an absolute requirement for fertilisation and/or syngamy may not be appropriate for a biological definition of human embryo.

4.3 SHOULD THE BIOLOGICAL DEFINITION OF HUMAN EMBRYO EXCLUDE TECHNIQUES COMBINING DNA FROM MORE THAN ONE SPECIES?

It is anticipated that the reproductive techniques described in Table 1 would result in the formation of embryos that would generally be considered to be unambiguously human. However, this could be complicated by emerging technologies that could theoretically result in an individual with a nuclear genome that is human while the mitochondrial genome could be derived from another species, such as a rabbit (reproductive technology 6).

The possibility of developmental problems resulting from mitochondrial heteroplasmy (the presence of different mitochondrial genomes within the same cell) is an unknown quantity (Brenner et al., 2004). This is an unresolved aspect of SCNT as it is possible that cloned embryos will contain mitochondria from different sources; ie associated with the transplanted donor nucleus and also from the recipient host enucleated oocyte.

Another possibility is an individual that contains cells from different species. Injection of genetically altered mouse embryonic stem cell lines into mouse blastocysts is used by researchers to generate transgenic and knockout mice (reproductive technique 10). Since the embryonic stem cell lines are from a different individual to the host blastocyst, a chimera is produced. It is not clear whether this technique could be applied to the generation of interspecific chimeras. Transplantation of whole rat inner cell masses or individual rat inner cell mass cells into mouse blastocysts did not produce any viable live births (Gardner and Johnson, 1975). Therefore, the developmental potential of chimeras created by injecting human embryonic stem cells into a blastocyst from a different species (reproductive technique 17) or

by injecting non-human embryonic stem cells into a human blastocyst (reproductive technique 16; DeWitt, 2002) is unknown.

Some of the techniques included in Table 1 have the potential to produce a new individual with DNA from more than one species. Any technique that could result in a live birth would likely involve the formation of an embryo at some point in the early development process. Therefore, a biological definition of human embryo should not specifically exclude an entity created with DNA from two species.

4.4 SHOULD THE BIOLOGICAL DEFINITION OF HUMAN EMBRYO INCLUDE A DEVELOPMENTAL TIME POINT?

For the past several decades embryologists have been unable to settle on a universally acceptable definition of a ‘human embryo’ or indeed the term ‘embryo’ itself. Given this ongoing dilemma it might be counterproductive to develop yet another definition of ‘human embryo’ that relies solely on a specific developmental time point to pinpoint the demarcation between ‘embryo’ and ‘not-embryo’. Nevertheless, it is questionable whether it is possible to define ‘human embryo’ without making some reference to a developmental time point.

It has been previously argued that the potential for continued development should be a key consideration for any definition of ‘embryo’ (Latham and Sapienza, 2004). It is clear that the discussion presented in this part of the discussion paper fully supports this view.

A more productive approach to the development of a biological definition of ‘human embryo’ may be one that does include a reference to a specific developmental time point, but in the context of the potential for continued development. From a purely biological perspective, the primitive streak is the first appearance of a cluster of cells that will uniquely give rise to the new individual. However, given that the objective of this paper is to develop a definition of ‘human embryo’, the ‘human’ element needs to be reflected in the definition. Based on the biology of the early mammalian developmental processes (see Figure 1) the term ‘human embryo’ is not applicable before the completion of fertilisation of a human oocyte by a human sperm (ie. syngamy) because this is when the new genome of the new individual is created. Prior to syngamy the maternally and paternally inherited genomes exist as two separate genomes.

A definition of ‘human embryo’ based on syngamy, however, excludes reproductive technologies that do not involve the fertilisation of a human egg by a human sperm. Although some of these technologies might result in live births if applied to the human, it is clear from animal studies that others would not. From a biological perspective, setting the definitive time point at syngamy would include entities that have no potential to form a live human individual. A more appropriate approach would be to assess the potential of such entities to develop to, or beyond the appearance of the primitive streak.

The above discussion suggests that a definition of ‘human embryo’ may need to be separated into two components: one for early developmental processes resulting from the fertilisation of a human egg by a human sperm and the second for those resulting by other means.

A final consideration is whether the definition should refer to syngamy, which cannot be visually confirmed until the initiation of the first mitotic division. Given that the aim of this paper is to develop a biological definition of ‘human embryo’ it may be preferable to include a measurable event, such as the first mitotic division.

5. BIOLOGICAL DEFINITION OF HUMAN EMBRYO

After consideration of the issues raised in the preceding discussion, the following biological definition of ‘human embryo’ is proposed:

A human embryo is a discrete entity that has arisen from either:

- (a) the first mitotic division when fertilisation of a human oocyte by a human sperm is complete; or**
- (b) any other process that initiates organised development of a biological entity with a human nuclear genome or altered human nuclear genome that has the potential to develop up to, or beyond, the stage at which the primitive streak appears;**

and has not yet reached eight weeks of development since the first mitotic division.

This definition attempts to combine the aspects of observed stages of development, developmental potential and origin of the DNA contributing to the new individual. It is recognised that this definition creates the possibility of an anomaly whereby an entity which arose from completion of fertilisation of a human egg by a human sperm and, for whatever reason, lacked the potential for future development would be considered as an embryo, while an identical entity that was artificially created would not have the status of an embryo. However, completion of fertilisation of a human oocyte by a human sperm is sufficient to define an entity as a human embryo regardless of any potential, or lack thereof, for future development.

Having arrived at a biological definition of human embryo it is instructive to apply this definition to the emerging technologies in Table 1.

If reproductive techniques 1 – 3, 5, 6 and 11 -13 were to be conducted using human material, then the entities created by these techniques would meet the biological definition of human embryo.

As demonstrated in the mouse model, the entities created by altered nuclear transfer (reproductive technique 9) have no potential to develop to, or beyond the stage at which the primitive streak appears. Therefore, the application of these techniques to the human (reproductive techniques 14 and 15) would not produce entities that would meet the biological definition of human embryo.

From animal experiments it is known that entities produced by reproductive techniques 4, 7 and 8 are able to implant. However, no live births have been produced by androgenetic (reproductive technique 8) or parthenogenetic (reproductive technique 4) means. Although there is a report describing the live birth of gynogenetic mice (Kono et al., 2004), this was successful only after a significant amount of genetic manipulation of the mice. It is not clear whether the entities

produced by these techniques without genetic modifications fail to develop to, or beyond the stage at which the primitive streak appears. Until further information becomes available, it is not possible to conclude whether or not the application of these techniques to the human system would result in entities that would meet the above definition of a human embryo.

There are no reports in the scientific literature of the successful conduct of reproductive techniques 16 and 17 in humans. Although the technique is routinely carried out to generate mice with genetic modifications (reproductive technique 10), this involves different strains of mice, not different species. It is presently unclear whether blastocysts containing embryonic stem cells from a different species will implant and/or develop to the stage at which the primitive streak appears.

6. CONCLUSION

Naturally occurring early human developmental processes as well as emerging technologies in reproductive sciences have been considered in this discussion paper. The deliberations focused on the biology of these processes and technologies. Based on these facts a biological definition of ‘human embryo’ was arrived at. The definition specifies that the term ‘human embryo’ cannot be applied prior to the completion of syngamy, or after eight weeks of development. This definition is also based on the potential to develop to or beyond the first time point at which a multicellular structure that will uniquely give rise to the new individual is formed.

The definition does not specify how much human genetic content an entity must possess before it can be considered to be a human embryo. It is felt that this issue would be more effectively addressed in the future as at present there is limited biological information. Until such time the ‘humanness’ of a genome should be considered on a case-by-case basis.

Many historical definitions of an embryo have been based on appearance of the primitive streak but in the past twenty years the term ‘embryo’ has, by common usage, also encompassed the earlier conceptus. The term ‘human embryo’ has thus been applied to those human conceptuses that have arisen from the completion of fertilisation of a human oocyte by a human sperm.

The biological definition of ‘human embryo’ presented in this discussion paper also acknowledges that emerging reproductive technologies may one day provide alternatives to the presently available reproductive techniques (eg. *in vitro* fertilisation, intra-cytoplasmic sperm injection). From a purely biological perspective it is clear that the application of such technologies would produce new individuals that at some point in the developmental process would have been a human embryo. It was beyond the scope of this discussion paper to consider legal, ethical and moral ramifications of these emerging technologies. However, it is hoped that when relevant experts undertake such considerations they may use this paper as a source of information.

7. REFERENCES

- Alikani, M. and Willadsen, S. M. (2002). Human blastocysts from aggregated mononucleated cells of two or more non-viable zygote-derived embryos. *Reproductive BioMedicine Online*, **5**, 56-58.
- Allen, N.D., Barton, S.C., Hilton, K., Norris, M.L. and Surani, M.A. (1994). A functional analysis of imprinting in parthenogenetic embryonic stem cells. *Development*, **120**, 1473-1482.
- Bailey, R. (2001). Calling Hippocrates! When it comes to human cloning, President Bush should remember: First, do no harm. *Reason Magazine*, November 28.
- Barton, S.C., Surani, M.A.H. and Norris, M.L. (1984) Role of paternal and maternal genomes in mouse development. *Nature*, **311**, 373-376.
- Brenner, C.A., Kubisch, H.M. and Pierce, K.E. (2004). Role of the mitochondrial genome in assisted reproductive technologies and embryonic stem cell-based therapeutic cloning. *Reproduction, Fertility and Development*, **16**, 743-751.
- Bukovsky, A., Svetlikova, M. and Caudle, M.R. (2005). Oogenesis in cultures derived from adult human ovaries. *Reproductive Biology and Endocrinology*, **3**, 17.
- Chan, A.W., Dominko, T., Luetjens, C.M., Neuber, E., Martinovich, C., Hewitson, L., Simerly, C.R, and Schatten, G.P. (2000) Clonal propagation of primate offspring by embryo splitting. *Science*, **287**, 317-319.
- Chang, K.H., Lim J.M., Kang, S.K., Lee, B.C., Moon, S.Y. and Hwang, W.S. (2004). An optimised protocol of a human-to-cattle interspecies somatic cell nuclear transfer. *Fertility and Sterility*, **82**, 960 – 962.
- Chen, Y., He, Z.X., Liu, A., Wang, K., Mao, W.W., Chu, J.X., Lu, Y., Fang, Z.F., Shi, Y.T., Yang, Q.Z., Chen da, Y., Wang, M.K., Li, J.S., Huang, S.L., Kong, X.Y., Shi, Y.Z., Wang, Z.Q., Xia, J.H., Long, Z.G., Xue, Z.G., Ding, W.X. and Sheng, H.Z. (2003). Embryonic stem cells generated by nuclear transfer of human somatic nuclei into rabbit oocytes. *Cell Research*, **13**, 251-263.
- Cibelli, J.B., Kiessling, A.A., Cunniff, K., Richards, C., Lanza, R.P., and West, M.D. (2001). Somatic cell nuclear transfer in humans: pronuclear and early embryonic development. e-biomed: *The Journal of Regenerative Medicine*, **2**, 25 – 31.
- Daar, A.S. and Sheremeta, L. (2002). The Science of Stem Cells: Some Implications for Law and Policy. *Health Law Review*, **11**, 5-13 (see Footnote 25).
- Dawson, K. (1993) Introduction: An outline of scientific aspects of human embryo research. In: *Embryo Experimentation: Ethical, legal and social issues*. Eds.: Singer, P., Kuhse, H, Buckle, S., Dawson, K. and Kasimba, P. Cambridge University Press, New York. 3 - 13.
- DeWitt, N. (2002). Biologists divided over proposal to create human-mouse embryos. *Nature*, **420**, 255.

- El-Maarri, O., Seoud, M., Coullin, P., Herbiniaux, U., Oldenburg, J., Rouleau, G. and Slim, R. (2003). Maternal alleles acquiring paternal methylation patterns in biparental complete hydatidiform moles. *Human Molecular Genetics*, **12**, 1405-1413.
- El-Maarri, O., Seoud, M., Riviere, J.B., Oldenburg, J., Walter, J., Rouleau, G. and Slim, R. (2005). Patients with familial biparental hydatidiform moles have normal methylation at imprinted genes. *European Journal of Human Genetics*, **13**, 486-490.
- Fisher, R.A., Hodges, M.D. and Newlands, E.S. (2004). Familial recurrent hydatidiform mole: a review. *Journal of Reproductive Medicine*, **49**, 595-601.
- Gardner, R.L. and Johnson, M.H. (1975) Investigation of cellular interaction and deployment in the early mammalian embryo using interspecific chimaeras between the rat and mouse. In *Cell Patterning*. Ciba Foundation 29. Elsevier, Excerpta Medica, North Holland. 183-200.
- Geijsen, N., Horoschak, M., Kim, K., Gribnau, J., Eggan, K. and Daley, G.Q. (2004). Derivation of embryonic germ cells and male gametes from embryonic stem cells. *Nature*, **427**, 148-154.
- Geller, E. (Ed) (2003). *McGraw-Hill Dictionary of Scientific and Technical Terms*. 6th edition. McGraw-Hill Book Company, U.S.A.
- Gook, D.A., Edgar, D.H., Borg, J., Archer, J., Lutjen, P.J. and McBain, J.C. (2003). Oocyte maturation, follicle rupture and luteinisation in human cryopreserved ovarian tissue following xenografting. *Human Reproduction* **18**, 1772-1781.
- Hochedlinger, K., Blueloch, R., Brennan, C., Yamada, Y., Kim, M., Chin, L. and Jaenisch, R. (2004). Reprogramming of a melanoma genome by nuclear transplantation. *Genes and Development*, **18**, 1875-1885.
- Hubner, K., Fuhrmann, G., Christenson, L.K., Kehler, J., Reinbold, R., De La Fuente, R., Wood, J., Strauss, J.F., 3rd, Boiani, M. and Scholer, H.R. (2003). Derivation of oocytes from mouse embryonic stem cells. *Science*, **300**, 1251-1256.
- Hurlbut, W.B. (2005a). Altered nuclear transfer. *New England Journal of Medicine*, **352** 1153-1154.
- Hurlbut, W.B. (2005b). Altered nuclear transfer as a morally acceptable means for the procurement of human embryonic stem cells. *National Catholic Bioethics Quarterly*, **5**, 145-51.

- Johnson, M.H. and Everitt, B.J. (2000). *Essential Reproduction*. 5th edition. Blackwell Science Ltd, Oxford.
- Johnson, M.H. and Selwood, L. (1996). Nomenclature of early development in mammals. *Reproduction, Fertility and Development*, **8**, 759-764.
- Jones, R.E. (Ed) (1997). *Human Reproductive Biology*. 2nd edition. Academic Press, San Diego.
- Kono, T., Obata, Y., Wu, Q., Niwa, K., Ono, Y., Yamamoto, Y., Park, E.S., Seo, J.S. and Ogawa, H. (2004). Birth of parthenogenetic mice that can develop to adulthood. *Nature*, **428**, 860-864.
- Lacham-Kaplan, O., Chy, H., and Trounson, A. (2005) Differentiation of ES cells into ovarian like structures. *Human Reproduction*, **20 (Supplement 1)**, i5-i6.
- Latham, K.E and Sapienza, C. (2004) Developmental potential as a criterion for understanding and defining embryos. *Connecticut Law Review* **36**: 1171-1176.
- Larsen, W.J. (2001). *Human Embryology*. 3rd edition. Churchill Livingstone, Philadelphia.
- Lee, R.G. and Morgan, D. (2001). *Human Fertilisation and Embryology: Regulating the Reproductive Revolution*. Blackstone Press, London.
- Mann, J.R., Gadi, I., Harbison, M.L., Abbondanzo, S.J. and Stewart, C.L. (1990). Androgenetic mouse embryonic stem cells are pluripotent and cause skeletal defects in chimeras: implications for genetic imprinting. *Cell*, **62**, 251-260.
- McFadden, D.E. and Kalousek, D.K. (1991). Two different phenotypes of fetuses with chromosomal triploidy: correlation with parental origin of the extra haploid set. *American Journal of Medical Genetics*, **38**, 535-538.
- McLaren, A.L. (1986). Embryo research. *Nature*, **320**, 570.
- McLaren, A.L. (1986). Why study early human development? *New Scientist*, **109**, 49-52.
- McLaren, A.L. (1987). Pre-embryos? *Nature*, **328**, 10.
- Meissner, A. and Jaenisch, R. (2006) Generation of nuclear transfer-derived pluripotent ES cells from cloned Cdx2-deficient blastocysts. *Nature*, **439**, 212-215.
- Melton, D.A., Daley, G.Q. and Jennings, C.G. (2004). Altered nuclear transfer in stem-cell research - a flawed proposal. *New England Journal of Medicine*, **351**, 2791-2792.

- Moore, K.L. and Persaud, T.V.N. (2003). *The Developing Human: Clinically Oriented Embryology*. 7th edition. Saunders, Philadelphia.
- Morgan, D. and Ford, M. (2004). Cell phoney: human cloning after Quintavalle. *Journal of Medical Ethics*, **30**, 524-526.
- Mulkay, M. (1994). The triumph of the pre-embryo: interpretations of the human embryo in parliamentary debate over embryo research. *Social Studies of Science*, **24**, 611-39.
- Pacholczyk, T. and Hurlbut, W.B. (2005). The substantive issues raised by altered nuclear transfer. *National Catholic Bioethics Quarterly*, **5**, 17-22.
- Pickering, S.J., Johnson, M.J., Braude, P.R. and Houlston, E. (1988) Cytoskeletal organization in fresh, aged and spontaneously activated human oocytes. *Human Reproduction*. **3**: 978 - 989.
- Pollard, I. (Ed) (1994). *A Guide to Reproduction: Social Issues and Human Concerns*. Cambridge University Press, Cambridge.
- Reiss, H. (Ed) (1998). *Reproductive Medicine from A – Z*. Oxford Medical Publications, Oxford.
- Sadler, T.W. (2004). *Langman's Medical Embryology*. 9th edition. Lippincott Williams and Wilkins, Philadelphia.
- Strain, L., Dean, J.C.S., Hamilton, M.P.R. and Bonthron, D.T. (1998). A true hermaphrodite chimera resulting from embryo amalgamation after in vitro fertilisation. *The New England Journal of Medicine*, **338**, 166-169.
- Surani, M.A.H. (1986) Evidences and consequences of differences between maternal and paternal genomes during embryogenesis in the mouse. In: *Experimental approaches to mammalian embryonic development*. Eds: Rossant, J. and Pedersen, R.A. Cambridge University Press, Cambridge. 401-435.
- Toyooka, Y., Tsunekawa, N., Akasu, R. and Noce, T. (2003). Embryonic stem cells can form germ cells in vitro. *Proceedings of the National Academy of Science U S A*, **100**, 11457-11462.
- Vrana, K.E., Hipp, J.D., Goss, A.M., McCool, B.A., Riddle, D.R., Walker, S.J., Wettstein, P.J., Studer, L.P., Tabar, V., Cunniff, K., Chapman, K., Vilner, L., West, M.D., Grant, K.A. and Cibelli, J.B. (2003) Nonhuman primate parthenogenetic stem cells. *Proceedings of the National Academy of Science*, **100 (Supplement 1)**, 11911-11916.
- Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J., and Campbell, H.S. (1997). Viable offspring derived from fetal and adult mammalian cells. *Nature*, **358**, 810-813.

Yu, N., Kruskall, M.S., Yunis, J.J., Knoll, J.H.M., Uhl, L., Alosco, S., Ohashi, M., Clavijo, O., Husain, Z., Yunis, E.J., Yunis, J.J. and Yunis, E.J. (2002). Disputed maternity leading to identification of tetragametic chimerism. *The New England Journal of Medicine*, **346**, 1545-1552.

Zhang, J., Zhuang, G., Zeng, Y., Acosta, C., Shu, Y., Grifo, J. and Yat-Sen, S. (2003). Pregnancy derived from human nuclear transfer. *Fertility and Sterility*, **80 (Supplement 3)**, S56.

APPENDIX I BIOLOGICAL DEFINITION OF EMBRYO WORKING PARTY MEMBERSHIP

Biological Definition of Embryo Working Party

Chairperson	Professor Jock Findlay AM (Vic)
Members	Professor Peter Illingworth (NSW) Dr Steven Junk (WA) Dr Graham Kay (Qld) Dr Adrienne Pope (Vic) Dr Leeanda Wilton (Vic)
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The NHMRC Act provides four statutory obligations:

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- to foster medical research and training and public health research and training throughout Australia; and
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The activities of the NHMRC translate into four major outputs: health and medical research; health policy and advice; health ethics; and the regulation of research involving donated IVF embryos, including monitoring compliance with the ban on human cloning and certain other activities. A regular publishing program ensures that Council's recommendations are widely available to governments, the community, scientific, industrial and education groups. The Council publishes in the following areas:

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